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The Genetic Variation and Genetics of the Resistance of Maize (*Zea mays* L.) to the Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte)

Thesis submitted to the
School of Graduate Studies and Research
University of Ottawa
in partial fulfillment of the requirements for the
Ph.D. Degree in the
Ottawa-Carleton Institute of Biology

Thèse soumise à
L'Écolé des Études Superieures et de la Recherche
Universite d' Ottawa
En vue de l'obtention du *Philosphia doctorae* a
L'institut de Biologie d ' Ottawa-Carleton

Acknowledgments

I wish to express my deepest gratitude to all of the people both professionally and personally who made this work possible. To Dr. John T. Arnason whose patience, guidance and profound knowledge provided a beacon along a path often strewn with obstacles. To Dr. Bernard Philogene, who provided many useful suggestions on the final draft. To Dr. Bob Hamilton and Dr. Lana Reid from the Central Experimental Farm who helped design and conduct the field portions of the study. To Rafik Assabgui, Rob Nicol and Dr. Francois Lorenzetti who were an enormous help in the molecular marker field study and who provided many useful suggestions in all aspects of this work. To Luc St-Laurent who translated the abstract into French. To Dr. David Hoisington, Dr. Diego Gonzalez-de-Leon, Dr. Mireille Khairallah, Dr. Jean Marcel Ribault, and all of the staff at CIMMYT Mexico who had the patience and skill to assist me in the molecular marker part of the study. To Dr. Antonio Serratos who was an invaluable help in my acclimatization to a different culture. To Dr. David Bergvinson, who helped me understand maize and maize pests throughout my studies. To my parents and sister who, throughout my life have shown me the value of understanding the world and who instilled in me the deepest respect for all life that shares this blue sphere with us. To my wife Leticia, who left her beloved family and country to join me in life's journey. Thank you all, I could not have done it without you.

ABSTRACT

This study examines the genetics and genetic variation of maize resistance to *Diabrotica virgifera virgifera* LeConte (rootworm). Rootworm resistance is a complex, multi-trait characteristic with inheritance mechanisms not fully understood or explained. In order to better understand the mechanisms of rootworm resistance and the genetic parameters that condition this trait, three studies were undertaken.

The first study examined the genetic variation of components of resistance in twenty three DEKALB Genetics Corporation hybrids. They were assessed for hydroxamic acid content under uniform greenhouse conditions and compared with field assessments over two growing seasons for resistance to the Western Corn Rootworm in terms of root pull resistance, root damage ratings and root lodging. Hydroxamic acid levels of all hybrids were found to be moderately low resulting in low antibiosis. Pearson correlation coefficients determined between all field traits and hydroxamic acid levels failed to show a consistent relationship. The only significant correlation was found for the 1992 growing season between hydroxamic acid levels and root pull resistance after root recovery ($r = 0.83$, $P = 0.04$). It appears that rootworm resistance in these hybrids is achieved mainly through tolerance, not antibiosis. The study suggests an opportunity for improvement of resistance in commercial inbreds through selection for antibiosis and hydroxamic acid content.

In the second study, the combining ability, additive and dominance effects and the heritability of several traits important to rootworm resistance in maize were determined utilizing a set of seven elite inbred lines from Agriculture Canada's breeding program in a diallel study and a set of two inbred lines in a generation means analysis. GCA and SCA effects were found to be significant in the inheritance of hydroxamate content, root weight and plant height. GCA effects were

found to be significant in relation to low root damage ratings. Heritability in a narrow sense as determined by the midparent/offspring regression was found to be 0.34 for hydroxamate content, 0.26 for root damage ratings and 0.35 for plant height. Additive genetic effects were found to be significant in the inheritance of root damage ratings, both additive and dominance effects contribute to the inheritance of plant height and dominance effects were found to be most important in the expression of root weight.

In the third study, two inbred lines were used to develop an F_2:F_3 population to further investigate the genetic parameters governing some of the traits important to rootworm resistance. A generation means analysis was also conducted with this population. Variance components of the F_3 generation were used to determine the heritability in a narrow sense and RFLP molecular markers were used to determine genomic locations that are associated with rootworm resistance traits. The inbred lines used were 93n445 (susceptible) and 118.31 (resistant). 270 F_2:F_3 families were developed for genotyping and phenotyping. Dominance effects were found to be significant in the inheritance of plant height, additive effects were found to be significant in the inheritance of root size and additive and dominance effects were found to be significant in the inheritance of root damage ratings. Heritability in a narrow sense was found to be 54% for root size, 55% for root damage ratings, 84.7% for stalk circumference and 89% for plant height for the inbreds used in this study. The RFLP marker npi239 was found to be significantly associated to stalk circumference, csu58 and csu48 were found to be associated to root size and umc2 was found to be associated with root damage ratings. Rootworm resistance is demonstrated to be a multi-characteristic trait which is inherited in a quantitative manner. It is hoped that the information obtained in this work will aid in the development of more effective rootworm resistance breeding strategies in maize.

RÉSUMÉ

Cette étude porte sur la génétique de la résistance du maïs à *Diabrotica virgifera* LeConte (la chrysomele de l'ouest), un caractère complexe à plusieurs facettes dont les mécanismes ne sont pas compris ou expliqués entièrement. Afin de mieux comprendre les mécanismes de la résistance à la chrysomele et les facteurs génétiques influançant ce caractère, trois études ont été entreprises.

La première étude examine la variation génétique de composantes de la résistance chez 23 cultivars résistants au ver des racines provenant entre autre de la DEKLAB Genetics Corp. Le contenu en acide hydroxamique de ces cultivars a été évalué sous des conditions uniformes en serres et comparé avec des résultats sur la résistance au ver des racines du maïs occidental obtenus en champs sur deux saisons de croissance en termes de résistance à l'arrachage des racines, des estimations de dommages racinaires et de la capacité des racines à maintenir la plante à la verticale. On a constaté que les niveaux d'acide hydroxamique de toutes les variétés sont modérément bas, ce qui se traduit par la faible antibiose observée, celle-ci ayant été déterminée par les coefficients de corrélation de Pearson entre tous les caractères en champs et les niveaux d'acide hydroxamique. La seule corrélation significative, entre les niveaux d'acide hydroxamique et la résistance à la traction des racines après leur rétablissement ($r = 0,83$, $p = 0,04$), a été observé pour l'année de croissance 1992. Il semble que la résistance a la chrysomele dans ces variétés se manifeste principalement par tolérance et non pas par antibiose. Cette étude

suggère que l'amélioration de la résistance de ces variétés commerciales est possible par sélection de caractères reliés à l'antibiose et au contenu en acide hydroxamique.

Dans la deuxième étude, la capacité combinatoire, les effets additifs et de dominance ainsi que l'héritabilité de plusieurs caractères importants de la résistance du maïs au ver des racines a été déterminée d'une part par une étude diallèle en utilisant un ensemble de sept lignées élite pures d'Agriculture Canada et d'autre part par analyse de moyennes de génération en utilisant un ensemble de deux lignées pures. Les effets de capacité combinatoire générale (CCG) et de capacité combinatoire spécifique (CCS) sont significatifs quant à l'héritabilité du contenu en hydroxamate, du poids des racines et de la hauteur de la plante. Il a été constaté que les effets CCG sont significatifs relativement aux estimations de faibles dommages racinaires et que l'héritabilité, déterminée de façon simple par la régression parent / descendant, est de 0,34 pour le contenu en hydroxamate, 0,26 pour les estimations de dommages racinaires et de 0,35 pour la hauteur de la plante. On a observé que les effets génétiques additifs sont significatifs quant aux estimations des dommages racinaires, que les effets additifs et de dominance contribuent à la transmission de la hauteur de la plante et que les effets de dominance sont les plus importants dans l'expression du poids des racines.

Dans la troisième étude, deux lignées pures ont été utilisées pour développer une population F_2/F_3 de façon à étudier en détail les facteurs génétiques contrôlant quelques uns des caractères importants de la résistance au ver des racines. Une analyse de moyennes de génération a également été effectuée sur cette population. Les composantes de la variance des caractères de la génération F_3 mesurés dans le

champ ont été utilisées pour déterminer de façon simple l'héritabilité et des marqueurs moléculaires PLFR ont servi à déterminer les localisations génomiques corrélées avec les caractères de résistance au ver des racines. Les lignées pures 93n445 (susceptible) et 118,31 (résistante) ont été utilisées. Deux cent soixante-dix familles F_2/F_3 ont été développées aux fins de génotypification et phénotypification. On a observé que les effets de dominance sont significatifs dans la transmission de la hauteur de la plante, les effets additifs dans la transmission de la taille des racines et les effets additifs et de dominance dans la transmission des estimations des dommages racinaires. L'héritabilité, déterminée de façon simple, est de 54% pour la taille des racines, 35% pour les estimations des dommages racinaires, 84,7% pour la circonférence de la tige et de 89% pour la taille de la plante dans le cas des variétés utilisées pour cette étude. Des corrélations significatives ont été constatées entre le marqueur PLFR npi239 et la circonférence de la tige, entre les marqueurs csu58 et csu48 et la taille des racines ainsi qu'entre le marqueur umc2 et les estimations des dommages racinaires. Il a été démontré que la résistance à la chrysomele est un trait à plusieurs facettes transmis de manière quantitative. Il est souhaité que les informations obtenues ici aideront au développement de stratégies de sélection de la résistance du maïs à la chrysomele plus efficaces.

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ABBREVIATIONS USED

ANOVA- Analysis of variance

DIMBOA-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one

DIM_2BOA-2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3(4H)-one

DNA- Deoxyribonucleic acid

$F_{1..x}$ -The first and subsequent filial generation in a set of crosses

GCA- General Combining Ability

GLM- General linear model

h^{2-} Heritability in a narrow sense

HMBOA-2-hydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one

HPLC- High pressure liquid chromatography

IPM- Integrated Pest Management

MBOA- 6-methoxy-benzoxazin-3-one

$P_{1..x}$ -The parental generation in a set of crosses

P -The probability of a given event

PCR- Polymerase chain reaction

QTL -Quantitative Trait Loci

r -The Pearson correlation coefficient

RAPD- Random amplified polymorphic DNA

SAS- Statistical analysis software

SCA- Specific Combining Ability

SNK -Student Newman-Kuels multiple comparison test

SSR- Simple sequence repeat

WCR -Western Corn Rootworm

1. Introduction

1.1 The Importance of Maize

Modern agricultural methods have produced substantial gains in the standard of living that humans enjoy, particularly in developed countries. The improvements in agriculture have produced major shifts in the demographics of farming. It is estimated that 80% of the population in North America farmed in the year 1800 and today it is only 4% (Galanat 1992). After wheat and rice, the third most important food crop in the World is maize. Maize provides food for humans and agricultural animals as well as providing a component of a large number of other commodities as diverse as beer, candies, breakfast cereals, wallboard, paints, paper products and pharmaceuticals (OCPA, Ontario Corn Producers Association database). The importance of maize can not be overstated, from a random sample of 10,000 grocery store items, it is estimated that 2,500 of those items utilize maize either directly or indirectly in their production (Statistics Canada, 1997).

Maize production has reached a very high efficiency largely due to the efforts of maize breeders to produce high yield hybrids as well as the introduction of pesticides and nitrogen fertilizers and other agronomic practices. In order to maintain high yields, pesticides have been employed in very large amounts to control several pests of maize. The large scale use of pesticides is now recognized as being very costly, both to the environment and to the farmer. Among the most important insect pests of corn in North America are several species of rootworms; *Diabrotica spp.* The Western Corn Rootworm (WCR); *Diabrotica virgifera virgifera* LeConte is the most damaging species in the United States and Canada (Krysan et. Al, 1986). It is often said that the corner stone of integrated pest management is host plant resistance since it provides pest management in an environmentally acceptable way and in a convenient genetic package, however

relatively few WCR resistant maize inbreds are available (Assabgui et. al 1993). Some of the mechanisms important to WCR resistance have been identified and these traits include hydroxamic acid content of roots (Xie et. al, 1992) and root morphological traits such as root size and recovery after damage (Reidell 1993, Branson, 1989). Integrated pest management is a pest control strategy that utilizes an economic threshold determination in order to ascertain when a pest population is sufficiently dense to justify the use of control procedures. IPM is a strategy that also relies on natural control mechanisms such as natural enemies, weather and crop management. This concept was first developed by Picket (1965) in Nova Scotia, Canada.

This thesis examines some of the genetic parameters of rootworm resistance in maize including genetic variation in rootworm resistance mechanisms of commercial inbreds. In addition, the inheritance of some of the factors important in rootworm resistance is studied through an examination of combining abilities and generation means analyses. An study identifying RFLP molecular markers that are associated with some of the traits important in rootworm resistance is presented and a strategy for developing and breeding for rootworm resistance in maize is proposed.

1.2 Literature Review

1.2.1 Maize

Maize (*Zea mays*:Gramineae) is a major food crop with over 100 million hectares of maize planted worldwide each year. It is Canada's third most important crop after wheat and barley and the most important crop in Ontario. Corn has been grown in Canada for over 800 years with archeological evidence showing the earliest known location of corn cultivation in Cambellville, Ontario. Canada produces a moderate amount of maize with 3.08 million hectares of land devoted to it's production and the majority (70%) of the maize grown in Canada is grown in Ontario. It is estimated that in 1908 Canadian corn

productivity was approximately 3935 kilograms per hectare and today it is roughly 6870 kilograms per hectare (Statistics Canada).

The ancestral origins of maize was the topic of some debate with various species being proposed as that which was domesticated by Mexican-American peoples some 8000 years ago, however the Mexican wild grass; *teosinte* is now generally accepted as being the wild species from which all modern maize was derived (Galanat, 1992). Teosinte and maize have many similarities including: separate male and female flowers, cobs enclosed in protective sheath and a chromosome number of $2n = 20$. As well as the morphological similarities, molecular evidence indicates that the teosinte; *Zea mays parviglumis* from the Central Balsas region of Mexico is the direct ancestor of modern maize (Doebley, 1990). The domestication of maize is so complete that it can not disperse it's own seed, depending on humans to propagate it. Modern maize is an extremely productive species with a single ear producing approximately 1000 kernels (Galanat 1992).

Along with the monoculture planting of maize has come the problem of pests. Humans have to compete with many insects that damage maize and reduce it's yield. These insects include stored grain pests *Sitotroga cerealella* and *Sitophilus zeamais*, borers such as *Ostrinia nubilalis* and *Diatraea spp* insects that damage the maize ears such as *Heliothis zea*, insects that damage the leaves such as *Spodoptera spp.* and *Rhopalosiphum maidis*, and insects that feed on the roots that belong to the *Diabrotica* spp.

Management of the insect pests of maize has been accomplished with the use of insecticides. After substantial increases in pesticide use in the 1960's and 1970's there has been a decline in pesticide use in Ontario over the past two decades with 145.2 tonnes of insecticides per thousand hectares used in 1983 reduced to 60.7 tonnes per thousand hectares in 1993 (Statistics Canada). These reductions are mainly due to a better understanding of insect populations and the ability to determine when and how much

insecticides should be used based on the probability and level of infestation in a given growing season and location.

1.2.2 Maize Root System

The root system of maize develops from the embryonic root or radicle of the kernel. The emerge of the radicle is the result of the elongation of existing cells in the presence of water, and involves few cell divisions initially. After radicle emergence, cells in the apical meristem of the root tip undergo mitosis. The development of the root results in the production of several distinct cell types, the epidermis, cortex, endodermis, pericycle, phloem and xylem. Lateral roots are produced a few millimeters distal from the apical meristem. The maize root system consists of nodes, or distinct rings of root growth around the circumference of the developing root system. In a mature maize plant, the root system typically has seven to ten nodes of roots. Above the surface of the soil, the maize plant also produces brace roots, these large, woody roots provide additional support for the plant, particularly during windy days (Ritchie and Hanway 1984).

1.2.3 Rootworms

Corn rootworms belong to the genus *Diabrotica* of the family Chrysomelideae. There are three main species in North America including *D. undecimpunctata, D. longicornis barberi* and *D. virgifera* (Figure 1-1). The Western Corn Rootworm (*Diabrotica virgifera virgifera)* is the most economically important pest of maize in North America and has become a serious problem in Canada (Chiang, 1973). This beetle is native to North America and was first identified in Kansas in 1868, and was recognized as a pest of corn in 1912 (Krysan, 1986). The Western Corn Rootworm (WCR) adult is a

small beetle about one centimeter in length. A study done in 1986 found that crop yield loss and the use of soil insecticides cost farmers in North America, over one billion dollars in that year (Metcalf 1986). One study revealed that a single insect can reduce a maize plant's yield by one percent and with 30 to 50 insects per plant being a typical infestation it is apparent that potential crop losses could be enormous (Chiang 1973). A study in Ontario found that with even with insecticide use, yield losses due to Western and Northern rootworm averaged 2.5% per larva. In untreated fields over a three year period the yield loss due to rootworm was 0.7 t/ha (Smith 1979). There are costs to the environment as well because the soil insecticides used to control rootworm species may have detrimental effects on non target organisms such as vertebrates (Brandenburg et. al, 1991) and non target arthropods (Weissling et. al, 1991). The initial migration of corn rootworm into Ontario involved the Northern Corn Rootworm; *D. longicornis barberi*, but in areas where Northern Corn Rootworm is found, it has been observed that populations have been replaced or accompanied by the Western Corn Rootworm; *Diabrotica virgifera virgifera.* The Western Corn Rootworm appears to be the more aggressive of the two species, causing more damage to maize plants by the feeding larvae, as well as exhibiting higher fecundity than the Northern corn rootworm (Krysan et. al, 1986).

Western Corn Rootworms are a highly productive univoltine species (Figure 1-2). A single female can lay between 300 and 1500 eggs over it's reproductive life (about 12 days). The eggs are laid in the soil in the late summer to early fall where they diapause. The eggs hatch the following spring when the soil temperature is at least 15 °C. When the eggs hatch the rootworm larvae must immediately find a food source or they will desiccate. The larvae find corn roots by a chemosensory mechanism and are attracted to CO_2 and other volatile molecules produced by maize roots. Rootworms will feed on other plant species but they do not perform as well on plants other than corn, showing a higher mortality and an impaired ability to develop into a reproductively successful adult. The

adult beetles are not as specific in their requirements having the ability to feed on pollen and leaves from plants of the families; Cucerbitaceae, Gramineae and Leguminoseae (Krysan et. al, 1986).

The larvae go through three instar stages during which time they feed on the corn roots causing crop losses. The damage ranges from surface scarring of the roots to complete consumption of the root mass, killing the plant. If the plant is not killed, the root damage may lead to the lodging of the plant, primarily due to lack of sufficient support during windy days (Figure 1-3). The feeding activity of the larvae may also enable pathogenic fungi and bacteria to have access to damaged cells and cause the roots to decay. The length of time from hatching to adult emergence is 10 to 12 weeks depending on the temperature of the soil. Once the adults emerge they feed on the leaves, silks and pollen of the corn plant. The damage done by the adult beetles is not as severe as that done by the larvae but can lead to yield losses when the beetles eat the silks required for pollination (Figure 1-4).

Figure 1-1. An adult *Diabrotica virgifera virgifera* beetle on the edge of a maize leaf. Actual size of beetle is approximately one centimeter in length.

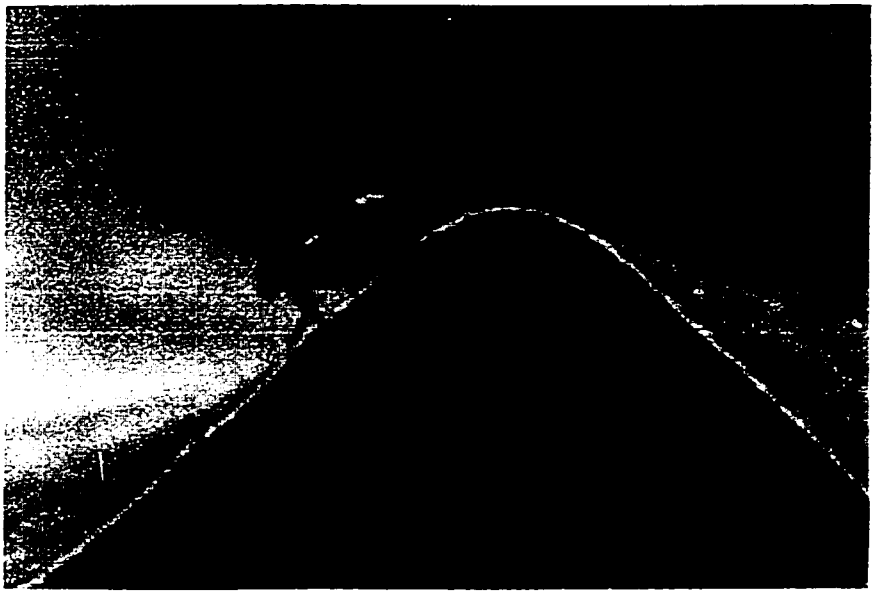


Figure 1-2. The Life

Cycle of the Western Corn Rootworm

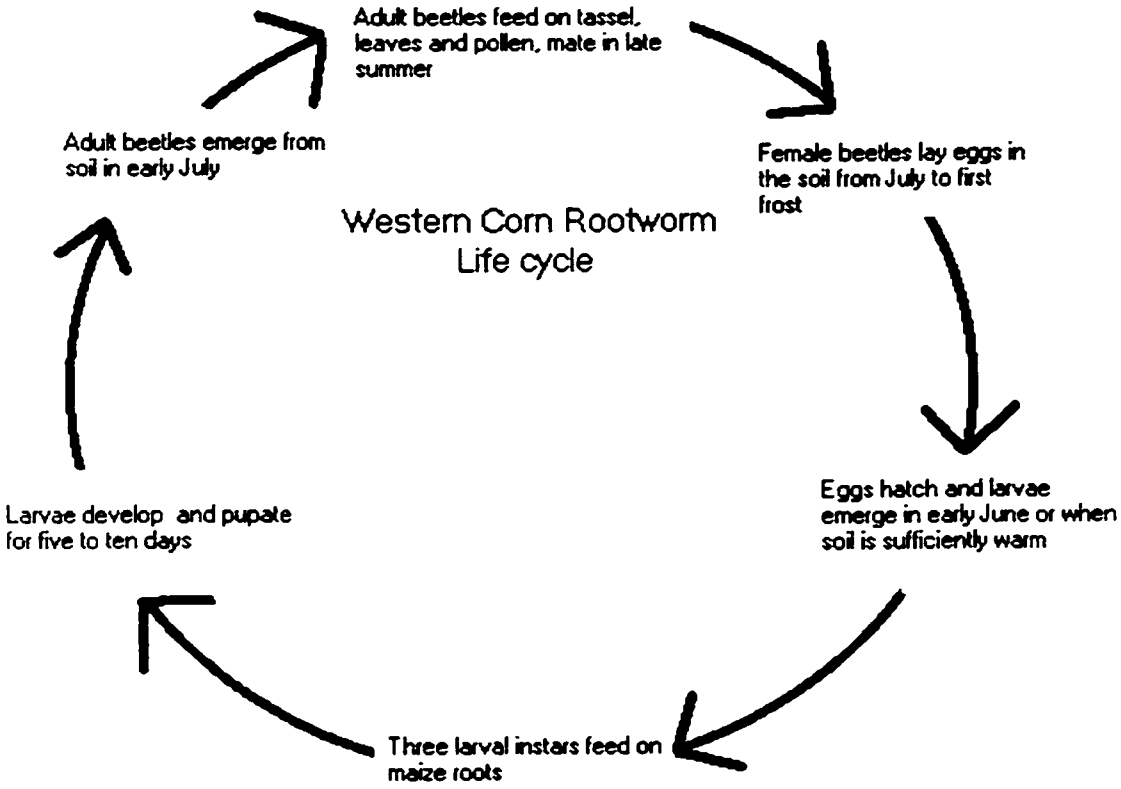

Adult beetles feed on tassel, leaves and pollen, mate in late summer
Female beetles lay eggs in the soil from July to first frost
Eggs hatch and larvae emerge in early June or when soil is sufficiently warm
Three larval instars feed on maize roots
Larvae develop and pupate for five to ten days
Adult beetles emerge from soil in early July
Western Corn Rootworm
Life cycle

Figure 1-3. The damage to a maize root system done by larvae *of Diabrotica virgifera virgifera*. The root system on the left has no root damage, corresponding to a root damage rating of 1. The root system on the right is extensively damaged, and corresponds to a root damage rating of 9 on the scale of Welch (1977).

Figure 1-4. The progression of damage causing failure of seed set due to consumption of silks required for pollination. The ear on the left suffered no silk damage, the ear on the right suffered greater than 90% seed set failure due to consumption of silks by adult Western Corn Rootworm.

1.2.4 Rootworm Control Strategies

Large quantities of soil insecticides are used to control rootworm, in fact more insecticide is used to control Western and Northern corn rootworms in North America than any other insect (Pike et. al 1991, Suguiyama and Carlson 1985). Resistance to insecticides develops fairly quickly in rootworms and many insecticides are no longer used due to their lack of efficacy (Meinke et. al 1998, Chio et. al 1979). The magnitude of the rootworm problem observed today is most probably a direct result of the introduction of chemical insecticides. When the WCR was first identified as a pest of maize the insects did not have a migratory habit, and the fecundity of the females was approximately 300 eggs per insect. With the advent of insecticide control, an insecticide resistant phenotype emerged that also expressed a migratory behavior and large increases in egg production, so this insect can be viewed as a 'man made pest' with the aggressive pest we now observe being a direct result of selection pressure due to insecticide use (Krystan et. al, 1986).

Along with the use of insecticides, crop rotation is recommended as an effective means of rootworm control, Rootworms that emerge in a field where there is no maize will have a poor chance of reaching maturity and mating. This system is becoming less effective due to changes in the biology of the insect. Many reports have demonstrated rootworm's ability to extend their diapause from one to two years of overwintering (Gray et. al 1996, Ostlie 1987, Krysan et. al, 1986), thus effectively circumventing crop rotation practices. This extended diapause is a response to the photo/thermoperiod experienced by the parental generation through mechanisms not well understood (Beck 1980).

Rootworms have a few natural enemies. Birds, nematodes, parasatoids and a species of fungus are known to utilize rootworms as a food source. There is presently no system in place that utilizes these natural enemies as control agents (Levine et. al 1991) . Recently a toxin produced by *Bacilus thuringensis* (*Bt*) has been discovered that is specific to Coleoptera (Slaney et. al 1992). The crystal protein *Cry*IIIA is effective against rootworm but seems to have less toxicity than Lepidopteran *Cry* proteins. There has been much interest in the toxins produced by *Bt* and these toxins appear to be quite effective, at least for the short term, but concerns over the development of insect resistance to *Bt* toxins suggests that this strategy should be applied carefully within a control program that minimizes insect adaptation. A stable *Bt* system for rootworm control is under investigation and there is good potential for developing rootworm resistant maize inbreds utilizing *Bt* toxins in transformed maize (Rupar et. al 1991, Donovan et. al, 1992, Kuo et. al 1996). Transformation of maize, as with other species involves the introduction of foreign genes into the genome of the maize plant in order to express a gene product not normally present. In the case of maize transformed with the *Bt* toxin, the maize plant would express the toxin and insects attempting to feed on the plant would suffer mortality from the presence of the toxic protein.

The lack of an effective biocontrol and the problems associated with insecticide resistance in rootworms and the decline in the efficacy of crop rotation has stimulated research into the maize plants own natural defenses as a possible control strategy.

1.2.5 Plant Resistance to Insect Pests

The natural mechanisms that plants use to defend themselves against insect pests were defined by Painter (1951) to consist of three mechanisms; 1) tolerance is the ability of the plant to survive insect damage by withstanding the damage or recovering after damage has been done. 2) antibiosis results in a negative effect on the insect that feeds on the plant through some type of toxin that interferes with the metabolism, digestion or reproduction of the insect and 3) antixenosis is the quality that makes the insect prefer not to utilize the plant as a food source or oviposition site. Tolerance is considered to be due to morphological characters such as waxiness, hairiness, tissue toughness, or other traits that interfere with the capability of the insect to feed upon the plant. Antibiosis and antixenosis are considered to be due to secondary metabolites that have anti-feedant, toxic or anti-oviposition quality which are produced by the plant as an evolutionary response to insect feeding.

The role of the secondary metabolites in plants was the topic of some debate with various explanations given for their existence. It was thought in the past that they were simply byproducts or waste products of primary metabolism but many well reasoned arguments demonstrate that these compounds have a clear function as plant defense chemicals, for example Ehrlich and Raven's (1964) important work on the coevolution of butterflies and plant secondary metabolites.

The chemical defenses of plants represent a truly diverse array of compounds that are generally classified as nitrogenous and non-nitrogenous toxins for the purpose of ecological theories of defense (Harborne, 1988). The nitrogenous toxins include molecules such as hydroxamic acids, non-protein amino acids, cyanogenic glycosides,

peptides, glucosinolates, and alkaloids. Some molecules belonging to this class include caffeine, cocaine and what is thought to be one of the most toxic compound known to science; ricin (fatal to humans in nanogram quantities). The non-nitrogenous toxins include molecules such as the sesquiterpene lactones, cardiac glycosides, saponins, furanocoumarins, polyacytalenes and the phenolics. Some of the toxins in this group include rotenone and tetrahydrocannabinol.

An understanding of the chemical defenses of plants in general and maize plants in particular assists in the development of integrated pest management strategies which are essential for the continued utilization and development of sustainable agricultural systems.

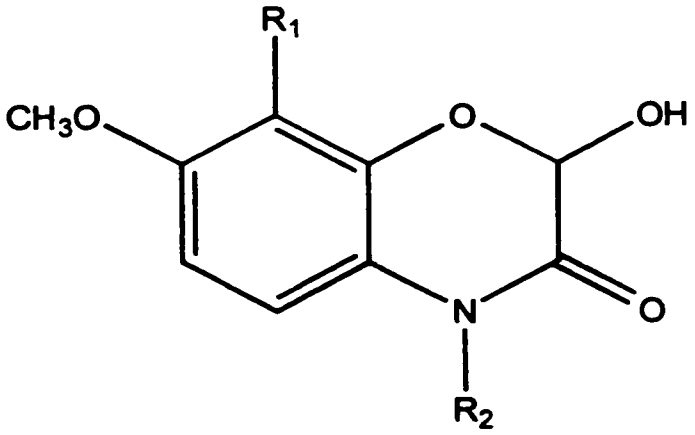
1.2.6 Mechanisms of Rootworm Resistance in Maize

Maize is known to have several strategies for resisting rootworm damage. Branson (1983) investigated maize root morphology and physiology as important factors in rootworm tolerance. It was found that maize plants with large roots that regrow quickly after damage are more likely to survive an attack. Branson also suggested that there was some unidentified antibiosis factor in resistant inbreds (Branson, 1983).

The antibiosis factor referred to by Branson was identified as being due to hydroxamic acids (Xie et. al 1990). Hydroxamic acids are a large group of compounds that all possess the hydroxamic group or -CON(OH)-. These compounds are most commonly found in microorganisms where they act as antibiotics (Niemeyer 1988), but hydroxamates are also synthesized by higher organisms such are plants belonging to the grass family. In corn root extracts there are approximately 15 hydroxamates known (Woodward et. al, 1979) including 1,4-benzoxazin-3-ones and the benzoxazolinones (Klun 1967). The most active of these compounds is 2,4-dihydroxy-7-methoxy-1,4-

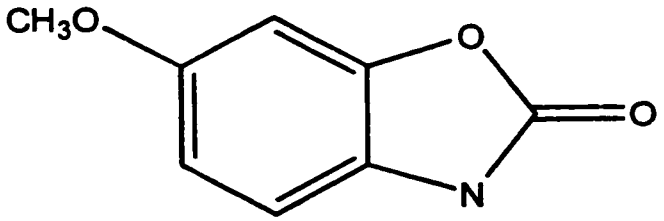
benzoxazin-3-one (DIMBOA). The main degradation product of DIMBOA is 6-methoxybenzoxazolin-3-one (MBOA). High pressure liquid chromatography analysis of corn root extracts reveals the presence of the four main hydroxamate compounds related to insect resistance (Figure1-5). DIMBOA glycoside is stored in vacuoles in plant cells. When the cells are damaged by the feeding action of insects the glycoside is released from the vacuole and is converted to the aglycone by the enzyme ß-glucosidase (Niemeyer 1988). It is the aglycone which is the active form. Many studies have demonstrated that DIMBOA acts as a feeding deterrent and toxin in maize against European Corn Borer (Housman et. al 1992, Klun et. al 1967). When DIMBOA is fed to *Ostrinia nubilalis* (corn borer) larvae in prepared diets the insects weight gain is slowed and it has been shown that DIMBOA inhibits the activity of the gut proteolitic enzymes chymotrypsin and trypsin. MBOA has also been shown to inhibit trypsin. When insects feed on plants containing these compounds the insects have difficulty obtaining proper nutrition from the meal due to the fact that their digestive enzymes have been compromised.

Figure 1-5. The major 1,4-benzoxazin-3-ones and the benzoxazolin-2-one found in maize root extracts



1,4-BENZOXAZOLIN-3-ONES

R1	R2	Abbreviation
H	OH	DIMBOA
MeO	OH	DIM_2BOA
H	H	HMBOA



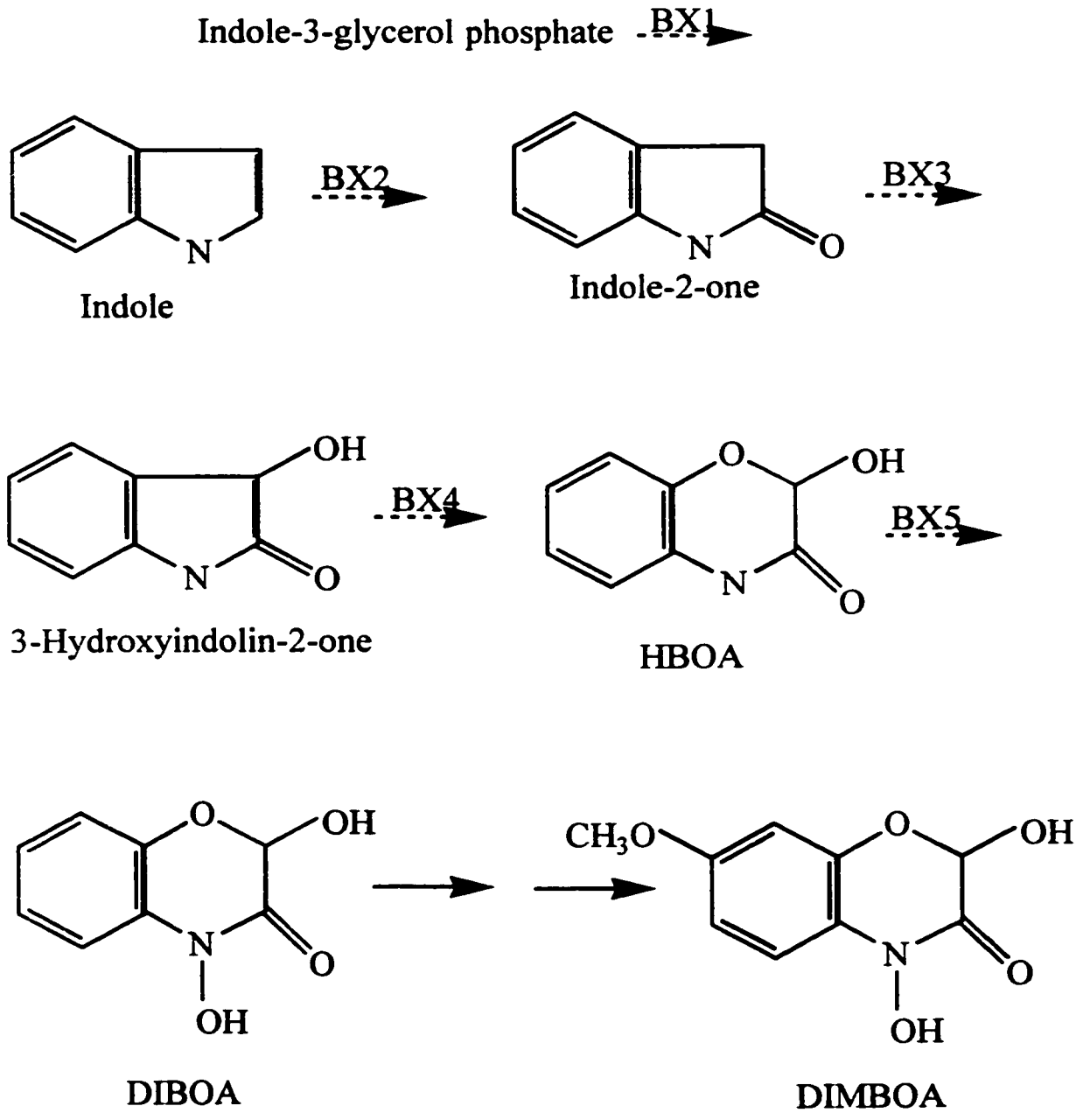
BENZOXAZOLIN-2-ONE

(MBOA)

The biosynthesis of the hydroxamates in maize is now fairly well defined, with recent publications establishing the synthetic origin of the atoms that make up these molecules (Figure 1-6). The hydroxamates obtain a large portion of their structure from the shikimic acid pathway, a well defined biosynthetic pathway that plays a major role in plants and is responsible for the synthesis of many essential amino acids as well as secondary metabolites such as phenolic compounds (Niemeyer 1988). The oxygen atoms in the hydroxamates have been demonstrated to originate from molecular O_2 (Glawischnig et. al, 1997). Five genes have been found to be essential in the biosynthesis of the hydroxamates that all cluster on the short arm of chromosome 4. These *Bx* genes code for products that are each involved in a step in the conversion of the various precursors to DIMBOA, starting with Indole-3-glycerol phosphate, a molecule common to both primary metabolism and the hydroxamic acid pathway (Frey et. al, 1997). *BX1* produces an enzyme that results in indole from indole-3-phosphate, *BX2* through *BX5* all code for enzymes in the CYP71C family of P-450 dependant monooxygenases that each play a role in adding and modifying oxygen atoms in the DIMBOA molecule (Frey et. al. 1997).

Several studies have established that those maize inbreds high in DIMBOA exhibit resistance to WCR (Xie et. al 1992, Assabgui et. al 1995). When trials were conducted with greenhouse grown plants infested with WCR, the high hydroxamate inbred resulted in a higher mortality rate, longer development time, inferior adult emergence, weight, and size of beetles that had fed on low hydroxamate plants (Xie et. al 1992). When DIMBOA is applied exogenously to the insect the effects are toxic, with an LC_{50} of 150 ppm.

Figure 1-6. The biosynthesis of DIMBOA. Figure adapted from Frey et. al. 1997


Indole-3-glycerol phosphate
BX1
BX2
BX3
N
O
Indole
Indole-2-one
OH
BX4
BX5
3-Hydroxyindolin-2-one
HBOA
CH3O
DIBOA
DIMBOA

1.2.7 Genetics of Host Plant Resistance

The study of the genetic aspects of host plant resistance has focused mainly on agricultural plants due to their economic importance. The resistance alleles that agricultural plants possess were inherited from wild ancestors so conclusions about the genetics of resistance in plants of importance to humans may, in some cases legitimately be extended to wild plants.

Numerous studies have been conducted on the genetics of resistance characteristics in agricultural plants (see Kennedy and Barbour, 1992). The phenotypes that contribute to resistance include both physiological and phytochemical factors as described in section 1.2.5 of this thesis, and the inheritance follow patterns consistent with most of the known types of genetic action including; dominance, overdominance, additive effects, and epistasis. The inheritance of resistance traits has been investigated in many plant species including; alfalfa, beans, maize, sorghum, sunflower, tomato and wheat. Studies of this type range from conventional genetics studies of single dominant genes to quantitative genetics studies utilizing diallels, generation means analysis and more detailed genetic dissections utilizing molecular markers. In most cases the resistance trait is not the result of a single, dominant gene but is inherited in a quantitative manner, often showing additive genetic action and multiple genes that condition the trait.

The genetic dissection of resistance traits is only as good as the measurement of the trait will allow. There are many factors which influence the plants expression of resistance including the age of the plant, the level of biotic and abiotic stress the plant is enduring and environmental effects which includes factors such as soil type, macro/micro nutrient levels, moisture availability and temperature. The behavior of the insect will also effect the response of the plant. Insects are influenced by environmental and other factors, so it is important to be aware that much of the variation observed is not genetic in origin, but is the result of factors which can not be controlled by the experimenter.

One of the most essential requirements of utilizing host plant resistance in the development of insect resistant plants is that the resistance characteristics demonstrate heritability in a narrow sense. It would be useless to attempt breeding for insect resistance if beneficial traits could not be transmitted from generation to generation. Many studies have demonstrated that resistance characters are heritable in crops of agronomic importance (Table 1-1).

Table 1-1 Heritability in a narrow sense (h^2) of plant resistance to insects. (Adapted from Berenbaum and Zangerl 1992).

Plant	Insect	Heritability (h^2)
Brassica oleracea	*Plutella xylostella*	0.81
Lycopersicon esculentum	*Heliothis armigera*	0.73
Medicago sativa	*Empoasca fabae*	0.43
Vigna unguicalata	*Lygus herperus*	0.60
Pastinaca sativa	*Depressaria pastinacella*	0.94
Solidago altissima	*Philaenus spumarius*	0.62

1.2.8 Breeding Rootworm Resistant Maize

Some progress has been achieved in breeding for rootworm resistance by screening and selecting for resistance characteristics in maize that is grown under rootworm stress. Branson et. al (1989) describe the method that is generally used to assess an inbred's or hybrid's performance under rootworm stress. The process involves growing maize in fields that either have a high natural infestation of insects or in fields artificially infested with rootworms. Artificial infestations are preferable since natural infestations are highly variable. Performance of the plants can be assessed by several criteria including; row rating, root size, root lodging resistance, plant height, vertical-pull resistance and visual root damage ratings. The most precise measurement of damage can be obtained by the visual root damage ratings since the other methods may be influenced

by factors such as soil type and moisture content. Damage ratings measure only non-preference, or antibiosis, not tolerance. Plants that are not lodged and have fairly large root systems may however be compromised by the feeding of rootworms since scarred roots have an impaired ability to absorb water and nutrients (Branson et. al 1980).

Some success has been achieved in the development of rootworm resistance by conventional selection, and several inbreds have been identified as being tolerant to rootworm damage, including the inbreds: A251, B14, B69, Mo22, N38A, NG72317, NG72312, NG72227, Oh05, SD10, SD30, SD1-1261 and W202 (Bigger, 1941). Resistance in these inbreds was observed to be the result of large, branching root systems that recovered quickly after insect damage. Welch (1977) identified several sources of rootworm resistance from synthetic and exotic open pollinated varieties and used recurrent selection to produce resistant inbreds, open pollinated, exotic and synthetic varieties. Since this work, a few programs have released rootworm resistant synthetics, for example NGSDCRS1(S2)C4 and SDCRW1CO (Kahler 1985).

Crosses of *Zea diploperennis* with *Tripsacum* species produce hybrids that are rootworm resistant, showing a good ability to tolerate rootworm damage by root recovery and lodging resistance (Eubanks et. al, 1993), however the use of wide crosses for the development of improved maize is only feasible in a long term breeding program due to the large amount of genetic drag associated with such crosses.

A recent study (Reidell et. al 1993) assessed the type of resistance mechanisms that exist in commonly used inbreds and it appears that root size is the most important resistance characteristic that now exists in the majority of important germplasm used today. Root recovery after damage is an important factor in resistance, however it has been suggested that compensatory root growth may have a detrimental effect on grain yield (Gray et. al 1998), so selecting for this trait alone may offset gains from reductions in insect damage.

Research on the genetics of rootworm resistance in maize has left many unanswered questions with resistance breeding programs devoted to selecting for resistance traits without possessing a detailed understanding of the genetic parameters governing the inheritance of these traits, or the quantitative trait loci (QTL) involved. Selection for rootworm resistance could be improved with a better understanding of genetic parameters governing resistance traits. Rootworm resistance seems to be under the control of multiple genes each contributing to tolerance or antibiosis. Resistance therefore can be seen as a trait that is inherited in a quantitative manner. Quantitative traits are considered those phenotypes that can not be categorized into discrete groups such as Mendel's classical work on "wrinkled" or "smooth" pea seeds. Most traits of agronomic importance are the result of multiple genes working together while being influenced by the environment. This can be seen in traits such as plant height or seed yield where there are not clearly distinguishable groups or categories but a distribution of phenotypes across some range of values (Falconer 1996). This is also the case in traits of disease or insect resistance. There is seldom a clear delineation between resistant and susceptible phenotypes but a range of different responses.

1.3 Quantitative Trait Loci and the use of Molecular Markers

The methods applied by Gregor Mendel to dissect the inheritance of discrete characters are not adequate to provide detailed information on the inheritance of continuous characters. Such complex traits do not conform to standard conceptions of simple dominant or recessive modes of inheritance due to single loci but are a result of the interaction of multiple loci that each contribute to a greater or lesser degree to a trait, interact in unpredictable ways, and to complicate matters further, are also under the

influence of environmental factors (Lander and Schork, 1994). The complex traits are considered to be due the presence of quantitative trait loci or QTL's.

One of the major advances of genetics has been the use of molecular markers to quantify and locate regions of the genome that condition complex traits. Restriction fragment length polymorphisms (RFLP's) are a commonly used molecular marker and more recently PCR based markers are being employed such as random amplified polymorphic DNA, simple sequence repeats and amplified length polymorphisms. Whichever marker is used the basic approach is to utilize statistical methods to determine which markers are segregating with a particular trait, and since the genomic location of the marker is known, the region containing the QTL can be identified (Falconer, 1996).

The most basic type of statistical analysis used to identify QTL's is to employ a simple ANOVA test in order to determine which markers have distinguishable marker classes, that is homozygous or heterozygous loci that show statistically significantly differences in the expression of the trait. If such an analysis shows differences between marker classes, and the markers are sufficiently dense across the organisms genome then a maximum likelihood method can be applied that establishes the LOD score, or the likelihood that a particular marker to marker interval contains a locus or loci conditioning the trait. A LOD score of 3.0, or the probability of declaring a QTL when none exists of 0.001 is generally accepted as being a sufficiently stringent confidence level (Gonzalez de Leon et. al 1996).

The use of molecular markers to reveal QTL's is a powerful method of localizing and quantifying loci that contribute to a quantitative trait, however the method can only identify putative regions and even with a very dense marker map, it is not possible to

distinguish between a single locus that exerts a large effect, or multiple loci, each exerting a small effect that sum to produce the observed QTL (Gonzalez de Leon et. al 1996). A recent paper (Kearsy and Farquhar 1998) suggests that identifying QTL locations with high precision is facilitated by a dense linkage map as well as a very high heritability of the traits being measured.

1.4 Hypothesis and Objectives

1.4.1 Hypothesis

The factors which are the basis for rootworm resistance are well understood, but the genetic mechanisms which govern these traits are not as well understood. The fact that there are many traits which together are expressed as resistance indicates that this is a quantitatively inherited trait that is conditioned by multiple genes. These facts lead to the following hypotheses;

a) No single resistance trait will account for all of the variation observed in rootworm resistance.

b) Additive genetic effects will be of major importance in the inheritance of rootworm resistance characteristics.

c) More than one chromosomal region will contain genes important to rootworm resistance.

1.4.2 Objectives

The primary objectives of this research is to:

1) Investigate genetic variation in rootworm resistance as it exists in some maize hybrids of commercial importance.

2) Elucidate some of the genetic components of the inheritance of resistance by diallel and generation means analysis.

3) Identify molecular markers associated with resistance characteristics.

4) Develop a strategy that can be applied to breeding for rootworm resistance in maize.

2. Materials and Methods

In order to study the characteristics that contribute to rootworm resistance in maize, some basic methodologies are used throughout this work, including;

1) Hydroxamic acid quantification

2) Root damage ratings

3) Root size ratings

2.1.1 Hydroxamate Extraction

The levels of total hydroxamic acids were determined on 3 week old maize seedlings, this is the time at which these compounds are at their highest levels and coincides with the emergence of 1st instar rootworm larvae in the field (Xie et. al, 1992). Plants were grown and hydroxamates extracted and analyzed with high pressure liquid chromatography (HPLC) according to Xie et. al (1992) with some modification of the HPLC gradient to obtain more efficient separation of the peaks as described below. Seed was germinated and grown in the greenhouses at Carleton University, Ottawa. Each genotype was grown in 3 pots of 10 seedlings per pot in a 50/50 mixture of perlite and vermiculite, each pot representing a single replicate. A minimum of 1 gram of fresh root tissue was harvested from each replicate and frozen for no greater than one month at -20 °C. The frozen tissue was thawed, and one gram samples were weighed out. The tissue was ground with mortar and pestle with 15 ml of distilled water and allowed to stand overnight to provide time for enzymatic conversion of the hydroxamate glucoside to the aglucone, the active form of hydroxamates. After standing overnight the suspension was filtered through cheese cloth, acidified to pH 2 with 1 N HCL, heated to 60 °C in a water bath for one minute and cooled on ice for 10 minutes then extracted with 4 x 20 ml of

ethyl acetate. The 80 ml of ethyl acetate extractives was dried to residue on a rotary evaporator and stored under 99.9% nitrogen gas at -15 °C until analyzed by HPLC. For HPLC analysis, samples were resuspended in 1 ml of chromatography grade MeOH.

2.1.2 HPLC Analysis

HPLC analysis was performed with a Perkin-Elmer binary pump equipped with a model 250 scanning diode array detector set to 265 nm wavelength. The column used was a C18 reverse phase column of dimensions 25 cm x 4.6 cm and particle size of 5 nm. The solvent system was a gradient of MeOH (solvent A) and 10 mM orthophosphoric acid (Solvent B) proceeding from 0% MeOH to 100% MeOH over 35 minutes. The gradient was as follows Step 1) 0% A to 15% A in the first five minutes, Step 2) 15% A to 80% A over the next 20 minutes. Step 3) 80% A to 100% A over the next five minutes and Step 4) 100% A to 0% A over the next five minutes. Hydroxamates were identified in extracts by comparison of retention times and online spectra from the diode array with authentic standards (Figures 2-1 to 2-4). Standards were synthesized according to Atkinson et. al, (1989). All of the hydroxamates have a maximum absorbance at approximately 265nm with the exception of MBOA, which has a maximum absorbance at 230 nm. This may introduce a small error into the quantification of MBOA, however this error is expected to be minimal since both the standard curves, and experimental samples were analyzed at the same wavelength of 265 nm. Another small error may have been introduced in the standard curves since the regression equations were calculated through the data points, and may not go through the value corresponding to zero absorbance with 0 μg of

hydroxamate used. Once again, this error is expected to be minimal since the deviation from zero is small. All standard errors are shown, in some cases the error is too small to appear on the figure. Under these conditions the HPLC chromatograms show four major peaks representing 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA), it's main degradation product; 6-methoxy-benzoxazin-3-one (MBOA), the lactam of DIMBOA; 2-hydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one (HMBOA) and 2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3(4H)-one (DIM_2BOA). Extraction efficiencies varied from 73% to 92% for these compounds. (Figures 2-5 to 2-7) A sample chromatogram (Figure 2-8) as well as the absorption spectra (Figures 2-9 to 2-12) for these compounds are given in this chapter. The three major hydroxamates have all been shown to exhibit some toxic activity towards rootworm (Xie et. al, 1992) and since MBOA is the major degradation product of DIMBOA the values given in this paper for total hydroxamates represents the sum of all 4 of these compounds as quantified with HPLC. Several previous studies utilize this method (Assabgui et. al, 1993, 1995. Xie et. al 1990, 1991, 1992) and strong correlations have been found between total hydroxamate content and maize resistance traits (Assabgui et. al 1995). No studies have established the existence of strong correlations between the individual compounds and field traits such as root damage ratings, lodging resistance or root size. A second justification for analyzing total hydroxamates is that DIMBOA, and it's degradation product MBOA together represent greater than 60% of the total hydroxamate mass found in the maize root extracts, the remaining hydroxamates; HMBOA and DIM_2BOA are less bioactive and account for a smaller percentage of the hydroxamate mass found in the maize root extracts (Atkinson, 1989). A third reason for analyzing total hydroxamates, as opposed to

each compound individually can be explained by considering the evolution of this defence mechanism in maize. All of the hydroxamate compounds are present in the fresh root tissue, with the exception of the degradation product; MBOA. When the insect feeds upon the root system, it is challenged by all compounds acting together. It is the combined effect of all of the bioactive compounds that constitute this phytochemical defence mechanism, so the level of total hydroxamates is relevant from an ecological point of view.

Figure 2-1. DIM_2BOA Calibration Curve. The relationship between peak absorbance at 265 nm is plotted against concentration of injected standard

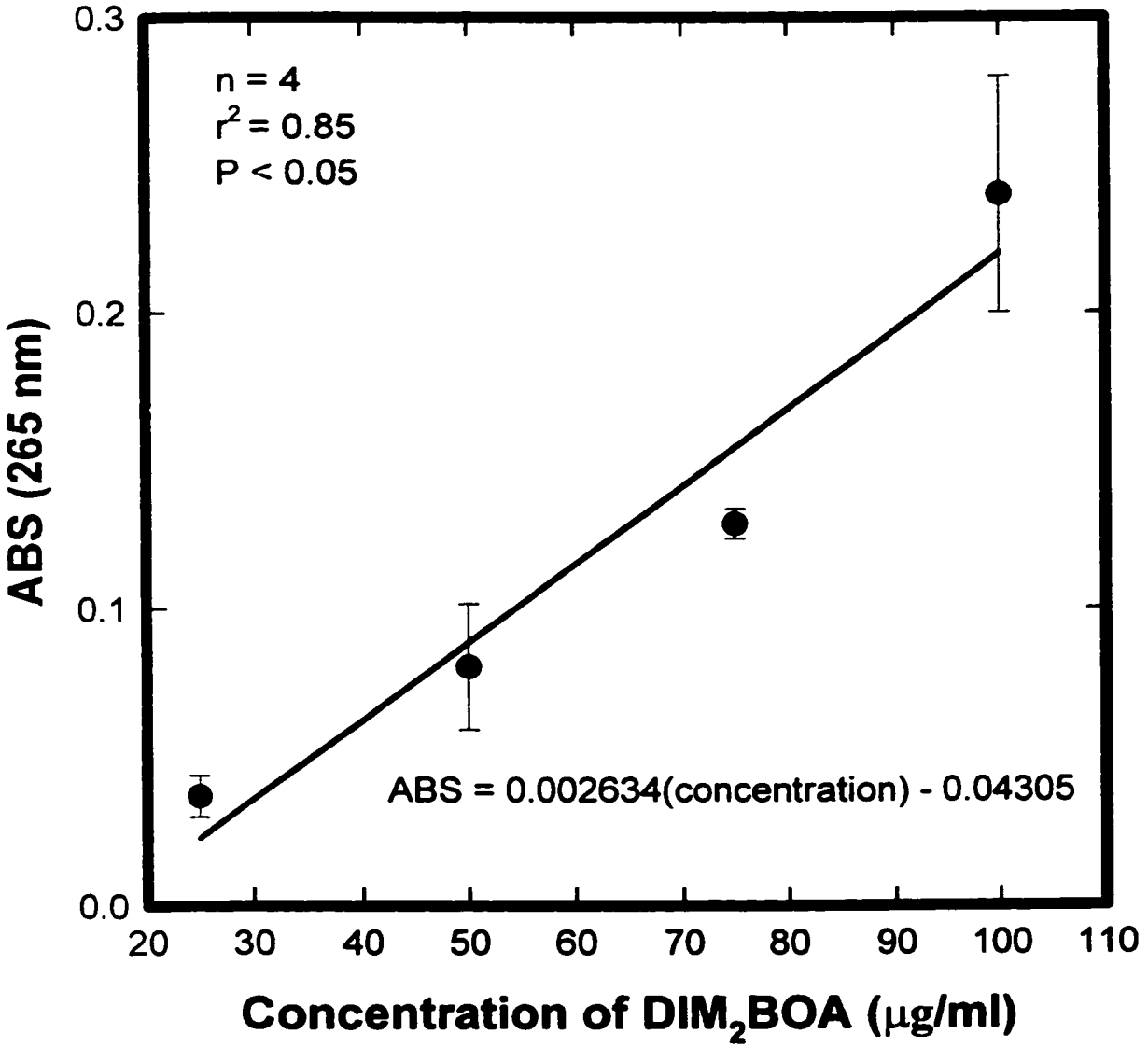

n = 4
r² = 0.85
P < 0.05
ABS = 0.002634(concentration) - 0.04305
ABS (265 nm)
0.0
0.1
0.2
0.3
20
30
40
50
60
70
80
90
100
110
Concentration of DIM2BOA (μg/ml)

Figure 2-2. DIMBOA Calibration Curve. The relationship between peak absorbance at 265 nm is plotted against concentration of injected standard

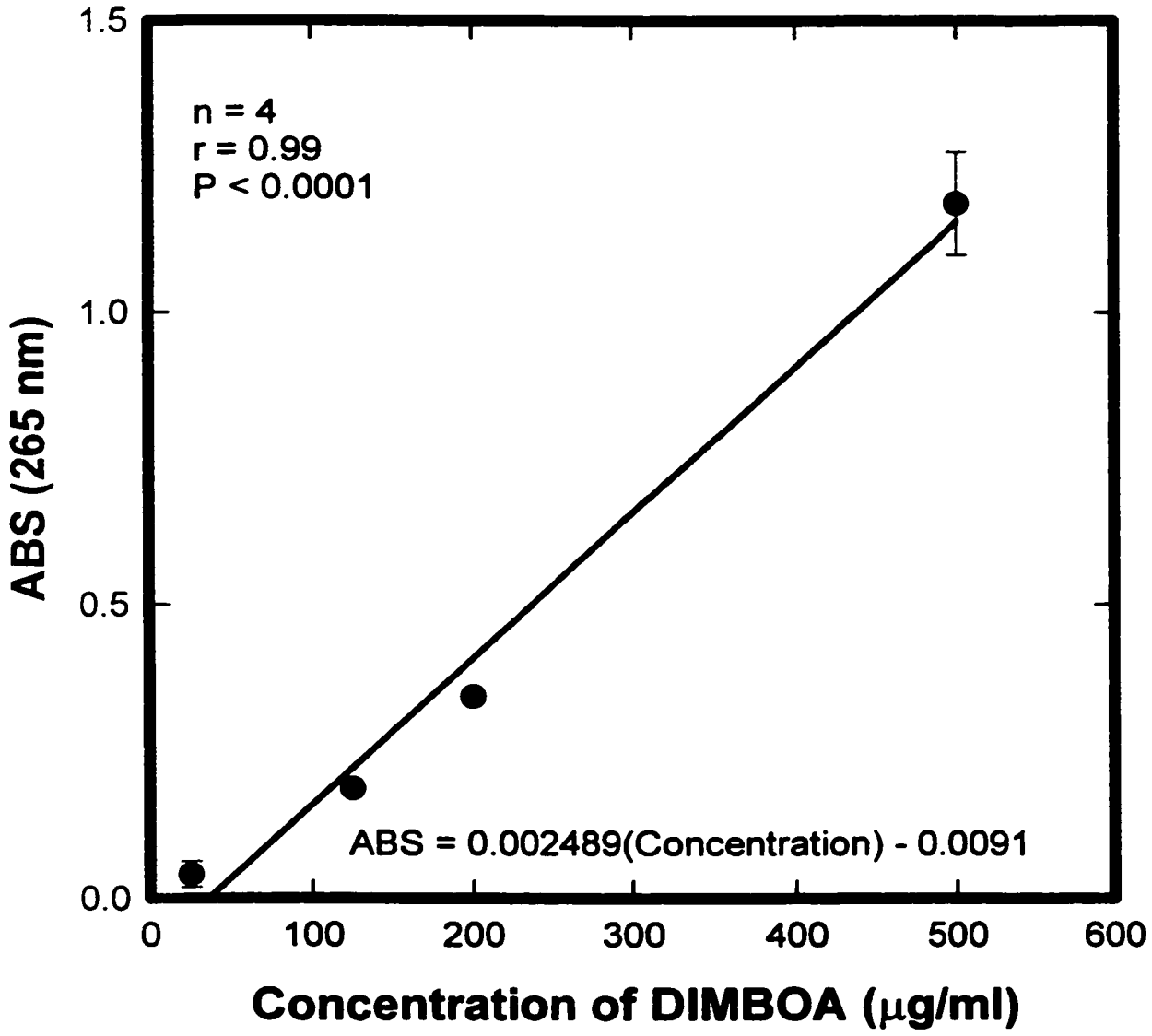

n = 4
r = 0.99
P < 0.0001
ABS = 0.002489(Concentration) - 0.0091
ABS (265 nm)
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
Concentration of DIMBOA (μg/ml)

Figure 2-3. HMBOA Calibration Curve. The relationship between peak absorbance at 265 nm is plotted against concentration of injected standard

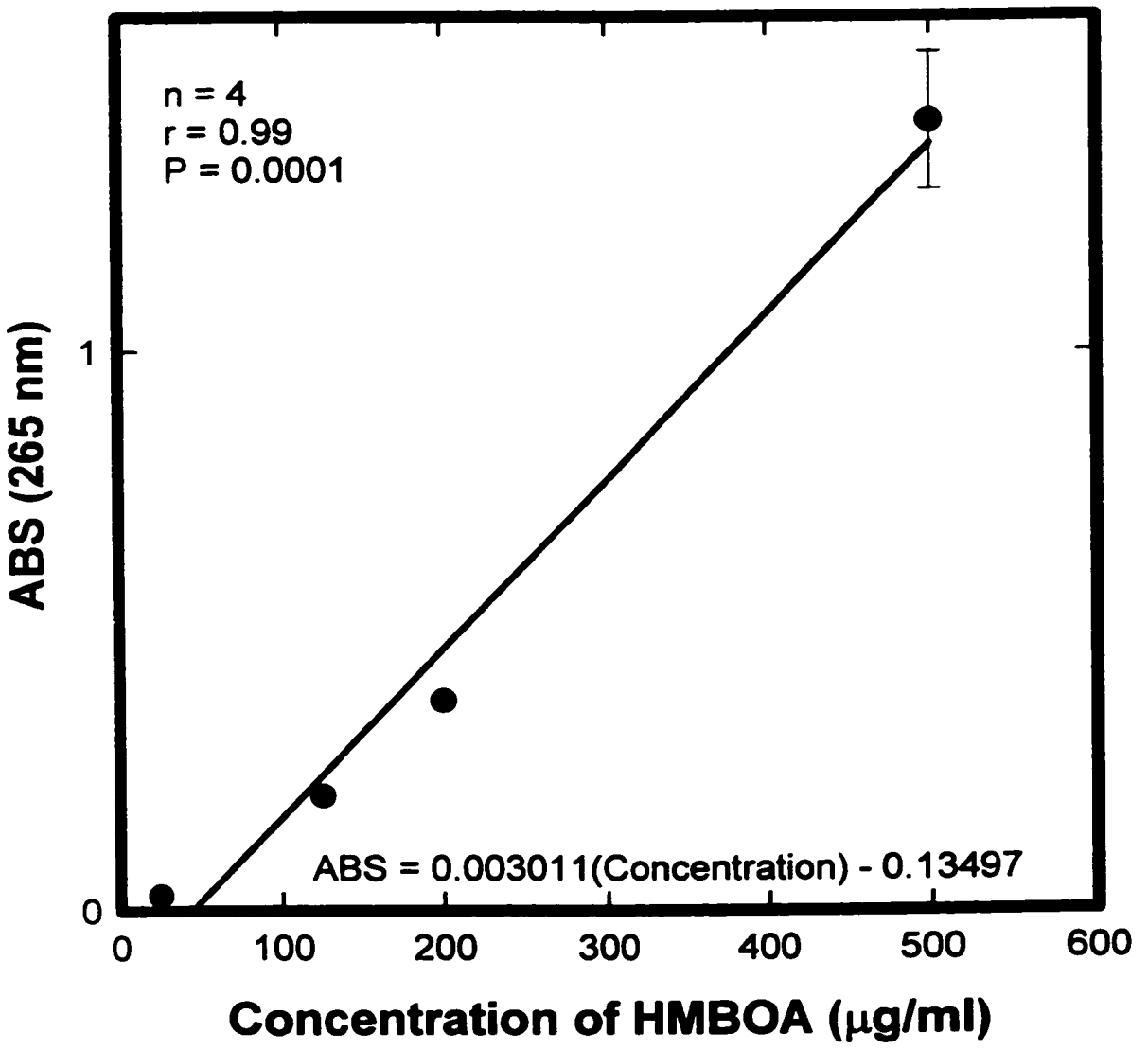

n = 4
r = 0.99
P = 0.0001
ABS = 0.003011(Concentration) - 0.13497
ABS (265 nm)
0
1
0
100
200
300
400
500
600
Concentration of HMBOA (µg/ml)

Figure 2-4. MBOA Calibration Curve. The relationship between peak absorbance at 265 nm is plotted against concentration of injected standard

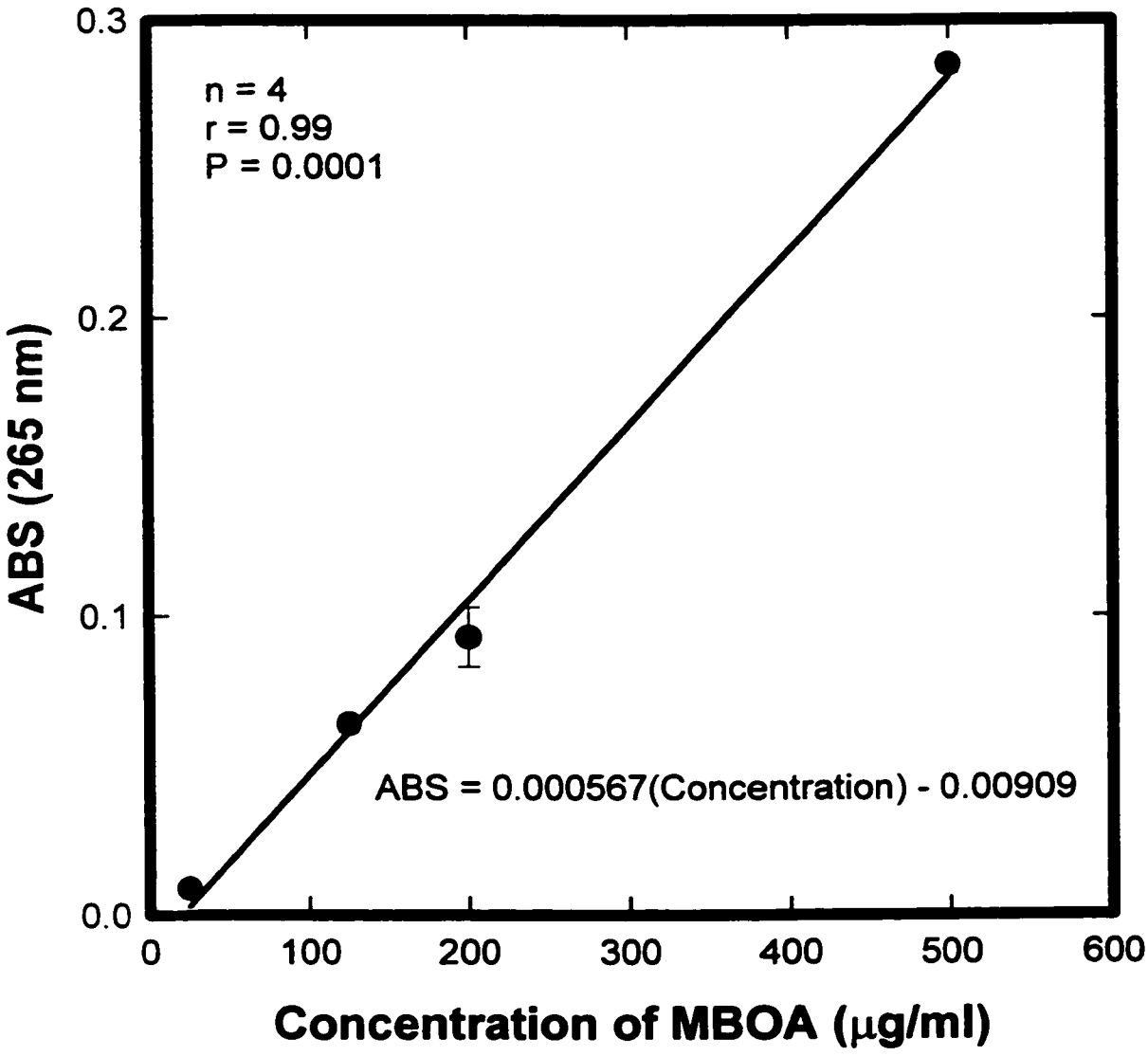

n = 4
r = 0.99
P = 0.0001
ABS = 0.000567(Concentration) - 0.00909
ABS (265 nm)
0.0
0.1
0.2
0.3
0
100
200
300
400
500
600
Concentration of MBOA (µg/ml)

Figure 2-5. Extraction Efficiency of DIMBOA. The hydroxamate is added to a sample of maize root extract and subjected to the hydroxamate extraction and analysis protocol as described in the text. The level of native hydroxamates are subtracted from total hydroxamate levels found and then plotted as amount of hydroxamate added in relation to the amount recovered. The slope of the curve gives the extraction efficiency.

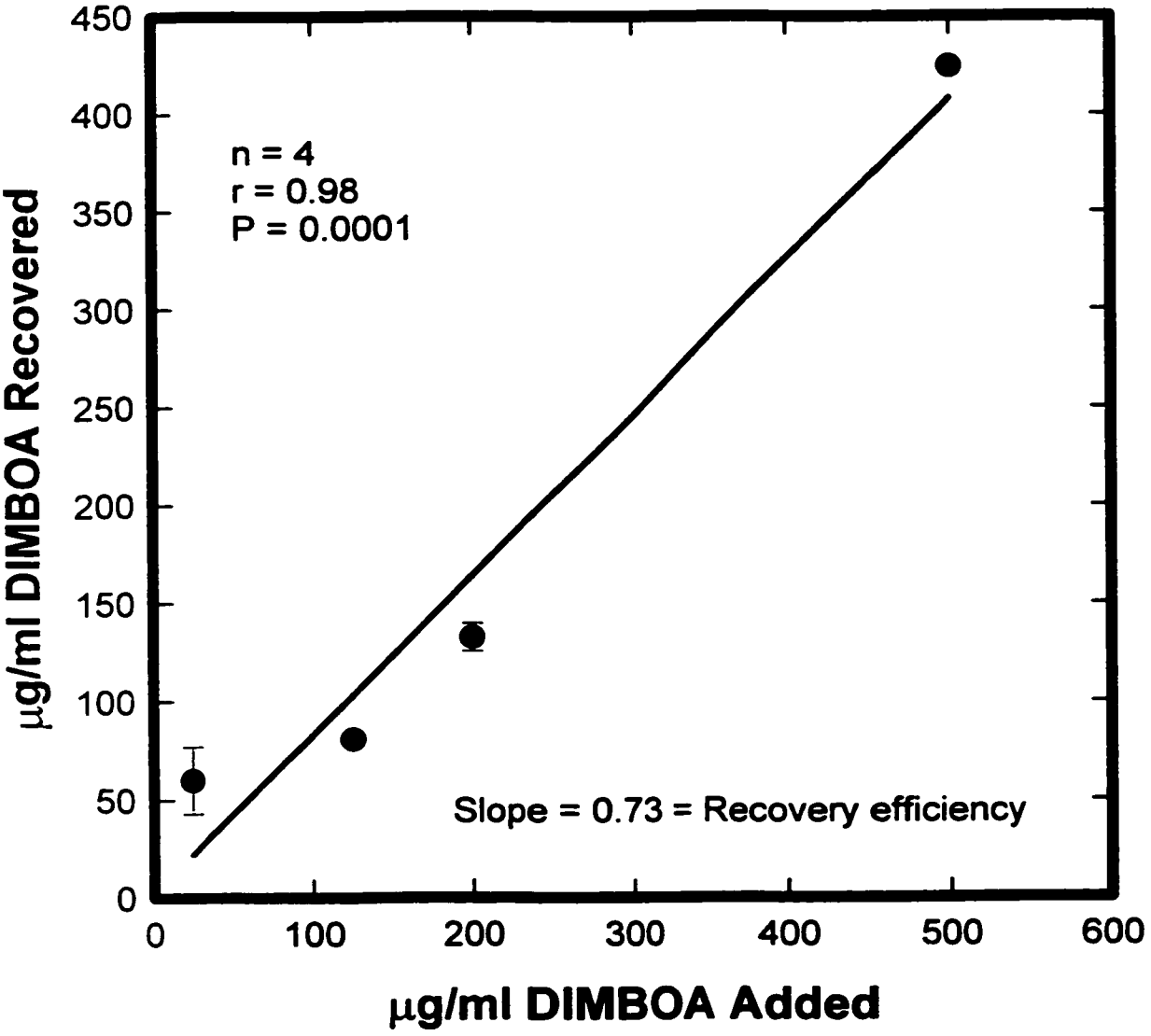
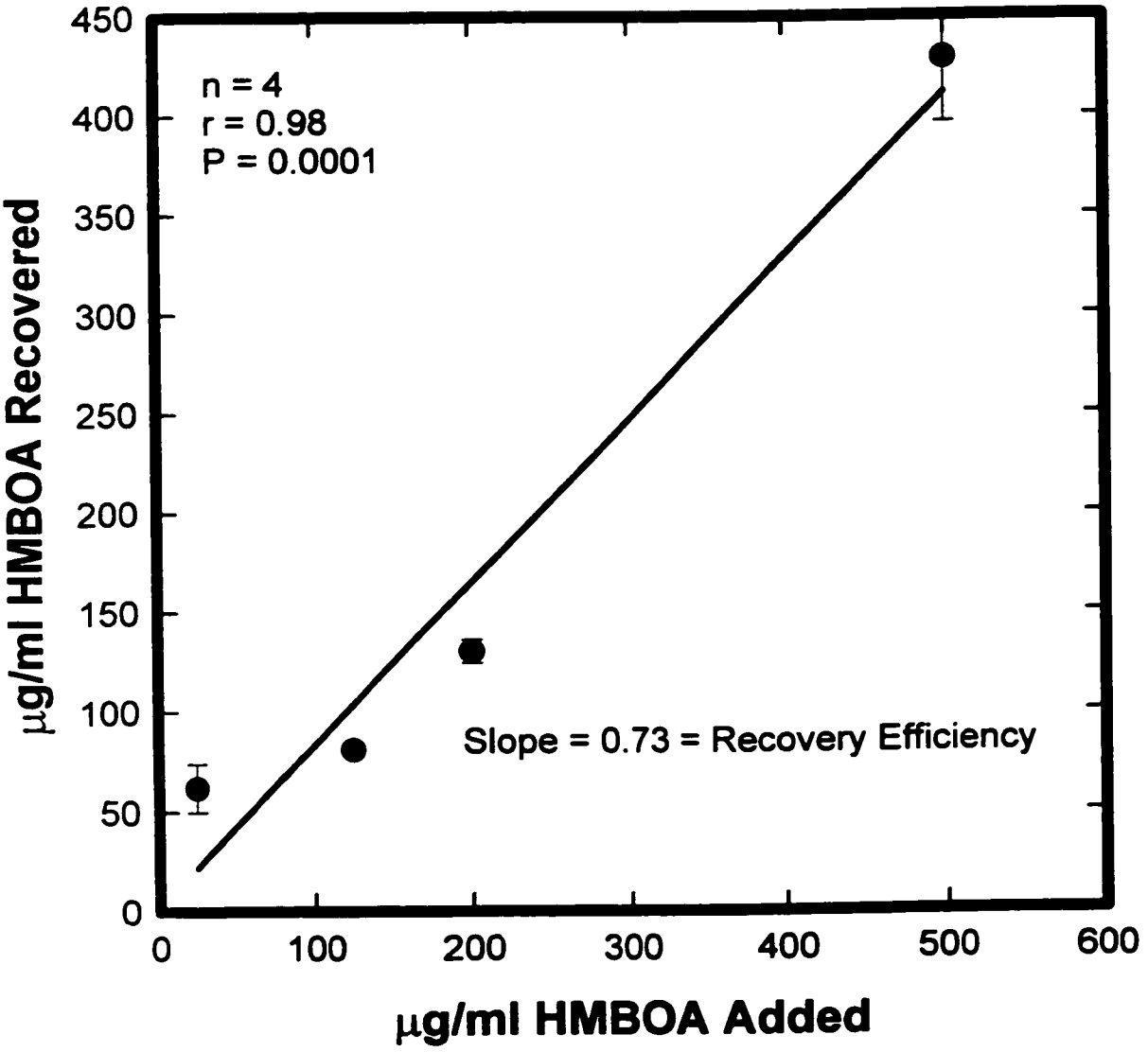

n = 4
r = 0.98
P = 0.0001
Slope = 0.73 = Recovery efficiency
µg/ml DIMBOA Recovered
µg/ml DIMBOA Added
0
50
100
150
200
250
300
350
400
450
0
100
200
300
400
500
600

Figure 2-6. Extraction Efficiency of HMBOA. The hydroxamate is added to a sample of maize root extract and subjected to the hydroxamate extraction and analysis protocol as described in the text. The level of native hydroxamates are subtracted from total hydroxamate levels found and then plotted as amount of hydroxamate added in relation to the amount recovered. The slope of the curve gives the extraction efficiency.


n = 4
r = 0.98
P = 0.0001
Slope = 0.73 = Recovery Efficiency
µg/ml HMBOA Recovered
µg/ml HMBOA Added
0
50
100
150
200
250
300
350
400
450
0
100
200
300
400
500
600

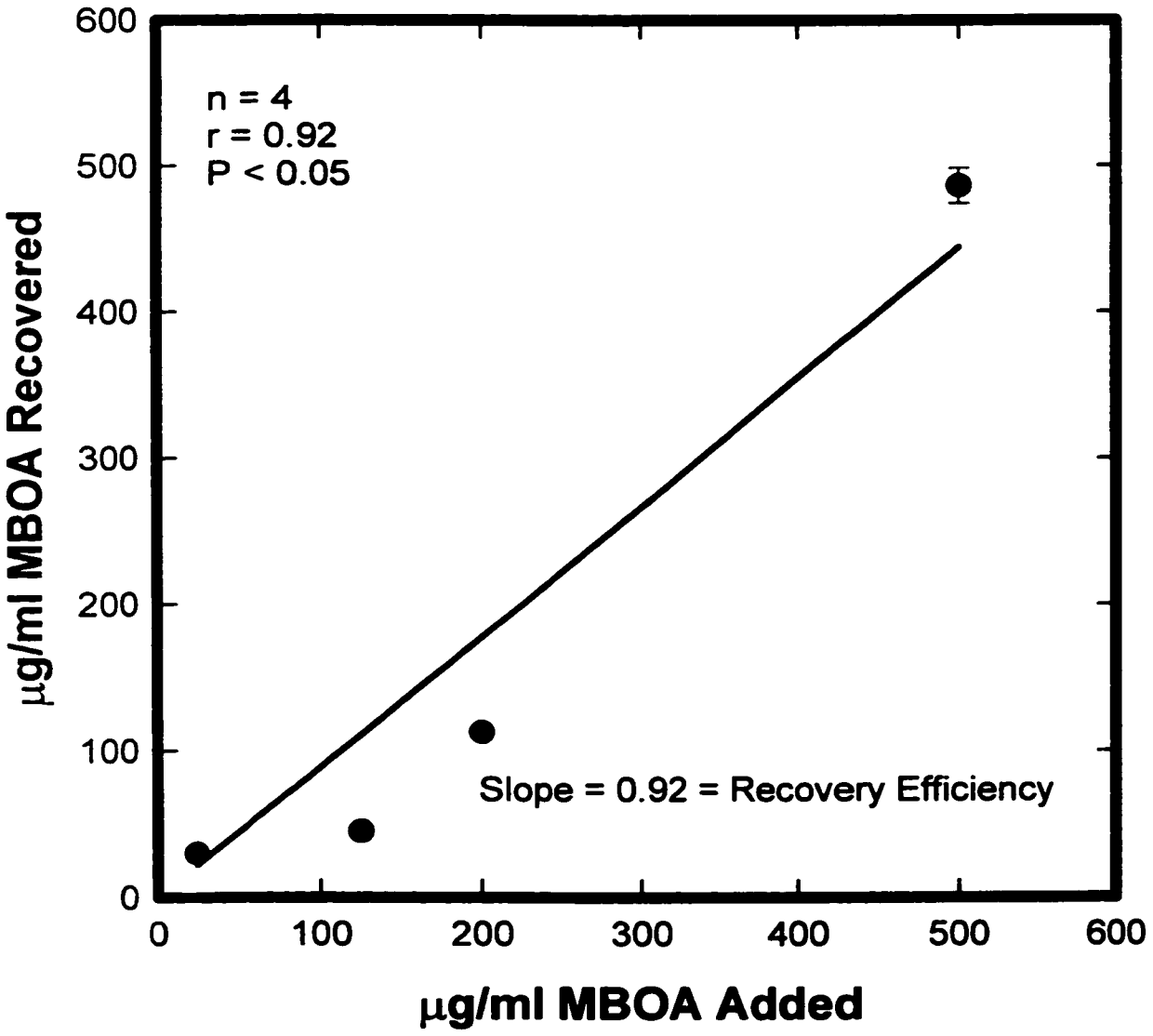

n = 4
r = 0.92
P < 0.05
Slope = 0.92 = Recovery Efficiency
µg/ml MBOA Recovered
µg/ml MBOA Added
0
100
200
300
400
500
600

Figure 2-8. Example of an HPLC Chromatogram of Maize Root Extract (wavelength 265 nm). The peaks were scanned from the output of the Perkin-Elmer printer/plotter

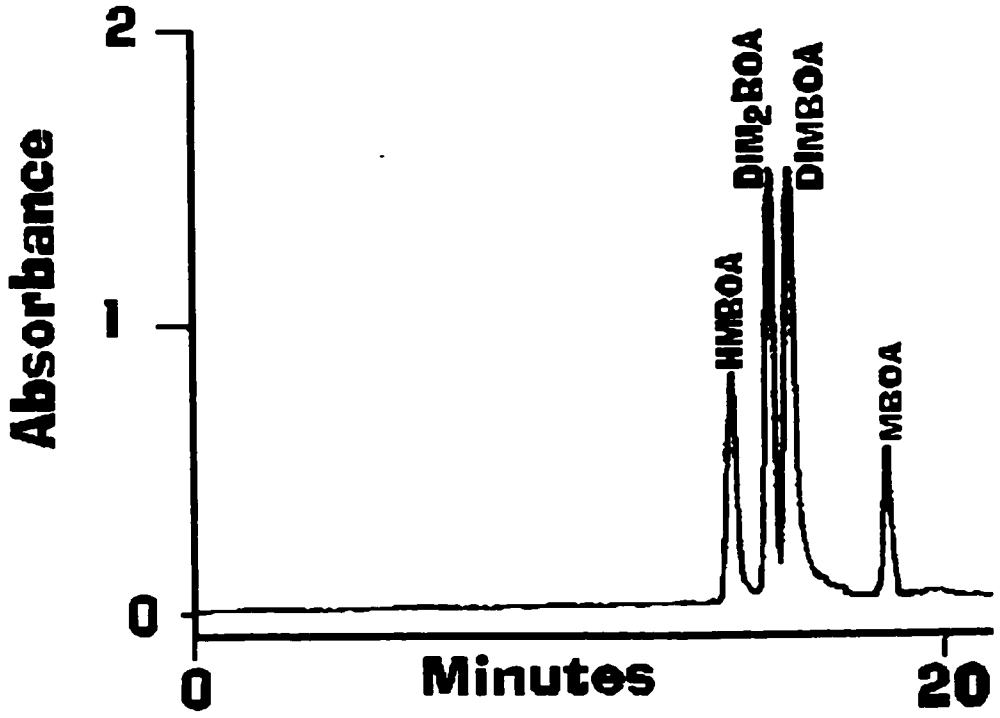

Absorbance
2
1
0
HMBOA
DIM2BOA
DIMBOA
MBOA
0
Minutes
20

Figure 2-9. Absorption Spectrum of HMBOA as produced by the Scanning Diode Array Detector

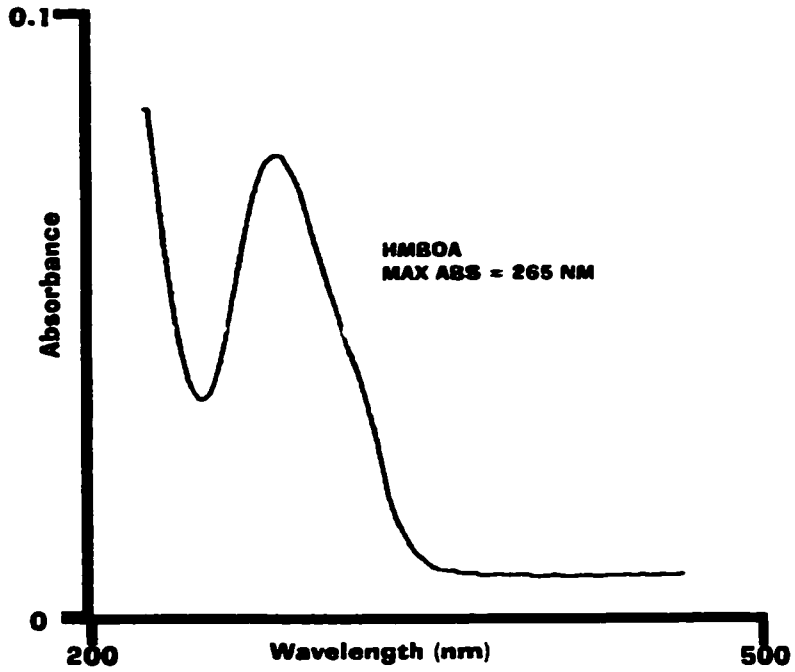

0.1
Absorbance
HMBOA
MAX ABS = 265 NM
0
200
Wavelength (nm)
500

Figure 2-10. Absorption Spectrum of DIM_2BOA as produced by the Scanning Diode Array Detector

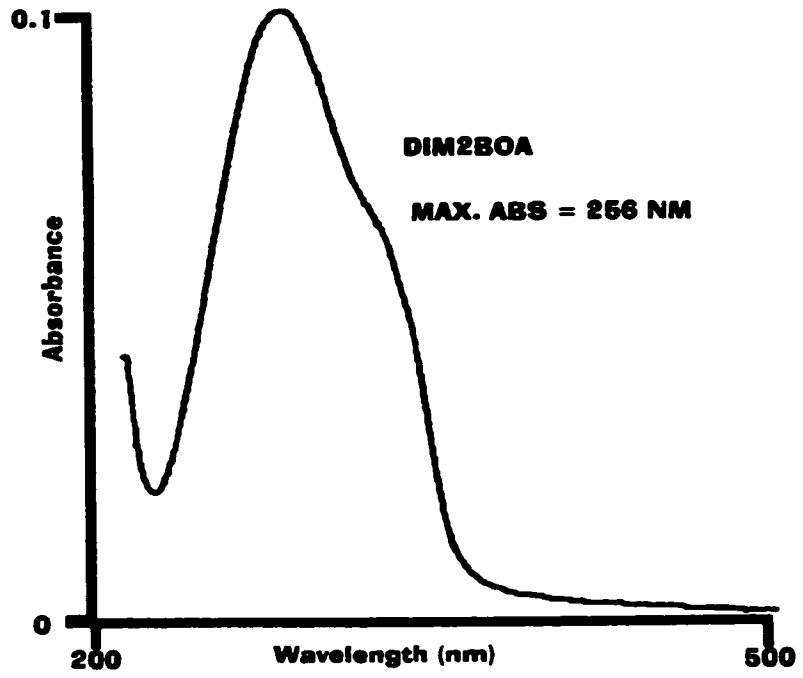

0.1
Absorbance
0
DIM2BOA
MAX. ABS = 256 NM
200
Wavelength (nm)
500

Figure 2-11. Absorption Spectrum of DIMBOA as produced by the Scanning Diode Array Detector

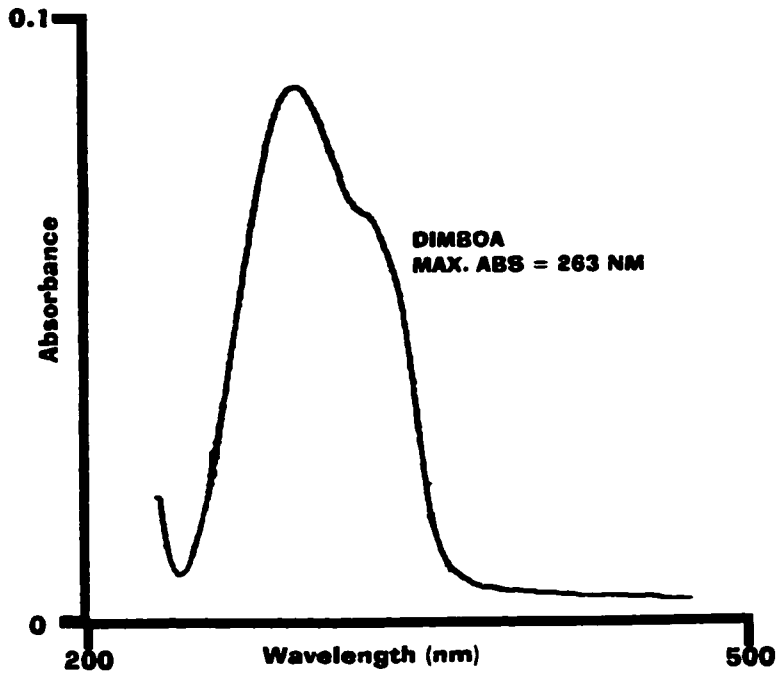
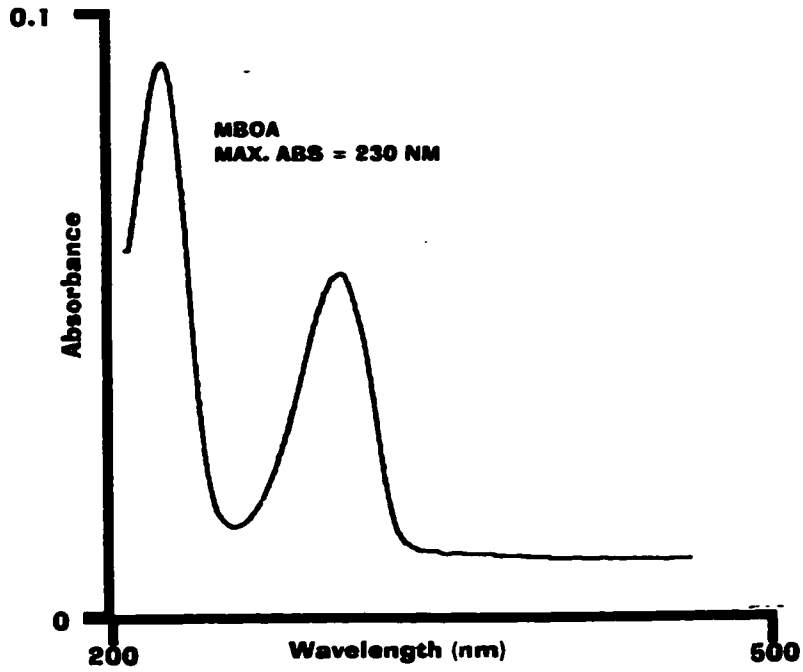

0.1
Absorbance
0
DIMBOA
MAX. ABS = 263 NM
200
Wavelength (nm)
500

Figure 2-12. Absorption Spectrum of MBOA as produced by the Scanning Diode Array Detector


0.1
MBOA
MAX. ABS = 230 NM
Absorbance
0
200
Wavelength (nm)
500

2.1.3 Root Damage Ratings

Root damage ratings were evaluated on the 9 point scale according to Welch (1977). Plants were grown in soil artificially infested with rootworm eggs and harvested at the point of maximum root damage, approximately 8 weeks post planting. Root systems were removed from the ground with shovels and then soaked in buckets of water. After the roots soaked for a brief period of time, the remaining soil was removed by cleaning with a jet of water. Root systems were inspected and assigned a rating from 1 to 9 based on the following guidelines;

1. No noticeable feeding damage.
2. Few feeding scars, but no root pruning.
3. Many noticeable feeding scars on outer portion of root, but no root pruning.
4. One to three roots pruned but less than an entire node pruned; outer portions with a moderate amount of feeding damage.
5. More than three roots pruned but less than a full node pruned; considerable feeding damage on outer portion of root system.
6. Less than a full node of roots pruned but with extensive feeding on outer portion of root system.
7. At least one full node of roots pruned, but with fewer than two full nodes.
8. At least two full nodes of roots pruned, but with fewer than three nodes.
9. Three or more full nodes of roots pruned.

2.1.4 Root Size Ratings

Root size ratings were evaluated based on the 6 point rating scale of Rogers (1977) which sets a scale based on the size of the root system. Slight modifications to this system were used in this study as described here. Root size ratings were done on a row basis with a rating given based on the average size of a root system within a particular row. Ratings were assigned as follows;

1) Very large root system, > 50 cm in width.
2) Large root system 40 to 50 cm in width.
3) Medium root system 30 to 40 cm in width.
4) Medium root system 20 to 30 cm in width.
5) Small root system 10 to 20 cm in width.
6) Very small root system, < 10 cm in width.

2.1.5 Statistical Methods

Statistical analyses were performed using the SAS computer program running on a 80486 class IBM compatible computer. The statistical models used were taken from various published sources indicated in the text of this thesis and the SAS programs were written with the aid of the SAS software manual (SAS 1982). Additional analysis was performed with the diallel analysis computer program accompanying the text by Christie et. al (1988). Randomization of the field design for the diallel and generation means analysis was performed with the computer program 'Scramble' written by Don Gratton (1993).

3. Genotypic Variation in Hydroxamic Acid Content and Rootworm Resistance of Some DEKALB Maize Hybrids

3.1 Introduction

The phenotypic characters that contribute to rootworm resistance in maize are fairly well understood, but the relative importance of all of the factors that contribute to rootworm resistance has not been clearly established. Dr. J.T. Arnason's laboratory at the University of Ottawa has identified the hydroxamates as the compounds that provide the antibiosis component of rootworm resistance (Xie et. al, 1992), but resistance due to hydroxamates is only one of many mechanisms that provide some protection to the maize plant from rootworm. Previous studies conducted by our laboratory examined hydroxamate content in recommended Ontario commercial hybrids and inbreds of different latitudinal pools. This study examines DEKALB commercial hybrids, one of the few programs with interest in rootworm resistance. The purpose of the present study is to 1) assess the genotypic variation in hydroxamate level of the DEKALB hybrids 2) predict the levels of antibiosis of these hybrids based on their hydroxamate levels and 3) determine if correlations exist between the field data and the hydroxamic acid level for these hybrids.

3.2 Materials and Methods

Variation in hydroxamic acid content was determined at the University of Ottawa using the method described in chapter 2. A field study was undertaken in Brookings South Dakota by the United States Department of Agriculture (USDA) with material provided by the DEKALB Genetics Corp. The germplasm is available through Dr. W. Reidell (USDA) or Dr. Dan Palmer (DEKALB genetics corp.). Further information on the pedigree of these hybrids was not available to the author. The statistical analysis was performed at the University of Ottawa. In 1992 and 1993 two field trials were performed to assess rootworm resistance in terms of root pull force, root damage rating and lodging

resistance. The hybrids were planted in a randomized block design consisting of five replicates at 10 plants per replicate and infested at rates of 0, 400, 800 or 1200 rootworm eggs per foot of row. The hybrids tested in the field for rootworm resistance were assessed based on the following criteria; the percentage of plants that were root lodged, the force required in kilograms to pull a plant system from the ground at the point of maximum root damage and after root recovery, and root damage ratings based on the 9 point scale of Welch (1977). Along with the 6 hybrids assessed in the field, several others were evaluated in our lab for their levels of hydroxamic acids. Determination of the level of antibiosis of these hybrids was performed by utilizing the LC_{90} (917pm) and LC_{50} (150 ppm) determined for hydroxamates by Xie (1992).

3.3 Results and Discussion

The twenty-three hybrids that were analyzed for hydroxamate content (Table 3-1) range from a low of 273.6 μg/g of total hydroxamates per gram of fresh root tissue (DK524) to a high of 484.1 μg/g (8149). The average total hydroxamate content for these hybrids is 344.7 μg/g. Using the DIMBOA LC_{50} of 150 ppm and the LC_{90} of 917 ppm, an arbitrary range of predicted antibiosis was established. Varieties possessing between 0 μg/g and 150 μg/g of hydroxamates would be predicted to have no antibiosis, between 150 μg/g to 500 μg/g to have low antibiosis, between 500 μg/g to 900 μg/g to have high antibiosis and hybrids having over 900 μg/g of hydroxamates would be predicted to have very high levels of antibiosis. It is concluded from their levels of hydroxamates that these DEKALB hybrids would have a low degree of antibiosis since they all have between 150 μg/g and 500 μg/g of hydroxamates.

The field data was obtained for six of the twenty three hybrids reveals that a moderate level of resistance is present. At the highest level of infestation (1200 eggs/foot row) the average root damage rating is 5.4 in 1992 and 6.8 in 1993. The economic threshold is 4.3, so these hybrids have not been completely destroyed by the rootworm larvae. Root pull force is seen to increase in all hybrids when values are compared between maximum damage and after root recovery, demonstrating the ability of these hybrids to recover after damage. The low levels of hydroxamates in these hybrids, along with the moderate level of field resistance and moderately high root damage ratings indicates that resistance in these hybrids is achieved mainly through tolerance and not antibiosis. Root damage ratings are considered to be the only measure of antibiosis since it provides information on the desirability of the root system as a food source for the larvae. Rootworm resistance in these hybrids may be improved by selecting for high hydroxamate content or introducing this trait through conventional breeding methods utilizing a high hydroxamate donor line.

Pearson correlation coefficients and Student Newman-Kuels (SNK) multiple comparison tests were performed with the SAS computer program (SAS, 1982). The correlations between hydroxamic acid levels and the field data (Tables 3-4 to 3-7 and 3-9 to 3-12) indicate some loose relationships exist, but the correlations are weak and non-significant with the exception of a single strong correlation ($r = 0.83$, $P = 0.04$) between the root pull force after recovery at an infestation level of 1200 rootworm eggs per foot row and the total hydroxamic acid content of three week old maize seedlings (Figure 3-1). It should be noted that if a P value of 0.05 or less is accepted as a statistically significant result then one would expect to obtain a significant correlation by chance in at least one

out of twenty plots. The lack of statistical significance in these correlations seems to be due to two main factors; 1) lack of significant differences between the hybrids for the field traits measured and 2) high variability in also as demonstrated by the standard errors associated with the data. The high variability in the field data is demonstrated by the fact that, although there appear to be large differences between the data presented, these differences were not statistically significant as demonstrated in the multiple comparison tests. The field data also exhibited relatively high standard deviations. 3) Poor choice of germplasm since it did not show large, significant differences between hybrids for the traits measured.

In this study, it was observed that the genotypic differences for the traits measured was small. This may be due to the fact that all of these varieties have similar pedigree backgrounds. In order to improve the results of this study, it would be necessary to choose hybrids with a much more diverse ancestral history.

Table 3-1. Concentration of compounds found in extracts of 23 DEKALB maize hybrids. All values given are μg per gram of wet weight maize root. Each value is the mean of three reps done on each hybrid. Differences between hybrid for a given compound were determined with an SNK multiple range, values in a column with the same letter do not differ significantly at P = 0.05

Variety	DIM2BOA	HMBOA	DIMBOA	MBOA	TOTAL
4116	52.8 ab	93.3 ab	146.3 b	60.7 ab	353.1 ab
4137	57.8 ab	100.3 ab	111.9 b	48.9 ab	319.0 ab
4246	52.8 ab	86.9 ab	142.3 b	41.9 b	323.9 ab
4317	37.4 ab	82.5 ab	141.8 b	66.6 ab	328.3 ab
4322	48.1 ab	78.9 ab	126.1 b	54.8 ab	307.9 ab
5119	44.4 ab	86.2 ab	211.8 ab	48.9 ab	391.4 ab
8149	50.8 ab	104.3 a	253.1 a	76.0 ab	484.1 a
DK397	52.8 ab	77.4 ab	127.3 b	65.4 ab	322.8 ab
DK403	56.4 ab	94.0 ab	210.7 ab	70.1 ab	430.3 ab
DK447	37.9 ab	83.3 ab	144.4 b	53.7 ab	319.3 ab
DK464	32.5 b	86.5 ab	106.1 b	53.6 ab	278.8 b
DK471	51.5 ab	90.9 ab	208.7 ab	78.3 ab	429.5 ab
DK485	45.7 ab	71.5 b	125.6 b	61.9 ab	304.7 ab
DK493	31.5 b	80.9 ab	124.9 b	70.1 ab	307.4 ab
DK524	33.0 b	75.4 ab	106.8 b	58.3 ab	273.6 b
DK535	42.1 ab	81.1 ab	159.9 b	48.9 ab	332.2 ab
DK547	37.9 ab	87.6 ab	146.0 b	51.5 ab	323.2 ab
DK527	34.3 ab	82.2 ab	116.3 b	58.3 ab	291.2 ab
DPGCRW-871	34.7 ab	79.8 ab	147.9 b	51.3 ab	313.7 ab
NGSDCRW	66.4 a	98.8 ab	180.1 ab	94.8 a	440.2 ab
RW17	44.4 ab	91.2 ab	182.5 ab	39.5 b	357.6 ab
RW17*NGSCRW	39.9 ab	93.5 ab	139.6 b	63.1 ab	336.1 ab
RW17 '92	44.8 ab	100.8 ab	164.5 b	51.3 ab	361.5 ab

Table 3-2. 1992 Field Observations Made in Brookings S.D. After Infestation With Western Corn Rootworm Eggs at Various levels of Infestation. Means Across a Row With the Same Letter Do Not Differ Significantly in the SNK Multiple Comparison Test (P = 0.05). Pull Force in Kilograms, Root Damage Ratings on a 1-9 Scale.

	DK397	DK464	DK485	DK524	DK535	DK547
0 Egg Infestation						
Pull Force (Max Dam)	142.0a	117.7a	137.0a	106.3a	108.3a	127.0a
Pull Force (At Recovery)	204.0a	217.0a	213.7a	175.7a	215.3a	208.0a
% Lodging	0a	0a	0a	0a	0a	0a
Rt. Dam. Rating	1a	1a	1a	1a	1a	1a
400 Egg Infestation						
Pull Force (Max Dam)	87.3a	121.3a	117.7a	100.7a	99.7a	126.3a
Pull Force (At Recovery)	184.7a	191.3a	185.0a	208.7a	179.3a	204.7
% Lodging	0a	0a	0a	0a	0a	7.3a
Rt. Dam. Rating	3.7a	3.5a	3.0a	3.5a	4.0a	2.7a
800 Egg Infestation						
Pull Force (Max Dam)	106.3ab	117.0ab	102.0ab	60.3b	109.7ab	139.3a
Pull Force (At Recovery)	172.3a	190.3a	195.0a	154.7	177.3a	205.3a
% Lodging	11.0a	33.3a	0a	12.3a	11.3a	7.3a
Rt. Dam. Rating	3.5a	3.3a	3.83a	5.0a	4.3a	3.3a
1200 Egg Infestation						
Pull Force (Max Dam)	93.0a	78.0a	91.3a	53.3a	92.7a	67.0a
Pull Force (At Recovery)	183.0a	146.7a	129.3a	112.0a	164.7a	169.0a
% Lodging	13.7a	61.7a	31.3a	52.0a	66.7a	39.0a
Rt. Dam. Rating	5.1a	4.2a	5.7a	6.7a	5.2a	5.8a

Table 3-3. Correlations Between the 1992 Field Traits at 0 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are given and the probability (in brackets) of Ho (No Correlation exists) n = 6. Na = Not able to calculate. (Max = maximum damage, Rec = after root recovery) *Indicates significant correlation (P=0.05) (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamates (mg/g)
Pull Force (Max)	0.34(0.51)	Na	Na	0.33(0.52)
Pull Force (Rec)		Na	Na	0.52(0.29)
Damage Rating (1-9)			Na	Na
Lodging (%)				Na

Table 3-4. Correlations Between the 1992 Field Traits at 400 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are given and the probability (in brackets) of Ho (No Correlation exists) n = 6. (Max = maximum damage, Rec = after root recovery) *Indicates significant correlation (P = 0.05). (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamates (mg/g)
Pull Force (Max)	0.32(0.53)	-0.75 (0.09)	0.56(0.24)	-0.22(0.67)
Pull Force (Rec)		-0.51(0.30)	0.51(0.30)	-0.55(0.26)
Damage Rating (1-9)			-0.74(0.09)	0.11(0.74)
Lodging (%)				0.31(0.55)

Table 3-5. Correlations Between the 1992 Field Traits at 800 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are Given and the Probability (in brackets) of Ho (No Correlation exists) n = 6. *Indicates significant correlation (P = 0.05). (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamate (mg/g)
Pull Force (Max)	*0.87(0.02)	*-.85(0.03)	0.08(0.90)	0.60(0.21)
Pull Force (Rec)		-0.78(0.07)	-0.08(0.87)	0.32(0.53)
Damage Rating (1-9)			-0.22(0.67)	-0.33(0.53)
Lodging (%)				-0.42(0.40)

Table 3-6. Correlations Between the 1992 Field Traits at 1200 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are Given and the Probability (in brackets) of Ho (No Correlation exists) n = 6. *Indicates significant correlation (P = 0.05). (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamates (mg/g)
Pull Force (Max)	0.53(0.28)	-0.60(0.21)	-0.26(0.61)	0.63(0.17)
Pull Force (Rec)		-0.50(0.31)	-0.32(0.53)	*0.83(0.04)
Damage Rating (1-9)			-0.14(0.79)	-0.20(0.70)
Lodging (%)				-0.24(0.64)

Figure 3-1. Correlation between Root Pull Force after Recovery and Hydroxamic Acid Levels in Three Week Old Maize Seedlings infested at 1200 rootworm eggs per foot row. 95% confidence intervals are shown

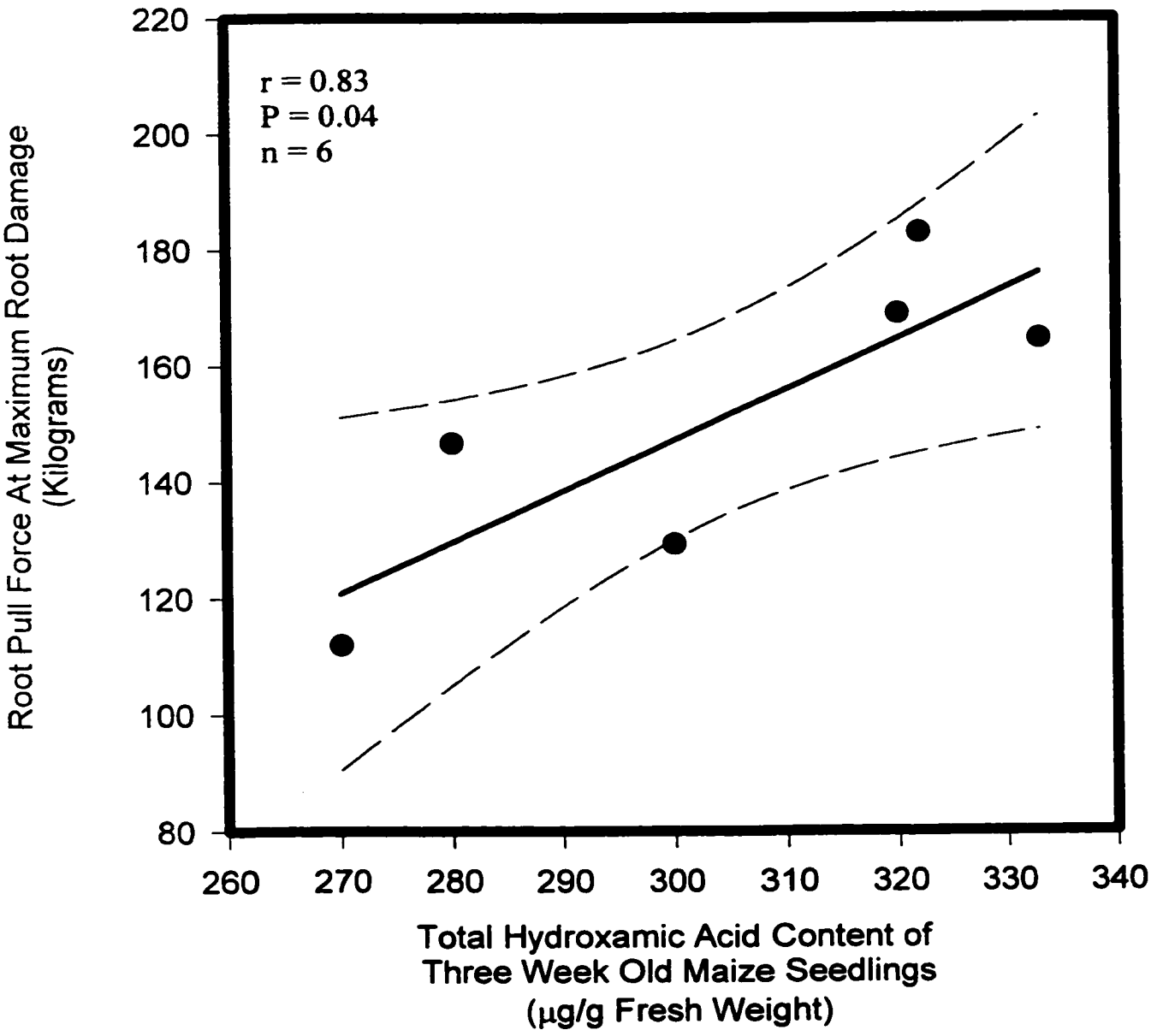

r = 0.83
P = 0.04
n = 6
220
200
180
160
140
120
100
80
260
270
280
290
300
310
320
330
340
Root Pull Force At Maximum Root Damage (Kilograms)
Total Hydroxamic Acid Content of Three Week Old Maize Seedlings (µg/g Fresh Weight)

Table 3-7. 1993 Field Observations Made in Brookings S.D. After Infestation With Western Corn Rootworm Eggs at Various Levels of Infestation. Means Across a Row With the Same Letter Do Not Differ Significantly in the SNK Multiple Comparison Test (P = 0.05). Pull Force in Kilograms, Root Damage Ratings on a 1-9 Scale.

	DK397	DK464	DK485	DK524	DK535	DK547
0 Egg Infestation						
Pull Force (Max Dam)	135.0a	111.5a	114.0a	98.2a	113.5a	104.5a
Pull Force (At Recovery)	167.5a	143.5	165.7a	137.0a	143.2a	150.0a
% Lodging	0a	0a	0a	0a	0a	0a
Rt. Dam. Rating	1a	1a	1a	1a	1a	1a
400 Egg Infestation						
Pull Force (Max Dam)	68.7a	60.5a	85.7a	74.0a	54.0a	73.7a
Pull Force (At Recovery)	113.7a	124.0a	124.0a	138.5a	116.0a	128.7a
% Lodging	33.25a	25.0a	33.2a	34.5a	42.5a	20.7a
Rt. Dam. Rating	4.7a	4.5a	4.6a	4.7a	53.4a	4.6a
800 Egg Infestation						
Pull Force (Max Dam)	55.0ab	54.0ab	74.7a	40.7b	66.5a	82.0a
Pull Force (At Recovery)	133.7a	96.0b	104.7a	88.2b	94.0b	121.2ab
% Lodging	31.0a	36.7a	33.2a	58.0a	63.5a	50.5a
Rt. Dam. Rating	6.5a	6.9a	6.0a	8.9a	5.0a	5.2a
1200 Egg Infestation						
Pull Force (Max Dam)	50.0ab	51.5a	77.5a	54.5ab	34.5b	55.7ab
Pull Force (At Recovery)	111.2a	90.2a	137.0a	93.2a	91.5a	137.2a
% Lodging	65.0a	80.5a	59.2a	56.2a	72.2a	56.2a
Rt. Dam. Rating	6.6a	6.2a	5.9a	7.1a	7.1a	7.7a

Table 3-8. Correlations Between the 1993 Field Traits at 0 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are given and the probability (in brackets) of Ho (No Correlation exists) n = 6. Na = Not able to calculate. *Indicates significant correlation (P = 0.05). (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamates (mg/g)
Pull Force (Max)	0.71(0.12)	Na	Na	0.79(0.06)
Pull Force (Rec)		Na	Na	0.55(0.26)
Damage Rating (1-9)			Na	Na
Lodging (%)				Na

Table 3-9. Correlations Between the 1993 Field Traits at 400 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are given and the probability (in brackets) of Ho (No Correlation exists) n = 6. (Max = maximum damage, Rec = after root recovery) *Indicates significant correlation (P = 0.05). (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamates (mg/g)
Pull Force (Max)	0.44(0.38)	-0.38(0.46)	-0.30(0.58)	-0.26(0.61)
Pull Force (Rec)		-0.05(0.93)	0.11(0.84)	-0.71(0.11)
Damage Rating (1-9)			0.78(0.06)	0.53(0.28)
Lodging (%)				-0.07(0.89)

Table 3-10. Correlations Between the 1993 Field Traits at 800 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are given and the probability (in brackets) of Ho (No Correlation exists) n = 6. (Max = maximum damage, Rec = after root recovery) *Indicates significant correlation (P = 0.05). (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamates (mg/g)
Pull Force (Max)	0.72(0.11)	-0.20(0.69)	-0.15(0.77)	0.47(0.35)
Pull Force (Rec)		-0.11(0.83)	-0.45(0.37)	0.56(0.24)
Damage Rating (1-9)			-0.73(0.10)	-0.55(0.25)
Lodging (%)				0.15(0.77)

Table 3-11. Correlations Between the 1993 Field Traits at 1200 Rootworm Egg Infestation Level and Hydroxamate Content. Pearson Correlation Coefficients are Given and the Probability (in brackets) of Ho (No Correlation exists) n = 6. (Max = maximum damage, Rec = after root recovery) *Indicates significant correlation (P = 0.05). (Max = maximum damage, Rec = after root recovery)

	Pull Force (Rec) (Kilograms)	Damage Rating (1-9)	Lodging (%)	Hydroxamates (mg/g)
Pull Force (Max)	0.70(0.12)	-0.51(0.30)	-0.49(0.32)	-0.38(0.46)
Pull Force (Rec)		0.001(0.99)	-0.63(0.18)	0.30(0.56)
Damage Rating (1-9)			-0.36(0.48)	0.32(0.54)
Lodging (%)				0.01(0.97)

There appears to be a general trend indicating that those hybrids which have higher levels of hydroxamates require more force to pull the root systems from the ground, particularly in the data from 1992. Another general trend can be seen in the data for the root damage ratings, hybrids with higher hydroxamate content tend to have lower root damage ratings, again the 1992 data is more consistent in this respect. The same trend exists for root lodging, with the higher hydroxamate roots suffering less root lodging, although the data is not convincing since the correlations are not statistically significant in most cases. (The term 'trend' here is only meant to indicate that the direction of the relationship is as would be expected if a true relation were to exist between the parameters under measure, although the levels of significance are not sufficient to declare a correlation.)

The DEKALB hybrids studied under rootworm stress show low to moderate levels of resistance to rootworm and relatively small differences in their performance under rootworm stress. Root damage ratings are the only measure of antibiosis and no statistically significant differences were observed between the hybrids in the study for this trait, exhibiting low to moderate resistance to rootworms and giving an average value of 5.4 for the 1992 field trial and 6.8 for the 1993 field trial. The low level of antibiosis is expected based on the level of hydroxamic acids seen in these hybrids. All of the hybrids exhibited some degree of resistance, showing moderate root lodging and the ability to regrow root mass after the rootworm larvae have pupated. These factors indicate that the resistance trait that is most prevalent in these DEKALB hybrids is that of tolerance, not antibiosis.

The levels of hydroxamates in the DEKALB hybrids is low and the lack of correlation between the hydroxamates and the field data indicates that hydroxamic acids are not a major resistance factor in these hybrids over the range of hydroxamate concentrations measured. Other studies using inbreds have found good correlations between hydroxamates and field traits, but this study utilizing hybrids did not reveal strong correlations between hydroxamate content and rootworm resistance.

3.4 CONCLUSIONS

Low to moderate levels of resistance were observed in the DEKALB hybrids tested in the field. Based on the levels of hydroxamates, antibiosis in these hybrids is predicted to be low, and the resistance observed is more due to tolerance than antibiosis. This is supported by the root damage ratings seen in the field portion of the study since root damage ratings are the only direct measure of antibiosis. Root damage ratings may be improved if selection for hydroxamic acid content was performed on these hybrids, but the low levels of hydroxamates indicates that an alternative sources of alleles for hydroxamate content may be required. Rootworm resistance in these hybrids seems to favor tolerance over antibiosis, but in order to maximize rootworm resistance traits, it is important to breed and select for all of the factors know to contribute to rootworm resistance. Combining ability and generation means analyses are useful methods to determine maize inbreds that are the best contributors of hydroxamates and rootworm resistance in general. In the following chapter, diallel and generation means analysis are presented with data on some inbreds of commercial importance in Canada to determine

the combining ability, heritability, the relative levels of rootworm resistance, as well as the levels of hydroxamic acid content present in the inbreds used in the study.

4. Inheritance of Resistance to the Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte) in Maize

4.1 Introduction

The diallel and generation means analyses are frequently used to assess the performance of a set of inbreds and their hybrid combinations (Mather and Jinks, 1982). These analyses have been used to assess some potential resistance traits including hydroxamic acid content as it relates to corn borer resistance (Klun et. al 1970), root pull force (Kahler et. al, 1985) and root lodging resistance (Hile, 1987).

The exploitation of host plant resistance and the development of a more effective resistance breeding strategy requires that some of the basic genetics of these characteristics be understood. This information may help maize breeders to choose appropriate inbreds for the development of inbreds better able to cope with or avoid rootworm damage, thereby reducing the need for insecticide use. Towards this goal, diallel and generation means analyses were conducted to assess the general combining ability (GCA), specific combining ability (SCA), the relative importance of additive and dominance genetic effects and the heritability of some of the traits related to rootworm resistance in a group of maize inbreds of importance in Canada.

4.2 Materials and Methods

4.2.1 Genotypes

Seven maize inbreds: CO272, SD10, NTR4034, NTR3983, ITR3872, ITR3865 and CM7 and their 21 F_1's (without reciprocals) were evaluated for hydroxamate content,

root damage ratings, root mass and plant height in a diallel study. CO272 was developed at the Central Experimental Farm in Ottawa, Canada. The NTR (northern temperate region) and ITR (intermediate temperate region) inbreds were developed at the International Maize and Wheat Improvement Centre (CIMMYT) and are synthetic latitudinal germplasm. The NTR inbreds were developed using germplasm from Mexico, Peru, Bolivia, Pakistan, China, Hungary and the United States and adapted to 46-52 degrees north/south of the equator. The ITR inbreds were produced using lines from the United States and Europe and adapted to 40-46 degrees north/south of the equator. Two of these inbred lines (Xie et. al, 1992), ITR3872 (high hydroxamates) and NTR4034 (low hydroxamates) were chosen to produce a set of reciprocal backcrosses to evaluate the root damage ratings, plant height and root weight in an analysis of generation means. The other inbreds in the diallel analysis were chosen for a variety of reasons: CO272, because it has demonstrated very good resistance to *Fusarium graminearum* colonization (Reid et. al 1992), and as part of an integrated pest management approach, inbreds that demonstrate a wide range of resistance characteristics would be useful to study. SD10, a rootworm resistant inbred (Shank et. al, 1965) and CM7 were chosen as checks.

4.2.2 Field Design

At the Central Experimental Farm in Ottawa, Canada in 1993, the performance of the study material was assessed using a randomized block design (Zar, 1984), with 4 replicates of 12 plants per replicate. Due to the relatively high cost of rootworm field trials, only a single year of data was obtained. Seed was placed 0.5 metres apart and rows were 1 metre apart. The seed was infested at the time of planting with 1200 rootworm

eggs per plant. Rootworm eggs were obtained from French Agricultural research (Lamberton, Minnesota). The eggs were suspended in a 0.25% agar solution at a concentration of 1200 eggs/ml and applied to the soil with a pipetter (Palmer et. al 1976). After 8 weeks of growth, plant height was measured and then the plants were removed from the soil with minimal disturbance of the roots. The roots were washed clean of soil with water and the feeding damage assessed on the visual 9 point rating scale (Welch, 1977). A rating of 4.3 is considered the economic threshold of damage with quantifiable economic losses occurring with damage greater than this. The roots were then dried for 5 days in an oven at 60 °C and the root mass of plants was obtained and averaged from each genotype and replicate.

4.2.3 Diallel and Generation Means Analysis

The diallel cross is a method that gives information on which inbred performs the best and which hybrid combination is the most suitable for the trait of interest. The data for the diallel in this study was analyzed by Griffings' method two, model one (Griffing, 1956). Griffings' method gives information about the combining ability of a set of inbreds and their hybrids in all possible combinations. It is split up into two components; GCA and SCA. GCA refers to the average performance of a parent in hybrid combinations. SCA refers to those instances when the performance of a hybrid is relatively better or worse than would be expected on the basis of the average performance of the parents involved. Griffings' analysis was accomplished using the diallel analysis computer program accompanying the text by Christie et. al (1988).

Generation means analysis was performed based on the model of Mather and Jinks (1982) and assesses the relative contribution of additive and dominance genetic effects. The model for the expected phenotypic value of a generation can be shown as; $P_i = M + A + D$, where P_i is the phenotypic value of a given generation, M is the midparent value, A is the contribution of additive genetic effects and D is the contribution of dominance effects. The specific crosses are described in Table 4-1 with the coefficients used for each generation and the data given in Table 4-2. The SAS GLM procedure (SAS Institute 1982) was used to establish the confidence levels associated with the coefficients for the additive and dominance parameters in the model.

4.3 Results and Discussion

4.3.1 Field and hydroxamate Data

The results of the field data and hydroxamate data are given in Table 4-3 which gives the mean value obtained across replicates. The data indicate that the inbred CO272 has both the highest level of hydroxamates as well as the lowest root damage rating. The inbred with the lowest level of hydroxamates was NTR4034, which had relatively high level of rootworm damage. The mean value of the inbreds for total hydroxamates is 332.7 μg/g fresh weight. This value places these inbreds in the intermediate to low level of hydroxamates typically found in maize. The mean values of root damage ratings, root weight and plant height for the inbreds of the study were 5.2, 187.5 grams and 146 cm respectively.

Table 4-1. The Specific Crosses Performed to Generate the F_1 and Backcross Generations of the Generation Means Analysis.

Generation	Specific Cross
P_1	ITR3872
P_2	NTR4034
F_{11}	ITR3872 X NTR4034
F_{12}	NTR4034 X ITR3872
BC_{11}	(ITR3872 X NTR4034) X ITR3872
RBC_{11}	ITR3872 X (ITR3872 X NTR4034)
BC_{12}	(NTR4034 X ITR3872) X ITR3872
RBC_{12}	ITR3872 X (NTR4034 X ITR3872)
BC_{21}	(ITR3872 X NTR4034) X NTR4034)
RBC_{21}	NTR4034 X (ITR3872 X NTR4034)
BC_{22}	(NTR4034 X ITR3872) X NTR4034
RBC_{22}	NTR 4034 X (NTR4034 X ITR3872)
F_{21}	(ITR3872 X NTR4034)SELFED
F_{22}	(NTR4034 X ITR3872)SELFED

Table 4-2. Hydroxamate Concentration (μg/g Fr. Wt.), Root Damage Rating (1-9), Root Weight (grams) of the inbreds and F1 progeny used in the diallel study. Field Data represents the Mean Values Across 4 Replicates of 12 Plants per Replicate. Hydroxamate Levels given as μg/g. fr. wt. and is the mean of 3 replicates extracted and quantified. Values with the same letter in a column do not differ significantly by the SNK multiple comparison test (df = 3 for hydroxamate data, 4 for root damage ratings and root weight, P = 0.05). Standard Error of the mean in the preceding column is indicated as S.E.

Genotype	Hydroxamate	S.E.	Damage Rating	S.E.	Root Weight	S.E.
CO272	482.2 (a)	2.4	3.5 (ij)	0.6	180.0 (ij)	5.9
SD10	275.0 (f-i)	37.9	4.1 (g-i)	0.5	222.1 (e-f)	27.8
ITR3872	291.9 (d-h)	1.9	5.5 (a-d)	0.8	153.2 (j)	1.8
NTR3983	276.1 (e-i)	4.6	5.4 (b-d)	0.4	274.7 (a-g)	24.7
NTR4034	224.1 (g-j)	3.9	5.4 (b-d)	0.3	131.4 (k)	21.8
CM7	391.4 (a-e)	12.7	6.0 (ab)	0.3	216.8 (g-j)	32
ITR3865	388.2 (a-f)	33.5	6.2 (a)	0.3	134.5 (k)	3.2
CO272 X SD10	362.0 (b-f)	43.4	4.2 (f-i)	0.4	212.2 (g-j)	40
CO272 X ITR3872	172.1 (ij)	24.8	4.4 (e-h)	0.3	247.6 (b-g)	24.4
CO272 X NTR3983	308.8 (c-h)	26.6	3.6 (a-d)	0.4	196.5 (h-j)	2.9
CO272 X NTR4034	316.1 (c-h)	20.6	4.8 (d-g)	0.1	320.3 (a)	2.2
CO272 X CM7	404.0 (a-d)	43.2	4.8 (d-g)	0.2	150.0 (j)	16.8
CO272 X ITR3865	315.9 (c-h)	54	3.4 (j)	0.2	210.8 (h-j)	34.7
SD10 X ITR3872	282.8 (e-i)	12.8	4.4 (e-h)	0.5	220.2 (e-g)	10.5
SD10 X NTR3983	329.1 (b-h)	21.8	4.4 (e-h)	0.4	174.6 (ij)	16.8
SD10 X NTR4034	289.8 (d-h)	36.6	5.0 (c-e)	0.4	304.5 (a-d)	3.4
SD10 X CM7	245.5 (g-i)	2.9	5.9 (ab)	0.4	270.7 (a-h)	27.2
SD10 X ITR3865	333.3 (b-g)	64.9	4.9 (d-g)	0.3	197.6 (h-j)	0.4
ITR3872 X NTR3983	414.2 (a-c)	48.1	5.5 (a-d)	0.2	236.8 (c-i)	0.6
ITR3872 x NTR4034	378.2 (a-f)	48.5	5.8 (ab)	0.1	268.1 (a-h)	3.7

Table 4.2. Continued

Genotype	Hydroxamate	S.E.	Damage Rating	S.E.	Root Weight	S.E.
ITR3872 X CM7	221.5 (g-j)	25.6	5.8 (a-c)	0.3	173.6 (ij)	14.9
ITR3872 X ITR3865	227.7 (g-j)	43.5	4.5 (e-h)	0.2	236.8 (c-g)	43.2
NTR3983 X NTR4034	130.9 (j)	22.7	4.2 (f-i)	0.4	176.9 (ij)	25.6
NTR3983 X CM7	238.8 (g-j)	17.3	4.9 (d-f)	0.3	249.6 (b-i)	13.5
NTR3983 X ITR3865	326.1 (b-h)	60.7	5.0 (h-j)	0.3	266.0 (a-h)	0.8
NTR4034 X CM7	215.9 (h-j)	17.7	5.5 (a-d)	0.2	278.6 (a-h)	42.3
NTR4034 X ITR3865	287.9 (e-h)	21.4	3.6 (ij)	0.5	278.6 (a-h)	51.6
CM7 x ITR3865	304.9 (c-h)	49.7	4.8 (d-g)	0.5	218.1 (g-i)	6.3

Table 4-3. Root Damage Rating (1-9 Scale), Root Weight (Weight in grams), Plant Height (Centimeters) and Coefficients of Mean Parent Value (M), Additive effects (A) and Dominance effects (D) of the Inbreds, F_1 and Backcrosses used in the Generation Means study. Field Data Represents the Mean Values Across 4 Replicates of 12 Plants per Replicate. Parent 1 (P_1) is ITR3872. Parent 2 (P_2) is NTR4034. The specific crosses are described in Table 2a. Values with the same letter in a column do not differ significantly by the LSD multiple comparison test. Standard Error of the mean in the preceding column is indicated as S.E.

Generation	Damage Rating	S.E.	Plant Height	S.E.	Root Weight	S.E.	M	A	D
P_1	6.37ab	0.5	132e	25.6	160.9b	9.9	1	1	0
P_2	5.39b	0.3	175a-e	17.6	197.1ab	28.8	1	-1	0
F_{11}	5.70ab	0.4	191a-c	10.5	228.2ab	16.3	1	0	1
F_{12}	5.70ab	0.2	212ab	6.3	272.1a	2.3	1	0	0
BC_{11}	7.06a	0.2	175a-e	10.4	199.8ab	11.3	1	1/2	1/2
RBC_{11}	6.33ab	0.4	170a-e	18.6	202.2ab	16.7	1	1/2	1/2
BC_{12}	6.19ab	0.2	163b-e	8.3	168.5b	4.6	1	1/2	1/2
RBC_{12}	6.85a	0.2	149de	15.6	107.0b	18.8	1	1/2	1/2
BC_{21}	5.93ab	0.4	141de	2.1	111.7b	13.7	1	-1/2	1/2
RBC_{21}	5.77ab	0.2	217a	9.5	109.0b	20.2	1	-1/2	1/2
BC_{22}	6.13ab	0.4	206a-c	14.5	89.7b	43.8	1	-1/2	1/2
RBC_{22}	5.84ab	0.3	213ab	15.1	125.7b	40.6	1	1/2	1/2
F_{21}	5.92ab	0.3	159c-e	15.9	157.1b	4.3	1	0	1/2
F_{22}	5.06b	0.9	152ed	18.9	188.3ab	5.4	1	0	1/2

4.3.2 Diallel Analysis

GCA effects were found to be significant and SCA effects were found to be non-significant in the inheritance of root damage ratings (Table 4-4). The genotype with the highest negative GCA was CO272 (Table 4-5).

Table 4-4. Anova Table for the Griffings Diallel Analysis of Root Damage Ratings

Source	D.F.	S.S.	M.S.	F
G.C.A	6	9.17	1.53	7.38*
S.C.A.	21	8.72	0.42	2.00**
ERROR	54	16.77	0.21	

*Value of F (P = 0.05) for GCA = 2.40
** Value of F (P = 0.05) for SCA = 2.02

Table 4-5. GCA (Diagonal) and SCA Effects (Off Diagonal) from the Griffings Diallel Analysis of Root Damage Ratings.

Parent	NTR4034	SD10	ITR3865	ITR3872	CM7	CO272	NTR3983
NTR4034	0.011	0.278	-1.282	0.412	-0.032	0.602	-0.854
SD10		-0.194	0.118	-0.125	0.708	0.207	-0.381
ITR3865			0.016	0.094	-0.607	-0.778	-0.036
ITR3872				0.342	-0.76	-0.079	0.876
CM7					0.531	0.283	0.282
CO272						-0.76	-0.597
NTR3983							0.053

Based on the GCA, the performance of the hybrids is predictable on the basis of the performance of the parents. Varieties that have the best (lowest) root damage ratings have the best (most negative) GCA so those inbreds that express low damage ratings under rootworm infestation can effectively pass this trait on to their progeny (Figure 4-1).

Figure 4-1. The Correlation Between the GCA for Root Damage Ratings and the Root Damage Rating

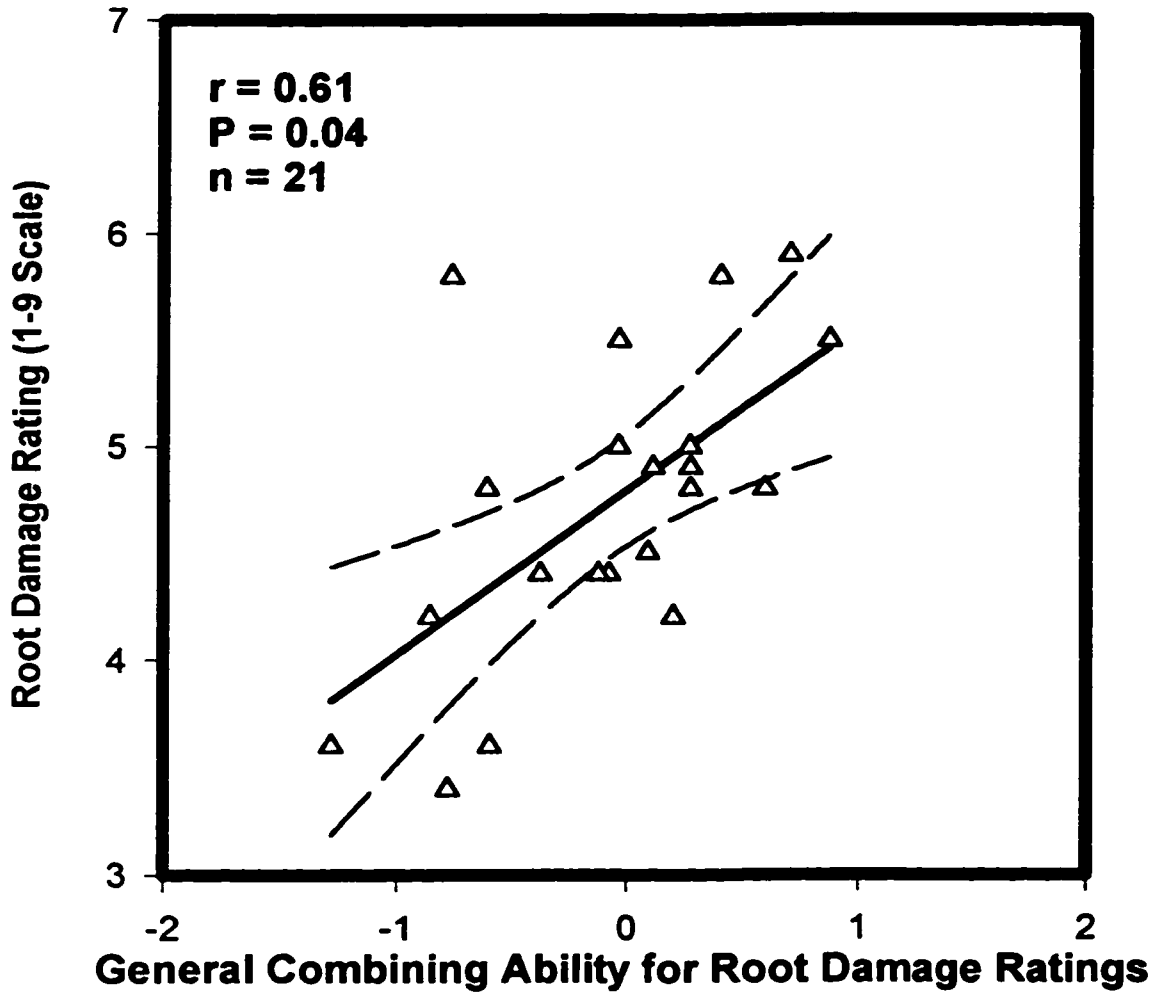

r = 0.61
P = 0.04
n = 21
Root Damage Rating (1-9 Scale)
7
6
5
4
3
-2
-1
0
1
2
General Combining Ability for Root Damage Ratings

Figure 4-2. The Correlation Between the GCA for Total Hydroxamic Acids and the Hydroxamic Acid Concentrations in Three Week Old Maize Seedlings

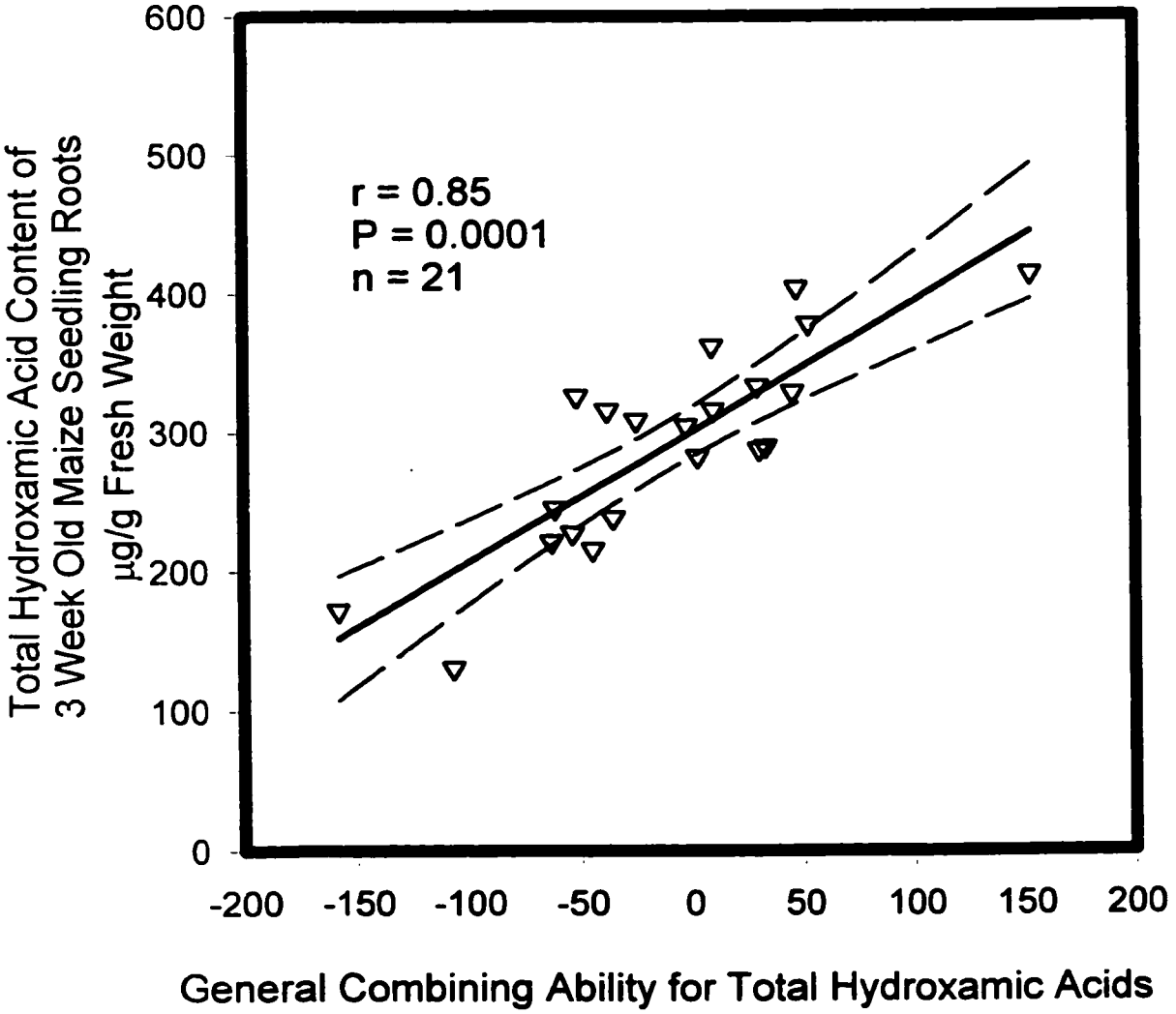
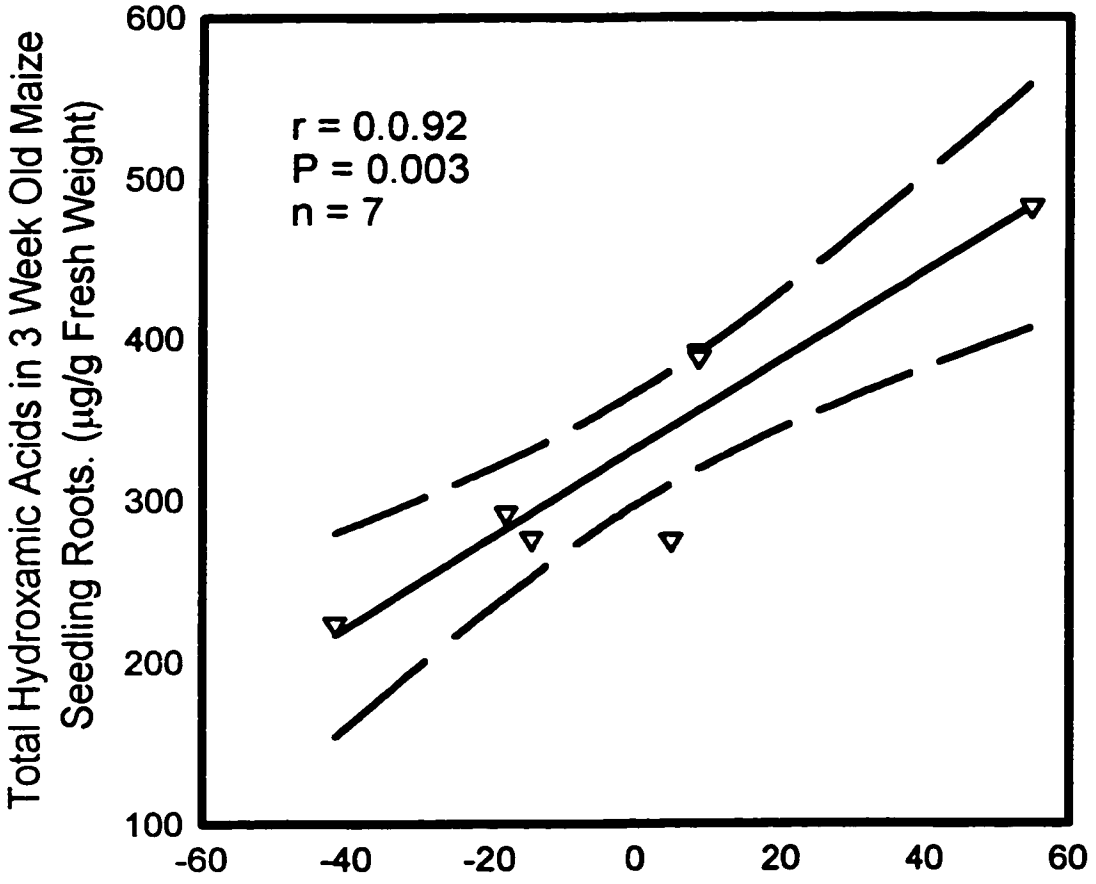

Total Hydroxamic Acid Content of
3 Week Old Maize Seedling Roots
µg/g Fresh Weight
600
500
400
300
200
100
0
r = 0.85
P = 0.0001
n = 21
-200
-150
-100
-50
0
50
100
150
200
General Combining Ability for Total Hydroxamic Acids

Figure 4-3. The Correlation Between the SCA for Hydroxamic Acid Content and the Hydroxamic acid content of three week old Maize Seedlings


r = 0.0.92
P = 0.003
n = 7
600
500
400
300
200
100
-60
-40
-20
0
20
40
60
Total Hydroxamic Acids in 3 Week Old Maize Seedling Roots. (µg/g Fresh Weight)
Specific Combining Ability for Total Hydroxamic Acids in 3 Week Old Maize Seedling Roots (µg/g Fresh Weight)

GCA and SCA effects are both significant in the inheritance of hydroxamate content (Table 4-6) and the genotype with the highest positive GCA was CO272 (Table 4-7).

Table 4-6. Anova Table for the Griffings Diallel Analysis of Root Hydroxamic Acid Content

Source	D.F.	S.S.	M.S.	F
G.C.A	6	48543.55	8090.59	4.45*
S.C.A.	21	105490.25	5023.345	2.76**
ERROR	54	98180.836	1818.16	

*Value of F (P = 0.05) for GCA = 2.40
** Value of F (P = 0.05) for SCA = 2.02

Table 4-7. GCA (Diagonal) and SCA Effects (Off Diagonal) from the Griffings Diallel Analysis of Root Hydroxamic Acid Content

Parent	NTR4034	SD10	ITR3865	ITR3872	CM7	CO272	NTR3983
NTR4034	-41.59	32.90	30.10	52.28	-44.79	9.17	-106.86
SD10		4.76	29.15	2.21	-61.55	8.66	45.05
ITR3865			5.57	-53.75	-3.01	-38.27	-51.87
ITR3872				-17.88	-62.94	-158.55	152.79
CM7					8.57	46.99	-35.77
CO272						54.86	-25.58
NTR3983							-14.38

Although the performance of the hybrids can be predicted based on the performance of the parents, those F_1's that have CO272 as a parent perform better than would be predicted based on the performance of the parents. The regression of hydroxamate content on the GCA of hydroxamate content (Figure 4-2), indicates that those inbreds highest in hydroxamates effectively pass this trait on to the F_1 hybrid. The regression of the SCA for hydroxamic acid content on the hydroxamic acid content is also significant (Figure 4-3.)

Both general and specific combining ability are significant in the inheritance of root weight and plant height (Tables 4-8 and 4-10), although the regression of SCA and GCA for these two traits on the traits themselves did not show a significant correlation. This

suggests that those inbreds that combine well for root weight and plant height were not those inbreds that expressed the traits of the heaviest root systems or the tallest plants.

The parent with the highest positive GCA for root weight was NTR4034 (Table 4-9), so this inbred combines well for large root mass but has low hydroxamate levels. The parent with the highest positive GCA for plant height (Table 4-11) was CM7, which has an intermediate level of hydroxamates.

Table 4-8. Anova Table for the Griffings' analysis of Root Weight

Source	D.F.	S.S.	M.S.	F
GCA	6	12708.4	2118.1	2.73*
SCA	21	37647.6	1792.7	2.31**
Error	54	41979.3	777.4	

*Value of F (P = 0.05) for GCA = 2.4
** Value of F (P = 0.05) for SCA = 2.02

Table 4-9. GCA (Diagonal) and SCA Effects (Off Diagonal) from the Griffings Diallel Analysis of Root Weight

Parent	CO272	NTR4034	ITR3872	CM7	SD10	NTR3983	ITR3865
CO272	7.22	51.76	16.18	-30.73	57.17	24.33	56.57
NTR4034		12.42	31.49	24.32	27.48	-11.30	13.91
ITR3872			-29.01	41.77	-15.43	-3.71	13.60
CM7				-11.33	17.36	-2.52	-22.78
SD10					11.44	-12.52	33.88
NTR3983						10.22	3.51
ITR3865							-0.96

Table 4-10. Anova Table for the Griffings' analysis of Plant Height

Source	D.F.	S.S.	M.S.	F
GCA	6	10423.11	1737.18	7.51*
SCA	21	30209.25	1438.54	6.22**
Error	54	12489.25	231.28	

*Value of F (P = 0.05) = 2.4
**Value of F (P = 0.05) for SCA = 2.02

Table 4-11. GCA (Diagonal) and SCA Effects (Off Diagonal) from the Griffings Diallel Analysis of Plant Height

Parent	CO272	NTR4034	ITR3872	CM7	SD10	NTR3983	ITR3865
CO272	-4.72	-18.46	13.35	14.39	-12.81	8.75	26.27
NTR4034		7.09	2.42	25.44	49.64	22.33	29.70
ITR3872			-18.59	29.17	29.61	13.73	15.65
CM7				17.56	21.40	16.96	15.07
SD10					-8.27	30.09	18.04
NTR3983						16.49	4.16
ITR3865							-9.56

The offspring, mid-parent regression utilizing a least squares regression estimates the heritability in a narrow sense (Falconer, 1996). When an offspring, mid-parent regression is applied to the crosses in this study, it results in h^2 values of 0.34 for hydroxamic acid content, 0.26 for root damage ratings and 0.35 for plant height (Figures 4-4 to 4-6), but due to the 'scatter' in the data these results may not be an accurate measure of the heritability of these traits and a more precise measurement of h^2 could be obtained with a segregating population, instead of the F_1, an F_2 or F_3 generation could be used to estimate heritabilities by utilizing the components of variance associated with the replicates and crosses, an analysis of this type is presented in chapter 5.

Figure 4-4. Heritability in a Narrow Sense of Root Damage Ratings by Midparent/offspring Regression

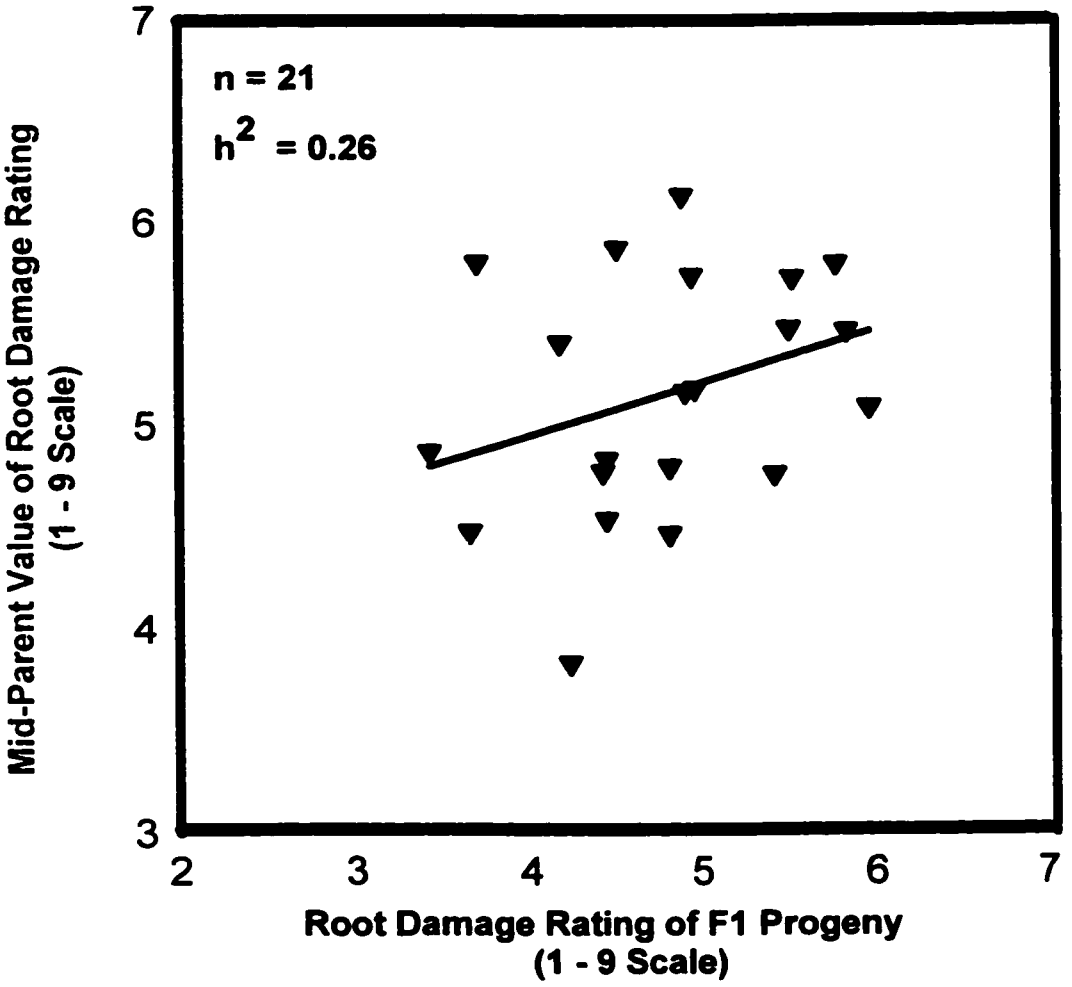

n = 21
h2 = 0.26
Mid-Parent Value of Root Damage Rating
(1 - 9 Scale)
Root Damage Rating of F1 Progeny
(1 - 9 Scale)
7
6
5
4
3
2
3
4
5
6
7

Figure 4-5. Heritability in a Narrow Sense of Hydroxamic Acid Content by Midparent/Offspring Regression

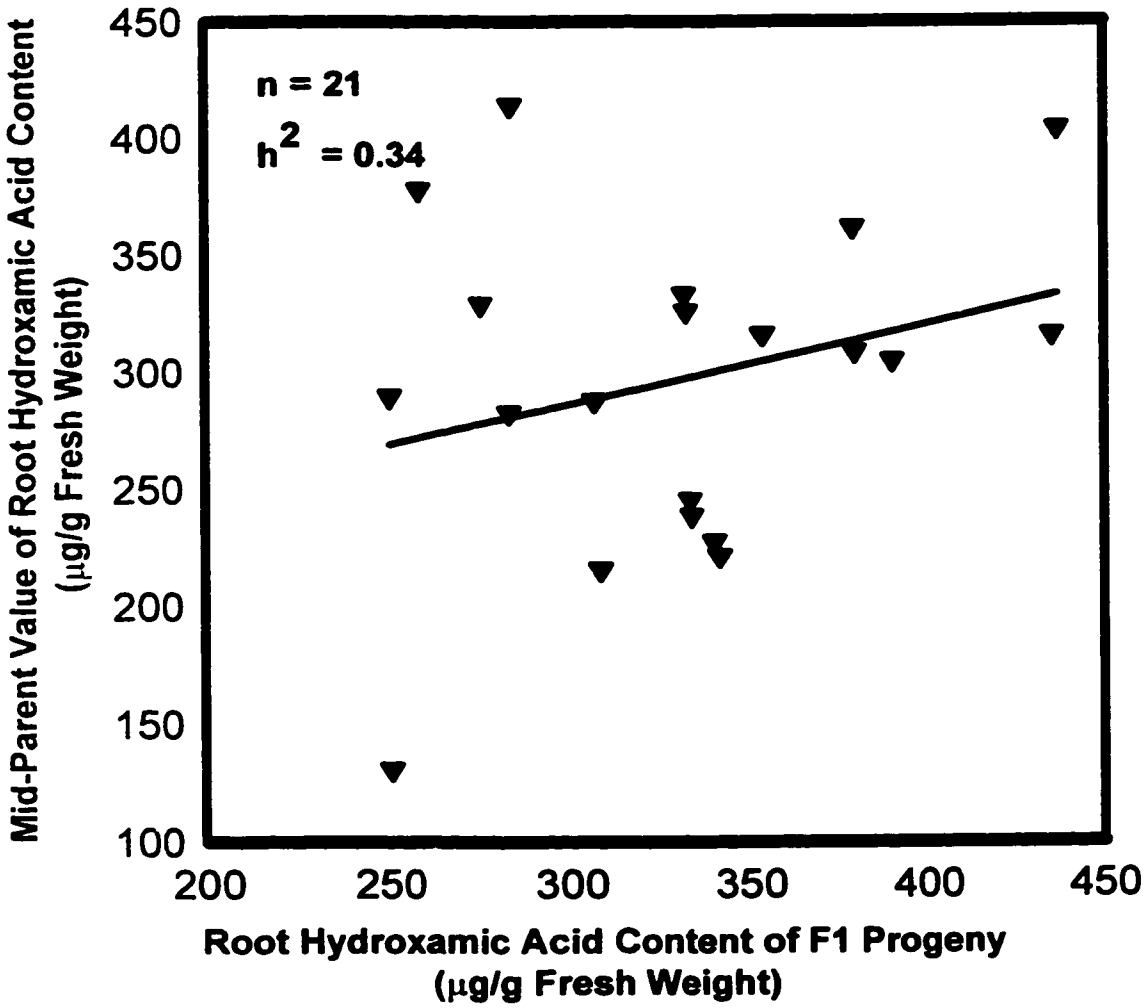
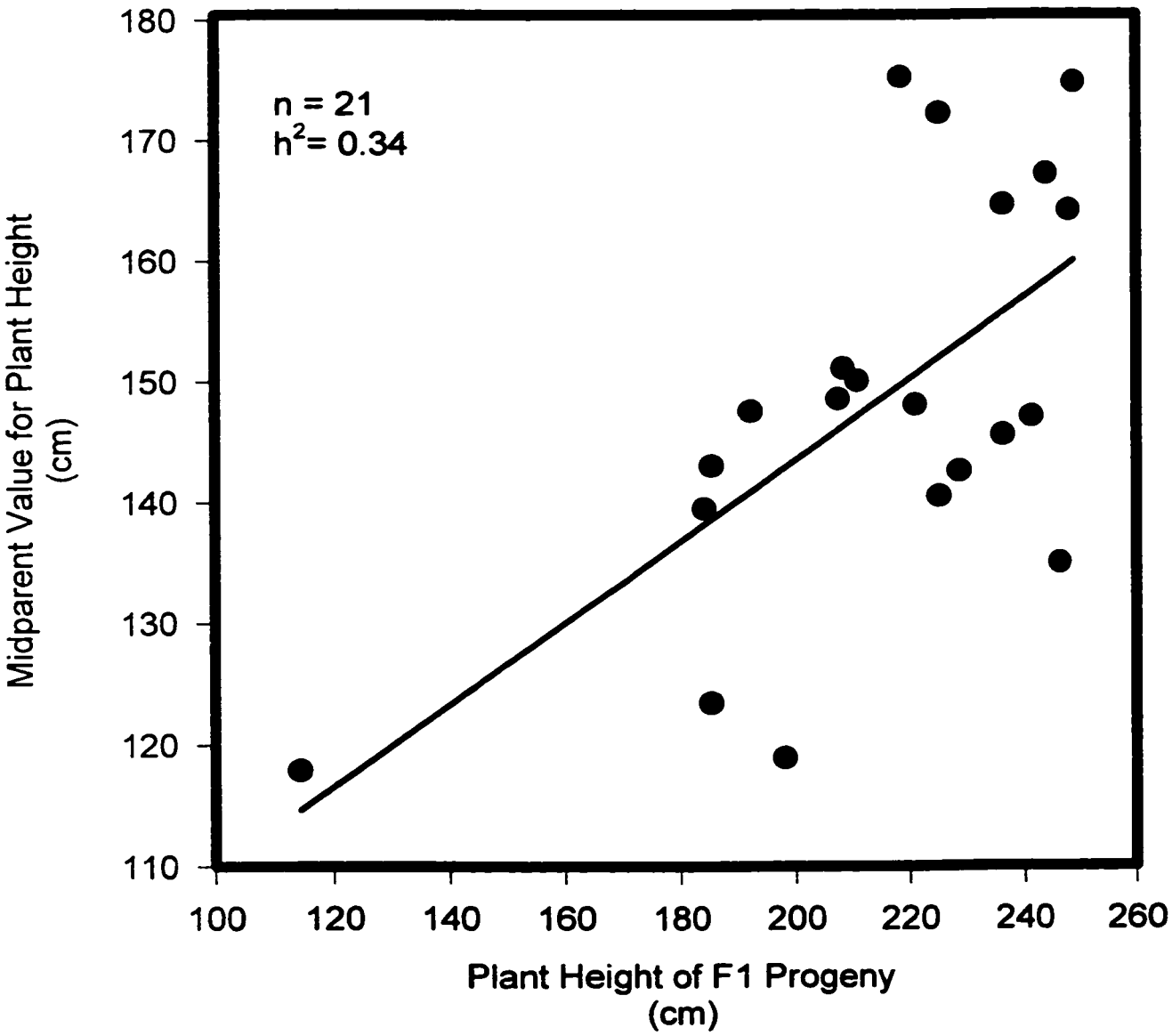

n = 21
h2 = 0.34
450
400
350
300
250
200
150
100
200
250
300
350
400
450
Mid-Parent Value of Root Hydroxamic Acid Content
(µg/g Fresh Weight)
Root Hydroxamic Acid Content of F1 Progeny
(µg/g Fresh Weight)

Figure 4-6. Heritability in a Narrow Sense of Plant Height by Midparent/Offspring Regression


n = 21
h^2= 0.34
Midparent Value for Plant Height (cm)
180
170
160
150
140
130
120
110
100
120
140
160
180
200
220
240
260
Plant Height of F1 Progeny (cm)

4.3.3 Generation Means Analysis

It was found that additive effects are significant (P = 0.04) and dominance effects are non-significant (P = 0.7) in the inheritance of root damage ratings. Additive and dominance effects were both found to be significant in the inheritance of plant height with P values of 0.01 and 0.04 respectively. The lower P value associated with additive effects indicates this parameter may be the more important genetic factor involved in the inheritance of plant height. Additive effects were found to be non-significant (P = 0.2) and dominance effects were found to be significant (P = 0.05) in the inheritance of root weight.

The significance levels for additive and dominance effects for these traits indicates that they are amenable to selection and improvement although the heritabilities may be low as shown in the parent/offspring regressions. Root damage ratings are an expensive and difficult means of measuring an inbred's performance under rootworm infestation and the significant dominance effects for root weight indicates a low probability of improving rootworm tolerance by selecting for root size instead of root rating. This would necessitate removing the root system from the ground for weighing, but could avoid costly rootworm egg infestation. One drawback to selecting for root size is that one may exclude genotypes with high hydroxamate content, so selecting for hydroxamate levels and root size at the same time may have the biggest impact against rootworm damage.

4.4 Rootworm Resistance in Maize

Rootworm resistance in maize can not be thought of as a single trait, it is a result of many factors, both biochemical and physiological that work together to provide some measure of protection to maize plants being attacked by rootworm. The qualities of host plant resistance; tolerance and antibiosis are observed in maize resistance to rootworm and it is clear that inbreds that express one resistance trait well, may not express the other traits to the same degree. No strong correlation between hydroxamic acid content and resistance or tolerance characteristics was found in this study, although previous studies using inbreds (Xie et. al 1990, Assabgui et. al 1995) support the concept that hydroxamates are antibiosis factors that contribute to much of the rootworm resistance seen in some maize inbreds. CO272, the best performer in terms of the inheritance of low root damage ratings and high hydroxamate content does not have good combining ability for root weight or plant height. These facts indicate that rootworm resistance is a quantitative trait controlled by numerous genes. A more complete picture of the genetic parameters controlling resistance may be obtained from other types of genetic dissection including QTL analysis utilizing molecular markers to identify and quantify loci contributing to the expression of resistance traits.

4.5 Conclusions

The inbred CO272 had the lowest root damage ratings as well as the highest level of hydroxamates. CO272 also had the highest negative GCA for root ratings and the highest positive GCA for hydroxamate content This inbred is also highly resistant to *Fusarium graminearum* disease (Reid et. al, 1992). These factors indicate that CO272 is a good source of resistance characteristics and could be a potential candidate for the development of maize with multiple resistance characteristics for use in Canada. It would also be beneficial if the root mass of CO272 could be improved to achieve a more complete set of resistance characteristics in this inbred, however the effect of increased root mass on yield must be assessed more fully due to possible negative effects on grain yield. Rootworm resistance is the result of the interaction of many factors which work together to produce resistant or tolerant maize. Further study is needed to reveal the relative importance of all of the factors that contribute to rootworm resistance in maize.

Diallel and generation means analysis are a useful way to determine which inbreds should be used in a breeding program, but it tells little of the underlying genetic origin of the resistance characteristics. Recently, many studies have utilized quantitative trait loci (QTL) analysis to identify the number and location of loci that contribute the traits of agronomic importance. The following preliminary study was performed to identify RFLP molecular markers that are associated with rootworm resistance traits.

5. Molecular Markers Associated to Rootworm Resistance Traits

5.1 Introduction

Many studies have utilized molecular markers for the identification of loci that significantly condition a trait. The traits investigated include resistance of a wild tomato species; *Lycopersicon hirsutum* to several arthropods (Nienhuis et. al 1987), and several traits in maize including yield, stress responses, morphological characters (Edwards et. al 1987, Stuber et. al 1987, Abler et. al 1991) and resistance to *Gibberella zea* infection (Pe et. al 1993). Byrne et. al (1998), and Lee et. al (1998) identified several QTL's that are significant in expression of the maize silk defense compound; maysin. Schön et. al (1993) investigated the loci significantly affecting resistance to European Corn Borer (ECB) in maize. They identified seven loci on 5 different chromosomes that are likely contributing to ECB resistance. In addition to insect resistance, the QTL's conditioning secondary metabolic pathways can also be identified with molecular markers. McMullen et al (1998) identified QTL's conditioning enzymes in the metabolic pathway of maysin. These studies illustrate that molecular markers can be used to attempt to identify and quantify chromosomal regions in maize that are important in the expression of agronomic traits.

Once genomic regions are identified that contribute in a significant manner to the trait of interest, marker assisted selection (MAS) can be utilized along with conventional selection to produce lines with desirable characteristics in a much shorter period of time than with conventional selection alone (Khairallah et. al, 1997). The breeding program at CIMMYT, Mexico has studied the feasibility of utilizing MAS in the development of

drought resistant lines. They concluded that additional gains in drought resistance will not be realized unless MAS is applied to the problem (Hoisington et. Al 1996).

In the present study, RFLP molecular markers were used to identify those markers that are significantly associated with the characteristics; root damage ratings, root size, stalk circumference and plant height. Field portions of this study were conducted at the Central Experimental Farm in Ottawa, Canada and the molecular marker study was performed in Mexico at the International Centre for Maize and Wheat Improvement (CIMMYT).

5.2 Materials and Methods

5.2.1 Germplasm and Population Development

In order for the RFLP's associated with resistance traits to be identified, a family of maize plants segregating for rootworm resistance was produced. This was accomplished by utilizing two inbreds that differ significantly for resistance, and crossed to produce F_1's, F_2's and F_3's. Two inbred lines were developed at the Central Experimental Farm in Ottawa, Ontario, Canada and were used as the parents for the study based on a 1994 screening of several inbred lines. Fourteen inbred lines under development at the Central Experimental Farm were planted in two replicates of 15 plants per replicate in a field with a high natural infestation of WCR and harvested at eight weeks post planting for rootworm resistance assessment (Table 5-1). Two inbreds were chosen that have statistically significant differences in their rootworm damage ratings. The inbreds were designated as 93n445 (Rootworm susceptible) and 118.31 (Rootworm resistant). 270 F_2:F_3 families were produced for the analysis. 93n445 is also known as

MBR622-5 and was developed at CIMMYT, Mexico as part of their multiple borer resistance breeding program. 118.31 is the fifth cycle of selfing of the cross TL89-6760-115 on record in the breeding program at CIMMYT, additional pedigree information on these varieties is not available. F_1 and F_2 generations were grown and selfed in Ottawa, Canada and the F_2:F_3 families were grown and selfed in Tlaltizipan, Mexico at the CIMMYT field station. The material was produced by Dr. Robert Hamilton, the corn breeder (Retired) from the Central Experimental Farm in Ottawa, Canada.

5.2.2 Generation Means Analysis

Generation means analysis was conducted on the generations used in this part of the study to determine if a similar result can be obtained for confidence levels associated with additive and dominance genetic affects obtained in the earlier study (Chapter 4). No back crosses were performed in this study so the generation means analysis was conducted utilizing only the inbreds, the F_1 and the F_3 generations.

5.2.3 Leaf harvesting

All protocols for the preparation of gels, Southern blots and lumonigraphs were performed according to the CIMMYT Applied Biotechnology Laboratory manual and only a summary of materials and methods is presented. (The laboratory manual is available to the public and may be referred to if the reader requires further information or a more detailed description of the protocols used. In some cases the original citation for a particular protocol is given).

Leaf tissue was taken from young, pre-anthesis plants with no necrotic areas or lesions, grown from December 1995 to March 1996 in Tlaltizipan, Mexico. The midribs

were removed and samples were frozen under liquid nitrogen and freeze dried. The samples were then ground to a fine powder with a mechanical mill and stored at -20° C.

5.2.4 Extraction of DNA

For extraction of genomic DNA (Sanghai-Maroof et. al 1994), 400 mg of freeze dried, milled tissue was extracted in 9 ml CTAB (mixed alkyltrimethylammonium bromide) for 1.5 hours. The slurry was then extracted with 2X 4.5 ml chloroform/octanol and centrifuged at 1500 g. The aqueous layer was pipetted off into tubes containing 50 µl of 10mg/ml RNAse A and allowed to incubate for 30 minutes. Ice cold 2-propanol was added and the DNA was precipitated with EtOH and washed twice, first with 76% EtOH/NaOAc (0.2M) and then with 76% EtOH/ NH4OAc (10mM). The DNA was then dissolved in 0.5 ml of TE buffer and stored at 4 °C. The DNA was quantified using a Beckman DU-65 spectrophotometer and the concentration was adjusted to 0.2 µg/ul. DNA was run on mini gel to check for quality to ensure high molecular weight DNA had been isolated.

5.2.5 Agarose Gel Electrophoresis

Double gels were prepared by melting 4.62 grams of Seakem agarose in 660 ml of 1X TE gel buffer in a microwave oven. The solution was cooled to 55°C and the first layer of 280 ml was poured into the gel mold and 4-30 tooth combs were inserted. The gel was allowed to cool to room temperature for 20 minutes, then the 380 ml second layer was poured onto the first layer and allowed to solidify. 200 µl of 0.2 µg/µl DNA/TE was restriction digested according to Helentjaris et. al (1986) with either *HINDIII* or *EcoRI* in

a restriction buffer consisting of ddH_2O, 10X restriction buffer, 0.1 M spermidine and 10U of enzyme for 4 hours at 37 °C. The reaction was stopped with 0.25M NaCl and DNA precipitated with 2.5 volumes of EtOH. The gel was loaded with 40 µg of DNA with 10 µl of SGB and run beside a *HINDIII* digested, digoxigenin end labeled Lambda DNA molecular weight marker. The gel was run in at 100 mA for 5-10 minutes, then at 15-20mA for 18 hours. The gel was then separated into two layers, stained with ethidium bromide, rinsed in water and photographed on a UV transilluminator with type 667 Polaroid film at f8 for 1 second.

5.2.6 Southern Blotting

Southern blots were performed in accordance with the protocols of Helentjaris et. al (1986). Magnagraph nylon membrane with 0.45 µm pore size was used as the blotting matrix. The DNA in the gel was denatured in a mixture of 0.4 N NaOH and 0.6 M NaCl for 30 minutes and then neutralized with a mixture of 0.5 M Tris (pH 7.5) and 1.5 M NaCl for 30 minutes. The gel was then placed on top of blotting paper and sponges and allowed to transfer onto the nylon membrane for 20 hours. Membranes were then UV crosslinked with a Stratagene UV crosslinker set at 120,000 µjoules/cm and then baked at 95 °C for 3 hours.

5.2.7 Hybridization and Detection of Labeled Probes.

Probes were chosen from the clone library at CIMMYT that were fairly evenly spaced across the 10 maize chromosomes that showed good polymorphism between the two parents The loci were typed; bnl6.25, bnl7.65, bnl8.45, csu48, csu58, npi114, npi239, umc2, umc133, umc147, umc17, umc21, umc7, umc34, umc58, umc59 (Gonzalez de Leon et. Al 1996). Probes were prepared in the following manner: plasmid inserts of cDNA or genomic DNA were amplified by polymerase chain reaction (PCR) and then

digoxigenin-dUTP was incorporated into the inserts, also with PCR. Unincorporated dNTP's were removed by spin column. The digoxigenin non-radioactive protocol exploits the specificity of antidigoxigenin (anti-dig). Anti-dig was labeled with alkaline phosphatase that removes a phosphate group from a substrate such as 3-(2'-Spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy)-phenyl-1-2-dioxetane (AMPPD) which then produces light and exposes X-ray film.

Blots were pre-hybridized for 3 hours in hybridization solution and then hybridized with labeled probes for 18 hours at 65°C. Blots were then washed in high stringency washes (0.15X SSC, 0.1% SDS) incubated in anti-dig for thirty minutes and then incubated in AMPPD for thirty minutes. Membranes were then placed into cassettes and exposed to Kodak XAR-5 X-ray film for 18 hours and developed in Kodak developer and fixer. Probes were removed so that the membranes could re-used. Films were read into the software program 'Hypermap' and data exported to the SAS statistical analysis program for establishment of markers associated with field traits by the GLM procedure.

5.2.8 Experimental Design and Field Study Method

The parental inbreds, F_1's, and 270 F_2:F_3 families were assessed for resistance to rootworm in a clay soil at the Central Experimental Farm in Ottawa, Canada in the summer of 1996. Three replicates of 10 plants per replicate were planted in the field in a an 0,1 alpha lattice design (Cochran and Cox, 1957) and artificially infested with 200 rootworm eggs/plant with the same protocol as described in chapter three. Resistance performance was assessed by measuring several physiological parameters including plant height, stalk circumference, root size on a 1 to 6 scale and root damage ratings on a 1 to 9 scale as described previously. Measurements were taken eight weeks post planting. Harvesting of plants and observations were taken in the same manner as described previously.

5.3 Results and Discussion

5.3.1 Field Observations

In 1994, a screening of inbred lines was undertaken at the Central Experimental Farm in Ottawa, Canada to determine which inbreds would be used for the development of the F_2:F_3 population. Fourteen inbreds being developed for rootworm resistance were planted in a field of high natural rootworm infestation in two replicates of 15 plants per replicate and assessed for rootworm damage after eight weeks of growth (Table 5-1). Large, statistically significant differences between the inbreds of this study were observed and resulted in the choice of 93n445 and 118.31 as the parental inbreds for the development of the F_2:F_3 population. It is interesting to note that the hydroxamate deficient mutant *bxbx* had an intermediate level of root damage, possibly indicating the role of another factor or secondary metabolite in the resistance of maize roots to rootworm.

Table 5-1. Inbred Lines Screened for Differences in Root Damage Ratings Under Natural Heavy Rootworm Infestation. Means With the Same Letter Do Not Differ Significantly by the SNK Multiple Comparison Test (P = 0.05)

Inbred Line	Root Rating
93n445	8.44a
116-16	8.34a
93n418	7.63ab
116-6	7.0bc
116-14	6.50cd
93n416	5.86ed
Bxbx	5.69ed
119-11	5.30ed
118-20	5.10e
122-26	5.04e
116-25	4.92e
118-31	3.73f
120-11	3.30f

Statistically significant differences were found between generations across the parental, F_1 and F_2:F_3 mapping population from the cross of 93n445 and 118.31 for plant height, stalk circumference, root size and root damage ratings as observed in the field and demonstrated by ANOVA analyses (Tables 4-2 to 4-5). All data sets are normally distributed and no F_3 families had phenotypic values that could be considered 'outliers'. This suggests that epistasis or overdominance were not confounding the analyses. The complete data set is presented in the appendix (Table 7-1).

Table 5-2. ANOVA Table showing significance of differences in Stalk Circumference of F_3 Individuals

Source	DF	S.S.	MS	F value	P
Model	268	1887.74	7.04	2.16	0.0001
Error	1826	5945.10	3.25		
Corrected Total	2094	7832.84			

Table 5-3. ANOVA Table showing significance of differences in Plant Height of F_3 Individuals

Source	DF	S.S.	MS	F value	P
Model	269	3185041.96	11840.30	1.2	0.0001
Error	5930	49619511.81	8367.54		
Corrected Total	6199	52804553.77			

Table 5-4. ANOVA Table showing significance of differences in Root Size Ratings of F_3 Individuals.

Source	DF	S.S.	MS	F value	P
Model	269	5855.05	21.77	18.40	0.0001
Error	5930	7013.23	1.18		
Corrected Total	6199	12868.28			

Table 5-5. ANOVA Table showing significance of differences Root Damage Ratings of F_3 Individuals.

Source	DF	S.S.	MS	F value	P
Model	269	14500.04	53.09	14.51	0.0001
Error	5929	22025.85	3.71		
Corrected Total	6198	36525.90			

5.3.2 Generation Means Analysis

Generation means analysis was conducted utilizing the parents, F_1 and F_3 generation. The model used for the analysis was that of Jinks and Mather (1982) as described earlier in this thesis. The coefficients used in the model are given in Table 5-6.

Table 5-6. Coefficients used for each of the parameters in the model used to test the significance of additive and dominant genetic effects.

Generation	M	A	D
P_1 (93n445)	1	1	0
P_2 (188.31)	1	-1	0
F_1 (P_1 X P_2)	1	0	1
F_3 (F_2 selfed)	1	0	0.25

Additive and dominant genetic effects were both found to be non-significant in the inheritance of stalk circumference with P values of 0.28 and 0.24 respectively. Additive genetic effects were found to be non-significant (P = 0.28), and dominant effects were found to be significant (P = 0.004) in the inheritance of plant height. Additive effects are significant (P = 0.0001) and dominance effects were found to be non-significant (P = 0.34) in the inheritance of root size. Both additive and dominance effects were found to be significant in the inheritance of root damage ratings with P values of 0.0001 and 0.02 respectively.

The results for the root traits obtained in this study are very similar to the generation means study presented in chapter four. In the earlier study, the inheritance of root size shows the same pattern as that found here, however the results for root damage rating reveal one small difference; dominance effects were found to be non-significant in the earlier study, but were found to be significant in the present study. The similarity in the two studies indicates that comparable genetic mechanisms are found to condition rootworm resistance in different maize inbreds, but some differences may exist that can be exploited in breeding maize for resistance to rootworm.

5.3.3 Heritability

Heritability in a narrow sense (h^2) for the field traits was calculated from the variance components for the traits using the field data for the F_3 and the SAS GLM, considering that $h^2 = \delta^2_g/\delta^2_p$ where δ^2_g is the genetic variance and δ^2_p is the phenotypic variance. The genetic variance can be calculated as δ^2_g = [mean square(entry) - mean square(error)]/[number of replicates] and the environmental variance can be calculated as; $\delta^2_e = \delta^2_g$ + mean square(error) and the phenotypic variance can be calculated as $\delta^2_p = \delta^2_g + \delta^2_e$. Using the data from Tables 4-2 to 4-5, the heritabilities of stalk circumference, plant height, root size and root ratings were found to be 84.7%, 89%, 54% and 55% respectively for the inbreds used in this study.

The heritabilities found in this study are much higher than those found in chapter four. As stated earlier, the parent offspring regressions (Figures 4-4 to 4-6) had a fair amount of scatter in the data which may have resulted in less than reliable results. Utilizing the variance components to determine levels of heritability as was done in the present study, may provide a more accurate measurement of h^2.

5.3.4 Molecular Marker Correlations to Field Traits

The data obtained from the lumonigraphs (example given in Figure 5-1) was analyzed as described earlier. The distribution of markers used was not sufficiently dense to permit the use of maximum likelihood analysis and the determination of LOD scores. In order to determine associations between markers and quantitative trait variation, a regression analysis was performed utilizing the model $y_j = m + bx_j + dz_j + e_j$ as described in Gonzalez de Leon et. al (1996) where j = 1,2...n, where y_j is the value of the trait for the jth individual, m is the mean, x_j and z_j are dummy variables for the jth individual, b is the simple regression coefficient and e_j was a random residual variable for the jth individual. Let $\ddot{y}_{MiMi}$, $\ddot{y}_{Mimi}$, $\ddot{y}_{mimi}$ be the observed phenotypic trait means of individuals with marker genotypes; M*i*M*i*, M*i*m*i*, m*i*m*i* for marker i in the F_2 population. Significant

differences among trait values for a given marker can be determined with the F statistic which tests the significance of the regression coefficient in the model as follows;

$$F = [n_{MiMi}(\ddot{y}_{MiMi} - \ddot{y})^2 + n_{Mimi}(\ddot{y}_{Mimi} - \ddot{y})^2 + n_{mimi}(\ddot{y}_{mimi} - \ddot{y})^2]/[2s^2/n-3]$$

where $\ddot{y}$ is the sample mean, s^2 is the pooled sample variance and n_{MiMi}, n_{Mimi} and n_{MiMi} are corresponding sample sizes in each marker class. The analysis was performed with the SAS GLM procedure.

Significant associations were found between markers and the traits; stalk circumference, root size and root damage ratings (Tables 5-7 to 5-10), the chromosomes on which these RFLP markers are located is indicated in Table 5-11.

Figure 5-1. Example of Lumonigraph with Segregation Pattern of RFLP marker umc133. Genomic DNA was restriction digested with *HindIII*. The *HindIII* digested λ molecular weight marker was used. Under these gel conditions, bands at the top of a run contain DNA of approximately 23130 base pairs and bands at the bottom of the run contain DNA of approximately 560 base pairs.

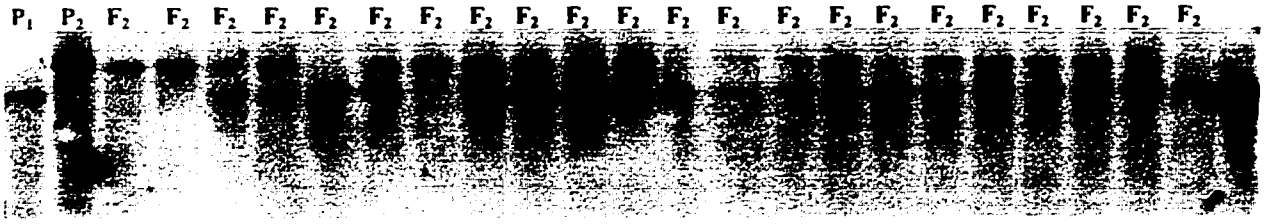

P1 P2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2 F2

Table 5-7. Anova Table for Significant Association between stalk circumference and marker designated npi239. (Corr. Tot. is the Corrected Total)

Source	D.F.	S.S.	M.S.	F	P
Model	1	5.71	5.71	6.49	0.01
Error	95	83.57	0.88		
Corr. Tot	96	89.27			

Table 5-8. Anova Table for Significant Association between Root Damage Ratings and marker designated csu58. (Corr. Tot. is the Corrected Total)

Source	D.F.	S.S.	M.S.	F	P
Model	1	11.15	11.15	5.05	0.03
Error	95	210.04	2.21		
Corr. Tot	96	221.19			

Table 5-9. Anova Table for Significant Association between Root Size and marker designated umc2. (Corr. Tot. is the Corrected Total)

Source	D.F.	S.S.	M.S.	F	P
Model	1	4.05	4.05	7.24	0.009
Error	65	36.41	0.56		
Corr. Tot	66	40.46			

Table 5-10. Anova Table for Significant Association between Root Size and marker designated csu48. (Corr. Tot. is the Corrected Total)

Source	D.F.	S.S.	M.S.	F	P
Model	1	4.34	4.34	6.38	0.01
Error	91	61.87	0.68		
Corr. Tot	92	66.22			

The statistically significant P values in the general linear model for the three traits indicates that molecular markers exist that are significantly associated to some traits related to rootworm resistance. The detected QTL's account for less than 10% of the total phenotypic variation for the traits measured. This limited analysis suggests that the regions in which the markers map to, contain genes contributing to characteristics important to rootworm resistance. More detailed information could be obtained by

choosing those chromosomal regions mapping near the markers of significance and saturating the local area with additional markers.

The identity or number of genes that may be within the regions identified is not known, nor is it known what their gene products might be, or how they relate to rootworm resistance. This study suggests that these regions are important to rootworm resistance, particularly the terminal portion of chromosome 3, which contains two molecular markers that are associated to the root characteristics of large size and low root damage ratings. It is possible that this region conditions for some physical quality and/or phytochemical defense that contributes to resistance by producing larger root masses and inhibiting root feeding, but the identity of the gene(s) awaits further investigation.

Table 5-11. The location of the RFLP markers used that show significant association with a phenotypic trait.

RFLP Marker	Trait	Chromosomal Location
npi239	Stalk Circumference	2
csu58	Root Size Ratings	3
umc2	Root Damage Ratings	3
csu48	Root Size Ratings	10

5.4 CONCLUSIONS

The QTL's that condition maize for rootworm resistance are contribute to the traits known to be involved in resistance including the physiological and phytochemical factors already discussed. These traits each contribute to a greater or lesser extent to resistance. The associations found in this study provide evidence that multiple genes contribute to rootworm resistance, particularly root physiology traits. Future work on the QTL's that contribute to rootworm resistance could concentrate on the loci near the markers that were found to be associated in a statistically significantly manner with resistance traits in this study, particularly on chromosome 3. The short arm of chromosome 4 is known to be important in the biosynthesis of hydroxamic acids so this region should be considered when conducting marker assisted selection for resistance traits. This was a preliminary examination of molecular markers that are associated with resistance traits and further work is necessary for a more complete understanding of the QTL's that contribute to resistance. A study utilizing a denser distribution of markers is required to produce a saturated linkage map in order to justify the use of marker assisted selection to improve rootworm resistance. It is also possible to exploit the newer PCR based markers such as random amplified polymorphic DNA (RAPD's) and simple sequence repeats (SSR's). These two methods have the potential to be even more powerful than RFLP based markers because they are less expensive and require less time to produce linkage maps.

6. General Discussion

The studies presented here demonstrate the mechanisms of rootworm resistance that exist in maize, some of the genetic parameters that govern these traits as well as evidence for the existence of QTL's that condition the resistance traits. Rootworm resistance was shown to be the result of many traits, including root physiological characters and root phytochemical factors (Chapter 3). Additive genetic effects were shown to be of importance in the inheritance of resistance characters (Chapter 4), and this fact indicates the potential to improve resistance traits through selection programs. Four RFLP molecular markers were found that are significantly associated to traits of importance in rootworm resistance (Chapter 5).

The fact that few truly rootworm resistant maize varieties exist indicates a basic deficiency in the approaches used to develop resistant varieties. The resistant varieties that do exist achieve resistance mainly through root morphology and physiology as opposed to biochemical mechanisms. The development of rootworm resistant maize continues to be a problematic goal, with artificial infestation trials and recurrent selection procedures as the primary method of assessing and developing resistant varieties, however the very large numbers of plants that need to be screened makes this a labor intensive, time consuming and costly process. The data presented in the preceding chapters indicates that the resistance traits are heritable, it demonstrates conventional types of quantitative inheritance patterns that are conditioned by multiple loci. This information suggests that the possibility exists to breed for rootworm resistant maize, but indicates that only concentrating on one or two resistance characteristics may not achieve

the full potential of resistance. This work emphasizes the need to utilize the new strategies available in order to produce resistant maize varieties in a more efficient manner and the development of new technologies makes this objective more probable than ever. The remainder of this discussion describes a few possible strategies that could be applied to the development of rootworm resistant maize

Rootworm resistance in maize is a trait which is realized through the contribution and interaction of many factors, both physiological and phytochemical and as a result of this, the genetics of resistance is difficult to dissect. The quantitative and multi-factorial nature of the inheritance of rootworm resistance makes this a particularly problematic trait to select and screen for since it is necessary to assess many characters, none of which in itself contributes significantly enough to resistance in order to exclude all other traits from the selection process. Most of the programs dedicated to breeding for rootworm resistance have developed resistant lines by screening for resistance in existing inbreds and then selecting those lines for improved performance. This approach has produced some limited results but substantial gains such as those made in corn borer resistance has been elusive. This could be at least partially due to the fact that most of the rootworm resistance seen in agronomically important maize inbreds appears to be the result of tolerance, not antibiosis and consequently an important resistance factor is being underutilized. Many inbreds do not differ significantly in their moderate levels hydroxamic acids and do not exhibit antibiosis as a result, thus it appears that hydroxamate content has not been selected for when these lines were developed. From this it is clear that a new strategy is required for breeding rootworm resistance into maize. This strategy could employ all of the conventional breeding methodologies, but also

utilize newer technologies such as marker assisted selection and genetic transformation.

One of the main problems with rootworm resistance breeding is the large expense (approximately $2000 U.S. per million eggs) and labor intensive procedures for assessing the performance of a maize inbred under artificial rootworm infestation. If host plant resistance to rootworm is to be used to it's full potential, a more practical approach needs to be developed, one that reduces the size of a population under field selection. Presently recurrent selection is most commonly used for the development of resistant lines. Recurrent selection aims to increase the frequency of favorable alleles while maintaining an acceptable amount of variability in the population for repeated cycles of selection in which the superior progeny are intermated. One of the drawbacks of this method is the large number of progeny that need to be produced to have a high probability of improving the trait of interest, typically thousands of plants need to be screened in the field for trait improvement. Another approach would be to utilize a backcross breeding method in conjunction with marker assisted selection. Such an approach would involve crossing a resistant line to a susceptible line and producing an F_2 family consisting of approximately 200 individuals. The susceptible line would be the line that is targeted for improvement (recurrent parent) and the resistant line is used as the source of the desired alleles (donor parent). QTL's that are correlated to the resistance trait can be identified with molecular markers, such as RFLP or PCR based markers such as simple sequence repeats (SSR's). As with all projects of this type, useful enzyme/probe or primer combinations must be found that are polymorphic between the parents and show segregation in the F_2 and F_3 generations. Field data and molecular marker experiments would be conducted as described earlier (see chapter 5). QTL's could be identified as simple marker/trait

associations as in the preceding study, but a more detailed linkage map is required to identify chromosomal regions in the donor line that are contributing in a statistically significantly manner to the traits under study. It is important that the QTL's identified should account for most of the variation observed in resistance traits, so the most useful situation is one where a low number of QTL's account for a large percentage of the variation. The next step of this process involves transferring those chromosomal segments that were identified as being important to the trait back into the recipient line by a backcrossing strategy in order to eliminate 'genetic drag' or unwanted alleles from the donor. The real advantage of molecular markers can be seen at this stage since there is a direct measure of the relative genetic makeup of the crosses, or what proportion of each parental genome exists in the progeny. The ability to gauge how much genetic drag still exists in a cross has the potential to speed up development of resistant lines considerably over conventional selection that does not use molecular markers (Figure 6-1) .

Many of the inbreds and hybrids examined in this work have exhibited more tolerance than antibiosis, another strategy for improving existing lines would be to increase their production of hydroxamates. Such an approach could also use a backcrossing method to augment the expression of hydroxamates in otherwise acceptable inbreds if a high hydroxamate line is used as the donor, alternatively a line's hydroxamate content could be augmented through gene manipulation, or upregulation of existing genes. The *Bx1* locus located on the short arm of chromosome 4 was found to be a major gene in the expression of hydroxamates in maize (Couture, 1971). Melanson et. al (1997) have demonstrated that the *bxbx* mutant does not produce hydroxamates due to a deletion in the indole synthetase gene, therefore It may be possible to attach a strong promoter to

this gene in order to achieve a high level of hydroxamate expression in the roots of agronomically desirable but rootworm susceptible lines.

There has been much research into the use of *Bacilus thuringiensis* (*Bt*) toxins to control insect pests recently. There are many advantages to this approach since the toxins are highly specific so have little effect on non-target organisms. The proteins designated *CryI* have been demonstrated to be very effective against Lepidopteran pests when the *CryI* genes are expressed in transgenic maize plants (Estruch et. al, 1997). *Cry* proteins have also been found that are specifically toxic to Coleoptera species (Johnson et. al, 1993). The *CryIIIA, CryIIIB* and *CryIIIC* toxins are effective against *Diabrotica* species and the mode of action involves the binding of the toxin to membrane receptors on brush border vesicles of the midgut and producing ion channels that permit the efflux of ions, resulting in the death of the insect. It is possible to transform maize with genes encoding *CryIII* proteins to reduce rootworm damage, or to treat rootworm infested fields with *B. thuringiensis* suspensions and this work is now underway by some researchers. At present in the literature there is no indication that rootworm control in maize utilizing *Bt* is being field tested, however several United States patent applications (U.S. patents office) have been made that indicate the intentions of several companies, including Ecogen Inc. and Plant Genetic Systems (PGS). These companies have different approaches to exploit these *Bt* toxins. Ecogen has applied for U.S. patent number 05369027 with the intention of developing *Diabrotica spp.* resistant *Bt* maize through genetic transformation technology. PGS has applied for U.S. patent number 05378625 and proposes to utilize extracted, pure *Bt* toxin or bacterial suspensions to treat rootworm infested maize. The companies describe in detail the types of constructs, toxicity and toxin specificity of the

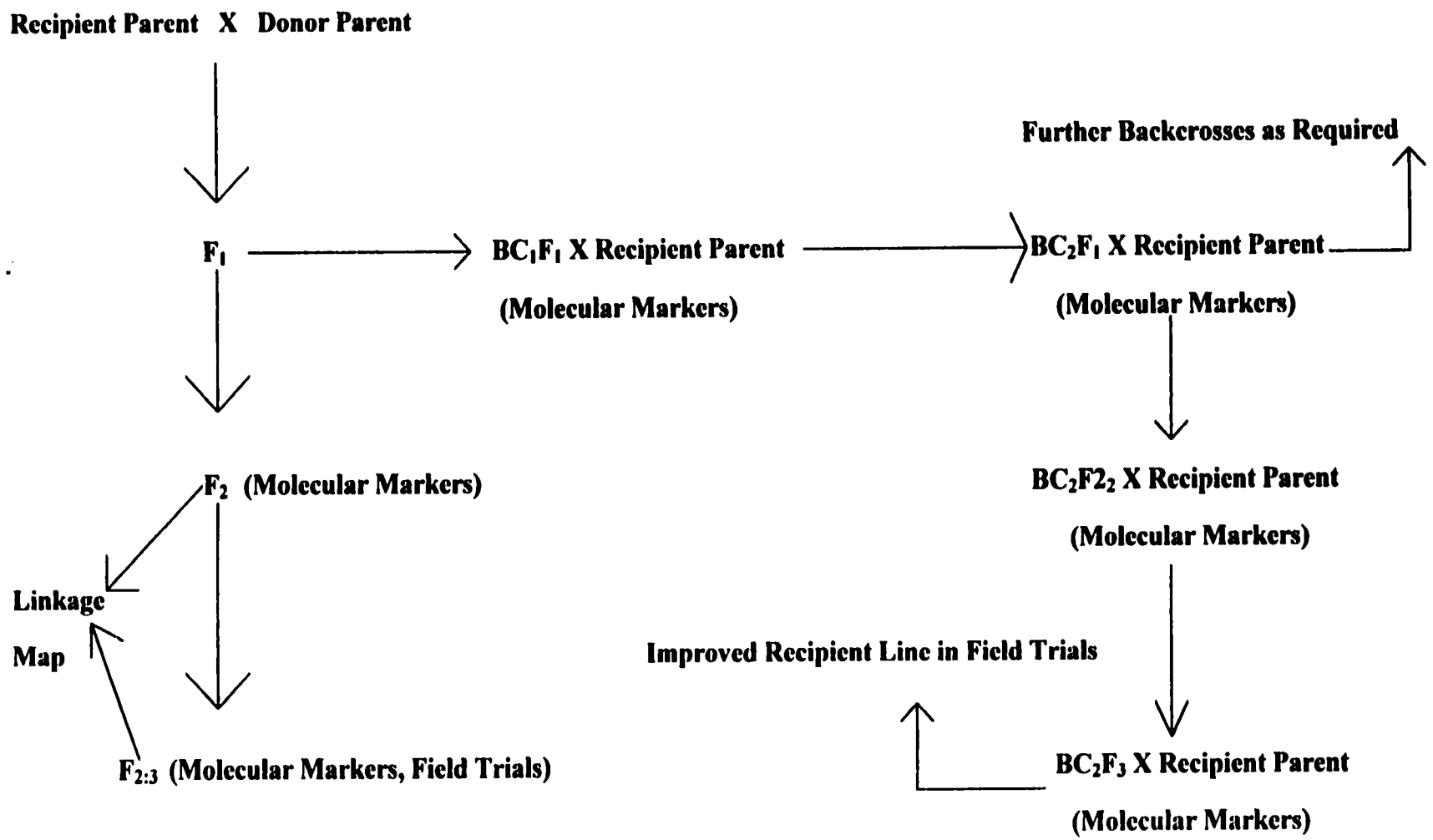
particular *B. thuringiensis* strain that they are utilizing in their patent applications and these systems have a good probability of succeeding, however the high toxicity of these compounds provides a strong selection pressure for the development of insect resistance. Along with the risk of the development of insect resistance, there is also the possibility of killing non-target Coleopterans such as lady bird beetles in the case of the PGS proposal or genetic flow from transformed inbreds grown in the field into native varieties in the case of the Ecogen proposal. Genetic flow would be particularly problematic in Mexico where maize is though to have originated (Serratos et. al, 1997). The existence of *teosinte*, *Tripsacum* and wild corn varieties in Mexico increases the likelihood that transformed maize genes will cross species barriers.

Considering these facts it is crucial to develop rootworm resistance in a manner that is both environmentally sound and sustainable, these are basic concepts of integrated pest management. In order to most effectively develop rootworm resistance in maize it is important to utilize all of the methods available to assess, breed and introduce resistance into important inbred lines. This pyramided resistance will help to prevent the development of resistance in the insects to the defenses of the maize plant and at the same time maintain a healthy interaction of trophic levels, provided resistance levels are at an intermediate level .

The bionomics of rootworms has been extensively documented as have the mechanisms of maize resistance to rootworm, but generally speaking these efforts have not produced substantial gains in terms of a commercial line release that exhibits good agronomic performance as well as acceptable rootworm resistance. This may be due to the fact that it is easier to apply insecticides to the field than invest the considerable

resources required to develop rootworm resistant maize. The development of rootworm resistant maize would be an important contribution to sustainable agriculture and environmentally sound pest control. The large scale use of insecticides presents a serious threat to the environment, and since such vast quantities of insecticides are used to control rootworm it is imperative that host plant resistance to rootworm be developed and exploited.

Figure 6-1. Proposed Scheme for Marker Assisted Selection for Improvement of Rootworm Resistance in Maize. (Adapted from Gonzalez-de-Leon et. al, 1996)


Recipient Parent X Donor Parent
F1
BC1F1 X Recipient Parent
(Molecular Markers)
Further Backcrosses as Required
BC2F1 X Recipient Parent
(Molecular Markers)
F2 (Molecular Markers)
BC2F2 X Recipient Parent
(Molecular Markers)
Linkage
Map
F2:3 (Molecular Markers, Field Trials)
Improved Recipient Line in Field Trials
BC2F3 X Recipient Parent
(Molecular Markers)

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8. APPENDIX

Table 8-1. 1996 Field data for the Parents, F_1's and F_3 families used in the correlation of RFLP molecular markers to field traits. Means of 3 Replicates Shown (10 plants/replicate) Standard Error Given in Brackets.

Generation	Stalk Circumference (cm)	Plant Height (cm)	Root Size Ratings (1-6 Scale)	Root Damage Ratings (1-9 Scale)
P_1 = 93n445	5.3(0.35)	52.7(4.40)	6(0)	8.5(0.18)
P_2 = 118.31	5.1(0.48)	49.17(2.2)	1.8(0.04)	1.1(0.1)
F_1	6.2(0.34)	79.83(4.8)	2.7(0.25)	1.8(0.14)
F_3 = 5	6.84(0.16)	68.47(5.32)	4(0.24)	5.9(0.56)
F_3 = 9	4.56(0.35)	44.57(2.67)	3.67(0.45)	4.2(0.66)
F_3 = 10	7.24(0.19)	49.01(5.85)	1.91(0.29)	1.65(0.20)
F_3 = 11	6.78(0.26)	66.53(6.65)	3.0(0.20)	3.32(0.30)
F_3 = 12	5.57(0.19)	45.83(4.21)	4.23(0.19)	7.15(0.33)
F_3 = 14	6.34(0.32)	53.38(2.9)	3.3(0.22)	4.1(0.28)
F_3 = 17	6.34(0.32)	53.38(2.9)	3.3(0.22)	4.1(0.28)
F_3 = 21	6.8(0.18)	72.26(4.2)	2.82(1.0)	4.39(0.48)
F_3 = 22	6.44(0.33)	63.35(3.83)	3.11(0.23)	4.5(0.48)
F_3 = 24	5.37(0.16)	59.63(2.81)	3.92(0.28)	3.73(0.42)
F_3 = 26	6.12(0.18)	63.60(5.88)	2.84(0.19)	3.26(0.54)
F_3 = 27	6.23(0.35)	59.33(3.44)	3.58(0.24)	4.42(0.47)
F_3 = 28	5.27(0.22)	50.1(2.61)	3.43(0.24)	4.21(0.455)
F_3 = 29	5.86(0.28)	73.09(5.55)	2.31(0.09)	4.38(0.40)
F_3 = 30	5.61(0.15)	57.47(4.39)	3.95(0.23)	5.21(0.50)
F_3 = 34	5.37(0.31)	78.03(4.18)	3.19(0.23)	2.46(0.44)
F_3 = 35	5.6(0.22)	53.56(3.3)	2.0(0)	3.13(0.36)
F_3 = 36	7.03(0.27)	105.68(6.99)	3.71(0.44)	4.05(0.30)
F_3 = 37	6.0(0.25)	65.21(3.03)	3.64(0.09)	3.79(0.20)
F_3 = 38	6.81(0.36)	50.64(2.81)	3.70(0.54)	5.6(0.72)
F_3 = 43	5.42(0.26)	59.41(5.01)	4.27(0.21)	5.04(0.46)
F_3 = 44	4.87(0.29)	54.29(3.87)	2.95(0.16)	4.20(0.43)
F_3 = 45	5.93(0.27)	50.93(2.67)	5.0(0)	2.5(0.18)
F_3 = 46	5.42(0.15)	63.79(4.38)	2.96(0.18)	3.88(0.30)
F_3 = 47	4.66(0.27)	40.14(2.77)	3.71(0.29)	3.19(0.68)
F_3 = 52	6.36(0.57)	73.10(2.84)	3.58(0.19)	3.33(0.25)
F_3 = 53	6.55(0.12)	79.66(4.46)	2.32(0.27)	4.0(0.32)
F_3 = 54	4.88(0.29)	28.62(1.71)	4.6(0.22)	4.55(0.32)
F_3 = 55	6.86(0.51)	51.75(2.61)	3.68(0.32)	4.47(0.53)
F_3 = 56	6.22(0.61)	51.72(3.51)	4.77(0.21)	4.45(0.59)
F_3 = 57	6.62(0.20)	56.35(2.72)	4.0(0)	6.61(0.34)
F_3 = 58	7.03(0.36)	48.08(2.78)	4.59(0.36)	4.91(0.32)
F_3 = 59	5.04(0.26)	43.71(3.46)	3.73(0.44)	6.19(0.44)
F_3 = 61	4.4(1.4)	56.61(3.89)	3.57(0.23)	4.5(0.59)

$F_3 = 62$	4.37(0.15)	55.32(3.83)	3.79(0.25)	4.17(0.51)
$F_3 = 63$	5.2(0.25)	86.87(5.73)	4.25(0.27)	3.35(0.52)
$F_3 = 64$	5.6(0.36)	64.05(4.15)	3.67(0.39)	7.17(0.36)
$F_3 = 65$	4.94(0.36)	64.44(7.44)	3.08(0.29)	4.96(0.47)
$F_3 = 66$	6.74(0.27)	85.81(3.84)	2.29(0.18)	4.29(0.29)
$F_3 = 67$	7.22(0.58)	58.39(4.52)	3.3(0.36)	4.5(0.29)
$F_3 = 69$	6.52(0.41)	65.23(3.65)	2.54(0.25)	3.68(0.36)
$F_3 = 71$	6.44(0.50)	70.69(4.55)	3.0(0.32)	3.62(0.28)
$F_3 = 77$	7.16(0.19)	55.70(3.80)	2.65(0.09)	4.77(0.24)
$F_3 = 78$	5.26(0.22)	57.80(2.35)	4.12(0.28)	5.21(0.64)
$F_3 = 79$	4.12(0.15)	39.34(2.10)	3.48(0.25)	4.09(0.68)
$F_3 = 83$	5.64(0.15)	37.11(2.42)	3.08(0.21)	4.81(0.25)
$F_3 = 84$	6.20(0.65)	45.30(2.56)	4.08(0.17)	4.21(0.36)
$F_3 = 85$	5.15(0.46)	46.96(2.84)	5.08(0.24)	5.85(0.88)
$F_3 = 89$	5.49(0.20)	53.36(3.21)	4.6(0.16)	5.64(0.43)
$F_3 = 90$	7.37(0.32)	52.04(8.01)	3.29(0.37)	5.21(0.64)
$F_3 = 91$	4.16(0.23)	43.85(6.93)	5.15(0.22)	7.92(0.18)
$F_3 = 92$	5.13(0.43)	50.42(7.35)	3.80(0.55)	3.90(0.97)
$F_3 = 94$	5.41(0.22)	54.25(6.90)	4.62(0.13)	5.43(0.32)
$F_3 = 95$	5.84(0.23)	43.73(2.17)	3.54(0.19)	3.45(0.48)
$F_3 = 96$	6.67(0.25)	61.96(4.29)	1.0(0.10)	1.70(0.15)
$F_3 = 97$	5.83(0.22)	58.86(3.17)	1.95(0.23)	1.79(0.26)
$F_3 = 101$	6.48(0.19)	59.41(2.50)	1.69(0.18)	3.41(0.26)
$F_3 = 102$	6.13(0.43)	85.0(7.79)	2.0(0)	2.50(0.21)
$F_3 = 103$	4.74(0.24)	49.28(3.29)	4.53(0.29)	7.32(0.49)
$F_3 = 104$	6.44(0.21)	75.40(5.03)	2.93(0.32)	1.68(0.15)
$F_3 = 107$	5.54(0.13)	55.52(3.17)	2.93(0.16)	4.15(0.36)
$F_3 = 108$	4.87(0.29)	40.24(2.16)	4.33(0.09)	5.26(0.35)
$F_3 = 110$	4.98(0.28)	61.74(4.58)	3.81(0.18)	4.86(0.54)
$F_3 = 112$	6.70(0.25)	60.71(4.13)	3.0(0)	3.06(0.52)
$F_3 = 116$	6.32(0.48)	59.43(4.84)	3.23(0.21)	4.09(0.27)
$F_3 = 117$	6.05(0.45)	63.94(6.51)	3.45(0.19)	3.18(0.31)
$F_3 = 120$	5.98(0.53)	74.06(7.04)	3.61(0.10)	5.35(0.33)
$F_3 = 122$	6.42(0.34)	61.40(4.26)	3.70(0.32)	6.04(0.399)
$F_3 = 123$	5.15(0.28)	66.34(5.11)	3.21(0.41)	4.68(0.59)
$F_3 = 125$	7.91(0.39)	105.61(6.31)	2.0(0.38)	1.50(0.20)
$F_3 = 127$	5.63(0.22)	61.66(4.39)	4.33(0.21)	5.09(0.57)
$F_3 = 128$	6.05(0.14)	57.44(2.94)	5.12(0.17)	7.28(0.36)
$F_3 = 129$	6.07(0.27)	61.86(3.35)	3.21(0.23)	4.71(0.40)
$F_3 = 130$	4.03(0.40)	48.35(3.14)	4.31(0.09)	6.28(0.41)
$F_3 = 133$	5.34(0.37)	51.66(3.08)	3.87(0.12)	6.31(0.53)
$F_3 = 136$	6.01(0.24)	51.03(2.25)	3.08(0.17)	2.48(0.23)
$F_3 = 137$	5.72(0.22)	65.54(4.42)	4.04(0.15)	3.85(0.32)
$F_3 = 138$	4.16(0.30)	44.45(5.01)	3.79(0.19)	6.79(0.39)
$F_3 = 139$	5.52(0.25)	54.48(3.23)	3.35(0.32)	3.18(0.49)

$F_3 = 141$	6.86(0.58)	58.29(6.51)	3.10(0.28)	2.16(0.21)
$F_3 = 142$	6.26(0.45)	63.31(3.54)	1.84(0.19)	3.42(0.28)
$F_3 = 143$	4.51(0.25)	42.57(3.29)	4.71(0.09)	5.71(0.36)
$F_3 = 145$	6.70(0.61)	76.42(4.63)	1.36(0.10)	2.40(0.22)
$F_3 = 147$	5.79(0.31)	51.30(3.44)	3.27(0.24)	4.65(0.60)
$F_3 = 149$	5.75(0.75)	57.58(3.71)	1.95(0.35)	3.26(0.54)
$F_3 = 152$	4.82(0.35)	64.41(28.53)	4.57(0.25)	6.21(0.71)
$F_3 = 154$	5.57(0.35)	61.46(4.49)	4.64(0.10)	6.72(0.32)
$F_3 = 156$	5.04(0.34)	41.27(2.52)	2.0(0.19)	3.92(0.47)
$F_3 = 157$	5.50(1.52)	67.26(4.16)	3.62(0.12)	4.0(0.52)
$F_3 = 160$	5.87(0.28)	62.68(2.72)	3.14(0.22)	4.81(0.42)
$F_3 = 161$	5.30(0.18)	56.38(3.11)	3.86(0.31)	5.11(0.63)
$F_3 = 162$	5.76(0.62)	46.11(3.26)	2.85(0.41)	4.05(0.68)
$F_3 = 163$	3.86(0.18)	82.61(4.44)	2.71(0.34)	3.0(0.32)
$F_3 = 164$	5.07(0.34)	59.40(4.76)	2.48(0.37)	4.0(0.59)
$F_3 = 165$	6.24(0.52)	49.89(3.01)	2.58(0.19)	4.46(0.35)
$F_3 = 166$	4.37(0.15)	67.78(4.35)	4.0(0)	3.44(0.25)
$F_3 = 168$	6.53(0.21)	256.88(174.22)	1.0(0)	2.62(0.32)
$F_3 = 170$	4.86(0.26)	57.86(4.0)	3.67(0.1)	3.25(0.26)
$F_3 = 171$	5.43(0.22)	51.37(3.29)	4.24(0.22)	5.29(0.54)
$F_3 = 172$	Na	57.51(3.04)	3.67(0.25)	4.73(0.79)
$F_3 = 178$	5.02(0.47)	41.74(3.66)	3.33(0.30)	3.06(0.42)
$F_3 = 179$	4.62(0.17)	49.56(2.47)	3.05(0.21)	5.35(0.41)
$F_3 = 181$	5.21(0.30)	45.55(2.85)	4.38(0.13)	5.95(0.33)
$F_3 = 182$	5.23(0.32)	51.16(2.59)	3.53(0.26)	4.47(0.43)
$F_3 = 183$	3.90(0.55)	42.55(3.32)	4.63(0.11)	3.37(0.37)
$F_3 = 186$	4.91(0.21)	60.53(3.43)	2.23(0.37)	3.19(0.48)
$F_3 = 188$	5.57(0.92)	39.22(3.75)	4.46(0.14)	4.69(0.75)
$F_3 = 189$	5.74(0.31)	289.99(232.33)	2.76(0.25)	2.48(0.31)
$F_3 = 190$	5.12(0.56)	61.33(5.27)	4.86(0.16)	4.32(0.64)
$F_3 = 191$	4.79(0.14)	44.85(2.39)	4.33(0.1)	3.29(0.52)
$F_3 = 192$	4.90(0.56)	61.58(4.45)	1.68(0.09)	2.46(0.41)
$F_3 = 194$	5.51(0.28)	40.19(4.24)	4.72(0.11)	6.11(0.50)
$F_3 = 196$	5.66(0.32)	63.67(3.55)	3.44(0.25)	4.48(0.37)
$F_3 = 199$	5.90(0.32)	68.39(4.17)	2.71(0.33)	4.81(0.54)
$F_3 = 202$	6.23(0.21)	81.83(4.09)	1.0(0)	2.37(0.28)
$F_3 = 203$	5.52(0.48)	66.81(5.01)	2.83(0.16)	2.78(0.18)
$F_3 = 204$	10.98(446)	75.05(3.78)	3.33(0.09)	4.47(0.29)
$F_3 = 208$	4.47(0.20)	38.66(2.02)	5.30(0.09)	6.67(0.52)
$F_3 = 209$	5.93(0.24)	65.50(3.87)	4.19(0.16)	7.29(0.35)
$F_3 = 212$	5.96(0.23)	53.22(3.19)	4.37(0.09)	5.59(0.46)
$F_3 = 213$	4.52(0.52)	76.31(6.13)	2.56(0.31)	4.22(0.31)
$F_3 = 214$	5.17(0.17)	50.54(3.39)	3.36(0.10)	3.64(0.46)
$F_3 = 215$	6.29(0.27)	72.0(3.50)	3.34(.24)	3.0(0.15)
$F_3 = 216$	5.32(0.26)	38.39(2.32)	3.0(0)	2.38(0.32)

$F_3 = 221$	6.79(0.35)	58.06(4.02)	1.72(0.26)	1.68(0.24)
$F_3 = 223$	7.22(0.13)	90.51(5.64)	3.04(0.18)	1.79(0.15)
$F_3 = 224$	6.7(0.27)	61.69(3.22)	4.5(0.34)	5.25(0.94)
$F_3 = 226$	5.89(0.37)	61.27(3.52)	1.67(0.17)	2.87(0.31)
$F_3 = 227$	6.0(0.30)	50.09(2.52)	3.41(0.18)	4.0(0.46)
$F_3 = 229$	6.2(0.23)	49.99(3.26)	3.08(0.17)	3.96(0.40)
$F_3 = 234$	6.75(0.25)	67.87(5.31)	2.83(0.33)	1.22(0.09)
$F_3 = 235$	6.05(0.36)	62.08(2.94)	2.38(0.23)	2.97(0.27)
$F_3 = 236$	5.06(0.20)	59.15(4.59)	3.83(0.08)	4.96(0.28)
$F_3 = 238$	6.49(0.16)	68.66(4.20)	2.11(0.16)	2.73(0.34)
$F_3 = 241$	5.8(0.28)	55.56(5.84)	2.68(0.09)	1.96(0.31)
$F_3 = 243$	6.05(0.36)	47.40(2.82)	4.31(0.09)	6.61(0.26)
$F_3 = 244$	6.52(0.30)	59.07(3.82)	3.76(0.19)	4.29(0.42)
$F_3 = 245$	6.3(0.34)	50.71(2.27)	1.71(0.14)	1.62(0.18)
$F_3 = 246$	5.28(0.40)	48.99(3.14)	3.71(0.36)	3.61(0.39)
$F_3 = 247$	5.43(0.24)	57.04(3.68)	4.25(0.27)	5.62(0.44)
$F_3 = 248$	4.6(0.38)	65.08(4.38)	2.28(0.25)	3.92(0.49)
$F_3 = 251$	5.32(0.59)	69.28(4.03)	4.0(0)	3.92(0.23)
$F_3 = 252$	6.3(0.35)	78.19(4.52)	4.0(0.15)	5.1(0.31)
$F_3 = 254$	6.61(0.33)	66.34(4.77)	2.36(0.17)	2.86(0.43)
$F_3 = 262$	4.75(0.28)	48.7(2.86)	2.94(0.06)	5.06(0.36)
$F_3 = 263$	5.92(0.20)	45.52(2.84)	2.92(0.17)	3.56(0.32)
$F_3 = 265$	5.9(0.28)	45.6(3.09)	3.9(0.19)	8.2(0.17)
$F_3 = 266$	5.19(0.38)	34.32(2.81)	5.17(0.11)	6.67(0.50)
$F_3 = 271$	6.21(0.40)	48.8(2.3)	4.62(0.11)	3.57(0.25)
$F_3 = 272$	6.08(0.29)	56.69(2.51)	4.23(0.09)	4.32(0.29)
$F_3 = 273$	5.77(0.25)	79.66(4.63)	3.97(0.15)	4.97(0.35)
$F_3 = 274$	5.4(0.20)	43.33(2.74)	4.24(0.22)	4.05(0.42)
$F_3 = 275$	5.61(0.22)	49.93(4.21)	2.59(0.12)	5.18(0.56)
$F_3 = 278$	6.79(0.23)	46.04(4.45)	3.92(0.17)	4.72(0.55)
$F_3 = 279$	5.5(0.8)	57.07(3.21)	4.33(0.14)	4.5(0.92)
$F_3 = 280$	6.12(0.37)	63.29(4.83)	2.69(0.09)	3.42(0.25)
$F_3 = 283$	5.57(0.11)	50.81(3.77)	3.44(0.14)	5.06(0.33)
$F_3 = 286$	4.6(0.37)	63.26(3.33)	3.67(0.32)	5.17(0.47)
$F_3 = 287$	5.59(0.47)	77.58(6.45)	2.23(0.11)	2.0(0.17)
$F_3 = 288$	5.07(0.20)	53.18(5.81)	4.0(0)	5.86(0.23)
$F_3 = 290$	5.58(0.22)	65.12(3.49)	3.37(0.09)	3.33(0.21)
$F_3 = 291$	6.09(0.28)	69.45(4.25)	2.38(0.11)	2.43(0.29)
$F_3 = 298$	5.67(0.27)	63.80(3.95)	1.89(0.15)	1.85(0.12)
$F_3 = 300$	6.57(0.63)	69.61(3.92)	2.04(0.17)	3.88(0.29)
$F_3 = 302$	4.92(0.22)	41.69(2.7)	5.19(0.09)	6.05(0.24)
$F_3 = 303$	4.52(0.21)	48.43(3.50)	4.68(0.24)	4.75(0.60)
$F_3 = 304$	5.34(0.15)	50.08(2.30)	5.68(0.09)	8.2(0.18)
$F_3 = 305$	6.01(0.25)	51.52(1.82)	1.92(0.16)	1.88(0.17)
$F_3 = 306$	4.4(0.87)	30.40(3.45)	4.0(0.30)	5.83(0.57)

$F_3 = 307$	5.44(0.31)	58.57(4.67)	3.35(0.10)	4.30(0.41)
$F_3 = 309$	5.04(0.27)	32.21(2.84)	4.85(0.10)	5.85(0.64)
$F_3 = 310$	4.86(0.47)	51.92(3.22)	4.11(0.34)	6.23(0.47)
$F_3 = 311$	5.94(0.13)	49.06(2.64)	4.5(0.28)	4.83(0.58)
$F_3 = 312$	6.61(0.38)	60.84(2.97)	4.36(0.09)	5.57(0.43)
$F_3 = 313$	5.65(0.33)	49.85(3.35	3.04(0.04)	2.79(0.24)
$F_3 = 317$	7.34(0.43)	75.31(3.82)	2.64(0.10)	3.04(0.21)
$F_3 = 319$	6.07(0.15)	52.15(2.32)	2.67(0.14)	2.95(0.43)
$F_3 = 321$	3.97(0.49)	40.59(2.76)	5.45(0.25)	7.73(0.57)
$F_3 = 323$	5.18(0.19)	54.35(3.24)	3.34(0.24)	4.90(0.40)
$F_3 = 325$	5.69(0.31)	51.76(2.46)	4.06(0.23)	2.59(0.37)
$F_3 = 326$	4.24(0.33)	34.33(2.45)	4.27(0.09)	5.0(0.48)
$F_3 = 329$	7.45(0.41)	64.10(5.73)	3.54(0.26)	4.19(0.45)
$F_3 = 331$	6.71(0.25)	64.08(4.07)	2.93(0.28)	3.71(0.29)
$F_3 = 334$	6.35(0.42)	53.69(4.36)	2.64(0.19)	2.40(0.22)
$F_3 = 337$	6.33(0.48)	60.44(4.26)	3.73(0.09)	3.04(0.35)
$F_3 = 338$	6.9(0.96)	61.19(5.70)	3.53(0.19)	3.71(0.48)
$F_3 = 342$	5.35(0.15)	55.53(4.15)	4.23(0.53)	5.21(0.81)
$F_3 = 343$	4.88(0.20)	41.95(2.78)	4.25(0.10)	5.9(0.40)
$F_3 = 345$	5.52(0.28)	81.85(4.67)	3.0(0)	5.58(0.41)
$F_3 = 346$	5.61(0.37)	49.23(2.40)	2.58(0.10)	1.29(0.11)
$F_3 = 347$	5.76(0.34)	34.37(3.77)	3.93(0.28)	4.0(0.40)
$F_3 = 348$	6.93(0.23)	75.68(3.56)	2.54(0.31)	2.09(0.16)
$F_3 = 350$	5.8(0.37)	70.86(4.23)	4.0(0.30)	6.3(0.35)
$F_3 = 351$	6.9(0.34)	64.55(3.26)	3.36(0.25)	2.54(0.35)
$F_3 = 355$	4.9(0.59)	81.69(5.63)	3.43(0.18)	4.78(0.41)
$F_3 = 356$	6.7(0.17)	53.71(1.93)	2.68(0.10)	3.18(0.39)
$F_3 = 358$	5.32(0.25)	57.37(3.99)	3.57(0.17)	2.89(0.31)
$F_3 = 359$	6.01(0.21)	63.71(5.26)	3.10(0.07)	4.65(0.48)
$F_3 = 360$	5.04(0.38)	45.35(3.09)	4.06(0.06)	3.59(0.58)
$F_3 = 362$	6.39(0.17)	66.99(3.47)	3.31(0.33)	5.72(0.40)
$F_3 = 363$	5.96(0.29)	60.03(3.44)	3.36(0.10)	1.92(0.21)
$F_3 = 364$	5.3(0.22)	49.09(2.81)	4.0(0)	5.70(0.44)
$F_3 = 365$	6.75(0.45)	54.41(3.40)	2.76(0.18)	3.24(0.20)
$F_3 = 367$	6.05(0.27)	42.80(1.87)	3.89(0.15)	3.07(0.39)
$F_3 = 368$	6.09(0.55)	66.96(3.26)	2.74(0.19)	2.63(0.21)
$F_3 = 369$	6.16(0.43)	52.47(4.08)	3.96(0.18)	3.30(0.26)
$F_3 = 370$	6.79(0.23)	61.14(3.48)	2.0(0.16)	3.38(0.30)
$F_3 = 371$	5.07(0.25)	47.09(1.80)	4.28(0.32)	6.28(0.45)
$F_3 = 372$	7.8(0.22)	77.92(3.33)	2.72(0.32)	3.86(0.44)
$F_3 = 375$	5.19(0.18)	53.97(4.05)	4.79(0.08)	4.79(0.41)
$F_3 = 376$	6.47(0.41)	58.92(4.03)	2.52(0.33)	3.16(0.36)
$F_3 = 384$	6.63(0.23)	74.79(5.0)	3.0(0.28)	4.18(0.52)
$F_3 = 385$	8.16(0.65)	67.07(4.03)	1.94(0.21)	1.50(0.15)
$F_3 = 386$	4.23(0.39)	56.58(5.02)	3.43(0.31)	4.52(0.50)

$F_3 = 388$	4.85(0.55)	42.02(3.37)	4.0(0.29)	5.04(0.63)
$F_3 = 390$	5.57(0.20)	58.02(4.19)	5.61(0.10)	8.77(0.11)
$F_3 = 391$	6.16(0.23)	67.28(4.06)	4.4(0.21)	6.25(0.64)
$F_3 = 392$	5.96(0.23)	49.58(2.37)	2.33(0.09)	3.17(0.29)
$F_3 = 393$	5.71(0.24)	49.65(4.33)	5.3(0.10)	4.7(0.76)
$F_3 = 395$	6.0(0.23)	51.06(3.30)	2.46(0.44)	3.31(0.64)
$F_3 = 396$	6.08(0.25)	51.32(4.55)	3.47(0.30)	3.68(0.43)
$F_3 = 398$	5.46(0.25)	43.11(2.08)	4.61(0.10)	6.42(0.30)
$F_3 = 399$	6.27(0.17)	68.06(4.79)	2.9(0.23)	4.95(0.51)
$F_3 = 401$	5.78(0.23)	49.40(4.13)	4.83(0.11)	7.17(0.34)
$F_3 = 403$	6.5(0.23)	57.65(3.68)	3.15(0.24)	3.77(0.53)
$F_3 = 404$	5.57(0.38)	45.82(3.01)	3.30(0.10)	3.48(0.52)
$F_3 = 406$	6.06(0.38)	57.17(3.29)	2.37(0.24)	3.0(0.41)
$F_3 = 407$	6.17(0.34)	80.19(4.38)	2.59(0.18)	2.48(0.40)
$F_3 = 408$	5.67(0.30)	58.63(5.21)	4.67(0.11)	5.78(0.65)
$F_3 = 411$	5.65(0.49)	67.11(5.21)	4.0(0.19)	5.12(0.51)
$F_3 = 413$	3.65(0.19)	38.50(2.45)	4.40(0.21)	4.05(0.44)
$F_3 = 417$	5.63(0.12)	72.07(3.99)	1.64(0.09)	2.46(0.36)
$F_3 = 418$	5.72(0.34)	79.59(4.04)	4.69(0.09)	5.65(0.38)
$F_3 = 421$	5.5(0.61)	60.90(3.77)	4.36(0.09)	3.57(0.25)
$F_3 = 423$	6.81(0.32)	86.55(5.83)	2.12(0.23)	2.36(0.22)
$F_3 = 425$	6.0(0)	83.33(6.66)	3.47(0.12)	5.32(0.44)
$F_3 = 428$	5.38(0.29)	53.38(2.73)	3.0(0.30)	3.43(0.47)
$F_3 = 429$	4.15(0.44)	50.02(6.06)	3.44(0.29)	4.33(0.67)
$F_3 = 431$	3.75(0.31)	59.74(5.01)	2.85(0.32)	3.26(0.48)
$F_3 = 432$	6.92(0.32)	63.54(5.0)	1.62(0.25)	1.92(0.15)
$F_3 = 433$	6.21(0.39)	69.62(3.42)	3.29(0.27)	2.25(0.33)
$F_3 = 435$	7.33(0.20)	74.58(3.35)	1.65(0.09)	1.73(0.24)
$F_3 = 436$	6.99(0.26)	62.91(4.50)	3.73(0.09)	5.65(0.24)
$F_3 = 440$	7.2(0.19)	64.73(3.39)	3.0(0)	3.33(0.35)
$F_3 = 441$	5.83(0.20)	54.06(2.70)	3.95(0.04)	2.95(0.17)
$F_3 = 442$	6.13(0.42)	50.98(4.69)	3.50(0.27)	3.60(0.66)
$F_3 = 443$	6.86(0.28)	67.14(3.28)	1.80(0.15)	1.76(0.13)
$F_3 = 451$	6.05(0.11)	57.54(1.96)	2.30(0.20)	4.09(0.33)
$F_3 = 455$	6.67(0.53)	57.81(4.65)	3.5(0.28)	5.54(0.48)
$F_3 = 456$	6.57(0.35)	61.35(3.11)	2.04(0.17)	3.04(0.30)
$F_3 = 458$	5.66(0.20)	61.44(3.23)	3.0(0)	6.18(0.29)
$F_3 = 459$	5.2(0.54)	54.47(4.74)	3.0(0.34)	2.50(0.20)
$F_3 = 460$	5.61(0.07)	48.01(1.78)	3.67(0.09)	3.40(0.29)
$F_3 = 461$	5.81(0.41)	68.14(5.56)	3.25(0.25)	5.37(0.38)
$F_3 = 462$	6.08(0.31)	38.71(5.13)	3.71(0.11)	5.41(0.30)
$F_3 = 463$	7.04(0.13)	57.42(2.95)	2.39(0.25)	2.56(0.33)
$F_3 = 464$	Na	52.28(5.81)	3.50(0.36)	3.28(0.27)