

THE GENETICS OF RESISTANCE TO THE CORN LEAF APHID,
RHOPALOSIPHUM MAIDIS (FITCH), IN CORN

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ABSTRACT

Genetic segregation for resistance to the corn leaf aphid, Rhopalosiphum maidis (Fitch), in a planting of AA8sh2 inbreds stimulated this study. Homozygous resistant and susceptible lines were derived from the AA8sh2 population. Appropriate crosses based on a resistant and a susceptible line were critically evaluated in the greenhouse and showed that the genetics of resistance was monogenic and recessive. The aphid resistant gene was designated as aph. The origin of this resistance gene is obscure, and the most probable source is W22.

Antibiosis was investigated in a number of lines; it was reflected in aphid weight, the number of days to reproduction, aphid longevity, the number of days reproductive and the rate of reproduction. Resistant AA8sh2 line 3360 showed significant antibiosis for all these parameters except aphid longevity and the number of days reproductive. The recessiveness of the antibiotic effects was in agreement with the recessive nature of its inheritance. Resistant line Mol7 showed significant antibiosis in all the parameters measured and unlike the resistance of 3660 was partially dominant or dominant.

Non-preference was investigated using "cafeteria-style" tests with aphids. High variability was obtained, and non-preference was detected only in the early growth stage of the corn plant. The inheritance of non-preference was not clear in the resistant lines derived from the AA8sh2 population. Mol7's non-preference was found to be dominant. A line much preferred by the aphid was the corn mutant, bxbx, which lacks DIMBOA, a compound generally regarded as a resistance factor against diseases and pests. Antibiosis was also lacking in this mutant.

Tolerance was unexpectedly detected as a result of an exceptionally severe infestation in which susceptible lines were killed while the resistant line 3660 was unaffected. This plant resistance mechanism is difficult to measure and has not been reported in corn before.

Resistance to the aphid was also studied using two diallel cross experiments growing under severe aphid infestations. High genotypic variance was obtained, indicating that the heritability for polygenic resistance is high and corn lines can be effectively improved by simple recurrent selection or even mass selection for greater resistance. Mol7, Oh545 and 3660 were inbreds showing good general combining ability for aphid resistance. Lines with poor GCA and hence, susceptible, included B37, Ant2D, CM104, CM105 and CM111. GCA mean square was found to be more important than SCA mean square, implying that the major portion of the genetic variance was additive. In general, the results of a greenhouse diallel were more reliable than those of a field diallel because of the greater uniformity of aphid infestation in the former.

A gas-liquid chromatography procedure was successfully adapted for highly accurate determination of individual analogs of the 2(3)-benzoxazolinones, improving upon earlier colorimetric methods. Individual toxicities of the three known analogs to various pests and fungi could now be studied.

MBOA distribution in etiolated corn seedlings was investigated. The concentration in the shoot and roots was high compared to that in the remainder of the embryo. A very small amount was found in the remainder of the endosperm. High MBOA concentration appears to be associated with tissues of high growth activity. Physiological implications of this are not fully understood as yet.

MBOA and dimethoxy-BOA were also found in the teosinte races from Balsas, Chalco and Jutiapa. MBOA was also present in Tripsacum dactyloides (4N), T. floridanum and T. laxum. All three analogs were detected in a detailed examination of T. dactyloides (2N). This group of unique compounds was not known previously in teosinte and the Tripsacum complexes, close relatives of corn. Studies of the individual analogs in appropriate interspecific crosses could help further our understanding of the origin of corn.

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1. INTRODUCTION

The corn leaf aphid, Rhopalosiphum maidis (Fitch), is present throughout the world, and sometimes causes extensive damage to corn. It is also a pest of sorghum and of particular concern because of its transmission of viruses like the sugarcane mosaic and the maize dwarf mosaic virus. Damage by the aphid is often not as obvious as that of other insect pests. Infestation usually results in the reduction or prevention of pollen shedding, while heavy infestation can cause serious reduction in yield.

In the autumn of 1972, a series of supersweet inbreds of AA8sh2 origin were found to be segregating for aphid resistance at the University of Hawaii Waimanalo Experiment Station. Preliminary trials positively identified a few highly resistant and susceptible lines. Observations indicated that the resistance was probably controlled by a single or a few major genes, as there were clear-cut segregations for resistance and susceptibility. A rare opportunity thus arose to further our understanding of the genetics of resistance of corn to this pest.

Broadly, the objectives of this study were to determine the genetics of resistance of corn to this aphid, and to study the major components of resistance, which are preference, antibiosis and tolerance based on these findings, to search for additional sources of resistance in commercial inbreds and other germplasm; finally, to devise a practical approach for the incorporation of the known resistant genes into usable corn stocks.

2. LITERATURE REVIEW

2.1 Insect resistance in crop plants

Differential responses of crop plants to insect attacks have been observed for more than a hundred years. One of the first outstanding examples of insect resistance as a major means of crop protection was the development resistance to Hessian fly (Mayetiola destructor (Say)) among wheat varieties in the United States. Over 10 million acres were planted with about 23 different Hessian fly resistant varieties in 34 states in 1972 (Maxwell et al., 1972). Another classical example is the introduction of American species of grapes, highly resistant to the grape phylloxera, Phylloxera vitifoliae (Fitch), to France which saved her wine industry. These and other early experiences demonstrate the importance and economic value of breeding for insect resistant crop varieties. With the present status of research and knowledge in this area, it is quite obvious that as a sole means of control, it is still unavailable to many crop. However, when insect resistant crops are available, conventional methods of insect control can combine even more effectively with it.

2.2 Definitions and terminology of the mechanisms of plant resistance

Painter (1951, 1958b) divided plant resistance mechanisms into three categories:

1. Preference and non-preference

In this case, the plant displays a degree of resistance by exerting an adverse effect on the insect's behavior. It denotes the group of plant characters and insect responses

that lead the insect to favor or reject particular plants or varieties for oviposition, food, shelter, or for combinations of the three.

2. Antibiosis

This is the tendency of the plant to prevent, injure or destroy insect life. Antibiosis was proposed for those adverse effects on the insect life history by a resistant host plant variety. The effects on the insect can take the form of death of the insect (often during the first instar), abnormal length of life, reduction in food reserves, followed sometimes by unsuccessful hibernation, smaller size, decrease in fecundity, and frequently, restlessness and other peculiar behavior.

3. Tolerance

A tolerant plant is capable of supporting an insect population without loss of vigor and without reduction in yield.

Beck (1965) considered plant resistance as 'being the collective heritable characteristics by which a plant species, race, clone, or individual may reduce the probability of successful utilization of that plant as a host by an insect species, race, biotype, or individual'. By such a definition, Painter's 'tolerance' class would be omitted as one of the mechanisms of resistance. Although tolerance is an important agronomic plant character, it implies a successful biological

relationship with the insect, and hence, in the strict sense of the resistance, it would not be included.

2.3 Crop plant resistance to aphids

Crop breeding for aphid resistance has probably been more common than breeding for resistance against any other plant-feeding insects. This is probably due to the abundance of occurrence of this insect in crop plants. Up to 1951, there were about 25 records of plant resistance to different species of aphids (Painter, 1958a). Since then, increasing number of reports have appeared in various research journals, reflecting the economic importance of these pests and successes in breeding resistance against them. A major advantage of aphids is that most of them can be raised easily and are adapted to study on plants under greenhouse conditions. As a result, greater control of various environmental factors can be achieved and experiments conducted this way are usually reliable. In addition, seedlings of most crop plants can be effectively tested and hence large numbers can be handled in a relatively small space.

2.4 Aphid resistance in selected crop plants

The spotted alfalfa aphid, Therioaphis maculata (Buckton), occurs in all alfalfa-producing areas in the United States (Smith, 1959). It is considered to be the most widespread and serious pest of alfalfa, causing losses of millions of dollars every year. Originally, the variety Lahontan and its parental clones had a high degree of resistance in the seedling stage, showing both antibiosis and tolerance (Howe and Smith, 1957). Subsequent evaluations of mature plants in

the field showed that Lahontan and also Moapa, Zia, Bam and Sirsa no. 9 were resistant (Howe and Pesho, 1960). By systematic selection, the resistant synthetic variety Cody was developed from the susceptible variety Buffalo in Kansas (Harvey et al., 1960). Two years after the release of Lahontan, high populations of aphids were found in some Lahontan plantings, suggesting a breakdown of resistance (Stanford and McMurty, 1959). The emergence of biotypes was responsible for the loss of resistance (Pesho and Lieberman, 1960). Nielson et al. (1971) screened numerous experimental lines and identified three lines highly resistant to all four known biotypes. Antibiosis, non-preference and tolerance have been found to confer resistance, either alone or in combination (McMurty and Stanford, 1960; Kishaba and Manglitz, 1965; Kircher et al., 1970; Sandmeyer et al., 1971).

Another major pest in alfalfa is the pea aphid, Acyrtosiphon pisum (Harris) which caused about 60 million dollars in losses annually (Carnahan, 1963). Painter (1958b) first reported the variety Ladak as resistant. Later, Ortman and Painter (1960) reported techniques for selection and evaluation of pea-aphid resistant alfalfa plants. Smith and Peadar (1960) increased the effectiveness in screening for resistant plants by excluding predators of the aphids. Others however rate the plants by counting the number of parasitized aphids on the upper surface of the leaves which appeared to be easier and more accurate (Harvey and Hackerott, 1967). The varieties Washoe (resistant to the spotted alfalfa aphid and the pea aphid), Dawson and Mesilla were the result of using these techniques and their modifications for the successful development of pea-aphid resistant plants

(Kehr et al, 1968; Melton, 1968). A yield trial using pea-aphid resistant variety Kanza and susceptible variety Cody showed that Kanza yielded 67% more forage than Cody, thus indicating that losses can be reduced dramatically by resistant varieties (Harvey et al., 1971). Though the genes controlling the inheritance of resistance in alfalfa to both the spotted alfalfa aphid and the pea aphid are unknown, breeding for resistance has been possible because selection methods were applied to alfalfa populations containing some resistant plants. Antibiosis and non-preference are the main mechanisms of resistance though in some instances tolerance is probably involved. As yet no specific chemical factors have been found to be related to pea aphid or spotted alfalfa aphid resistance. Auclair (1963) however observed that pea aphid resistant varieties of peas were generally deficient in some amino acids as compared to the susceptible varieties.

Another aphid, the greenbug, Schizaphis graminum (Rond), attacks a number of crop plants, among which are wheat, barley, sorghum, rye and oats. Screening for resistance has been successful with seedling populations. In wheat, the genetics of greenbug resistance was investigated by Daniels and Porter (1958); Curtis et al. (1960); and Porter and Daniels (1963). Resistance was monogenic and recessive. The appearance of biotypes as reported by Wood (1961); and Singh and Wood (1963) have complicated the picture. In oats, the genetics of resistance to the greenbug was determined to be monogenic (Gardenshire, 1964). A rye variety Insave F.A. was reported to be resistant to the two known greenbug biotype, B and C (Harvey and Hackerott, 1969), unlike the selected variety of Caribou which was only resistant to

biotype B. The difference in reaction may be due to the results of different alleles at a single locus or to two distinct genes at separate loci.

Prior to 1968, the greenbug, Schizaphis graminum, was not considered a major pest of sorghum in the United States. During the summer of 1968, widespread greenbug infestations of sorghum occurred in the Midwest and Southwest. Field damage ranged from slight injury of lower leaves to severe plant defoliation and reduced yields. In most cases where seedling sorghums were planted late for forage, severe infestation killed them. Harvey and Hackerott (1969) reported that the greenbug was a new biotype and named it C biotype. They found that 'Piper' sudangrass seedlings were resistant to greenbugs originating from wheat (B biotype) but susceptible to this biotype from sorghum (C biotype). Because of the widespread occurrence and severity of the 1968 greenbug attack, many studies were conducted in attempts to control this pest. Resistance to the C biotype was subsequently found in Sorghum virgatum (Hack) Stapf. (Hackerott et al., 1969). On the basis of seedling survival, tolerance appeared to be the major component of resistance and the genetics of resistance appeared to be controlled by dominant genes at more than one locus. Wood (1971) systematically screened a large number of varieties and hybrids among different species of sorghum. Eight entries were identified to have a high degree of resistance to the three biotypes of the greenbug. Development of resistant sorghums by transfer of resistant germplasm through breeding to adapted commercial varieties is thus possible. Harvey and Hackerott (1974) studied the type of damage that the greenbug caused

in a susceptible sorghum variety and found that heavy greenbug feeding caused reduced tillering, plant height and delayed maturity though seed weights were not affected. Yield losses of surviving plants appeared to be primarily due to the reduction in numbers of secondary culms. The components of host-plant resistance in sorghum were studied recently by Schuster and Starks (1973) using ten known resistant selections represented by five sorghum species and a susceptible check. In free choice tests some of the selections were highly non-preferred. Antibiosis was also an important component and was reflected in fewer nymphs with smaller weights on the resistant selections. The time for each individual to reach sexual maturity was also lengthened. Tolerance as measured by plant height differences between infested and non-infested plants of each entry and by plant injury ratings showed that it is the major component in some selections. Further tests identified several selections with comparatively high degree of all three resistant components. Teetes et al (1974) reported similar results, resistant lines being less preferred than susceptible lines, and the F_1 between resistant and susceptible lines was not as much preferred when compared to the susceptible parent. Non-preference in this case appears to be incompletely dominant. Duration of stadia was increased, and progeny per adult, adult longevity and length of reproductive period were decreased for greenbugs reared on resistant sorghum. Nymphal mortality however did not differ in resistant and susceptible sorghums.

The raspberry aphid, Amophorophora rubi (Kalt.), is a vector of several viruses and four strains are presently recognized (Briggs,

1965a,b). Genetic control in the aphid involves one dominant and one recessive gene. Aphids without these two genes are strain 1, the dominant gene gives strain 2, and the recessive gene gives strain 3. Presence of the two genes give strain 4. Strains 2 and 4 are rare and resistance to strains 1 and 3 are of major importance in Britain (Briggs, *ibid*).

Host selection by the cabbage aphid, Brevicoryne brassicae (L.), is influenced by color and intensity of irradiated light from the leaf surface (Radcliffe and Chapman, 1965a,b; 1966). Red was least preferred but was most favorable to aphid increase once infestation had occurred. In New Zealand 90,000 acres of rape resistant to the cabbage aphid are grown annually. Recently however, a new biotype has been found to attack formerly resistant lines (Lammerink, 1968).

Solanum penellii Correll with a heavy vesture of glandular hairs is resistant to the potato aphid, Macrosiphum euphoriae (Thomas). However, Lycopersicon peruvianum (L.) Mill. which has only sparse vesture of glandular hairs reacted similarly, probably due to physiological factors (Gentile and Stoner, 1968). Resistance to both the potato aphid and the green peach aphid, Myzus persicae (Sulzer), were found in wild species of Solanum indigenous to Mexico (Radcliffe and Lauer, 1966; 1970). Future development of aphid-resistant commercial potato would be feasible since crosses of the resistant wild parents to S. tuberosum L. do not present any particular obstacles.

A key pest of cultivated peas, Pisum sativum (L.) is the pea aphid, Acyrtosiphon pisum. It is also a pest of many other legumes and small grains. Maltias and Auclair (1957) reported that susceptible

varieties of peas contain more nitrogen and less sugar than resistant varieties at various stages of plant growth. Subsequent studies showed that the same susceptible varieties have higher concentration of free and total amino acids than the resistant ones (Auclair et al., 1957). Further observations indicated that aphids fed at a greater rate and a greater proportion of the sap ingested was excreted in susceptible varieties as compared to resistant varieties (Auclair, 1959). From the feeding data it was concluded that the aphids on resistant plants were in a semi-starved condition, and thus consumed less but used more of what was consumed. Three biotypes of the pea aphid are now recognized in Southern Quebec and the R₁ biotype is used as the standard biotype for testing pea varieties (Cartier, 1959, 1960).

Resistance to the green peach aphid on tobacco was tested by screening of introductions and wild Nicotiana species (Thurston, 1961). Nicotiana gossei Domin., N. repanda Willd. and N. trigonophylla Dun. were highly resistant but N. gossei x N. tabacum L. did not show the high level of resistance of N. gossei. Work by Guthrie et al. (1962) suggests that the green peach aphid was able to adapt to nicotine producing plant by feeding in the phloem and avoiding the nicotine-containing xylem. Thurston and Weber (1962) reported that resistance in Nicotiana species to the green peach aphid appeared to be the result of the production of toxic material on the aerial parts of the plants. Further studies of the toxic material showed that alkaloids were produced in the trichomes of seven Nicotiana species (Thurston et al., 1966). Nicotine, the major component, was found in all seven species,

anabasine and nornicotine were found in only two species. Aphids were killed by these secretions.

2.5 The corn leaf aphid, *Rhopalosiphum maidis* (Fitch)

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is distributed in many parts of the world, principally in the warmer regions. It occurs every summer in the North American cornbelt and sometimes causes extensive damage. Occasionally, corn breeders experience serious difficulty in obtaining enough pollen from certain plants because of extensive deposits of honey dew on the tassels. It is also a pest of sorghum and is of particular concern because of its transmission of viruses like the sugarcane mosaic and the maize dwarf mosaic virus. About 67 host plants has been recorded for this aphid, most of them in the Gramineae family (Patch, 1938; Everly, 1966). Cultivated crops include oat, barley, millet, rye, wheat, broomcorn, sorghum, sugarcane and sudangrass. Since the hosts include weed found around corn fields, early migrants can build up in great numbers without being apparent and subsequently migrate to corn.

The corn leaf aphid reproduces by parthenogenesis. No eggs have ever been found. It undergoes four instar stages before maturing into either apterate (non-wing) or alate (wing) form. It starts to reproduce when it is about 11 days old and can give birth to as many as 95 over a 21-day period, the average being 34 (Davis, 1909). Everly (1966) estimated that the aphid can produce about 11 generations each summer. Based on an average of 34 and 11 generations, one corn leaf aphid could produce 7.0188844×10^{16} progenies in one summer. This

tremendous biotic potential is the single most important factor in their abundance. Fortunately aphids are exposed to many environmental hazards and relatively few of this potential number survive.

Visually, damage by the aphid is not very evident compared to other pests. Infestations usually result in reduction or prevention of pollen shedding. Heavy infestation can cause serious reduction in yield (Snelling et al., 1940; Everly, 1960, 1966). Of greater significance however is that this insect can cause a measurable loss of yield with infestation levels below those required to produce barren plants. This is a consequence of partial prevention of pollination and also some serious physiological effects on the plant resulting in the retardation or prevention of the development of the ear shoot (Everly, 1960, 1966).

Usually the aphid has a relatively short period of about two or three weeks in which it can cause severe damage. When the tassel has matured and begun to dry out, the aphids then disperse to other more succulent parts of the plant such as the ears and leaf sheaths. When the entire plant matures, the apterate form dies and the alate form migrates to other more succulent plants. Several environmental factors influence the abundance of the corn leaf aphid. The biotic potential and high soil fertility, act towards increasing aphid population; and effective factors that reduce aphid abundance are predators, parasites and host plant resistance (Everly, 1966). High soil moisture has also been reported as conducive to aphid increase but colonies of aphid can and do persist during dry weather (Neiswander and Triplehorn, 1961). Triplehorn (1959, 1960) observed that if there is an abundance of soil

moisture the corn plants can tolerate aphid feeding and still produce a good crop. However in a field with low soil moisture, plants supporting large colonies of aphid at tassel emergence are usually barren.

Howitt and Painter (1956) tested many sorghums in the United States for resistance to the corn leaf aphid and identified Sudan type sorghum to be consistently more resistant. A single plant Piper Sudan 428-1 was found to be highly resistant to the general aphid population. The resistance appeared to be dominant and carried in a heterozygous condition in that plant, since some selfed progenies were fairly susceptible (Painter, 1958a). Biotypes of the corn leaf aphid were first reported by Cartier and Painter (1956) and designated KS-1 and KS-2. Subsequently two additional ones, KS-3 and KS-4 were also found (Pathak and Painter, 1958a,b, 1959). The four biotypes were however differentiated by sorghum varieties and not corn. Painter and Pathak (1960) discussed the significance and distinguishing features of the four biotypes. The biotypes react differently at different temperatures, being more alike at 70°F than at 60°F or 80°F (Singh and Painter, 1965). They also vary significantly in reproductive rate, body weight and length of life, and in their response to low and high levels of nitrogen, phosphorus, and potassium of the host.

2.6 The genetics of resistance to the corn leaf aphid

Most studies of the genetics of resistance of corn to the corn leaf aphid were due to natural outbreaks of the aphid rather than by planned experiments. Gernert (1917) reported that F₁ of Euchlaena mexicana (teosinte) x Yellow Dent corn as well as the teosinte parent

remained free of aphids in a greenhouse and field planting. A widespread outbreak of the corn leaf aphid occurred in 1938 and 1939 in the Central and Northern part of the cornbelt. Favorable weather conditions, low number of parasites and predators and the widespread use of inbreds and hybrids susceptible to the aphids were important contributing factors responsible for these epizootics (Painter, 1951).

As a result of these outbreaks several important observations were made in Illinois, Indiana and Ohio (Snelling et al., 1940; Walter and Brunson, 1940; Huber and Stringfield, 1940, 1942). Today they still stand as authoritative works, reflecting relatively little advance since then in the understanding of the genetics of resistance to this aphid. Inbred WF9 was reported as highly susceptible by itself and in hybrid combinations in Illinois and Ohio (Snelling et al., 1940; Huber and Stringfield, 1940). The susceptibility appeared to be dominant. Inbred 38-11 was reported as highly susceptible in Indiana and Illinois (Walter and Brunson, 1940; Snelling et al., 1940). Reactions in hybrid combinations indicated that the susceptibility was recessive. The inbred R4 was found to be very resistant in Indiana, no aphids developing on any plant, and only a few aphids were found when it was in hybrid combinations (Walter and Brunson, 1940). In Illinois R4 was also very resistant by itself and in all hybrid combinations tested except with WF9. In Ohio it was only rated as moderately resistant. The genetics of resistance appears to be complex (Painter, 1951). Later, evidence showed that there were clear indications that some of the inbred lines were not uniform in their resistance or susceptibility, thus further complicating the picture (Walter and Brunson, 1946).

No character or group of characters could be consistently correlated with aphid susceptibility. The peculiar tassel type in which the leaves closely enclose the tassel until pollen shedding was thought to be correlated with susceptibility but was found in the aphid resistant R4 (Haber and Gaessler, 1942; Coon, 1948; Coon et al., 1948). Huber and Stringfield (1940) found a correlation coefficient range of 0.29 - 0.50 between European corn borer damage and aphid infestation. It is generally accepted that resistance to the corn leaf aphid cannot be studied readily in the greenhouse because this aphid has been reported to be unable to maintain a population on corn seedlings (Forbes, 1905; Viale, 1950; Painter, 1956). As with the European corn borer, young corn plants are more resistant to aphids than older plants.

2.7. The role of DIMBOA in disease and pest resistance

Some disease and pest resistances of corn, rye and wheat are related to the presence of post-formed compounds, produced by a simple hydrolytic process upon invasion of the plant tissue. Collectively known as benzoxazolinones, these naturally occurring compounds and their precursor hydroxamic acids have been widely recognized as plant resistance factors. As a result of work done principally by Wahlroos and Virtanen (1959), the reactions responsible for the formation of benzoxazolinones can be summarized as shown in Figure 1.

The glucosides occur in uninjured plant tissue. On being injured by hyphal penetration or insect feeding, the glucosides are converted to their corresponding hydroxamic acids enzymatically. The hydroxamic acids then undergo slow chemical degradation to benzoxazolinones. It

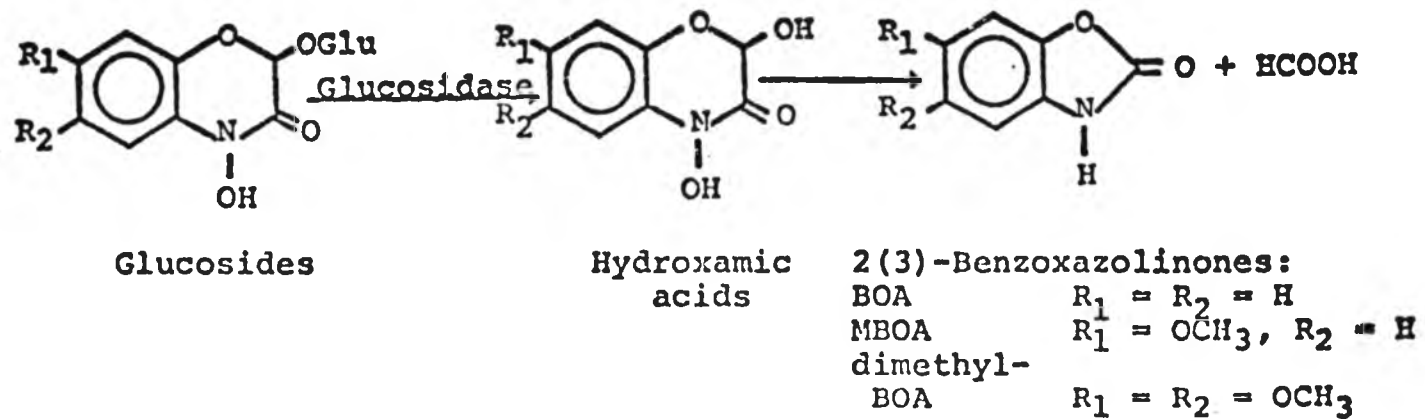


Figure 1. Formation of 2(3)-benzoxazolinones.

is now known that the active biochemical substance responsible for resistance is the hydroxamic acid as elaborately demonstrated by work on the resistance of corn to the European corn borer (Klun et al., 1967). Present thinking generally accepts this finding as applicable to other diseases too. The major analog among the hydroxamic acids is 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA). DIMBOA undergoes chemical degradation to 6-methoxy-2(3)-benzoxazolinone (MBOA).

Koyama et al. (1955) first reported the isolation and identification of MBOA in the roots of Jobs tears, Coix lacryma (L). In the same year, Virtanen and Hietala (1955) isolated 2(3)-benzoxazolinone (BOA) from rye, Secale cereale. MBOA was later reported in wheat, Triticum aestivum (Virtanen and Wahlroos, 1958). BOA and MBOA were found in corn (Loomis et al., 1957), and a dimethoxy-analog, 6,7-dimethoxy-2(3)-benzoxazolinone (dimethoxy-BOA) was recently identified in corn (Klun et al., 1970).

Early workers have shown MBOA to be active against the growth of Fusarium nivale, F. moniliforme, Penicillium spp. (Whitney and Mortimore, 1959a,b) and the bacterial wilt pathogen, Xanthomonas stewartii (Whitney and Mortimore, 1961). The precursor hydroxamic acids of BOA and MBOA were considered responsible for conferring resistance of wheat against stem rust, Puccinia graminis Pers tritici, as there were greater concentrations in the resistant varieties (Elnaghy and Linko, 1966). A significant correlation also exists between the concentration of benzoxazolinones and resistance to corn stalk rot, Diplodia zeae (Bemiller and Pappelis, 1965). More recently, the hydroxamic acid, DIMBOA has been directly implicated as partly

responsible for the resistance of corn to the Northern leaf blight, Helminthosporium turcicum (Couture et al., 1971).

In corn, the Bx gene controls the production of hydroxamic acids and related compounds. The presence of the recessive allele, bx results in the insignificant production of the hydroxamic acids and can be considered as a deficient allele. A single dominant gene, Ht conditions chlorotic lesion resistance to the Northern leaf blight. Couture et al. (1971) studied the interaction of Ht and Bx with respect to resistance to H. turcicum. The hydroxamic acid, DIMBOA was evaluated as to its role in the monogenic resistant reaction. Resistant normal (HtHtBxBx) and susceptible normal (hthtBxBx) were compared with resistant deficient (HtHtbxbx) and susceptible deficient (hthtbxbx) genotypes. Percent leaf infection was significantly higher in the bxbx genotypes as a result of an increase in the number and size of lesions. Extreme susceptibility was shown by the double recessive genotype, hthtbxbx. In addition, germination of H. turcicum spores were inhibited by DIMBOA isolated from corn at a low concentration of 1 to 10 ppm.

In an extension of this work, the leaf drop diffusate technique was applied to the above genotypes (Calub et al., 1974). Seedling leaves of the corn plant were inoculated with a spore drop suspension of H. turcicum in a humid chamber. The drop suspensions were then withdrawn after a determined length of time and centrifuged. The supernatant or diffusate now free of spores and germ tube debris was mixed with a fresh spore suspension. Diffusates which inhibited spore germination or delay growth of the germ tube were presumed to contain phytoalexin. As

expected, the greatest inhibition of spore germination was produced by the resistant normal genotype (HtHtBxBx) while the least inhibition was shown by the susceptible deficient genotype (hthtbxbx). The resistant deficient (HtHtbxbx) and susceptible normal (hthtBxBx) genotypes showed intermediate inhibition values. A similar trend was observed in the inhibition of germ tube growth. Comparisons of hthtbxbx with hthtBxBx, and HtHtbxbx with HtHtBxBx indicated that the Bx gene contributes significantly to greater resistance against H. turcicum. Unfortunately, the chemical basis for this inhibitory effect present in the diffusates of Bx lines has not been unequivocally demonstrated to be DIMBOA. Further work in this area will be fruitful if a highly accurate analytical method is available for the detection of minute amounts of DIMBOA.

Benzoxazolinones as important biochemical plant resistance factors were first recognized in the breeding for resistance of corn against the European corn borer, Ostrinia nubilalis (Hubner) (Beck and Stauffer, 1957). A positive correlation between the concentration of MBOA in dried whorl tissue of inbred corn lines and the resistance to leaf feeding by the first brood European corn borer was found (Klun and Brindley, 1966). However, incorporation of MBOA in artificial diet for the larvae showed that the compound is inhibitory only at a concentration that is twice the amount found in highly resistant inbred lines. By more elaborate bioassay, the hydroxamate precursor DIMBOA was later found to be highly toxic to the first instars of O. nubilalis and is now generally regarded as the major active fraction of the hydroxamates responsible for the resistance of corn to this pest (Klun et al., 1967).

Quite recently, Sullivan et al. (1974) reported that certain exotic corn lines resistant to the first and second brood European corn borer showed low DIMBOA content. It appears that these lines possess other antibiotic chemicals or that resistance is controlled by a morphological or preferential nature.

The bxbx genotype in corn is also very susceptible to the corn leaf aphid under greenhouse conditions (Day, 1974). Since aphid resistant and susceptible lines have the BxBx genotype, it can be again postulated that the hydroxamate precursors of benzoxazolinones play a supplementary role in the plant's defense mechanism, as is evident in the H. turcicum and O. nubilalis experiments.

Recently Long et al (1976) showed that aphid resistance is correlated to DIMBOA concentration in corn. A significant correlation of -0.72 was obtained between DIMBOA concentration and severity of aphid infestation indicating that corn lines containing high DIMBOA concentration are generally more resistant to the aphid. DIMBOA concentration in corn plants has been shown to be highest at the seedling stage and decrease progressively with age (Klun and Robinson, 1969).

Although there is great interest shown by researchers studying disease and insect resistance of benzoxazolinone-containing crops, no ideal method is available for the analysis of individual compounds. Several procedures have so far been adopted for the quantitative determination of either the hydroxamic acids or the benzoxazolinones in plant materials. They are the isotopic dilution technique (Klun and Brindley, 1966); the direct measurement of benzoxazolinones by A at 285nm (Beck et al., 1957); spectrofluorometry of benzoxazolinones

(Bowman et al., 1968); and finally the colorimetric procedure for the determination of hydroxamic acids using FeCl_3 as a chromogenic reagent (Hamilton, 1964). The isotopic dilution technique, besides being tedious and requiring the synthesis of C^{14} -benzoxazolinones, also needs a relatively large amount of plant material. The spectrophotometric methods are generally non-specific in nature and usually require clean-up of sample extracts prior to analysis. But Hamilton (1964) reported that the presence of interfering compounds in corn seedlings was insignificant when the FeCl_3 -colorimetric method was used. A rapid and simplified procedure for the estimation of hydroxamic acids in corn seedlings based on Hamilton's method was developed by Long et al. (1974). An attempt to use gas-liquid chromatography methods to determine BOA and MBOA was made by Bowman et al. (1968), but the results were not satisfactory.

3. MATERIALS AND METHODS

3.1 Location of experiments

All experiments were conducted at the Waimanalo Experiment Station or in the greenhouse facilities of the Department of Entomology and Plant Pathology, University of Hawaii.

The Waimanalo Experiment Station is located 21°N in Oahu, Hawaii. The soil is silty clay with a pH of 6.5. Fertilizer was applied at a rate of 600 lb per acre of 16-16-16 before plowing and 300 lb per acre urea as a side dressing after the plants were thinned. Plants were normally grown in single row plots, spaced 20 cm between plants and 75 cm between rows. Overhead sprinkler system was used twice weekly. Rainfall and temperature records at Waimanalo for 1973 are as follows:

Month	Rainfall (in inches)	Monthly Average Temperature (°F)	
		<u>Maximum</u>	<u>Minimum</u>
January	2.11	77.8	64.7
February	1.95	77.2	64.4
March	0.83	78.0	68.1
April	0.99	79.3	67.1
May	1.76	80.5	67.5
June	0.67	82.7	70.8
July	2.71	83.1	71.8
August	0.45	85.0	73.0
September	1.14	85.8	72.6
October	1.45	84.4	71.7
November	7.12	84.8	70.2
December	7.29	78.3	66.8

The greenhouses are located in the Pope Laboratory and the Magoon facilities at the Manoa Campus. The maximum temperatures of the greenhouses are generally 10°F higher than those of Waimanalo's. Plants were grown in 6" pots. Depending on the size of the plant, they were watered once or twice a day. Fertilizer was applied twice: a basic 16-16-16 at planting and urea when the plants were four weeks old.

3.2 Preliminary observations of genetic segregation for aphid resistance

In the Fall of 1972, the corn leaf aphid, Rhopalosiphum maidis (Fitch), was observed in a planting of numerous related lines of super-sweet corn, designated (AA8sh2 x (W22 x B14A))BC₃S₁ at the Waimanalo Experiment Station. Some lines were heavily infested with the aphid while other lines were not. There were also other lines which showed infestations in some plants and not in others. The distinction between the susceptible and resistant lines was clear-cut. Based on these observations, numerous paired crosses and selfs were made for a preliminary study of the inheritance of aphid resistance.

The seeds were planted at the Waimanalo Experiment Station, University of Hawaii, in March, 1973. The field in which they were planted was partially protected from strong wind by macadamia and casuarina trees. Apparently this was sufficient to enhance aphid build-up. Aphids from an adjacent infested field served as an excellent source of carry-over population.

Each entry consisted of a single row plot. The entries were planted in a systematic manner. No experimental designs were made as the purpose of this study was to confirm that the resistance is genetic, and to extract homozygous resistant and susceptible lines for more critical investigations. At tasseling time, plants in which the uppermost leaves and tassel were very lightly or not infested were scored as a resistant, while those that were moderately to severely infested were considered susceptible.

The (AA8sh2 x (W22 x B14A))BC₃S₁ population was derived by crossing a AA8sh2 line to (W22 x B14A), a rust resistant hybrid. It was then backcrossed three times to the AA8sh2 line and selfed. From this population, various lines were selfed and tested based on the field scores. Homozygous resistant and susceptible lines thus obtained were used in subsequent studies. The susceptible lines are designated as 3652 and 3655, and the resistant lines as 3660 and 3901.

3.3 Method for the study of the inheritance of resistance in the AA8sh2 population

To achieve greater control of the aphid predators and parasites of Hawaii, this experiment was conducted in a greenhouse. The plants were individually grown in 6" pots and rated during development. Four-week old plants were individually infested with aphids to ensure that every plant was adequately exposed to the pest. Large numbers of aphids were obtained by raising them on susceptible AA8sh2 lines. To achieve an initial uniform infestation, bits of susceptible tassels or leaves supporting heavy aphid populations were removed from the susceptible plants, and placed into the whorl of each plant.

At tasseling time, plants were rated as resistant or susceptible, as described in Section 3.2. Materials worked on in this experiment were from the AA8sh2 segregating population. A cross between the resistant line 3660 and susceptible line 3655, its backcrosses, the F₂ and the two parental lines were tested.

3.4 Leaf cage technique for investigation of the components of resistance

To critically evaluate corn leaf aphid resistance, light-weight leaf cages are needed to confine the aphids to the leaves of the test plants. The light-weight leaf cage, as described by Hughes et al. (1966), was found to be most suitable, after making a modification. The cage body was made up of a $\frac{1}{2}$ " section of a 1" o.d. plastic tubing ($\frac{1}{16}$ " wall). The top end of the tubing was covered by a removable lid while on the bottom, two nails were embedded in opposing positions (Figure 2).

In contrast to the original model devised by Hughes et al. (loc. cit.) where a backing plate for each individual cage was used, a floor support was constructed for the cages using a 3' x 4 $\frac{1}{4}$ "-thick plywood lined with a $\frac{1}{4}$ " cork sheet. This was, in turn, covered with a sheet of $\frac{1}{4}$ " polyurethane foam. Up to 12 cages could be spaced on each floor support. A maximum of six 6" pots of plants could be placed on each side of the floor support. The whole structure was shaded from direct sunlight by a 3'2" x 4" piece of plywood. With this arrangement, the selected leaf of each plant could be drawn over the floor support and pinned down on it by the leaf cage such that the leaf covered the entire floor of the cage. Aphids could then be introduced and confined to each cage by covering it with the removable lid. As the plants grew taller, the floor support was raised to the appropriate height to accommodate the selected leaves without bending the plants over (Figure 3).



Figure 2. The light-weight leaf cage.



Figure 3. Upper, general view showing the three floor supports and arrangement of the pots. Lower, view of leaves pinned by leaf cages in an antibiosis experiment.

3.5 The numerical rating system

Evaluations of aphid resistance of corn lines have been based on counts of aphid colonies or on visual rating systems. Walter and Brunson (1940) classified infestation as light, moderate or heavy. In order to obtain a single value to represent the infestation of each strain, an index number was calculated by adding the percentages of each class of infestation weighted to include the degree of infestation. This was done as follows: light x1, moderate x2, and heavy x3. Snelling et al. (1940) used a rating system identical to that of Walter and Brunson. Neiswander and Triplehorn (1961) rated the reactions on a 1 to 5 scale, 1 being the plot with the least infestation and 5 with the most infestation. Two - 4 were intermediate values. Dishner and Everly (1961) made actual counts of aphids by dissecting the plants. Rhodes and Luckmann (1967) also rated the degree of infestation in a subjective manner, as follows: 0, no aphids; 1 - 2, light; 3 - 4, moderate; 5 - 6, heavy; 7 - 8, severe; and 9 - 10, very severe. Criticisms have been leveled at these methods because of their limitations, and none of them were found to be satisfactory for the conditions in this study.

A visual rating system was developed, using a numerical scale of 1 - 6, based on the degree of aphid infestation and also the degree of plant damage. Plants were rated when the tassels fully unfolded, as follows:

1. Very resistant - no aphids on plant.
2. Resistant - 0 to 50 aphids, mostly on lower tassel.

3. Intermediate - 50 to 200 aphids, light infestation on leaves and tassel.
4. Susceptible - more than 200 aphids, moderate infestation on leaves and tassel. Reduced pollen shedding.
5. Very susceptible - heavy infestation on leaves and tassel. Little pollen shedding.
6. Extremely susceptible - heavy infestation on leaves and tassel. Lower leaves and stalk are also infested. Leaf tissue becomes necrotic. No pollen shedding.

3.6 Plant resistance mechanisms experiments

3.6.1 Antibiosis - based on aphid weight

Antibiosis was evaluated by two methods in the greenhouse. In antibiosis, based on aphid weight, each entry was planted individually in 6" pots. The pots were arranged alongside the floor support, six to each side, making a total of twelve pots for each floor support. Each experiment consisted of three entries and twelve replications in a completely randomized block design, except for experiment 4, which was made up of four entries and nine replications. An experiment, therefore, had a total of 36 pots and 3 floor supports.

Two weeks after germination, the plants were thinned to one per pot, and the last most fully expanded leaf of each entry was pinned onto the floor support, so that the leaf covered the entire floor of the cage. With the aid of a fine camel-hair brush moistened with water, 10 adult apterate aphids were transferred to each of the cages. The next day, all except 10 to 20 day-old or younger nymphs

were removed. Those nymphs were allowed to feed on the leaf for six days. A pair of scissors was used to cut off the section of the test leaf bearing the cage, taking care not to displace the aphids inside. The three floor supports, now freed from the plants, were then taken to the laboratory and 10 aphids from each cage were promptly weighed (Figure 4). The following day, the experiment was repeated so that the weekly schedule was maintained. A typical experiment would begin at the second week of the plant's growth, and continue until the eighth week.

Materials used in the four antibiosis experiments are shown in Table 1. Resistant line 3660, and susceptible lines 3652 and 3655,

Table 1. Materials used in the antibiosis experiments based on aphid weight

Experiment Number	Generation			
	P ₁ (Resistant)	P ₂ (Susceptible)	F ₁	
1	3660	CM105	3660 x CM105	
2	3660	3655	3660 x 3655	
3	Mol7	3655	Mol7 x 3655	
			Entry	
	1	2	3	4
4	3901	3660	3652	bxbx

are lines derived from the segregating population, AA8sh2. CM105 was bred in India, by prolonged sibbing of a Peruvian variety, Peru 330. It is also a susceptible line. Mol7 is a resistant inbred, released by the University of Missouri. The bxbx line is a mutant, derived



Figure 4. Three floor supports with leaf cages containing aphids on test leaves.

from a Northern flint (Couture et al., 1971). Most corn have the BxBx genotype, which provides normal amounts of benzoxazolinones. The bxbx genotype results in the inability of the plant to produce any significant amount of these biochemical compounds and can be considered to be a deficient allele. Day (1974) reported that this mutant appeared very susceptible to the corn leaf aphid.

3.6.2 Antibiosis - based on aphid reproductive parameters

Each entry was planted individually in 6" pots. An experiment was made up of three entries and 12 replications, in a completely randomized block design. The pots were arranged in a manner as described for antibiosis based on aphid weight (Sect. 3.6.1).

Two weeks after germination, the plants were thinned to one per pot, and the tip end of the last most fully expanded leaf was pinned onto the floor support, as was described (Sec. 3.6.1). Five to ten apterate adults were placed in each cage. The following day, the adults were removed, leaving nymphs 24 hours old or younger. After four days, the nymphs were reduced to one apterate aphid per cage. When reproduction started, the progenies were counted and removed every day until the adult died. Weekly transfer of the adult to the last fully expanded leaf was made so as to provide a fresh leaf surface. Materials used are shown in Table 2. Aphid reactions of these entries were described in Section 3.6.1.

Table 2. Materials used in the antibiosis experiments based on aphid reproductive parameters

Experiment Number	Generation		
	P ₁ (Resistant)	P ₂ (Susceptible)	F ₁
1	3660	CM105	3660 x CM105
2	3660	3655	3660 x 3655
3	Mo17	3655	Mo17 x 3655

3.6.3 Non-preference

The non-preference test was set up by caging together leaves of the three entries under one cage. This was achieved by taping together the three leaves with scotch tape in such a way that on placing the cage over them, approximately a third of the floor area in the cage was covered by each entry (Figure 5).

An experiment was made up of 12 pots to each floor support. Each test (or replication) was made up of the three entries. With three floor supports, there was a total of 12 tests (or replications) arranged in a completely randomized block design.

Plants were grown individually in 6" pots as described earlier (Sect. 3.6.1), and the test was initiated two weeks after germination. The last most fully expanded leaves of the three entries were placed together and pinned by the cages. Thirty adult apterate aphids were then placed randomly in each cage, using a moist camel-hair brush. The next day, the number of aphids on each leaf was counted. This was done each day for four days. Counts were discontinued after the fourth day



Figure 5. Non-preference experiment showing a leaf cage enclosing three test leaves.

because of the difficulty in distinguishing the adults from later instars.

The test was repeated every week and an experiment could go on until the eighth week, although some were terminated earlier. Materials used are shown in Table 3.

Table 3. Materials used in the non-preference experiments

Experiment Number	Generation		
	P ₁ (Resistant)	P ₂ (Susceptible)	F ₁
1	3660	CM105	3660 x CM105
2	3660	3655	3660 x 3655
3	Mo17	3655	Mo17 x 3655
4	3901	3655	3901 x 3655
		Entry	
	1	2	3
5	3660	3655	bxbx

Aphid resistant line 3901 is derived from the AA8sh2 population. Aphid reactions for the rest of the materials used have been described in Section 3.6.1.

3.7 Estimates of general and specific combining ability on corn infested with the corn leaf aphid (Diallel Cross method)

Two diallel crosses were evaluated by the numerical rating system, as described in Section 3.5, and analyzed with the usual analysis of variance as a precondition for further analysis. Griffing's (1956) method 2, models I and II for the diallel analyses were employed to

estimate general and specific combining abilities, as well as variance and covariance components based on the aphid ratings.

The four methods of analyzing and interpreting the results from the diallel crosses as described by Griffing (1956) are:

Method 1: utilizes parents, F_1 's and their reciprocals.

Method 2: utilizes parents, and F_1 's, excluding reciprocals.

Method 3: utilizes F_1 's and reciprocals, excluding parents.

Method 4: utilizes F_1 's, excluding parents and reciprocals.

In model I, or the "Fixed Model", parents are chosen as a fixed sample, and GCA and SCA effects are computed. In model II, or the "Random Model", parents are assumed to represent a random sample from the population. Genotypic and environmental components of the population variance are computed using this model.

3.7.1 The 9 x 9 diallel rated in the field

Nine corn lines, originally selected for the study of resistance to Puccinia sorghi, were grown at the Waimanalo Experiment Station, University of Hawaii in September, 1973. The inbred lines used in the experiment were Oh545, Mol7, 3660, AA25, B14, Ant2D, CM105, B37 and CM111.

A randomized complete block design with four replications was employed. Each plot had 15 plants, spaced 20 cm between plants and 75 cm between rows. As a result of a massive infestation of corn leaf aphid at tasseling time in October, the 9 parents and 36 F_1 's were rated individually using the 6 point rating system of Section 3.5. The abundance of aphids was probably due to the very favorable environmental

conditions for the aphids, among which were low parasite and low predator populations.

The data were then subjected to the statistical procedures described in Section 3.7.

3.7.2 The 8 x 8 diallel rated in the greenhouse

Four resistant and four susceptible corn lines were selected for this study. Four lines, Oh 545, Mo17, 3660 and AA25, had shown resistance to the aphid in previous studies. The 8 parents and the 28 F_1 's were grown in individual 6" pots in a greenhouse to achieve a greater control of parasites and predators of the aphid.

A randomized complete block design with three replications was used. Plants were spaced as close as the 6" pots would allow. Each replication consisted of 4 plants and a total of 12 plants were tested for each entry. Infestation was encouraged by transferring infested plant material into the whorl of each plant at the 4th week of growth (See Sec. 3.3). In addition, susceptible plants were grown at each end of each row to serve as a check and as another source of aphids.

At tasseling, the plants were rated individually, using the 6 point rating system of Section 3.5. The data were then subjected to the statistical procedures, described in Section 3.7.

3.8 Gas-liquid chromatography procedure for the determination of benzoxazolinones

As reviewed earlier (See Literature Review), there has been no ideal method for the analysis of individual compounds of the benzoxazolinones. The slow pace in the research of these unique compounds is the

direct result of a lack of a simple and sensitive method for their quantitative and qualitative analyses.

With this difficulty in mind, work was initiated to develop a simple but sensitive method of detection and quantification of benzoxazolinones in plants. The technique of gas-liquid chromatography, known for its high specificity and sensitivity to individual chemical compounds, was examined as a promising method. Corn and Coix were first subjected to the GLC determination, as they are well-known as benzoxazolinone-containing plants. As the techniques were refined, other plants including wheat, rye, sorghum, teosinte and Tripsacum were also analyzed.

The procedure involved two stages. In the first stage, samples were prepared in such a way as to get the maximum yield of the compound in question. The second stage was the actual determination of the compound by the use of an appropriate column and the gas chromatograph.

Sample preparation

Samples for the determination of 2(3)-benzoxazolinones were prepared following the guideline of Walroos and Virtanen (1959). The steps adopted after some preliminary experimentations were:

1. Etiolated corn seedlings germinated in the dark for one week were used as the plant material.
2. Shoots were weighed (ca 0.1 g) and homogenized in an all glass Ten Broeck homogenizer with 1 ml of distilled water.
3. The homogenate was transferred to a centrifuge tube with more distilled water and the final volume made up to 10 ml with water.

4. The sample was then incubated at room temperature for one hour, to allow enzymatic hydrolysis of the glucosides to the hydroxamic acids.
5. The hydroxamic acid aglucones were converted to benzoxazolinones by heating in a water-bath at 100°C for 30 minutes.
6. The aqueous suspension was then centrifuged and the supernatant extracted twice with 15 ml of redistilled CH_2Cl_2 . To facilitate extraction, the sample was shaken vigorously and then left standing for about 20 minutes.
7. The organic phases were combined and dried with anhydrous sodium sulfate for one-half hour.
8. The dried sample was finally transferred to a graduated tube (10 ml) and evaporated at 100°C under N_2 to a concentration suitable for GLC analysis (about 0.1 ml - 1.0 ml).

Gas chromatograph

Gas chromatography was performed with a Bendix 2500 Gas Chromatograph equipped with flame ionization detectors. A 1 m x 2 mm i.d. glass column packed with Silar-5CP (Supelco, Inc.) on 80/100 mesh high performance Chromosorb W AW-DWCS was prepared according to the procedure of Leibrand and Dunham (1973). A 0.5% solution of Silar-5CP in CHCl_3 with a three-volume excess of the solid support was used for coating. Flow rates were: N_2 , 25 ml/min; H_2 , 25 ml/min; air, 300 ml/min. The temperatures were: injector, 200°; detector, 210°; column, 175° for BOA and 200° for MBOA analyses. Quantitative measurements were determined by comparison of the peak heights of standard solutions and those of the samples.

Gas chromatograph - mass spectrometer (GC-MS)

A Finnigan Model 3000 peak identifier was used with a sensitivity of 10^{-6} A/V, electron multiplier high voltage -2.00 KV and electron energy -69.5V; mass spectrograms were usually taken at the apex of the peaks. The GLC column conditions were the same as described in the previous section.

Preparation of standard solutions

DIMBOA prepared from corn seedlings was obtained from Dr. Carl L. Tipton of Iowa State University. A standard MBOA solution was prepared from a 0.2 mM aqueous DIMBOA solution using a procedure similar to that described in the preparation of samples. Standard BOA solution was prepared from a commercial product (Aldrich Chemical Co., Inc.).

4. RESULTS

4.1 Preliminary observations of genetic segregation for aphid resistance

Field scores under severe aphid infestation in 1972 provided the basis for identification of a series of resistant and susceptible stocks used in this and subsequent studies (See Sect. 3.2). As was described earlier, a series of paired crosses and selfs were grown in single row plots at the Waimanalo Experiment Station. A heavy infestation had developed by tasseling time.

Plants in which the uppermost leaves and tassels were very lightly or not infested were scored as resistant, while those that were moderately to severely infested were considered susceptible. The AA8sh2 pedigree, its parental reaction and the ratings obtained are summarized in Table 4. Lines were identified with numerical codes. The first two digits denote the year of planting, while the next four digits the row number of each line. In some cases, an additional single digit helped identify the individual plant in a row. Inbred line 72-3881-1 selfed is thus interpreted as a 1972 planting in row number 3881 with plant number 1 being self-pollinated.

Of the three susceptible selfs, two gave all susceptible progenies. The third, line 72-3881-1 selfed, gave mostly susceptibles with a few resistant plants, possibly escaping infestation. All three susceptible x susceptible crosses produces susceptible progenies. Susceptible x resistant crosses segregated susceptible and resistant progenies in seven cases. Progenies in two crosses were all susceptible, suggesting that resistance was recessive. However, in three susceptible x

Table 4. Classification of some inbreds in the AA8sh2 population for aphid reaction*

AA8sh2 parents	Parental Reaction (1972)	Progeny Reaction (1973)	
		S	R
72-3881-1 selfed	S	42	5
72-3894 x 72-3881-1	S x S	64	0
72-3881-2 selfed	S	53	0
72-3894 x 72-3881-2	S x S	60	0
72-3881-3 selfed	S	78	0
72-3894 x 72-3881-3	S x S	33	0
72-3883-1 selfed	R	0	7
72-3893 x 72-3883-1	S x R	14	11
72-3883-2 selfed A	R	0	11
72-3883-2 selfed B	R	0	43
72-3893 x 72-3883-2	S x R	8	14
72-3883-4 selfed	R	0	12
72-3893 x 72-3883-4	S x R	9	0
72-3883-3 selfed	R	10	10
72-3892-1 selfed	R	0	17
72-3895 x 72-3892-1	S x R	3	50
72-3892-2 selfed	R	0	9
72-3895 x 72-3892-2	S x R	0	8
72-3892-3 selfed	R	0	13
72-3895 x 72-3892-3	S x R	1	21
72-3892-4 selfed	R	0	5
72-3895 x 72-3892-4	S x R	0	61
72-3898-1 selfed	R	0	7
72-3894 x 72-3898-1	S x R	5	37
72-3898-2 selfed	R	0	7
72-3894 x 72-3898-2	S x R	0	13
72-3898-3 selfed	R	0	41
72-3894 x 72-3898-3	S x R	18	37
72-3915-1 selfed	R	0	18
72-3914 x 72-3915-1	S x R	8	4
72-3915-2 selfed	R	0	31
72-3915-3 selfed	R	0	15
72-3914 x 72-3915-2	S x R	31	0

*S - Susceptible, R = Resistant.

resistant crosses, the progenies were found to be all resistant, suggesting that resistance was dominant. Resistant selfed in 14 cases gave all resistant progenies. Only in one case a resistant self segregated resistant and susceptible types.

No definite conclusion can be drawn from the results. The conflicting observations may be attributed to a misclassification of aphid reactions due to 'escapes'. In other words, a visually resistant-looking plant may have been a susceptible plant which had escaped infestation. Definitive homozygous resistant and susceptible lines were identified and selfed for more critical studies on the inheritance of resistance to the aphid.

4.2 Inheritance of resistance to the corn leaf aphid in the AA8sh2 population

This experiment was conducted in a greenhouse at the Magoon facilities of the University of Hawaii, Manoa Campus. To achieve a greater control of the aphid predators and parasites, the greenhouse was used for most experiments, rather than the field (See Appendix). The materials worked on in this experiment were derived from the AA8sh2 population which was found to be segregating for aphid resistance. Resistant line 3660 was derived from the selfed ear of 72-3883-2 selfed B, and susceptible line, 3655, was derived from the selfed ear of 72-3881-3 selfed. Both of these lines were determined to be homozygous for the aphid reactions. The cross between resistant line 3660, and susceptible line 3655, its backcrosses, the F_2 and the two parental lines were grown individually in 6" pots. Plants were thinned to one

per pot when they were three weeks old. One week later, the plants were individually infested with aphids to ensure full exposure.

At tasseling, plants were rated as resistant or susceptible, and the results are summarized in Table 5. The F_1 plants were susceptible, demonstrating the recessive nature of the resistance. Backcross data pointed to monogenic inheritance, though three plants in the susceptible parent backcross were classified as resistant where there should have been none. These were attributed to 'escapes' rather than actual resistance. The F_2 generation gave 42 susceptibles and 16 resistants. The chi-square of 0.0919 was not significant, indicating a good fit to the 3:1 ratio. The evidence shows that the genetics of resistance to the corn leaf aphid in the AA8sh2 population is monogenic and recessive.

4.3 Antibiosis based on aphid weight

Experiments were conducted between March 1975 and January 1976 at the Pope Laboratory greenhouse facilities, University of Hawaii. Four experiments were conducted to investigate this plant defense mechanism.

Day-old nymphs, born in individually caged leaves, were allowed to grow for six days. Aphid weights were obtained by weighing 10 aphids taken from each cage at the end of the sixth day (See Sect. 3.6.1). The weight is a function of the antibiotic effect of the plant tested and reflects its resistance or susceptibility.

Experiment 1: 3660, CM105 and F_1

Mean weights of 10 aphids for the three entries were obtained from the 2nd to the 8th week of the plants' growth and are summarized in Table 6. A completely randomized block design with 12 replications was

Table 5. Results of ratings of aphid reactions in the AA8sh2 population

Entry	Generation	Total Rated	Observed Res.	Observed Susc.	Expected Ratio Res : Susc
Resistant 3660	P ₁	10	10	0	All resistant
Susceptible 3655	P ₂	10	0	10	All susceptible
3660 x 3655	F ₁	10	0	10	All susceptible
(3660x3655) x 3660	B ₁	20	9	11	1 : 1
(3660x3655) x 3655	B ₂	20	3	17	All susceptible
(3660x3655) selfed	F ₂	58	16	42	1 : 3

Table 6. Weights of aphids grown on 3660 (P₁), CM105 (P₂) and F₁ in antibiosis tests with 12 replications

Entry	Week							Means		Visual Plant Rating**
	2	3	4	5	6	7	8	Week 2-8	Week 3-8	
----- Mean weight of 10 aphids in mg -----										
P ₁	1.17a*	1.93a	3.23a	3.22b	3.62b	4.22b	4.74a	3.16a	3.49b	1.4c
P ₂	0.39c	2.01a	3.07a	4.56a	4.49a	5.10a	5.11a	3.53a	4.06a	3.5a
F ₁	0.80b	2.20a	3.46a	3.54b	4.91a	4.85a	4.97a	3.53a	3.99a	2.5b
Bayes LSD	0.36	--	--	0.97	0.78	0.83	--	0.38	0.41	0.5

*means followed by same letter are not significantly different at 5% level.

**based on a scale of 1-6, 1 is very resistant, 6 is very susceptible.

Table 7. Analyses of variance of antibiosis tests of 3660, CM105 and F₁ data in Table 6

Source	df	Week							Means		Visual Plant Rating
		2	3	4	5	6	7	8	Week 2-8	Week 3-8	
----- Mean Squares -----											
Entry	2	1.82**	0.24	0.46	5.87*	5.24**	2.42	0.42	0.42	1.13*	13.04**
Rep	11	0.23	1.43	1.26	2.28	0.97	2.15*	0.72	0.13	0.18	1.69
Error	22	0.21	0.35	0.74	1.32	0.89	0.87	0.83	0.18	0.26	0.50

**significant at the 1% level; *significant at the 5% level.

utilized. The weight of each entry increased as the plant matured. Bayes LSD values at the 5% level of probability were computed for each week and comparisons were made among the entries within each week. Significant differences were detected in the 2nd, 5th, 6th and 7th week. In general, resistant parental line 3660 had lower weights than the susceptible parental line CM105 and the F_1 . The average weight for the seven weeks (2nd - 8th week) for 3660, CM105 and the F_1 were 3.16, 3.53 and 3.53 mg, respectively. No significant difference was detected in this case. Visual plant ratings were made possible due to natural infestations under greenhouse conditions that were favorable to aphid multiplication. Results of the 6-point numerical rating at tasseling for 3660, CM105 and the F_1 were 1.4, 3.5 and 2.5, respectively, and significantly different from each other. Linear correlation of the ratings and the mean aphid weights of the seven weeks was 0.30.

A comparison of the 2nd week and the 3rd week data suggested that the results of the 2nd week were aberrant. It was recalled that the fertilizer was inadvertently withheld at planting. Nutritional deficiency symptoms in the plants showed up during the 2nd week but were quickly corrected by subsequent fertilization. Nutritional deficient plants affect aphid biology to a significant extent (van Emden, 1966). New means based on six-week data (3rd - 8th week) were analyzed. Here, resistant line 3660 had significantly lower aphid weight than the susceptible line CM105 and the F_1 . These results support the view that aphid resistance in this case is recessive or intermediate in nature.

The analyses of variance of this experiment are summarized in Table 7. Significant differences for the Entry component were detected

in the 2nd, 5th and 6th week. Aphid mean weights based on the 3rd to 8th week and the visual plant ratings were also significant, with a linear correlation value of 0.33, significant at the 5% level. The regression equations for the aphid weight (Y) for each week (X) were calculated from the data in Table 6. They are as follows: 3660 $\hat{Y} = -0.49 + 1.02X - 0.12X^2$, $R^2 = 0.90$; CM105 $\hat{Y} = -3.71 + 2.37X - 0.16X^2$, $R^2 = 0.98$; F_1 $\hat{Y} = -2.64 + 1.93X - 0.12X^2$, $R^2 = 1.00$. The coefficients of determination (R^2) for the three equations were very high, indicating a very good fit of the data to these regression models. Based on these quadratic equations, fitted curves were constructed and are shown in Figure 6. Resistant parental line 3660 had lower weights than susceptible parental line CM105 and the F_1 throughout the plants' growth, depicting the recessive nature of the resistance to the aphid.

Data from all the six weeks were combined and analyzed as a single experiment. The means of the six weeks and the three entries are shown in Table 8. Mean weights of the aphid increased with plant age, and confirmed the results of the earlier analyses. As was determined, resistant line 3660 had significantly lower weight than susceptible line CM105 and the F_1 . A regression equation for the mean aphid weight (Y) for each week (X) was calculated from the data in Table 8, and is as follows: $\hat{Y} = -2.27 + 1.77X - 0.11X^2$, $R^2 = 1.00$. The coefficient of determination (R^2) indicated a perfect regression model. Based on this quadratic equation, a fitted curve was constructed and is shown in Figure 7. Previously, Viale (1950) and Painter (1958) found that corn leaf aphid cannot be maintained in corn seedlings and as a result, aphid resistance could not be studied readily in the greenhouse. Principally

Figure 6. Weekly mean weights of 10 aphids in 3660, CM105 and F_1 antibiosis tests (fitted curves).

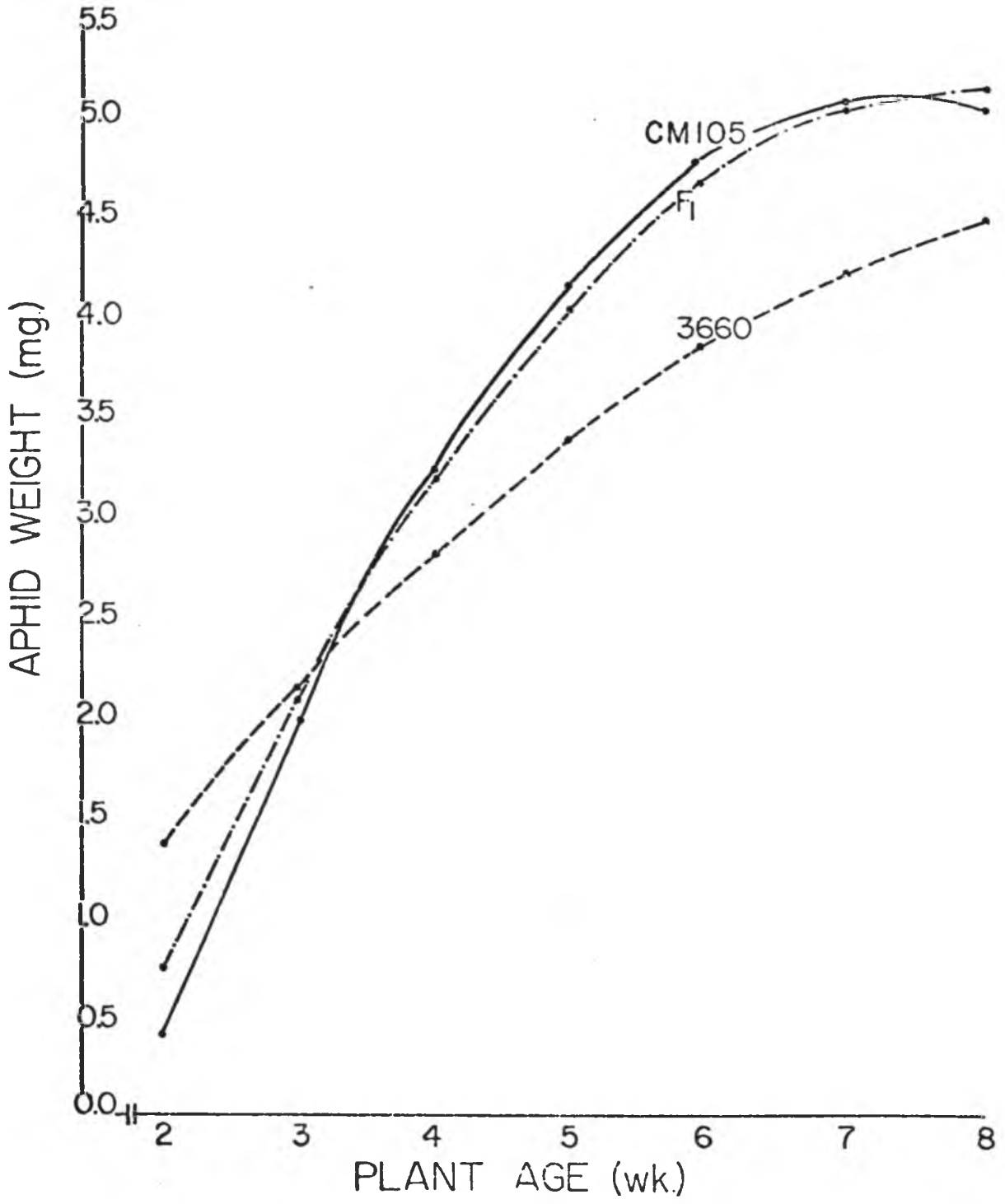


Table 8. Weights of aphids grown on 3660, CM105 and F₁ in antibiosis tests as mean of each week and mean of each entry over the weeks (analyzed with the 2nd week data deleted)

Week	Means of 3 entries	Entry	Means of 6 weeks
	mg		mg
8	4.94a*	CM105	4.06a
7	4.72ab	F ₁	3.99a
6	4.34b	3660	3.49b
5	3.77c		
4	3.25d		
3	2.05e		
Bayes LSD	0.49	Bayes LSD	0.28

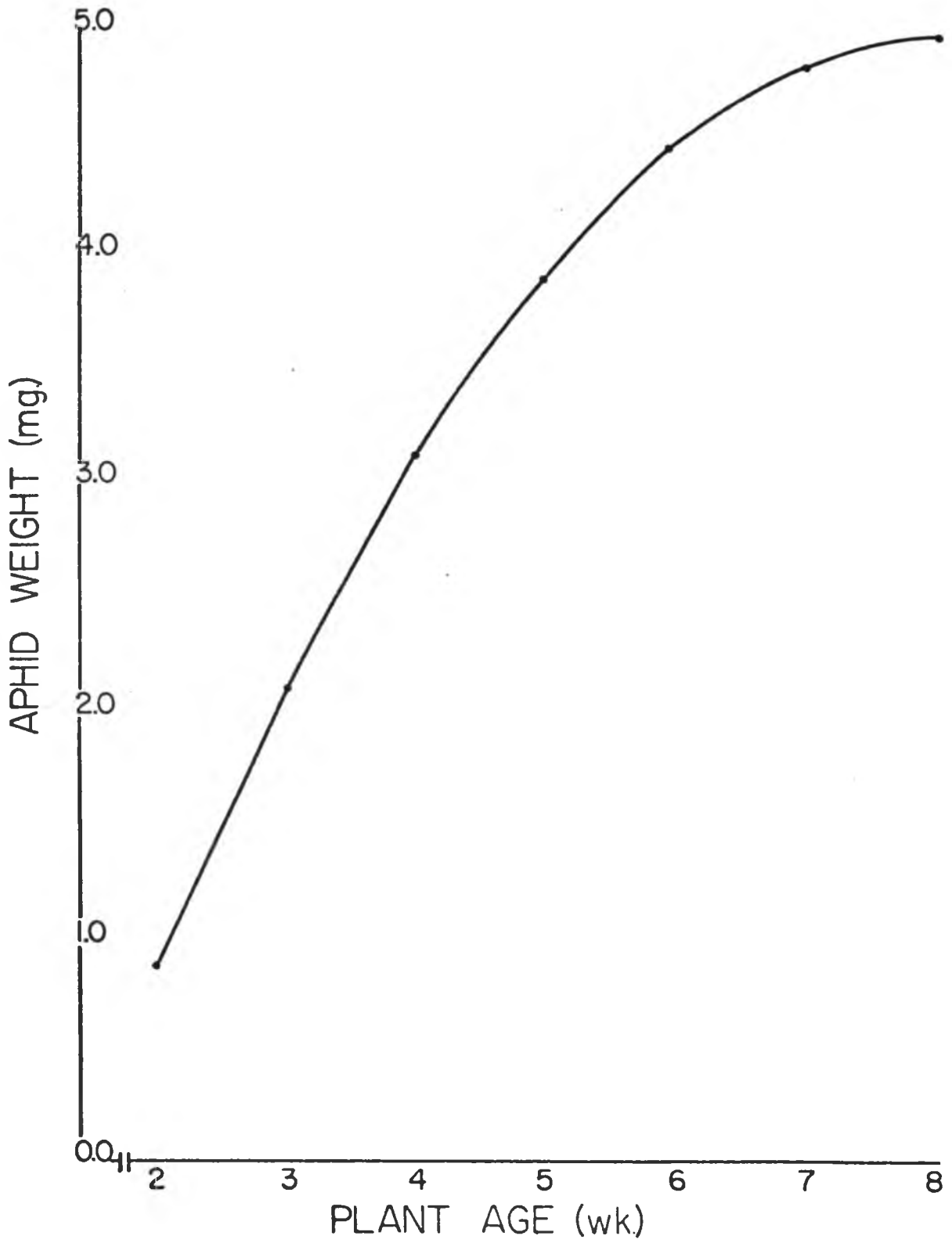
*means followed by same letter are not significantly different at 5% level.

Table 9. Combined analysis of variance of antibiosis tests of 3660, CM105 and F₁ data from Table 8.

Source	df	SS	MS	F
Week	5	209.16	41.83	31.22**
Rep	11	11.94	1.09	0.81
Error (a)	55	73.53	1.34	
Entry	2	13.51	6.75	8.13**
Entry x Week	10	15.66	1.57	1.89
Error (b)	132	110.02	0.83	
Total	215	433.82		

**significant at the 1% level of probability.
Coefficient of variability = 23.7%.

Figure 7. Weekly weights of 10 aphids based on the average weights of 3660, CM105 and F_1 antibiosis tests (fitted curve).



because of this reason, little is known about the details of the resistance of corn to this aphid. Figure 7 illustrates this phenomenon in a more precise term. Corn seedlings have strong antibiotic effects which were generally reduced as the plants matured. The rate of loss of this effect therefore differentiates resistant plants from susceptible ones (Figure 6).

Combined analysis of variance showed highly significant Week and Entry components (Table 9). This, again, confirmed the results of the earlier analyses.

Experiment 2: 3660, 3655 and F₁

Mean weights of 10 aphids for the three entries were obtained from the 2nd to 7th week and summarized in Table 10. A completely randomized block design with 12 replications was utilized. The weight of each entry increased from the 2nd week up to about the 5th or 6th week and then decreased as the plant matured. The regression equations for the aphid weight (Y) for each week (X) were calculated from the data in Table 10. They are as follows: 3660 $\hat{Y} = -1.75 + 2.44X - 0.26X^2$, $R^2 = 0.64$; 3655 $\hat{Y} = 1.08 + 2.48X - 0.32X^2$, $R^2 = 0.67$; and F₁ $\hat{Y} = -1.45 + 3.59X - 0.43X^2$, $R^2 = 0.71$. Based on these equations, fitted curves were constructed and are shown in Figure 8.

Bayes LSD values at the 5% level of probability were computed for each week and comparisons made among the entries within each week. Resistant parental line 3660 had significantly lower aphid weights than susceptible parental line 3655 and the F₁ in all weeks except the 7th. The average aphid weights for the 6 weeks for 3660, 3655 and the F₁ were 3.20, 4.82 and 4.73 mg, respectively. 3660 resistance is clearly

Table 10. Weights of aphids grown on 3660 (P₁), 3665 (P₂) and F₁ in antibiosis tests over 12 replications

Entry	Week						Means	Visual Plant Rating**
	2	3	4	5	6	7		
----- Mean weight of 10 aphids in mg -----								
3660	1.87c*	3.44b	4.22c	3.00c	4.14b	2.53a	3.20b	1.00b
3655	5.45a	4.36a	5.59b	6.10a	5.15a	2.24a	4.82a	4.33a
3660x3655	4.67b	4.33a	6.43a	5.46b	5.52a	1.94a	4.73a	4.17a
Bayes LSD	0.45	0.84	0.66	0.59	0.71	--	0.32	0.49

*means followed by same letter are not significantly different at 5% level.

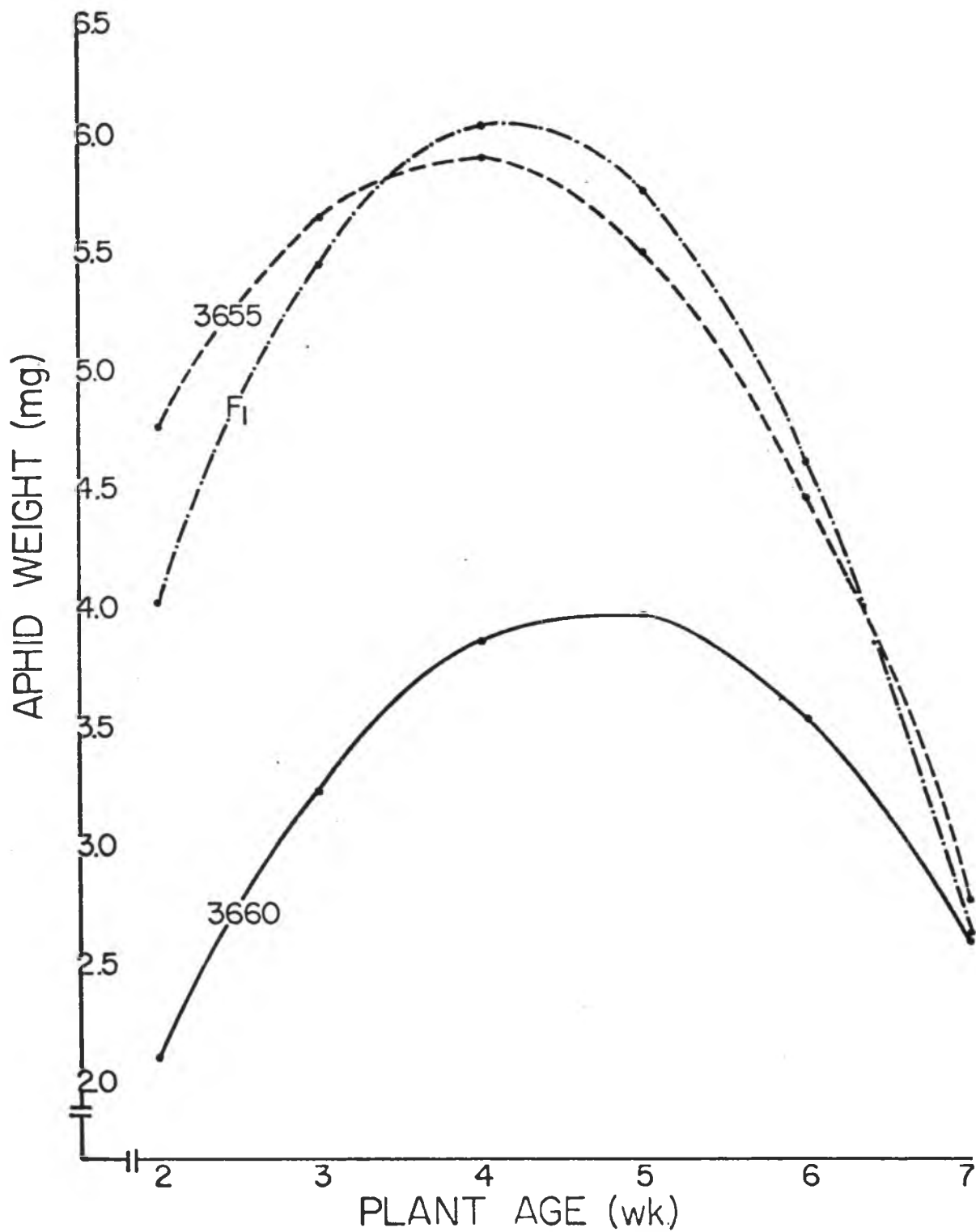
**based on a scale of 1-6, where 1 is very resistant, 6 is very susceptible.

Table 11. Analyses of variance of antibiosis tests of 3660, 3655, and F₁ data in Table 10.

Source	df	Weekly mean squares						Means	Visual Plant Rating
		Week							
		2	3	4	5	6	7		
Entry	2	43.55**	3.31*	14.95*	32.10**	6.12**	1.11	9.88**	42.33**
Rep	11	1.43**	0.27	0.41	1.53*	0.84	0.73	0.36	0.51
Error	22	0.37	0.94	0.76	0.64	0.79	0.47	0.21	0.48

**significant at 1% level; *significant at 5% level.

Figure 8. Weekly mean weight of 10 aphids in 3660, 3655 and F₁ antibiosis tests (fitted curves).



recessive and confirmed the results of Experiment 1 and its inheritance (See Sect. 4.2). Figure 9 shows a typical result of the 2nd week test and illustrates the effect of the recessive resistant gene in line 3660.

Ratings of the plants at the end of the 7th week (Table 10) were made possible due to natural infestation under greenhouse conditions that were favorable to aphid multiplication. Lines 3660, 3655 and the F_1 were rated as 1.00, 4.33 and 4.17, respectively. Resistant line 3660 had a significantly lower score than susceptible line 3655 and the F_1 . Linear correlation of the ratings and the mean of the six weeks was 0.71 and this is highly significant.

The analyses of variance of this experiment are summarized in Table 11. Significant differences were shown in the Entry component for all weeks except the 7th. The mean weights of the six weeks and the rating Entry component were also highly significant.

The data from all the six weeks were combined and analyzed as a single experiment. The means of the six weeks and the three entries are shown in Table 12. The mean aphid weights of the three entries for the six weeks also confirmed the results of the earlier analysis. Resistant line 3660 had significantly lower aphid weight than susceptible line 3655 and the F_1 , again indicating that aphid resistance is recessive in 3660. Weekly aphid weight for the 2nd week was 4.00 mg and it increased to a peak of 5.42 mg at the 4th week. Subsequently, there was a decline to a low of 2.23 mg at the 7th week. A regression equation for the mean aphid weight (Y) for each week (X) was calculated from the data in Table 12, and is as follows: $\hat{Y} = -0.64 + 2.84X - 0.34X^2$, $R^2 = 0.78$. The coefficient of determination (R^2) was quite high indicating



Figure 9. Antibiosis based on aphid weight. A typical result in the 2nd week test of 3655, 3660 and F_1 showing the recessiveness of aphid resistance.

Table 12. Weights of aphids in 3660, 3655 and F₁ in antibiosis tests, as mean of each week and mean of each entry over the weeks

Week	Means of 3 entries	Entry	Means over 6 weeks
	mg		mg
4	5.42a*	3655	4.82a
6	4.94b	F ₁	4.73a
5	4.86b	3660	3.20b
3	4.04c		
2	4.00c		
7	2.23d		
Bayes LSD	0.33	Bayes LSD	0.24

*means followed by same letter are not significantly different at 5% level.

Table 13. Combined analysis of variance of antibiosis tests of 3660, 3655 and F₁ data from Table 12.

Source	df	SS	MS	F
Week	5	229.57	45.91	74.26**
Rep	11	23.77	2.16	3.54**
Error (a)	55	33.61	0.61	
Entry	2	118.59	59.29	89.83**
Entry x Week	10	81.71	8.17	12.38**
Error (b)	132	87.12	0.66	
Total	215	574.37		

**significant at 1% level.

Coefficient of variability = 40.3%.

a good fit of the data to the regression model. Based on this quadratic equation, a fitted curve was constructed and is shown in Figure 10. This curve illustrates the generally high antibiotic effect of corn seedlings and its gradual loss with age. The steep drop after the 6th week is attributed to the rapid senescence of the plant and not resistance per se.

Combined analysis of variance showed highly significant Week, Replication, Entry and Entry x Week components (Table 13).

Experiment 3: Mol7, 6355 and F₁

Mean weight of 10 aphids for the 3 entries were obtained from the 2nd to 8th week and summarized in Table 14. A completely randomized block design with 12 replications was utilized. The weight of each entry increased progressively from the 2nd to the 6th week and then decreased thereafter. The regression equations for the aphid weight (Y) for each week (X) were calculated from the data in Table 14, and are as follows: Mol7 $\hat{Y} = -0.66 + 1.02X - 0.08X^2$, $R^2 = 0.61$; 3655 $\hat{Y} = 1.90 + 0.49X - 0.04X^2$, $R^2 = 0.33$; and F₁ $\hat{Y} = 1.42 + 0.28X - 0.02X^2$, $R^2 = 0.15$. The coefficient of determination (R^2) for these equations were low, indicating a poor fit of the data to the regression. The uncharacteristically high aphid weights in the 3rd week for all three entries undoubtedly contributed to the low R^2 . The data from the other weeks appeared to conform well to a quadratic regression model. Caution should therefore be exercised in interpreting these equations. Fitted curves based on these equations showed that Mol7 and the F₁ have much lower aphid weights than 3655 (Figure 11).

Figure 10. Weekly weights of 10 aphids based on the average weights of 3660, 3655 and F_1 antibiosis tests (fitted curve).

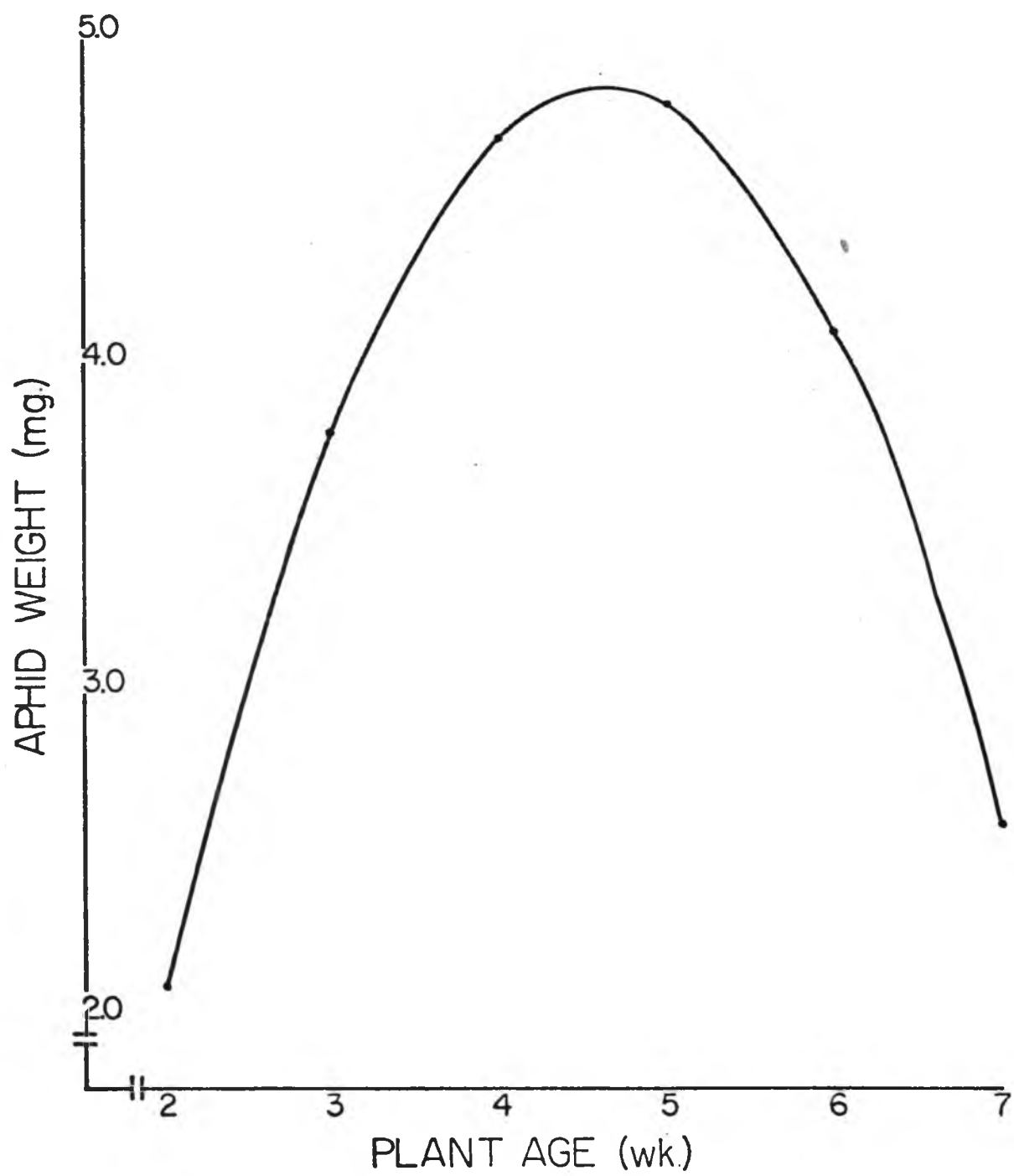


Table 14. Weights of aphids grown on Mol7 (P₁), 3655 (P₂) and F₁ in antibiosis tests over 12 replications

Entry	Week							Means
	2	3	4	5	6	7	8	
----- Mean weight of 10 aphids in mg -----								
Mol7	0.99b*	2.31b	1.35b	2.56a	2.97a	2.41b	2.27b	2.12b
3655	2.37a	3.57a	2.83a	3.05a	3.48a	3.78a	3.25a	3.19a
Mol7x3655	1.61ab	2.83ab	1.44b	2.55a	2.50a	2.27b	2.47b	2.24b
Bayes LSD	0.88	0.81	0.50	5.47	2.12	0.80	0.69	0.33

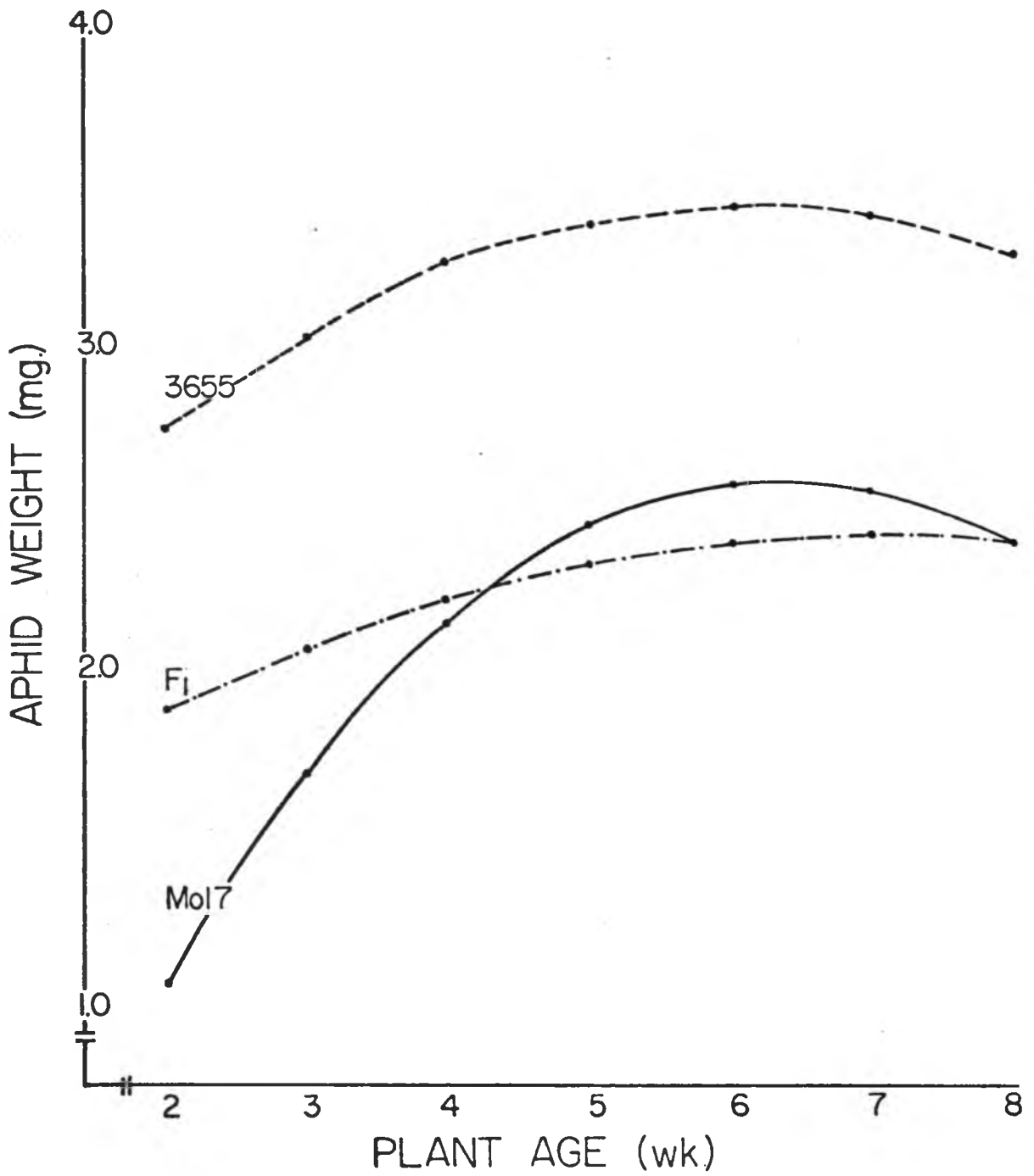
*means followed by same letter are not significantly different at 5% level.

Table 15. Analyses of variance of antibiosis tests of Mol7, 3655 and F₁ data in Table 14.

Source	df	Weekly mean squares							Means
		Week							
		2	3	4	5	6	7	8	
Entry	2	5.69*	4.84*	8.26**	0.97	2.86	8.35**	3.19*	4.11**
Rep	11	0.97	1.24	0.39	1.69	1.16	0.86	0.77	0.16
Error	22	1.12	0.95	0.43	0.85	1.64	1.00	0.68	0.21

**significant at 1% level; *significant at 5% level.

Figure 11. Weekly mean weights of 10 aphids in Mo17, 3655 and F₁ antibiosis tests (fitted curve).



Bayes LSD values at the 5% level of probability were computed for each week and comparisons made among the entries within each week (Table 14). Significant differences were detected in the 2nd, 3rd, 4th, 7th and 8th week. In all these cases the resistant parental line Mol7 and the F_1 had significantly lower aphid weights than the susceptible parental line 3655. Figure 12 illustrates a typical result of the 2nd week test. The average aphid weight for the seven weeks for Mol7, 3655 and the F_1 were 2.12, 3.19 and 2.24 mg, respectively. The susceptible parental line 3655 had significantly higher aphid weight than the resistant parental line Mol7 and the F_1 . Results obtained indicated that the Mol7 aphid resistance was dominant, a contrast to the 3660 resistance seen in Experiments 1 and 2.

The analyses of variance of this experiment are summarized in Table 15 and significant differences are shown in the Entry component for the 2nd, 3rd, 4th, 7th and 8th week. The Entry component for the average of the seven weeks was also highly significant.

The data from all the seven weeks were combined and analyzed as a single experiment. The means of the seven weeks and the three entries are shown in Table 16. The mean weight of the three entries averaged over the seven weeks also confirmed the results of the earlier analyses where the resistant line Mol7 and the F_1 showed significantly lower weights than the susceptible line 3655. In general, aphid weight increased from a low value in the 2nd week to a peak in the 6th week, and subsequently declined. A regression equation for the mean aphid weight (Y) for each week (X) was calculated from the data in Table 16, and is as follows: $\hat{Y} = 1.02 + 0.59X - 0.05X^2$, $R^2 = 0.33$. Based on this



Figure 12. Antibiosis based on aphid weight. A typical result in the 2nd week test of Mol7, F_1 and 3655 showing the dominance of aphid resistance.

Table 16. Weights of aphids in Mol7, 3655 and F₁ in antibiosis tests as mean of each week and mean of each entry over the weeks

Week	Means of 3 entries	Entry	Means of 7 weeks
	mg		mg
6	2.98a*	3655	3.19a
3	2.90a	F ₁	2.24b
7	2.82a	Mol7	2.12b
5	2.72a		
8	2.67a		
4	1.87b		
2	1.66b		
Bayes LSD	0.43	Bayes LSD	0.27

*means followed by same letter are not significantly different at 5% level.

Table 17. Combined analysis of variance of antibiosis tests of Mol7, 3655 and F₁ data from Table 16

Source	df	SS	MS	F
Week	6	60.30	10.05	10.15**
Rep	11	12.11	1.10	1.11
Error (a)	66	65.70	0.99	
Entry	2	57.41	28.70	30.21**
Entry x Week	12	10.92	0.91	0.96
Error (b)	154	146.93	0.95	
Total	251	353.37		

**significant at 1% level.
Coefficient of variability = 38.7%

quadratic equation, a fitted curve was constructed and shown in Figure 13. Though the R^2 was low, it basically depicts the high antibiotic effect of corn seedlings and its gradual loss with age. The apparent increase in antibiotic effect after the 6th week is a result of the plants' senescence and cannot be interpreted as resistance per se.

Combined analysis of variance showed highly significant Week component (Table 17).

Experiment 4: 3660, 3901, 3652 and bxbx

Mean weights of 10 aphids for the four entries were obtained from the 2nd to 7th week and are summarized in Table 18. A completely randomized block design with nine replications was utilized. The weight of each entry increased progressively from the 2nd week until the 4th week and then decreased thereafter. The regression equations for the aphid weight (Y) for each week (X) were calculated from the data in Table 18, and are as follows: 3660 $\hat{Y} = -4.09 + 3.85X - 0.42X^2$, $R^2 = 0.75$; 3901 $\hat{Y} = -3.96 + 3.16X - 0.32X^2$, $R^2 = 0.91$; 3652 $\hat{Y} = -5.00 + 4.89X - 0.55X^2$, $R^2 = 0.92$; and bxbx $\hat{Y} = -1.32 + 2.93X - 0.34X^2$, $R^2 = 0.68$. The coefficients of determination (R^2) for 3901 and 3652 were high while those for 3660 and bxbx were lower. Based on these quadratic equations, fitted curves were constructed and are shown in Figure 14. Bayes LSD values at the 5% level of probability were computed for each week. Comparisons made among the entries within each week showed that generally, resistant lines 3660 and 3901 had significantly lower aphid weights than susceptible lines 3652 and bxbx in all weeks except the 7th. A natural heavy infestation of aphids occurred in the 7th week.

Figure 13. Weekly weights of 10 aphids based on the average weights of Mo17, 3655 and F_1 antibiosis tests (fitted curve).

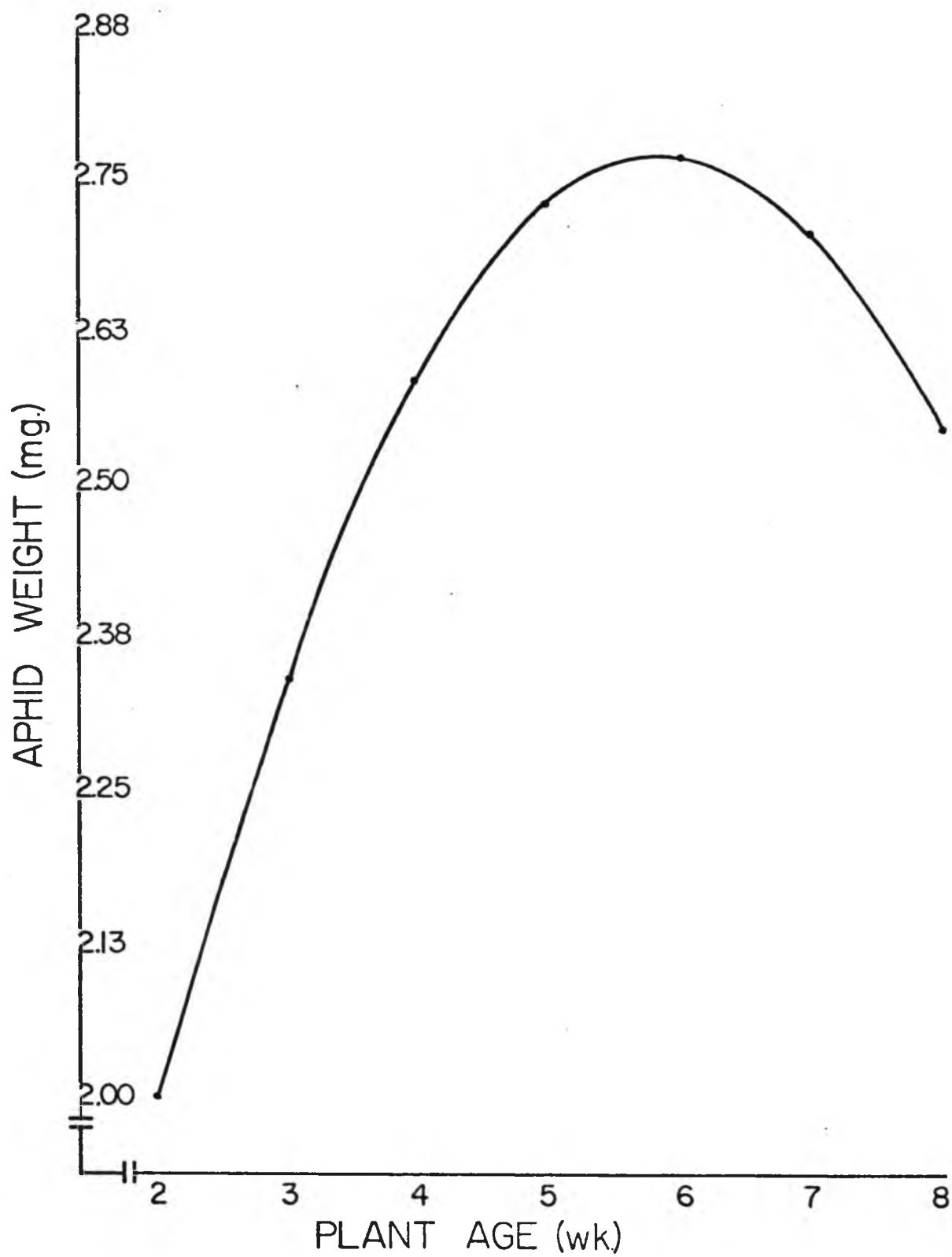


Table 18. Weights of aphids grown on 3660, 3901, 3652 and bxbx in antibiosis tests over 12 replications

Entry	Week						Means	Visual Plant Rating**
	2	3	4	5	6	7		
----- Mean weight of 10 aphids in mg -----								
3660	1.47bc*	4.41a	5.24a	3.83ab	3.41b	2.60a	3.50b	1.8c
3901	0.85c	3.05b	3.39b	3.51b	3.78b	2.48a	2.84c	1.9c
3652	2.49ab	4.49a	6.17a	4.95a	5.15a	2.23a	4.22a	4.4a
bxbx	3.22a	4.16a	5.26a	3.95ab	5.09a	2.21a	3.97ab	3.2b
Bayes LSD	1.06	1.11	1.28	1.12	0.86	--	0.56	0.5

*means followed by same letter are not significantly different at 5% level.

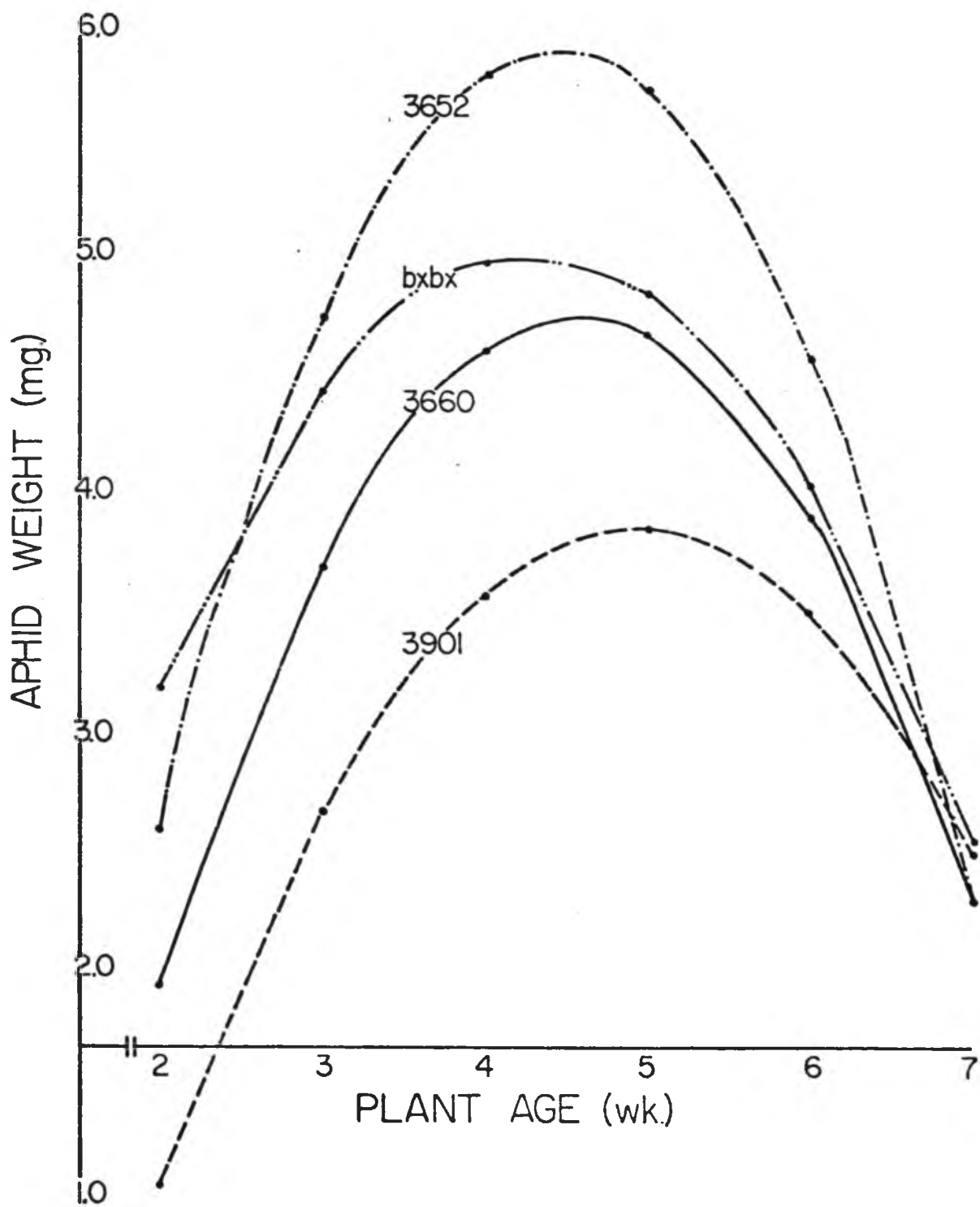
**based on a scale of 1-6, where 1 is very resistant, 6 is very susceptible.

Table 19. Analyses of variance of antibiosis tests of 3660, 3901, 3652 and bxbx data in Table 18

Source	df	Weekly mean squares						Means	Visual Plant Rating
		Week							
		2	3	4	5	6	7		
Entry	3	9.97**	4.03*	11.89**	3.48	6.85**	0.33	3.29**	12.96**
Rep	8	3.99*	0.68	1.00	2.16	0.59	0.55	0.29	0.27
Error	24	1.33	1.19	1.87	1.18	0.87	0.43	0.38	0.31

**significant at the 1% level; *significant at the 5% level.

Figure 14. Weekly mean weights of 10 aphids in 3660, 3901, 3652 and bxbx antibiosis tests (fitted curves).



Susceptible lines 3652 and bxbx were severely infested, suffered symptoms of aphid damage and began to senesce rapidly. Although the results in the 7th week were non-significant, the lower weights in 3652 and bxbx were undoubtedly due to the natural infestation of aphids.

Of special interest in the 2nd week's data is the high aphid weight seen in bxbx. This mutant corn has an insignificant amount of DIMBOA, a compound regarded as an insect and disease resistant factor. The concentration of DIMBOA is highest at the seedling stage (Klun and Robinson, 1969) and may be an important defense mechanism against the aphid. Lacking DIMBOA, bxbx may have become especially vulnerable at this stage. Long et al. (1976) reported that corn leaf aphid resistance was related to DIMBOA concentration, apparently supporting this contention.

The average aphid weight for the six weeks for 3901, 3660, bxbx and 3652 were 2.84, 3.50, 3.97 and 4.22 mg, respectively. Plant ratings for 3901, 3660, bxbx and 3652 were 1.9, 1.8, 3.2 and 4.4, respectively. The two resistant lines had significantly lower scores than the susceptible lines. Plant ratings were possible due to the severe natural infestation under greenhouse conditions that favored aphid multiplication. Linear correlation of the ratings and the mean aphid weight of the six weeks was 0.42 and significant at the 1% level of probability.

Plants were allowed to grow until the 8th week by which time the susceptible lines were killed by the aphids. The resistant lines were without any apparent damage suggesting the presence of tolerance as a

plant defense mechanism. Figure 15 shows the effect of the aphid on resistant line 3660 and susceptible line 3652. Tolerance, a difficult component to measure, is shown by 3660, in addition to its antibiotic effect.

The analyses of variance of this experiment are summarized in Table 19, showing significant differences in the Entry component for all weeks except the 5th and 7th. Significant differences at the 1% level for the Entry component were also found for the average of the six weeks and the plant rating.

Data from all the six weeks were combined and analyzed as a single experiment (Table 20). Resistant lines 3901 and 3660 had significantly lower aphid weights than susceptible lines 3652 and bxbx, thus confirming the results of the earlier analysis. Weekly mean weights of the aphid increased progressively from 2.01 mg in the 2nd week to a peak of 5.00 mg in the 4th week. Subsequently it decreased to 2.38 mg in the 7th week. A regression equation for the mean aphid weight (Y) for each week (X) was calculated from the data in Table 20 and is as follows: $\hat{Y} = -3.73 + 3.7X - 0.4X^2$, $R^2 = 0.89$. The coefficient of determination (R^2) was high, indicating a good fit of the data to the regression model. Based on this equation, a fitted curve was constructed and is shown in Figure 16. This curve illustrates the generally high antibiotic effect of corn seedlings and its gradual loss with age. As noted earlier, the drop after the 6th week is attributed to the rapid senescence of the plant and cannot be interpreted as resistance per se.



Figure 15. Aphid damage at the 8th week. Left, healthy resistant line 3660. Right, dead or drying susceptible line 3652.

Table 20. Weights of aphids in 3660, 3901, 3652 and bxbx in antibiosis tests, as mean of each week and mean of each entry over the weeks

Week	Means of 4 entries	Entry	Means of 6 weeks
	mg		mg
4	5.00a*	3652	4.24a
6	4.34b	bxbx	3.97a
5	4.06b	3660	3.50b
3	4.03b	3901	2.84c
7	2.38c		
2	2.01c		
Bayes LSD	0.51	Bayes LSD	0.37

*means followed by same letter are not significantly different at 5% level.

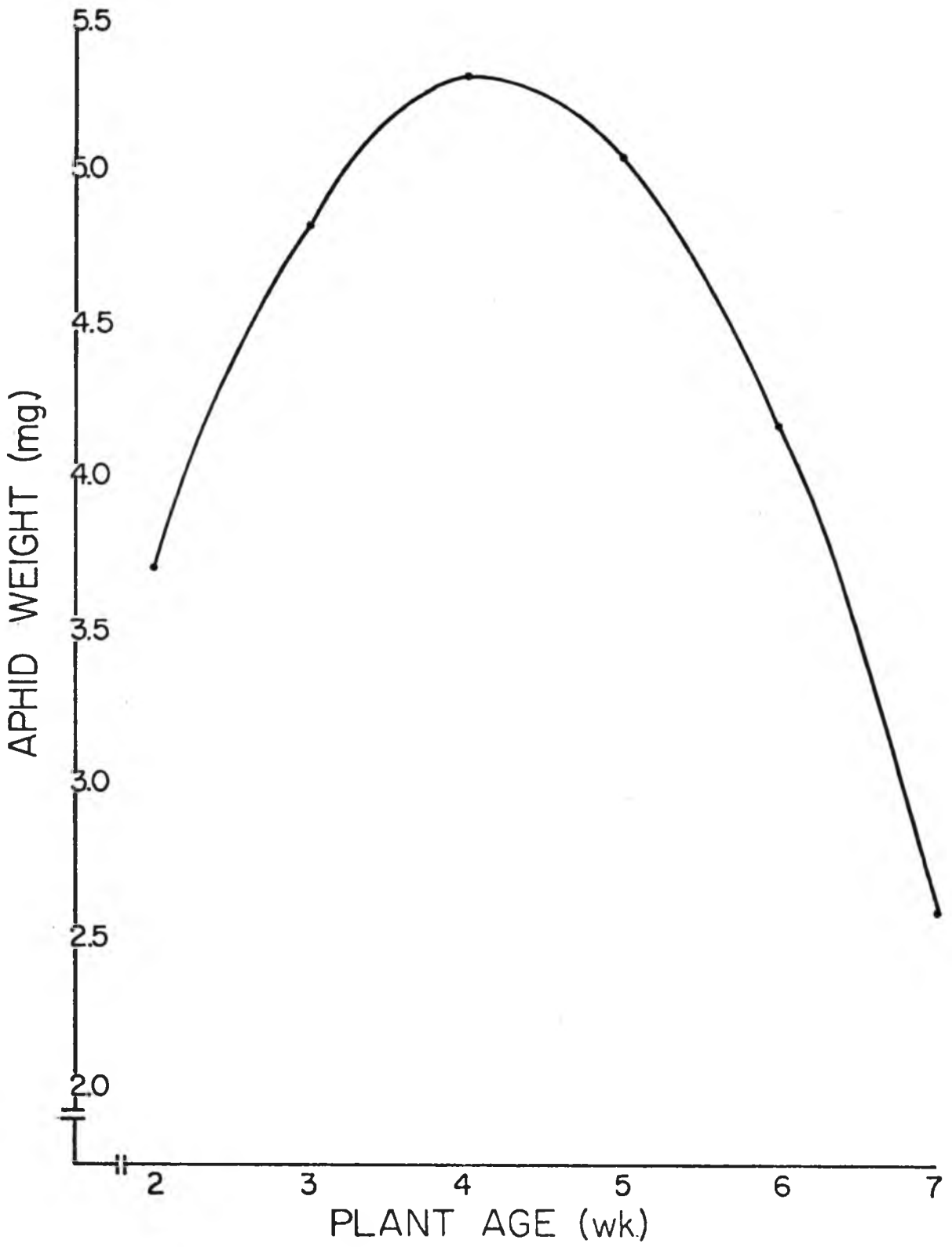
Table 21. Combined analysis of variance of antibiosis tests of 3660, 3901, 3652 and bxbx data from Table 20

Source	df	SS	MS	F
Week	5	249.62	49.92	34.91**
Rep	8	14.53	1.82	1.27
Error (a)	40	57.22	1.43	
Entry	3	60.72	20.24	17.60**
Extry x Week	15	48.94	3.26	2.83**
Error (b)	144	165.00	1.15	
Total	215	596.03		

**significant at 1% level.

Coefficient of variability = 29.4%.

Figure 16. Weekly weights of 10 aphids based on the average weights of 3660, 3901, 3652 and bxbx antibiosis tests (fitted curve).



Combined analysis of variance showed highly significant Week, Entry and Entry x Week component (Table 21).

4.4 Antibiosis based on aphid reproductive parameters

The three experiments were conducted between August 1975 and March 1976, at the Pope Laboratory greenhouse facilities, University of Hawaii. A completely randomized block design with 12 replications was utilized.

Day-old nymphs, born on individually caged 2-week old leaves, were allowed to grow for four days. After the fourth day all but one apterate aphid was left in each cage. When reproduction commenced, the progenies from each cage were counted every day and removed. Daily counts were continued until the aphid died.

Five reproductive parameters were computed to reflect antibiosis.

The parameters are:

1. Days to reproduction: the number of days a nymph takes to become a reproducing adult and produces its first progeny.
2. Aphid longevity: the number of days an aphid lives.
3. Days reproductive: the total number of days the aphid is reproductive.
4. Number of nymphs produced: the total number of nymphs produced in the life-span of an aphid.
5. Rate of reproduction: the average number of nymphs produced per reproductive day.

Experiment 1: 3660, CM105 and F₁

Means and Bayes LSD values (5% level) for the five reproductive parameters are summarized in Table 22. The number of days to

Table 22. Aphid reproductive parameters reflecting antibiosis in 3660, CM105 and F₁

Gener- ation	Entry	Days to Repro- duction	Aphid Longevity	Days Repro- ductive	No. of Nymphs Produced	Rate of Repro- duction
		----- Days -----				No./day
P ₁	3660	7.4a*	25.6b	14.2b	46.6b	3.3b
P ₂	CM105	6.3b	27.3ab	16.2ab	64.8a	4.0a
F ₁	3660x CM105	6.5b	29.3a	17.6a	70.2a	4.0a
Bayes LSD		0.6	3.2	2.5	9.0	0.5

*means followed by same letter are not significantly different at 5% level.

Table 23. Analyses of variance of 3660, CM105 and F₁ data from Table 22

Source	df	Days to Repro- duction	Aphid Longevity	Days Repro- ductive	No. of Nymphs Produced	Rate of Repro- duction
		----- Mean squares -----				
Entry	2	4.53**	42.25	35.36*	1835.36**	2.06*
Rep	11	0.60	21.46	4.09	211.24	0.63
Error	22	0.53	13.19	8.06	137.21	0.38

**significant at 1% level; *significant at 5% level.

reproduction for aphids caged on 3660, CM105 and the F_1 were 7.4, 6.3 and 6.5, respectively. Aphids raised on the resistant parental line 3660 took a significantly longer time to start reproduction as compared to the susceptible parental line CM105 and the F_1 . Aphid longevity and days reproductive on 3660 were significantly shorter than the F_1 but not CM105. The number of nymphs produced by the aphids grown on 3660, CM105 and the F_1 were 46.6, 64.8 and 70.2, respectively. A strong antibiotic effect in 3660 reduced the number of nymphs produced significantly as compared to CM105 and the F_1 .

In summary, strong antibiotic effects on the aphids were exerted by the resistant parental line 3660 on the number of days to reproduction, the number of nymphs produced and the rate of reproduction. The results obtained indicated that the antibiotic effects are recessive in nature.

The analyses of variance for this experiment are summarized in Table 23. Significant differences at the 1% level of probability were obtained in the Entry component for the number of days to reproduction and the number of nymphs produced. Significant differences at the 5% level of probability were detected in the Entry component for the number of days reproductive and the rate of reproduction.

Experiment 2: 3660, 3655 and F_1

Means and Bayes LSD values (5% level) for the five reproductive parameters are summarized in Table 24. The number of days to reproduction for aphids caged on 3660, 3655 and the F_1 were 7.9, 5.7 and 6.3, respectively. Aphids raised on the resistant parental line 3660 took a significantly longer time to start reproduction as compared to aphids

Table 24. Aphid reproductive parameters reflecting antibiosis in 3660, 3655 and F₁

Gener- ation	Entry	Days to Repro- duction	Aphid Longevity	Days Repro- ductive	No. of Nymphs Produced	Rate of Repro- duction
		----- Days -----				No./day
P ₁	3660	7.9a*	27.3a	15.8a	50.6b	3.2b
P ₂	3655	5.7b	27.9a	16.1a	66.0a	4.1a
F ₁	3660x 3655	6.3b	27.3a	16.1a	66.9a	4.2a
Bayes LSD		0.6	--	--	8.4	0.3

*means followed by same letters are not significantly different at 5% level.

Table 25. Analyses of variance of 3660, 3655 and F₁ data from Table 24

Source	df	Days to Repro- duction	Aphid Longevity	Days Repro- ductive	No. of Nymphs Produced	Rate of Repro- duction
		----- Mean squares -----				
Entry	2	15.44**	1.38	0.25	1010.58**	3.50**
Rep	11	0.76	33.06**	11.27	152.88	0.25
Error	22	0.60	3.85	6.25	114.19	0.18

**significant at 1% level.

raised on the susceptible parental line 3655 and the F_1 . No significant difference was detected for aphid longevity and the number of days reproductive. The number of nymphs produced by aphids grown on 3660, 3655 and the F_1 were 50.6, 66.0 and 66.9, respectively. A strong antibiotic effect exerted by 3660 significantly reduced the number of nymphs produced as compared to 3655 and the F_1 . The rate of reproduction was also significantly reduced in 3660 as compared to 3655 and the F_1 .

In summary, strong antibiotic effects on the aphids were exerted by the resistant parental line 3660 on the number of days to reproduction, the number of nymphs produced and the rate of reproduction. The results obtained indicated that the antibiotic effects are recessive in nature. Similar results were obtained in Experiment 1, reflecting the consistency of the resistance in 3660.

The analyses of variance for this experiment are summarized in Table 25. Significant differences at the 1% level of probability were detected in the Entry component for the number of days to reproduction, the number of nymphs produced and the rate of reproduction.

Experiment 3: Mol7, 3655 and F_1

Means and Bayes LSD values (5% level) for the five reproductive parameters are summarized in Table 26. The number of days to reproduction for aphids caged on Mol7, 3655 and the F_1 were 8.9, 6.3 and 8.7, respectively. Aphids raised on the resistant parental line Mol7 and the F_1 took a significantly longer time to start reproduction as compared to the susceptible parental line 3655. Aphid longevity and the number of days reproductive in Mol7 and the F_1 were significantly shorter than

Table 26. Aphid reproductive parameters reflecting antibiosis in Mol7, 3655 and F₁

Gener- ation	Entry	Days to Repro- duction	Aphid Longevity	Days Repro- ductive	No. of Nymphs Produced	Rate of Repro- duction
		----- Days -----				No./day
P ₁	Mol7	8.9a*	24.3b	11.7b	31.9b	2.8b
P ₂	3655	6.3b	30.2a	16.3a	64.7a	4.0a
F ₁	Mol7x 3655	8.7a	25.3b	11.6b	36.7b	3.2b
Bayes LSD		0.8	4.8	3.1	11.8	0.6

*means followed by same letter are not significantly different at 5% level.

Table 27. Analyses of variance of Mol7, 3655 and F₁ data from Table 26

Source	df	Days to Repro- duction	Aphid Longevity	Days Repro- ductive	No. of Nymphs Produced	Rate of Repro- duction
		----- Mean squares -----				
Entry	2	24.36**	116.78*	58.59	3770.78**	5.04**
Rep	11	0.94	40.05	23.55	288.63	0.68
Error	22	1.18	33.57	18.58	260.75	0.60

**significant at 1% level; *significant at 5% level.

that in 3655. The number of nymphs produced in Mol7, 3655 and the F_1 were 31.9, 64.7 and 36.7, respectively. Dominance of the antibiotic effect in this experiment resulted in a significant reduction of nymphs produced on Mol7 and the F_1 as compared to 3655. The rate of reproduction was also significantly reduced in Mol7 and the F_1 as compared to that in 3655.

In summary, strong antibiotic effects were exerted by the resistant parental line Mol7 and the F_1 on all five reproductive parameters. In contrast to Experiments 1 and 2, the antibiotic effects of Mol7 are dominant, reflecting a different mode of inheritance.

The analyses of variance for this experiment are summarized in Table 27. Significant differences at the 1% level of probability were found in the Entry component for all the parameters except the number of days reproductive.

4.5 Non-preference

The five experiments were conducted between March 1975 and January 1976 at the Pope Laboratory greenhouse facilities, University of Hawaii. A completely randomized block design with 12 replications was utilized.

Thirty apterate aphids were introduced into each cage containing approximately equivalent areas of leaves of the three entries. The next day the number of aphids on each leaf was counted. This was done daily for four days. Weekly tests were conducted from the 2nd week until the 8th week. Some experiments were terminated earlier.

Generally, the total number of aphids found on the three entries were greatest on the first day and decreased thereafter. This is attributed to the fact that some aphids tended to settle on the cages'

wall and roof after prolonged caging. Occasionally, a few aphids escaped.

Experiment 1: 3660, CM105 and F₁

Means and Bayes LSD values (5% level) for the 2nd to 8th week tests are summarized in Table 28. Based on the means of each, the resistant parental line 3660 was the least preferred among the three entries for all weeks except the 4th and the 8th week. However significant non-preference was only detected in 3660 as compared to the susceptible parental line CM105 in the 2nd and 3rd week. The F₁ was also significantly less preferred than CM105 in the 2nd week. No significant difference in preference was detected from the 4th to 8th week.

The analyses of variance of this experiment are summarized in Table 29. Highly significant differences for the Entry component in the 2nd and 3rd week confirmed the results of the earlier analyses that the resistant line 3660 was less preferred than the susceptible line CM105.

Means of the seven weeks were combined and analyzed as a single experiment. The mean number of aphids in each week and in the entries are shown in Table 30. No significant difference among the three entries was detected. Weekly average number of aphids in the three entries was lowest in the 2nd week. Non-preference was therefore strongest in this week. The 4th week was the most preferred week by the aphids. In general, the corn plant is least preferred in the seedling stage. The plant then becomes progressively more preferred until about the 4th to 6th week. Subsequently, senescence of the plant makes

Table 28. Number of aphids in 3660, CM105 and F₁ in non-preference tests with 12 replications

Week	Entry	Day				Means
		1	2	3	4	
2nd	3660	3.0b*	0.7b	0.1b	--	1.2b
	CM105	8.8a	3.1a	0.9a	--	4.3a
	F ₁	4.6b	1.2b	0.3ab	--	2.0b
	Bayes LSD	3.2	1.1	0.6	--	1.4
3rd	3660	6.9a	5.8b	5.4b	6.5b	6.2b
	CM105	9.8a	9.8a	9.4a	9.4a	9.6a
	F ₁	8.8a	8.6ab	7.4ab	5.7b	7.8ab
	Bayes LSD	6.8	3.4	2.6	2.1	2.1
4th	3660	10.2ab	9.1a	7.9a	--	9.1a
	CM105	11.9a	11.3a	6.8a	--	10.0a
	F ₁	6.5b	7.2a	7.6a	--	7.1a
	Bayes LSD	4.8	5.2	--	--	4.3
5th	3660	8.5a	7.3a	6.4a	--	7.4a
	CM105	7.3a	8.6a	9.1a	--	8.3a
	F ₁	10.8a	9.4a	6.3a	--	8.8a
	Bayes LSD	4.8	--	3.3	--	--
6th	3660	9.3a	7.0a	6.4a	6.3a	7.2a
	CM105	7.6a	9.8a	9.0a	9.5a	8.9a
	F ₁	8.6a	7.9a	8.5a	7.1a	8.0a
	Bayes LSD	--	--	11.1	3.3	--
7th	3660	9.5a	8.4a	6.8a	4.6a	7.3a
	CM105	7.0a	7.6a	8.6a	7.0a	7.6a
	F ₁	9.8a	8.7a	6.3a	7.2a	8.0a
	Bayes LSD	20.5	--	8.3	2.6	--
8th	3660	11.5a	10.6a	7.9a	--	10.0a
	CM105	6.5a	6.6a	6.6a	--	6.6a
	F ₁	8.8a	7.8a	5.7a	--	7.4a
	Bayes LSD	5.7	5.0	--	--	4.2

*means followed by same letter are not significantly different at 5% level.

Table 29. Analyses of variance of non-preference tests of 3660, CM105 and F₁ data in Table 28

Week	Source	df	Mean squares				
			Day				Means
			1	2	3	4	
2nd	Entry	2	105.86**	19.53**	2.19*	--	30.13**
	Rep	11	11.96	5.36*	0.18	--	4.08
	Error	22	15.35	2.01	0.53	--	3.24
3rd	Entry	2	24.78	50.78	48.00**	46.53**	34.87**
	Rep	11	6.94	4.09	2.61	2.39	2.70
	Error	22	19.60	15.29	9.64	6.56	6.53
4th	Entry	2	91.69	50.08	4.33	--	26.04
	Rep	11	0.88	1.42	22.25	--	2.52
	Error	22	29.91	25.96	12.79	--	15.33
5th	Entry	2	37.75	13.19	29.36	--	6.07
	Rep	11	2.21	3.23	1.26	--	1.60
	Error	22	20.87	21.50	12.48	--	11.30
6th	Entry	2	8.44	25.08	22.53	34.75	7.85
	Rep	11	3.60	3.58	4.69	2.73	2.60
	Error	22	43.11	26.42	20.65	13.11	15.82
7th	Entry	2	28.78	3.86	17.19	25.08	1.45
	Rep	11	5.47	3.47	3.60	4.49	2.74
	Error	22	27.84	26.65	15.28	8.51	14.76
8th	Entry	2	75.25	50.78	15.36	--	38.95
	Rep	11	2.25	2.82	2.78	--	1.58
	Error	22	35.25	25.51	18.00	--	18.72

**significant at 1% level; *significant at 5% level.

Table 30. Number of aphids in 3660, CM105 and F₁ in non-preference tests, as mean of each week and mean of each entry over the weeks

Week	Means of 3 entries	Entry	Means of 7 weeks
4	8.7a*	CM105	7.9a
5	8.2ab	3660	7.1a
6	8.1ab	F ₁	6.9a
8	8.0b		
3	7.9b		
7	7.6b		
2	2.5c		
Bayes LSD	0.6	Bayes LSD	1.3

*means followed by same letter are not significantly different at 5% level.

Table 31. Combined analysis of variance of non-preference tests of 3660, CM105 and F₁ data from Table 30

Source	df	SS	MS	F
Week	6	973.20	162.20	67.02**
Rep	11	36.79	3.34	1.38
Error (a)	66	159.41	2.42	
Entry	2	46.56	23.28	1.96
Entry x Week	12	300.15	25.01	2.11*
Error (b)	154	1829.67	11.88	
Total	251	3345.78		

**significant at 1% level; *significant at 5% level.
Coefficient of variability = 47.2%

it less palatable and therefore less preferred by the aphid. In a strict sense this senescence period cannot be reliably regarded as a non-preference effect and thus would have no value in the identification of non-preferred plants.

The combined analysis of variance is summarized in Table 31. Highly significant Week component was found and significant Entry x Week component generally reflects the complexity of this defense mechanism, and the high uncontrolled variability is evident in a CV of 47%.

Experiment 2: 3660, 3655 and F_1

Means and Bayes LSD values (5% level) for the 2nd to 5th week tests are summarized in Table 32. Based on the means of each week, the resistant parental line 3660 was the least preferred among the three entries in the 2nd and 3rd week but was only significantly so in the former. In the 4th week, the F_1 was the least preferred, 3660 being intermediate and 3655, the most preferred. However the F_1 was significantly different from only 3655. The 5th week's results showed that 3660 was most preferred by the aphids and significantly different from 3655 and the F_1 . At that time, there was a natural heavy infestation of aphids on susceptible line 3655 and the F_1 . It is thus not inconceivable that because of excessive feeding elsewhere in the plant, the portion of the test leaves of 3655 and F_1 were either depleted of nutrients or were senescing prematurely. As a result they became highly undesirable to the aphids.

The analyses of variance of this experiment are summarized in Table 33. Significant differences were detected in the 2nd, 4th and

Table 32. Number of aphids in 3660, 3655 and F₁ in non-preference tests with 12 replications

Week	Entry	Day				Means
		1	2	3	4	
2nd	3660	4.8b*	3.0b	3.7a	3.8ab	3.9b
	3655	10.3a	7.9a	4.8a	2.8b	6.6a
	F ₁	8.3ab	6.4a	5.7a	5.5a	6.5a
	Bayes LSD	4.6	2.8	10.7	2.5	2.4
3rd	3660	8.9a	6.5a	5.7a	4.3a	6.4a
	3655	7.8a	7.3a	6.9a	5.2a	6.8a
	F ₁	11.0a	8.7a	6.7a	4.0a	7.6a
	Bayes LSD	4.5	3.2	--	--	--
4th	3660	8.4a	7.3b	7.3ab	5.6a	7.1ab
	3655	9.0a	10.5a	8.4a	6.9a	8.7a
	F ₁	5.3a	6.2b	5.6b	5.9a	5.8b
	Bayes LSD	6.0	2.9	2.2	--	2.0
5th	3660	15.6a	15.1a	10.7a	7.8a	12.3a
	3655	5.6b	6.8b	7.2ab	7.7a	6.8b
	F ₁	5.5b	4.8b	5.3b	4.6b	5.1b
	Bayes LSD	5.0	4.0	4.5	2.9	3.3

*means followed by same letter are not significantly different at 5% level.

Table 33. Analyses of variance of non-preference tests of 3660, 3655 and F₁ data in Table 32

Week	Source	df	Mean squares				Means
			Day				
			1	2	3	4	
2nd	Entry	2	90.53	76.19**	12.11	22.03	28.81*
	Rep	11	6.88	19.84	20.90	14.15	13.39
	Error	22	28.04	11.80	11.51	7.97	7.55
3rd	Entry	2	31.08	14.53	5.25	4.33	4.56
	Rep	11	2.55	7.18	8.49	5.24	3.34
	Error	22	17.57	8.50	10.86	6.12	5.80
4th	Entry	2	48.86	61.03*	24.33*	5.78	26.60*
	Rep	11	26.93	7.79	3.83	3.06	4.41
	Error	22	29.22	11.79	6.64	9.05	5.86
5th	Entry	2	403.36**	354.25**	88.11	39.08*	169.33*
	Rep	11	4.02	11.52	3.02	3.21	3.86
	Error	22	40.30	27.25	26.54	10.93	17.72

**significant at 1% level; *significant at 5% level.

5th week's Entry component, confirming the results of the previous analyses.

Means of the four weeks were combined and analyzed as a single experiment. The mean number of aphids in each week and in the entries are shown in Table 34. No significant difference among the entries was observed. Weekly average number of aphids in the three entries for the 2nd week was the lowest and this increased to a high in the 5th week. Plants were therefore the least preferred by the aphids in the 2nd week and most preferred in the 5th.

The combined analysis of variance is summarized in Table 35. Highly significant differences were found for the Week and Entry x Week components.

Experiment 3: Mo17, 3655 and F₁

Means and Bayes LSD values (5% level) for the 2nd to 8th week tests are summarized in Table 36. Based on the means of each week, the resistant parental line Mo17 and the F₁ were less preferred than the susceptible parental line 3655 for the 2nd, 3rd and 4th week. The F₁ was significantly less preferred by the aphids as compared to 3655 for the 2nd, 3rd, 4th and 5th week. However Mo17 was significantly less preferred by the aphids, as compared to 3655, for only the 2nd week. Evidence here suggests that non-preference in Mo17 appears to be dominant. No significant difference in preference was detected from the 6th to 8th week.

The analyses of variance of this experiment are summarized in Table 37. Highly significant differences were detected for the 2nd, 4th and 5th week's Entry component.

Table 34. Number of aphids in 3660, 3655 and F₁ in non-preference tests, as mean of each week and mean of each entry over the weeks

Week	Means of 3 entries	Entry	Means of 4 weeks
5	8.1a*	3660	7.4a
4	7.2ab	3655	7.2a
3	6.9b	F ₁	6.2a
2	5.6c		
Bayes LSD		Bayes LSD	
	1.0		1.5

*means followed by same letter are not significantly different at 5% level.

Table 35. Combined analysis of variance of non-preference tests of 3660, 3655 and F data from Table 34

Source	df	SS	MS	F
Week	3	113.00	37.67	6.76**
Rep	11	88.72	8.07	1.45
Error (a)	33	183.70	5.57	
Entry	2	38.23	19.12	2.08
Entry x Week	6	418.59	69.77	7.58**
Error (b)	88	810.26	9.21	
Total	143	1652.49		

**significant at 1% level.

Coefficient of variability = 43.8%.

Table 36. Number of aphids in Mol7, 3655 and F₁ in non-preference test with 12 replications

Week	Entry	Day				Means
		1	2	3	4	
2nd	Mol7	4.9b*	5.1b	4.2b	4.6b	4.7b
	3655	14.3a	12.0a	11.2a	9.7a	11.8a
	F ₁	4.8b	3.0b	3.5b	4.1b	3.9b
	Bayes LSD	3.0	2.4	3.1	2.9	2.1
3rd	Mol7	9.2a	9.2ab	6.8a	7.6a	8.2ab
	3655	10.8a	11.8a	10.9ab	8.2a	10.5a
	F ₁	6.2a	5.3b	5.7b	6.2a	5.9b
	Bayes LSD	6.2	4.6	4.4	--	4.0
4th	Mol7	10.4a	7.8a	6.1b	5.2b	7.4ab
	3655	10.0a	10.2a	11.2a	10.4a	10.5a
	F ₁	5.7a	6.2a	4.9b	4.2b	5.3b
	Bayes LSD	5.1	8.5	4.4	3.6	4.1
5th	Mol7	11.0a	9.9a	10.2a	9.5a	10.2a
	3655	8.2ab	9.0a	9.9a	9.9a	9.3a
	F ₁	4.9b	5.0a	4.2b	4.0b	4.6b
	Bayes LSD	4.0	5.8	4.9	4.5	4.3
6th	Mol7	5.1a	4.9a	5.5a	5.6a	5.3a
	3655	9.7a	9.3a	7.5a	6.0a	8.1a
	F ₁	9.9a	9.6a	7.8a	6.8a	8.6a
	Bayes LSD	5.9	5.1	--	--	5.7
7th	Mol7	7.6a	5.2a	5.5a	4.1a	5.6a
	3655	6.0a	6.0a	6.2a	3.7a	5.5a
	F ₁	7.8a	6.7a	4.9a	3.2a	5.7a
	Bayes LSD	--	--	--	--	--
8th	Mol7	3.9a	4.5a	4.4a	3.9a	4.2a
	3655	9.9a	7.7a	6.6a	5.0a	7.3a
	F ₁	8.0a	6.2a	6.3a	4.2a	6.2a
	Bayes LSD	8.6	--	11.9	--	7.5

*means followed by same letter are not significantly different at 5% level.

Table 37. Analyses of variance of non-preference tests of Mol7, 3655 and F₁ data in Table 36

Week	Source	df	Mean squares				Means
			Day				
			1	2	3	4	
2nd	Entry	2	357.86**	266.36**	214.36**	118.11**	228.46**
	Rep	11	4.21	5.66	6.03	11.66	4.79
	Error	22	15.59	10.03	16.03	13.17	7.88
3rd	Entry	2	67.58	128.11*	91.19*	12.44	63.49
	Rep	11	7.10	5.35	8.09	10.39	6.75
	Error	22	36.34	29.23	26.10	17.78	20.24
4th	Entry	2	83.03	46.58	136.33*	133.86**	82.54*
	Rep	11	5.36	11.16	14.19	19.57	11.26
	Error	22	31.76	35.40	27.36	19.98	22.58
5th	Entry	2	111.36*	82.03	140.36*	130.86*	109.90*
	Rep	11	4.66	7.97	8.87	10.33	6.68
	Error	22	23.27	37.51	33.60	28.29	26.22
6th	Entry	2	88.86	82.69	19.11	4.86	38.22
	Rep	11	8.57	9.81	13.02	12.03	9.96
	Error	22	39.74	32.12	27.84	20.01	24.12
7th	Entry	2	11.86	6.78	4.69	2.08	0.13
	Rep	11	8.09	10.35	9.12	11.70	7.95
	Error	22	31.07	10.47	12.24	6.87	7.17
8th	Entry	2	112.69	30.19	16.86	3.86	29.44
	Rep	11	3.99	4.39	4.75	5.24	3.84
	Error	22	64.45	35.71	15.92	11.59	23.44

**significant at 1% level; *significant at 5% level.

Means of the seven weeks were combined and analyzed as a single experiment. The mean number of aphids in each week and in the entries are shown in Table 38. Mol7 and the F_1 were significantly less preferred than 3655, thus confirming the dominance of non-preference in this cross. Weekly average number of aphids in the three entries showed a trend similar to the previous experiments. In general corn seedlings were the least preferred and non-preference was gradually lost with age. The apparent increase in non-preference after the 6th week was due to the plants becoming less desirable to the aphids because of senescence. Strictly, this phase cannot be considered as non-preference per se.

The combined analysis of variance is summarized in Table 39. Highly significant Week, Entry and Entry x Week components were detected, reflecting the complexity of this defense mechanism.

Experiment 4: 3901, 3655 and F_1

Means and Bayes LSD values (5% level) for the 2nd to 6th week tests are summarized in Table 40. No test was conducted in the 3rd week as the greenhouse was undergoing fumigation. Based on the means of each week, the resistant parental line 3901 was the least preferred in the 2nd and 4th week. However significant non-preference was detected in 3901 as compared to 3655 and the F_1 only for the 2nd week. The 5th week's results showed that the F_1 was the least preferred, 3901 being intermediate and 3655 the most preferred. However the F_1 was only significantly different from 3655 and not 3901. No significant difference in preference was found in the 6th week.

Table 38. Number of aphids in Mol7, 3655 and F₁ in non-preference tests as mean of each week and mean of each entry over the weeks

Week	Means of 3 entries	Entry	Means of 7 weeks
3	8.2a*	3655	9.0a
5	8.0a	Mol7	6.5b
4	7.7ab	F ₁	5.7b
6	7.3ab		
2	6.8bc		
8	5.9cd		
7	5.6d		
Bayes LSD	1.1	Bayes LSD	1.2

*means followed by same letter are not significantly different at 5% level.

Table 39. Combined analysis of variance of non-preference tests of Mol7, 3655 and F₁ data from Table 38

Source	df	SS	MS	F
Week	6	224.21	37.37	5.96**
Rep	11	149.89	13.63	2.17*
Error (a)	66	413.68	6.27	
Entry	2	492.49	246.25	13.09**
Entry x Week	12	611.87	50.99	2.71**
Error (b)	154	2896.09	18.81	
Total	251	4788.24		

**significant at 1% level; *significant at 5% level.
Coefficient of variability = 61.3%.

Table 40. Number of aphids in 3901, 3655 and F₁ in non-preference tests with 12 replications

Week	Entry	Day				Means
		1	2	3	4	
2nd	3901	7.0a*	4.6b	3.6c	3.7b	4.8b
	3655	6.8a	8.7a	9.1a	6.7a	7.8a
	F ₁	9.6a	9.6a	6.3b	5.7ab	7.8a
	Bayes LSD	4.2	3.4	2.4	2.4	2.3
4th	3901	7.4a	5.6a	6.1a	4.7b	6.0a
	3655	11.0a	9.3a	7.4a	7.3a	8.8a
	F ₁	8.4a	9.2a	8.1a	6.7ab	8.2a
	Bayes LSD	--	8.0	6.0	2.2	3.9
5th	3901	8.9ab	6.3ab	3.6a	5.4a	6.1ab
	3655	12.2a	10.5a	5.1a	4.4a	8.1a
	F ₁	6.7b	4.9b	4.4a	3.5a	4.9b
	Bayes LSD	4.6	4.7	--	2.1	2.7
6th	3901	11.8a	9.3a	7.8a	6.3a	8.7a
	3655	11.6a	9.2a	10.1a	5.4a	9.1a
	F ₁	6.6a	10.5a	7.4a	6.4a	7.8a
	Bayes LSD	5.9	--	3.3	--	--

*means followed by same letter are not significantly different at 5% level.

The analyses of variance of this experiment are summarized in Table 41. Significant differences for the Entry component in the 2nd week confirmed the importance of non-preference as a defense mechanism in the early growth stage of the corn plant against the aphid.

Means of the four weeks were combined and analyzed as a single experiment. The mean number of aphids in each week and in the entries are shown in Table 42. The resistant parental line 3901 and the F_1 were significantly less preferred by the aphids than the susceptible parental line 3655. The results indicated dominance of non-preference. Due to a natural infestation of aphids in this experiment, plant ratings on the 6-point scale were taken and shown in Table 43. Ratings for 3901, F_1 and 3655 were 3.3, 4.1 and 4.7, respectively; and these were significantly different from each other. Linear correlation of the ratings and the mean aphid number of the four weeks was 0.48 and significant at the 1% level of probability. Weekly average number of aphids in the three entries showed a trend similar to the previous experiments. In general corn seedlings were least preferred and this non-preference was gradually lost with age.

The combined analysis of variance is summarized in Table 43. Highly significant Week and Entry components were found confirming the results of the earlier analyses.

Experiment 5: 3660, 3655 and bxbx

Means and Bayes LSD values (5% level) for the 2nd to 7th week tests are summarized in Table 44. Based on the means of each week, the resistant parental line 3660 was the least preferred of the three entries in the 2nd, 3rd, 4th and 6th week. However 3660 was significantly less

Table 41. Analyses of variance of non-preference tests of 3901, 3655 and F₁ data in Table 40

Week	Source	df	Mean squares				Means
			Day				
			1	2	3	4	
2nd	Entry	2	29.53	85.03*	90.75**	28.00*	37.72*
	Rep	11	5.66	8.47	12.54	14.67	8.16
	Error	22	16.13	17.06	9.30	7.70	7.74
4th	Entry	2	41.03	53.86	12.44	23.11*	26.76
	Rep	11	4.29	11.72	10.45	10.81	7.52
	Error	22	42.57	37.92	10.63	6.41	14.29
5th	Entry	2	90.57	103.94*	6.38	10.03	30.78
	Rep	11	2.29	12.31	7.91	8.26	5.46
	Error	22	27.80	29.11	6.96	4.73	9.46
6th	Entry	2	102.38	6.73	24.58	3.66	5.62
	Rep	11	0.96	3.67	4.99	10.54	1.66
	Error	22	42.22	26.76	11.75	5.78	8.66

**significant at 1% level; *significant at 5% level.

Table 42. Number of aphids in 3901, 3655 and F₁ in non-preference tests, as mean of each week and mean of each entry over the weeks; and rating at the eighth week

Week	Means of 3 entries	Entry	Means of 4 weeks	Visual Plant Rating**
6	8.5a*	3655	8.4a	4.7a
4	7.6ab	F ₁	7.2b	4.1b
2	6.8bc	3901	6.4b	3.3c
5	6.3c			
Bayes LSD	1.1	Bayes LSD	1.3	0.4

*means followed by same letter are not significantly different at 5% level.

**based on a scale of 1-6, where 1 is very resistant, 6 is very susceptible.

Table 43. Combined analysis of variance of non-preference tests of 3901, 3655 and F₁ data from Table 42

Source	df	SS	MS	F
Week	3	98.46	32.82	5.93**
Rep	11	68.20	6.20	1.12
Error (a)	33	182.62	5.53	
Entry	2	104.85	52.43	5.22**
Entry x Week	6	96.89	16.15	1.61
Error (b)	88	883.45	10.04	
Total	143	1434.47		

**significant at 1% level.

Coefficient of variability = 43.2%.

Table 44. Number of aphids in 3660, 3655 and bxbx in non-preference tests with 12 replications

Week	Entry	Day				Means
		1	2	3	4	
2nd	3660	7.2b*	7.3a	6.9a	6.6a	7.0a
	3655	7.7ab	9.4a	8.4a	7.9a	8.4a
	bxbx	10.5a	8.0a	6.6a	6.7a	8.4a
	Bayes LSD	3.2	4.5	2.8	4.6	2.9
3rd	3660	6.2b	5.8b	5.2b	3.7b	5.2b
	3655	7.7b	6.9b	7.2ab	6.7a	7.2ab
	bxbx	12.0a	10.5a	8.5a	8.1a	9.8a
	Bayes LSD	3.4	3.4	2.8	2.6	2.7
4th	3660	7.6b	7.3b	7.4a	6.5a	7.2b
	3655	9.8ab	9.4ab	8.4a	7.5a	8.8ab
	bxbx	11.8a	11.1a	9.0a	9.1a	10.3a
	Bayes LSD	2.7	3.5	--	2.9	2.3
5th	3660	7.6a	8.2a	7.7a	6.6a	7.5a
	3655	10.1a	7.8a	7.2a	6.1a	7.8a
	bxbx	8.6a	7.9a	6.6a	5.8a	7.3a
	Bayes LSD	--	--	--	--	--
6th	3660	7.7a	6.2a	6.4a	6.9a	6.8a
	3655	9.9a	8.7a	6.3a	5.8a	7.7a
	bxbx	9.4a	8.6a	5.8a	6.7a	7.6a
	Bayes LSD	--	7.6	--	--	--
7th	3660	12.5a	7.7a	5.4a	5.0a	7.7a
	3655	5.7b	5.3b	4.8a	5.3a	5.3b
	bxbx	6.5b	5.3b	5.6a	4.8a	5.6b
	Bayes LSD	3.8	2.4	--	--	1.2

*means followed by same letter are not significantly different at 5% level.

preferred to bxbx only in the 3rd and 4th week. No significant difference was found in the 5th and 6th week. In the 7th week, 3660 was significantly more preferred than 3655 and bxbx.

The analyses of variance of this experiment are summarized in Table 45. Significant differences for the Entry component in the 3rd and 4th week confirmed the results of the earlier analyses.

Means of the six weeks were combined and analyzed as a single experiment. The mean number of aphids in each week and in the entries are shown in Table 46. The entry, bxbx, was significantly more preferred to 3660 but not 3655. The resistant line 3660 was the least preferred but not significantly so from 3655. Weekly average number of aphids in the three entries showed that the 4th week was the most preferred and the 7th week the least preferred by the aphids. In general the preference trend was quite similar to the earlier experiments.

The combined analysis of variance is summarized in Table 47. Highly significant differences were detected in the Week and Entry x Week components, as well as the Entry component. The results here again reflect the complexity of this plant defense mechanism.

4.6 Aphid ratings of 9 x 9 diallel

Nine parents and their 36 F_1 hybrids were scored for aphids under natural epizootic conditions. Aphid ratings based on a scale of 1 - 6, 1 being highly resistant and 6 being extremely susceptible, are summarized in Table 48. Parent, F_1 and grand means were 2.62, 1.66 and 1.85, respectively. Highly significant differences in aphid

Table 45. Analyses of variance of non-preference tests of 3660, 3655 and bxbx data from Table 44

Week	Source	df	Mean squares				
			Day				Means
			1	2	3	4	
2nd	Entry	2	38.03	13.58	11.44	6.69	7.75
	Rep	11	1.72	2.37	2.69	6.11	3.40
	Error	22	13.00	10.25	6.60	5.79	5.29
3rd	Entry	2	110.11**	71.58*	34.03	61.03**	62.86*
	Rep	11	4.72	5.04	4.75	3.30	3.04
	Error	22	17.29	16.55	10.30	10.36	11.07
4th	Entry	2	54.25*	42.36	7.69	20.36	27.46*
	Rep	11	1.64	2.53	1.57	1.36	1.17
	Error	22	10.46	15.48	12.12	9.27	7.03
5th	Entry	2	19.00	0.58	3.58	1.75	0.99
	Rep	11	5.28	2.55	5.61	3.00	3.03
	Error	22	24.58	18.40	9.83	8.84	10.79
6th	Entry	2	15.44	25.08	1.19	3.86	2.88
	Rep	11	1.36	3.48	4.15	1.66	1.21
	Error	22	22.87	20.66	6.80	7.32	8.37
7th	Entry	2	162.02**	24.01	2.08	0.76	19.63**
	Rep	11	5.10	13.46	11.02	11.91	8.13
	Error	22	22.43	7.66	8.61	5.22	2.41

**significant at 1% level; *significant at 5% level.

Table 46. Number of aphids in 3660, 3655 and bxbx in non-preference tests, as mean of each week and mean of each entry over the weeks

Week	Means of 3 entries	Entry	Means of 6 weeks
4	8.8a*	bxbx	8.2a
2	7.9b	3655	7.5ab
5	7.5b	3660	6.9b
6	7.4b		
3	7.4b		
7	6.2c		
Bayes LSD	0.8	Bayes LSD	0.9

*means followed by same letter are not significantly different at 5% level.

Table 47. Combined analysis of variance of non-preference tests of 3660, 3655 and bxbx data from Table 46

Source	df	SS	MS	F
Week	5	127.51	25.50	7.80**
Rep	11	40.19	3.65	1.12
Error (a)	55	179.58	3.27	
Entry	2	55.76	27.88	3.72*
Entry x Week	10	187.40	18.74	2.50**
Error (b)	132	988.93	7.49	
Total	215	1579.37		

**significant at 1% level; *significant at 5% level.
Coefficient of variability = 36.3%.

Table 48. Aphid ratings of 9 parents and their 36 F₁ hybrids*

Parent	Mo17	Oh545	B37	CI21E	AA8	CM111	CM104	Ant2d	CM105	Avg. of Hybrid
Mo17	<u>1.00</u>	1.00	1.00	1.00	1.00	1.00	1.03	1.38	1.38	1.10
Oh545		<u>1.08</u>	1.20	1.00	1.00	1.00	1.00	1.25	1.00	1.06
B37			<u>1.85</u>	1.00	1.25	1.28	1.43	1.45	1.25	1.23
CI21E				<u>2.20</u>	1.48	1.25	1.90	1.43	1.00	1.26
AA8					<u>2.73</u>	2.00	2.88	2.28	1.73	1.70
CM111						<u>2.95</u>	2.38	3.38	2.93	1.90
CM104							<u>3.63</u>	4.38	3.35	2.29
Ant2D								<u>3.65</u>	3.50	2.38
CM105									<u>4.53</u>	2.02

*Grand mean = 1.85, Parental mean = 2.62, and F₁ mean = 1.66

Average % heterotic effect = -37.94 (more resistant)

se of any treatment means = 0.096

se of the difference between two treatment means = 0.136

ratings were found in both parents and the F_1 . The range of the nine parents was from 1.00 to 4.53, and that of the 36 F_1 hybrids was from 1.00 to 4.38.

Three parental lines, Mol7, Oh545 and B37, showed some degree of resistance to the aphids (Table 48). The best resistance was shown by Oh545 and Mol7. The yellow inbred dent, Oh545, was developed cooperatively by the Ohio Agricultural Research and Development Center and the United States Department of Agriculture, and was released to corn breeders in 1970. It is derived from ((M14 x CI187-2)Oh45 x Oh45) x ((Oh45T4 x Cash) x (M14 x CI187-2)Oh45 x Oh45) x Oh45A. Mol7, a very popular inbred, came out of CI187-2 x C103, and was released by the University of Missouri. B37, another much used inbred, is derived from the Iowa-Stiff Stalk Synthetic by the Iowa State University.

Five parents, AA8, CM111, CM104, Ant2D and CM105, were susceptible to the aphid. The most susceptible, CM105, was bred in India by prolonged sib-pollination from a Peruvian variety, Peru 330. CM104, a yellow Colombian flint, was also bred in India. Antiqua 2D, another notable susceptible, came out of the Coastal Tropical flint. AA8, a Hawaiian sweet inbred, and CM111, an Indian release derived from a Cuban yellow flint were moderately susceptible. CI21E, a USDA release derived from K577c x (Hy)², was intermediate in resistance. The ranking of the hybrids followed closely that of the more resistant parent. However, susceptible x susceptible gave susceptible hybrids. Heterotic effects in per cent were calculated from the hybrids over their respective mid-parent values and averaged out to -37.94% (Table 48).

The negative effect indicated that the hybrids were more aphid resistant than the parents.

The results of the randomized block analysis of variance for parental and hybrid aphid ratings are summarized in Table 49, based on Griffing's method 2 and model I (Griffing, 1956). Highly significant differences were detected among the genotypes for aphid ratings, a necessary condition for computation of the combining ability analysis, summarized in Table 50. GCA and SCA mean squares were both highly significant. From a variance component analysis of the aphid ratings, GCA and SCA variance components accounted for 42.5% and 56.3%, respectively (Table 51). Error variance was extremely small, accounting for 1.2% of the total variance, indicating that the replications were adequate.

Using model II (random model), the GCA and SCA mean squares were also highly significant (Table 52). The assumption in using this model is that we are dealing with random samples from a population and inferences can only be made about the parameters in the general population and not in the individual samples.

Estimations of the total phenotypic variance (V_P), total genotypic variance (V_G), additive genetic variance (V_A), non-additive genetic variance (V_D), and environmental variance (V_E), as well as the broad (bh^2) and narrow (nh^2) sense heritabilities are summarized in Table 53. The genotypic variation of 72.1% accounted for the major portion of the total variation. The additive and non-additive genetic variance were 44.4% and 27.7%, respectively, of the total variation.

Table 49. Analysis of variance of aphid ratings in Table 48 based on Griffing's method 2, fixed model I

Source	df	SS	MS	F
Genotype	44	1840.048	41.819	111.816**
Replication	3	8.911	2.970	7.941**
Genotype x Rep	132	456.539	3.459	
Error	1620	606.600	0.374	

**significant at 1% level.

Table 50. Combining ability analysis of variance based on method 2, model I

Source	df	SS	MS	F
GCA	8	29.627	3.703	411.444**
SCA	36	16.374	0.455	50.556**
Error	1620	15.065	0.009	

**significant at 1% level.

Table 51. Variance component analysis in per cent for GCA and SCA values in Table 50

Component	Variance	Per cent
GCA	0.336	42.5
SCA	0.445	56.3
Error	0.009	1.2

Table 52. Combining ability analysis of variance based on method 2, model II

Source	df	SS	MS	F
GCA	8	29.627	3.703	8.138**
SCA	36	16.374	0.455	5.291**
Error	132	11.413	0.086	

**significant at 1% level.

Table 53. Estimates of phenotypic variance and its components, and of broad (bh^2) and narrow (nh^2) sense heritabilities for aphid ratings

			Per cent
Phenotypic	(V_P)	1.331 ± 0.354	100.00
Genotypic	(V_G)	0.959 ± 0.354	(72.05)
Additive	(V_A)	0.591 ± 0.337	44.37
Non-additive	(V_D)	0.368 ± 0.108	27.68
Error	(V_E)	0.372 ± 0.013	27.95
	bh^2	72.1 (61.9 - 77.9)	
	nh^2	44.4 (25.9 - 55.1)	

Table 54. Estimates of general combining ability effects for aphid ratings based on Griffing's method 2, model I

Parent	Parental Mean	GCA Effects
Mol7	1.00	-0.70
Oh545	1.08	-0.72
B37	1.85	-0.45
CI21E	2.20	-0.37
AA8	2.73	0.05
CM111	2.95	0.23
CM104	3.63	0.64
Ant2D	3.65	0.71
CM105	4.53	0.61
pas		0.04

Broad and narrow sense heritabilities were calculated as:

$bh^2 = V_G/V_P$; $nh^2 = V_A/V_P$. The broad sense heritability was 72.1% and the narrow sense heritability was 44.4%. These high estimates suggest that the inheritance of aphid resistance in corn is controlled by major genes.

General combining ability effects for the nine parents are summarized in Table 54, using the fixed model. The GCA effects differed widely among the parents. Four lines, Oh545, Mo17, B37 and CI21E, had high negative GCA effects, indicating that these lines were good general combiners for increase in aphid resistance in hybrid combinations. The other five lines, in increasing order, AA8, CM111, CM105, CM104 and Ant2D, had significant positive GCA effects, indicating that these five parents were poor general combiners for resistance to the aphid and, in fact, contributed to susceptibility in hybrid combinations as is shown in Table 48.

The coefficient of linear determination (R^2) between the parental average and their GCA effects based on method 2 for aphid ratings was extremely high at 0.92 and subsequently, highly significant.

The specific combining ability effects, based on method 2, model I, for aphid ratings are summarized in Table 55. SCA effects represent those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved. The hybrid, CI21E x CM105, showed the highest negative SCA effects, indicating that this combination made the most use of non-additive genes for aphid resistance. At the other end of the scale, CM104 x Ant2D showed the highest positive SCA effects

Table 55. Estimates of specific combining ability effects for aphid ratings based on Griffing's method 2, model I

Parent	Mol7	Oh545	B37	CI21E	AA8	CM111	CM104	Ant2D	CM105
Mol7		0.57	0.30	0.22	-0.20	-0.38	-0.77	-0.48	-0.38
Oh545			0.52	0.24	-0.18	-0.36	-0.77	-0.59	-0.74
B37				-0.03	-0.20	-0.36	-0.62	-0.66	-0.76
CI21E					-0.06	-0.47	-0.22	-0.77	-1.09
AA8						-0.14	0.33	-0.34	-0.78
CM111							-0.35	0.58	0.23
CM104								1.17	0.25
Ant2D									0.33
CM105									

se_d between effects of two crosses having one parent line in common = 0.130

se_d between effects of two crosses having no parent line in common = 0.123

indicating that this combination had the least non-additive genes for aphid resistance. Other hybrid combination with high negative SCA effects were AA8 x CM105, B37 x CM105, Mol7 x CM104, Oh545 x CM104, OH545 x CM105 and Oh545 x Ant2D.

4.7 Aphid ratings of 8 x 8 diallel

Eight parents and their 28 F₁ hybrids were scored for aphids under epizootic conditions created in the greenhouse. Aphid ratings based on a scale of 1 - 6, 1 being highly resistant and 6 being extremely susceptible, are summarized in Table 56. Parents, F₁ and grand means were 2.83, 3.21 and 3.13, respectively. Highly significant differences in aphid ratings were found in both parents and the F₁. The range of the eight parents was from 1.42 to 4.04, and that of the 28 F₁ hybrids was from 1.00 to 4.21.

Four parental lines Oh545, Mol7, 3660 and AA25 showed good resistance to aphids (Table 56) and confirmed the 1973 field experiment of the high resistance of Oh545 and Mol7. 3660, a shrunken-2 super-sweet line derived from a segregating Hawaiian population designated AA8sh2, and AA25, a University of Hawaii version of B14, were additional sources of resistance.

The other four lines CM111, B37, CM105 and Ant2D were susceptible to the aphid. Their pedigrees have been discussed earlier (See 9 x 9 diallel). Most of the F₁ hybrids had aphid ratings biased towards the higher parent. Two exceptions were Oh545 x Mol7 and Oh545 x B37 where the ratings were lower than the lower parent. Heterotic effects in per cent were calculated from the hybrids over their respective

Table 56. Aphid ratings of 8 parents and their 28 F₁ hybrids*

Parent	Oh545	Mo17	3660	AA25	Ant2D	CM105	B37	CM111	Avg. of Hybrid
Oh545	<u>1.42</u>	1.25	1.79	2.42	3.04	2.88	1.00	3.54	2.27
Mo17		<u>1.88</u>	2.04	3.04	3.58	3.00	3.54	4.21	2.95
3660			<u>2.00</u>	2.54	3.08	3.50	3.46	3.54	2.85
AA25				<u>2.08</u>	3.21	3.33	4.00	4.00	3.22
Ant2D					<u>3.58</u>	4.04	3.75	4.00	3.53
CM105						<u>3.67</u>	4.13	3.96	3.55
B37							<u>3.96</u>	4.00	3.41
CM111								<u>4.04</u>	3.89

*Grand mean = 3.13, Parental mean = 2.83 and F₁ mean = 3.21

Average % heterotic effect = 13.96 (more susceptible)

se of any treatment means = 0.113

se of the difference between two treatment means = 0.160

mid-parent value and average out to 13.96% (Table 56), indicating that the F_1 's were more susceptible than the parents.

The results of the randomized block analysis of variance for parental and hybrid aphid ratings are summarized in Table 57, based on Griffing's method 2 and model I (Griffing, 1956). Highly significant differences were detected among genotypes for aphid ratings, a necessary condition for the computation of the combining ability analysis summarized in Table 58. GCA and SCA mean squares were both highly significant. From a variance component analysis of the aphid ratings, the GCA and SCA variance components accounted for 54.2% and 43.5%, respectively (Table 59). Error variance was very small, accounting for only 2.3% of the total variance, indicating that the replications were adequate and the results are reliable.

Using model II (random model), the GCA and SCA mean squares were also highly significant (Table 60). The assumption in using this model is that we are dealing with random samples from a population and inferences can be made only about the parameters in the general population and not in the individual samples.

Estimation of the total phenotypic variance (V_p), total genotypic variance (V_G), additive genetic variance (V_A), non-additive genetic variance (V_D) and environmental variance (V_E), as well as broad (bh^2) and narrow (nh^2) sense heritabilities are summarized in Table 61. The genotypic variation of 83.9% accounted for the major portion of the total variation. Additive and non-additive genetic variances were 58.9% and 25.0%, respectively, of the total variation. The broad sense heritability was 84.0% and the narrow sense heritability was 59.0%.

Table 57. Analysis of variance of aphid ratings in Table 56 based on Griffing's method 2, fixed model I

Source	df	SS	MS	F
Genotype	35	347.792	9.937	64.526**
Replication	2	0.691	0.346	2.247
Genotype x Rep	70	17.267	0.247	
Error	324	50.000	0.154	

**significant at 1% level.
ns non-significant.

Table 58. Combining ability analysis of variance based on method 2, model I

Source	df	SS	MS	F
GCA	7	21.677	3.097	238.231**
SCA	28	7.302	0.261	20.077**
Error	324	4.167	0.013	

**significant at 1% level.

Table 59. Variance component analysis in per cent for GCA and SCA values in Table 58

Component	Variance	Per cent
GCA	0.308	54.2
SCA	0.248	43.5
Error	0.013	2.3

Table 60. Combining ability analysis of variance based on method 2, model II

Source	df	SS	MS	F
GCA	7	21.677	3.097	11.866**
SCA	28	7.302	0.261	12.429**
Error	70	1.439	0.021	

**significant at 1% level.

Table 61. Estimates of phenotypic variance and its components, and of broad (bh^2) and narrow (nh^2) sense heritabilities for aphid ratings

			Per cent
Phenotypic	(VP)	0.962 ± 0.339	100.00
Genotypic	(VG)	0.807 ± 0.339	(83.96)
Additive	(VA)	0.567 ± 0.331	58.98
Non-additive	(VD)	0.240 ± 0.070	24.98
Error	(VE)	0.154 ± 0.012	16.04
	bh^2	84.0 (75.3 - 88.1)	
	nh^2	59.0 (37.9 - 69.1)	

Table 62. Estimates of general combining ability effects for aphid ratings based on Griffing's method 2, model I

Parent	Parental Mean	GCA Effects
Oh545	1.42	-0.94
Mol7	1.88	-0.37
3660	2.00	-0.42
AA25	2.08	-0.14
Ant2D	3.58	0.37
CM105	3.67	0.40
B37	3.96	0.37
CM111	4.04	0.72
$s_e d$		0.05

These high estimates suggest aphid resistance in corn is highly genetic. Usually when heritability is high major genes are suggested.

General combining ability effects for the eight parents are summarized in Table 62, using the fixed model. GCA effects differed widely among the parents. Three lines Oh545, Mol7 and 3660 had high negative GCA effects indicating that these lines were good general combiners for increase in aphid resistance in hybrid combinations. AA25 had a low negative GCA effect and therefore is not considered a good general combiner for aphid resistance. The remaining four lines, in increasing order, Ant2D, B37, CM105 and CM111, had significant positive GCA effects, indicating that these lines were poor general combiners for resistance to the aphid and in fact, contributed to increase in susceptibility when in hybrid combinations, as was shown in Table 56.

The coefficient of linear determination (R^2) between parental average and their GCA effects based on method 2 for aphid ratings was 0.92. This is extremely high and therefore highly significant.

The specific combining ability effects based on method 2, model I, for aphid ratings are summarized in Table 63. SCA effects represent those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved. Oh545 x B37 showed the highest negative SCA effects, indicating that this combination possessed a large number of non-additive genes for aphid resistance. Other resistant combinations were Oh545 x Mol7 and Mol7 x 3660. At the other extreme, AA25 x B37 had the highest positive SCA effects indicating that this combination

Table 63. Estimates of specific combining ability effects for aphid ratings based on Griffing's method 2, model I

Parent	Oh545	Mo17	3660	AA25	Ant2D	CM105	B37	CM111
Oh545		-0.57	0.02	0.37	0.48	0.28	-1.55	0.63
Mo17			-0.30	0.43	0.45	-0.16	0.42	0.73
3660				-0.02	0.00	0.39	0.38	0.11
AA25					-0.15	-0.05	0.65	0.30
Ant2D						0.14	-0.12	-0.22
CM105							0.23	-0.29
B37								-0.21
CM111								

se_d between effects of two crosses having one parent line in common = 0.152

se_d between effects of two crosses having no parent line in common = 0.143

had very few non-additive genes for aphid resistance. Oh545 x Ant2D also had high positive SCA effects.

4.8 Gas-liquid chromatography results for the determination of 2(3)-benzoxazolinones in plants

A typical gas chromatogram of the CH_2Cl_2 extract of macerated corn seedling is shown in Figure 17. Mass spectra obtained from peaks 1 and 2 are identical to those obtained from the standard BOA and MBOA, respectively (Figure 18). The mass spectrum of peak 3 had a molecular ion and base peak at m/e 195 and a characteristic peak at 180 (P- CH_3 , I% = 82%), corresponding to the reported values for dimethoxy-BOA (Klun et al., 1970). Differences of 30 mass units ($\text{OCH}_3\text{-H}$) were observed for the molecular ions of peaks 1, 2 and 2,3, supporting the evidence that they are in fact analogs with different numbers of methoxy substitutions.

Preliminary examinations of several benzoxazolinone-containing plants which included Coix, wheat and rye demonstrated the presence of BOA and MBOA in all of them. Sorghum was also assayed but no benzoxazolinones were detected.

The results of the determination of BOA and MBOA in one-week old etiolated corn seedlings are shown in Table 64. Dimethoxy-BOA was also detected and could be quantitatively determined if a standard was available. BOA concentration was determined for CI21E and CM105, and the values were 0.36 and 0.22 mg/g fresh weight, respectively. MBOA concentration for CI21E and CM105 were 2.61 and 2.55 mg/g fresh weight, respectively. As compared to that of BOA, MBOA concentration was about nine times as much. This confirmed a similar relationship reported previously (Klun and Robinson, 1969). BOA is therefore con-

Figure 17. A typical gas chromatogram of 2(3)-benzoxazolinones from corn seedlings. Peaks 1, 2 and 3 are BOA, MBOA and dimethoxy-BOA, respectively. Column temperature is 190°, and other conditions are described in Section 3.8.

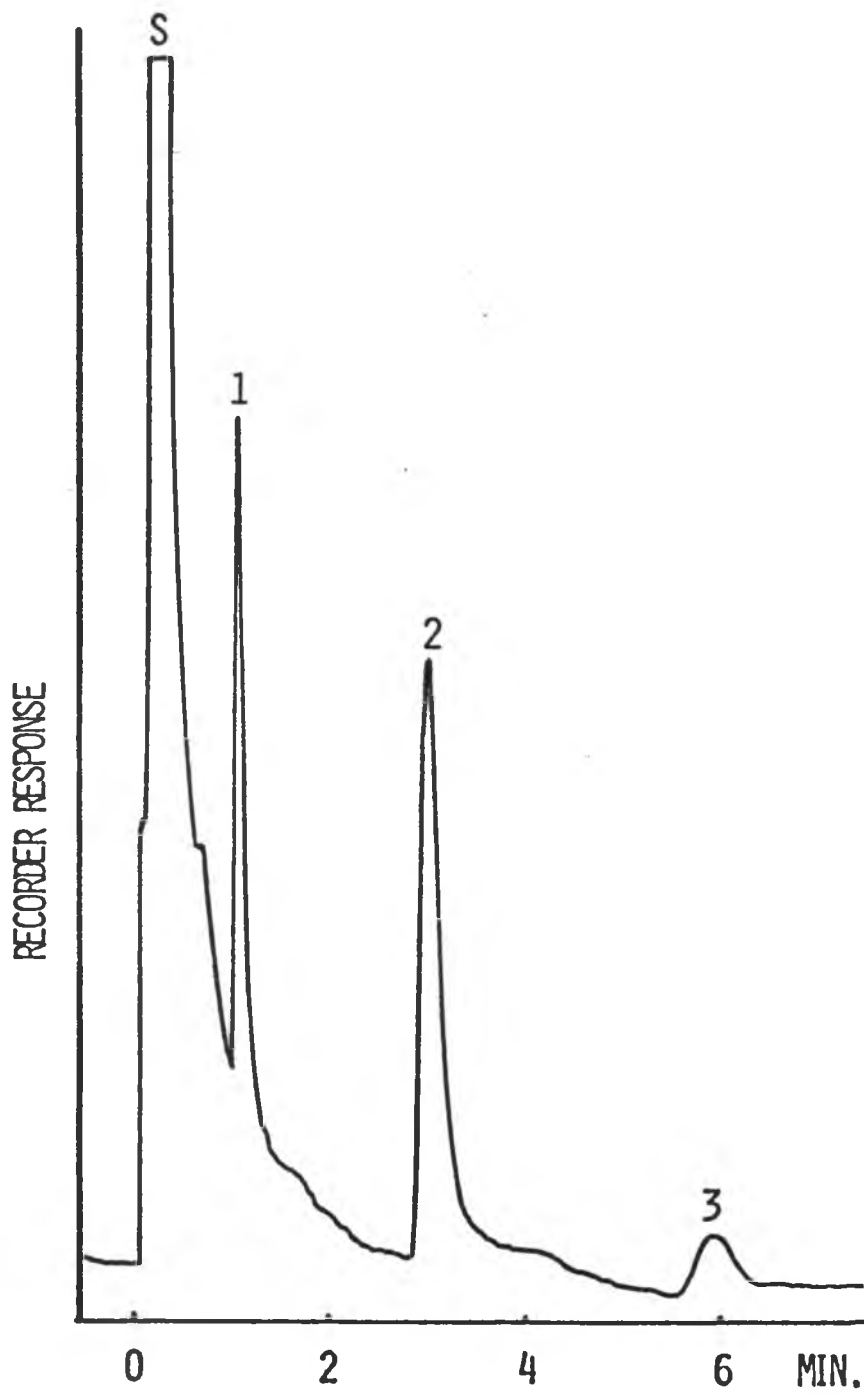


Figure 18. Mass spectra of BOA, MBOA and dimethoxy-BOA obtained from peaks 1, 2 and 3 in Figure 17 by GC-MS.

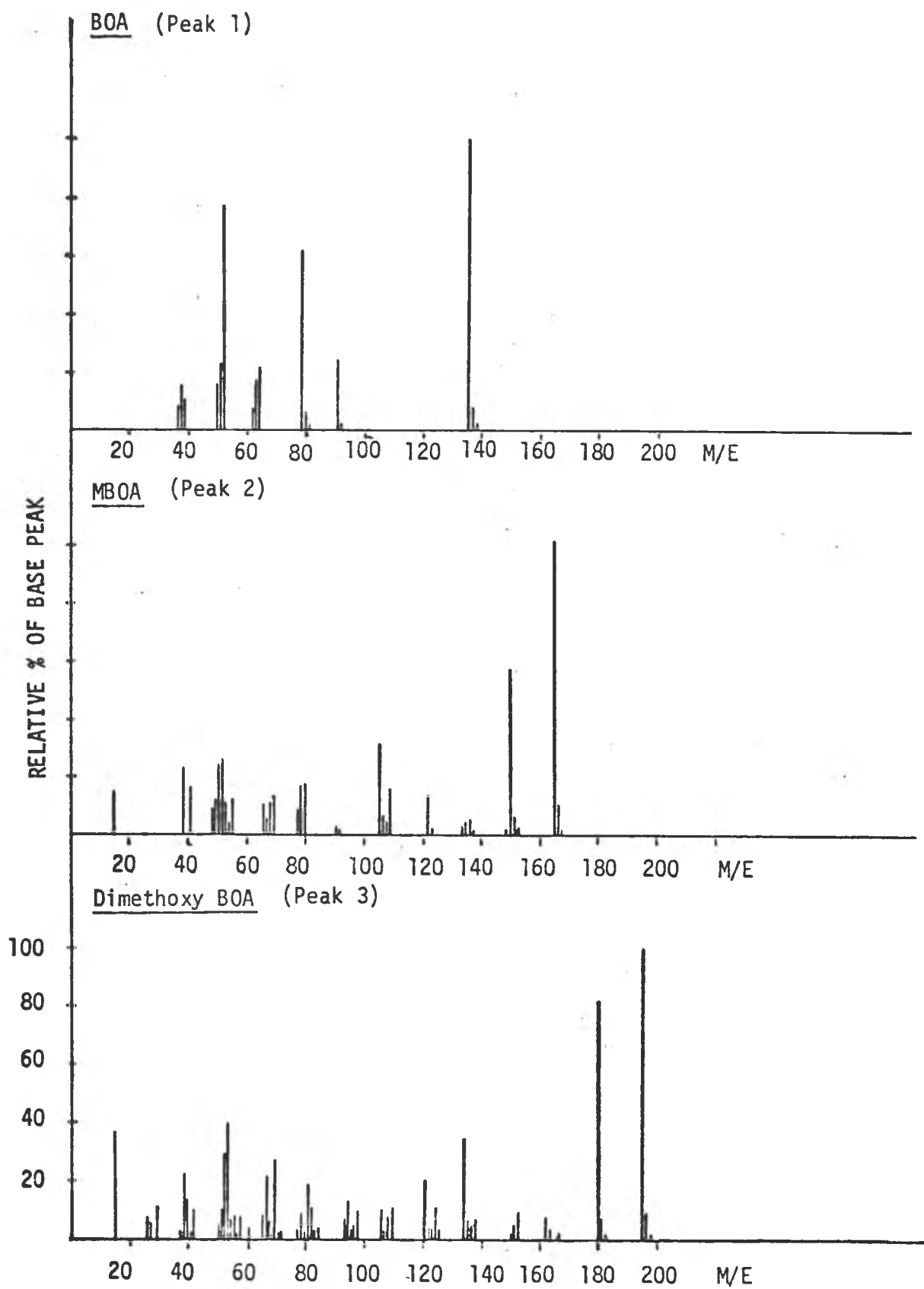


Table 64. BOA and MBOA concentrations in shoots of etiolated corn seedlings

Entry	BOA	MBOA
	mg/g fresh wt.	
CI21E	0.36	2.61
CM105	0.22	2.55
3660	*	0.95
3660 x CM105	*	2.82
bxbx	*	0.08

*no determination made.

Table 65. MBOA distribution in two etiolated corn seedlings

Inbred	Shoot	Root	Remainder of	Remainder of
			Embryo	Endosperm
mg/g fresh wt.				
CI31A	2.39	1.93	1.28	0.25
WF9	2.31	2.07	0.97	0.13

Table 66. MBOA concentration of some teosinte and Tripsacum seedlings

Teosinte	<u>Tripsacum</u>	
	mg/g fresh wt.	mg/g fresh wt.
Chalco	1.65	<u>T. dactyloides</u> (2N) 1.29
Balsas	1.00	<u>T. dactyloides</u> (4N) 1.37
Jutiapa	1.76	<u>T. floridanum</u> 2.57
		<u>T. laxum</u> * 1.23

*from mature leaves.

sidered a relatively minor component in corn and apparently does not play a significant part in the resistance to the European corn borer. It is now generally regarded as an unimportant plant resistance factor and little attention is paid to it.

The aphid resistant line 3660 had a low MBOA concentration of 0.95 mg/g fresh weight as compared to CM105. The cross 3660 x CM105 had a concentration of 2.82 mg/g fresh weight which was higher than the high parent, CM105. For the bxbx corn mutant, an extremely low concentration of 0.08 mg/g fresh weight was found, demonstrating the sensitivity of this technique. The accuracy of this procedure was shown by the fact that the standard deviation for three GLC determinations did not exceed 0.04.

MBOA distribution in one-week old etiolated corn seedling of CI31A and WF9 were examined and the results summarized in Table 65. One-week old tissues from the shoot, root and remainder of the embryo and endosperm were assayed. The concentration was highest in the shoot and lowest in the remainder of the endosperm. The root and the remainder of the embryo had the second and third highest concentration, respectively.

Teosinte, Zea mexicana (Schradler) Kuntze and Tripsacum, long considered relatives of corn, were also assayed for benzoxazolinones to determine if this group of unique compounds was present. MBOA and dimethoxy-BOA were detected in the teosinte races from Balsas, Chalco and Jutiapa. No BOA was detected. MBOA was found in Tripsacum dactyloides (2N), T. dactyloides (4N), T. floridanum and T. laxum. A closer examination of T. dactyloides (2N) showed that all the three

analogs, BOA, MBOA and dimethoxy-BOA were present. The results of the determination for MBOA concentrations in teosinte and Tripsacum are summarized in Table 66. Except in one case, only 0.1 to 0.2 gm fresh weight of shoots of 14 to 20-day old seedling were used. The mature leaves of T. laxum were assayed as seedlings were not available.

5. DISCUSSION AND CONCLUSION

Genetics and sources of resistance

Severe aphid infestation of a series of AA8sh2 stock in 1972 at the Waimanalo Experiment Station, University of Hawaii, first provided evidence of genetic differences for resistance to the corn leaf aphid, Rhopalosiphum maidis (Fitch). Initial observations of a series of paired crosses and selfs derived from this population indicated that the resistance was genetic in nature (Table 4). However conflicting observations, although occurring in the minority of the cases, prevented any definite conclusion to be reached. The deviant result is attributed to initial misclassification of the parental reaction and non-uniformity of the aphid infestation in the field. The sporadic nature of infestation, predators and parasites of the aphid and other insufficiently understood environmental factors affecting the aphid population reduce the reliability of field tests.

In order to provide a more uniform level of infestation and a greater control of the aphid predators and parasites of Hawaii, most of the experiments were conducted in greenhouses at the University of Hawaii. Homozygous resistant and susceptible lines were obtained from the AA8sh2 population based on the results of the field experiment. Crosses based on two of these lines were critically evaluated in the greenhouse to study the genetics of resistance. Plant ratings based on the two parental lines the F₁, the F₂ and the two backcrosses indicated that the genetics of resistance to the aphid was monogenic and recessive (Table 5). The gene symbol aph has been designated as the

aphid resistant gene (Brewbaker and Chang, 1974). In accordance with this symbol, the genotype for the resistant line would be aph/aph and that for the susceptible, Aph/Aph (Chang and Brewbaker, 1976). Work on the inheritance of resistance to this aphid is scarce and previous reports indicated that susceptibility could be dominant (WF9) or recessive (38-11, R4), depending on the inbred used (Huber and Stringfield, 1940; Snelling et al., 1940; Walter and Brunson, 1940). From the few studies made, the genetics of resistance appeared to be complex (Painter, 1951).

One rather intriguing question is the source of this resistance. The origin of the resistance is as yet obscure, occurring among Rp (rust), Ht (blight) and sh2 (supersweet) conversions of the U. H. sweet corn inbred, AA8. AA8 was derived from Hawaiian Sugar, first bred by Dr. Alfred Mangelsdorf of the Hawaiian Sugar Planters' Association. The Illinois line R839sh2 was the source of the supersweet gene. AA8sh2 was then in turn converted to Rp_1^d by crossing with (W22 x B14A). While nothing is known about the resistance of R839sh2 and Hawaiian Sugar, the inbred, W22, had been reported to be aphid resistant (Everly, 1965) and is probably the most likely source of the 3660 resistance. B14A, a popular inbred, is also a possible source as the U. H. version, AA25, was found to be moderately resistant in the 8 x 8 diallel experiment. Routine screening of these four stocks may help resolve this question.

Plant resistance mechanisms

Plant resistant mechanisms to insects can be broken down into antibiosis, non-preference and tolerance. Painter (1958) reported the

presence of non-preference, and antibiosis in the corn inbreds, K148, K150 and WF9, against the corn leaf aphid. However no details were given as to how they were measured.

Four experiments were set up to investigate antibiosis as reflected by the weight of the aphids raised on appropriate test materials. 3660, CM105 and the F_1 were evaluated in Experiment 1 and antibiosis was detected in the resistant line 3660 (derived from AA8sh2), based on the mean aphid weight of the 3rd to 8th week tests (Table 6). The results indicated that the effect was recessive. Correlation of the plant ratings with the mean weights was significant at the 5% level, showing that antibiosis is a major resistance component. In Experiment 2, 3660, 3655 and the F_1 were evaluated and antibiosis was again detected in 3660, confirming the consistency of the recessive nature of the resistance (Figure 9). Significantly lower aphid weights were found in the resistant parental line 3660, as compared to the susceptible parental line 3655, and the F_1 in all weeks except the 7th (Table 10). Plant ratings and the mean aphid weights gave a highly significant correlation of 0.71, indicating that aphid weights obtained in this manner was a good criterion to measure aphid resistance.

In Experiment 3, Mol7, 3655 and the F_1 were evaluated and the antibiotic effect was again seen. In this case, Mol7 and the F_1 had lower aphid weights as compared to the susceptible line 3655 in all weeks but significant difference was not detected in the 5th and 6th week due possibly to high experimental errors (Table 14). Antibiosis here was clearly dominant, in contrast to the recessive resistance in 3660 (Figure 12). A dominant resistance gene is much more attractive

than a recessive one especially in hybrid corn production since only one of the inbred parents need be converted to this gene. Unfortunately no natural infestation of the aphid occurred at the end of this experiment. No direct comparison of the antibiosis results with field infestation was therefore possible.

The weekly mean aphid weight of the three experiments appeared to follow a similar trend. In general, corn plants in the 2nd week have the highest antibiosis and this effect is gradually lost with age (Tables 8, 12, 16; Figures 7, 10, 13). The findings here explained in a more precise term the observations of Viale (1950) and Painter (1958) that aphids could not be maintained in corn seedlings. Greenhouse studies of the resistance to the corn leaf aphid were therefore severely restricted and as a result little is known about the details of the resistance. The rate of loss of this antibiotic effect differentiates resistant plants from susceptible ones (Figures 6, 8, 11). Plants are generally the most vulnerable to the aphid from the 4th to 6th week. After this stage, there appears to be an increase in antibiosis. Physiological maturity of lower leaves is reached at this stage, and shortly afterwards, greenhouse-grown plants begin to senesce, becoming less palatable to the aphid. Observations indicate that the rate of senescence is influenced by the degree of aphid infestation. Plants can also be brought into early senescence by early and heavy infestation. This apparent increase in resistance due to the plants' decline cannot be strictly considered as antibiosis and therefore has no value as an indicator of resistance. From the standpoint of aphid control in corn without genetic resistance,

insecticides should therefore be applied prior to the 4th week, the most vulnerable growth stage.

Experiment 4 was set up to compare the resistant lines 3660 and 3901 with the susceptible lines 3652 and bxbx. The corn genotype, bxbx, lacks DIMBOA (a precursor of the benzoxazolinone, MBOA) as compared to normal corn, and has been observed to be very susceptible to the aphid (Day, 1974). Results of the antibiotic tests showed that generally the resistant lines 3660 and 3901 had lower aphid weights than the susceptible lines (Table 18). Mean aphid weights for the six weeks showed that 3901 was more resistant and had significantly lower weights than 3660. Since they have presumably the same genotype with respect to aphid resistance, i.e. aph/aph, the difference may be the result of minor genes acting to increase the overall resistance in 3901. The correlation of the plant ratings and the mean aphid weights was 0.42 and highly significant, indicating that antibiosis based on aphid weight was a good parameter for measuring aphid resistance.

Of special interest is the high aphid weight obtained for bxbx in the 2nd week as compared to the others; although not significantly different from 3652. DIMBOA, which is lacking in the bxbx genotype, has been generally regarded as an insect and disease resistant factor in corn. DIMBOA concentration is highest in corn seedlings and decrease with plant age (Klun and Robinson, 1969). There seems to be a strikingly parallel relationship of the decreasing antibiotic effect obtained in the previous experiments and the decreasing DIMBOA concentration with plant age. Lacking DIMBOA, the bxbx may have become especially susceptible to the aphid at the seedling stage. Recently,

Long et al. (1976) reported that the inbred corn lines with high concentrations of DIMBOA generally have improved resistance to the aphid, thus supporting this contention. Since aphid susceptible lines like 3652 and 3655 have the BxBx genotype (i.e. containing DIMBOA at varying concentrations), it becomes evident that DIMBOA may serve a supplementary role in the plant's defense against the aphid, especially at the seedling stage. The increase in aphid weight from the 2nd - 4th week for the bxbx line further suggests that other factors may be responsible for aphid resistance. A report that may be relevant is the discovery of exotic corn lines with low DIMBOA content, resistant to the first and second brood European corn borer (Sullivan et al., 1974).

An exceptionally heavy infestation of aphids at the end of Experiment 4 killed the two susceptible lines 3652 and bxbx. In contrast, the two resistant lines 3660 and 3901 appeared to be undamaged in spite of the large number of aphids found on them. In addition to the antibiotic effects, the resistant lines, especially 3660, thus showed a high degree of tolerance (Figure 15).

Antibiosis was also tested by evaluating the plant's effect on the reproductive biology of the aphid. Strong antibiosis was expressed by the resistant line 3660 by increasing the number of days it takes for the first instar aphid to commence reproduction. In addition, the total number of nymphs produced and the rate of reproduction of aphids raised on the resistant line 3660 were significantly reduced as compared to the susceptible lines CM105 and 3655, as well as the 3660 x CM105 and 3660 x 3655 F₁ hybrids (Tables 22, 24). The recessiveness of

these antibiotic effects are in agreement with that based on aphid weight and also the results of the inheritance study.

Strong antibiosis was similarly shown by the resistant parental line Mol7 and the F_1 as compared to the susceptible parental line 3655 on all the five reproductive parameters studied. They act to increase the number of days it takes for the first instar aphid to commence reproduction, and decrease aphid longevity, days reproductive, the number of nymphs produced and the rate of reproduction (Table 26). The dominance of antibiosis in this experiment is in agreement with the results obtained based on aphid weight.

Non-preference was measured by providing three choices to 30 apterous aphids. Five experiments were conducted to study this component of resistance. This turned out to be a difficult parameter to evaluate as there was considerable variability even with 12 replications. Significant non-preference was shown by the resistant line 3660 for the 2nd and 3rd week in some cases (Tables 28, 32, 44). For the resistant line 3901 significant non-preference was found in the 2nd and 5th week (Table 40). However the inheritance of non-preference was not very clear. In some weeks, non-preference was dominant and in others, recessive. The mechanism of non-preference does not appear to be simple, and variability inherent in the aphids together with environmental variation undoubtedly complicate the situation. Non-preference in Mol7 was shown in the 2nd week as a dominant effect (Table 36). The bxbx line was found to be the most preferred in the 3rd and 4th week in comparison with 3660 and 3655 (Table 44). The bxbx genotype lacking DIMBOA may have become more attractive to the aphid

even in comparison to the aphid susceptible line 3655. Non-preference was not detected in all five experiments after the 4th or 5th week. Similar findings by Rhodes and Luckmann (1967) showed the loss of non-preference resistance in corn from the 4th to 7th week.

In general, the non-preference experiments though not as clear-cut as the antibiosis tests revealed that this component of resistance is present only in the early stages of plant growth.

Suggested procedure for breeding aphid resistant corn

For the incorporation of the monogenic recessive gene, the conventional backcross method is recommended. The critical stage is in the identification of the resistant segregants (aph/aph) which occur in about 25% of the population in the F₂ generation. Inbred lines under conversion therefore need only be screened every selfed generation. Plants of the segregating generation are preferably screened by massive infestation of aphids in an insect-proof greenhouse. It is essential to keep out the predators and parasites of the aphid to ensure a heavy aphid population. Of particular concern are the two parasitic wasps, Aphelinus maidis Timberlake and Lysiphlebus testaceipes (Cresson), that parasitize the aphids.

If an insect-proof greenhouse is not available, plants may be screened from the 2nd - 4th week by the leaf cage technique. About 12 plants from the segregating generation can be screened and theoretically, three would be resistant. A resistant and susceptible check should also be tested at the same time. The test plants supporting aphids with low weights would be aph/aph plants.

Diallel crosses

While the inheritance of resistance to the aphid is monogenic and recessive in the AA8sh2 population, relatively little is known about the genetics of resistance in other corn stocks.

The 9 x 9 diallel (field)

Highly significant differences in aphid ratings in the field were found on the nine different inbreds and their F_1 hybrids. Mo17 and Oh545 were found to be the best sources of resistance. CM105, Ant2D and CM104 were some of the outstanding susceptibles (Table 48). The aphid ratings of the hybrids followed closely that of the more resistant parent but susceptible x susceptible combinations gave susceptible reactions.

In the combining ability analysis of variance, GCA mean square was about eight times as large as the SCA mean square, indicating that the additive gene action was more important than the non-additive gene action in the inheritance of resistance to the aphid (Table 50). The high genotypic variance obtained for aphid resistance in this study suggests that resistance is highly heritable and can be improved effectively by simple recurrent selection or mass selection. The high estimate of the coefficient of linear determination (R^2) between the parental aphid rating averages and their GCA effects suggests that selection of parental lines for resistance will effectively utilize their GCA values (Table 54).

Specific combining ability effects appear to be important in aphid resistance. CI21E had good SCA effects in combination with CM105 and

Ant2D as shown by their high negative values. Similarly, AA8 combines well with CM105 for increased resistance. CI21E and AA8, with relatively poor GCA, should be tested more thoroughly in order that maximum use of their superior SCA can be made (Table 55).

Approximately 72% of the total phenotypic variance for aphid resistance was due to genetic causes, expressed as a high broad sense heritability. Narrow sense heritability was about 44%, indicating that a substantial portion of the genetic variance is additive. Non-additive variance accounted for about 28% of the total genotype variance (Table 53).

The 8 x 8 diallel (greenhouse)

Highly significant differences in aphid ratings were found in the eight different inbreds and their F_1 hybrids. Oh545, Mo17 and 3660 were found to be the best sources of resistance. Ant2D, CM105, B37 and CM111 were the susceptible lines used. AA25, although not susceptible, was not considered a resistant line (Table 56). The aphid ratings of the hybrids were biased towards the more susceptible parent in most cases. Two exceptions were Oh545 x Mo17 and Oh545 x B37, where the ratings were similar to or were lower than that of the more resistant parent.

In the combining ability analysis of variance, the GCA mean square was about 12 times as large as the SCA mean square. This meant that the additive gene action was more important than the non-additive gene action in the inheritance of resistance to the aphid (Table 58). The high genotypic variance observed suggests that resistance is highly

heritable and can be improved effectively by simple recurrent selection or mass selection. The high estimate of the coefficient of linear determination (R^2) between the parental aphid rating averages and their GCA effects suggests that selection of parental lines for resistance will effectively utilize their GCA values (Table 62).

Specific combining ability effects appear to be important in aphid resistance. Oh545 had good SCA effects when combined with B37 and Mol7. B37, with relatively poor GCA effects, should be tested more thoroughly with other combinations in order to make a greater use of its SCA effects. Mol7 had good SCA effects with 3660. AA25 x B37 gave the highest positive SCA effects and therefore had the least number of non-additive genes for aphid resistance (Table 63).

Approximately 84% of the total phenotypic variance for aphid resistance was due to genetic causes, expressed as a high broad sense heritability. The narrow sense heritability was about 59%, implying that a good portion of the genetic variance is additive. Non-additive genetic variance accounted for about 25% of the total genotypic variance (Table 61).

The results from the two diallel experiments revealed that polygenic resistance to the corn leaf aphid is probably important especially in areas where biotypes of this aphid are present. Comparisons of the results from the two diallels confirmed the high genetic variation present in the corn populations which is potentially very useful. Some discrepancies also occurred in the results. Evaluation of the 9 x 9 diallel in the field was possible due to a heavy infestation of aphids. Unfortunately, the infestation did not occur early enough to allow a

sufficient build-up of aphids in the early growth stages of B37 and some hybrids before the plants senesced. Consequently, the results of the better controlled 8 x 8 diallel in the greenhouse are more reliable.

The results of the 8 x 8 diallel and the antibiosis study of the resistant line Mol7 were not consistent. Antibiosis study of Mol7 with the susceptible AA8sh2 line 3655 indicated that its resistance was dominant. However in the diallel study with other corn lines, Mol7's resistance was recessive. Comparison of its average hybrid performance with that of the resistant AA8sh2 line 3660 showed that the level of resistance in hybrid combinations was similar for both (Table 56).

GLC determination of 2(3)-benzoxazolinones

A successful GLC procedure was developed for the determination of 2(3)-benzoxazolinones. GLC determination of synthetic carbamate insecticide residues in plant material had been reviewed by Williams (1971). Many of these compounds are known to decompose readily in GLC columns. Since 2(3)-benzoxazolinones are naturally-occurring cyclic carbamates, care must be taken in column preparation to prevent decomposition. It was found that columns prepared according to the procedure of Leibrand and Dunham (1973) were consistently satisfactory. With their procedure, active sites on the supporting material were effectively coated, thus preventing decomposition. Further, it was found to be essential to use an all-glass GLC system in order to avoid catalytic decomposition of the benzoxazolinones by any metal surface. The Bendix 2500 Gas Chromatograph equipped with flame ionization detectors was found to be suitable for this purpose.

Gas chromatograph of the CH_2Cl_2 extract of macerated corn seedlings showed peaks for BOA, MBOA and dimethoxy-BOA. This was confirmed by mass spectra (Figures 17 and 18). The concentrations of BOA and MBOA were measured by comparing their peak heights with those obtained from BOA and MBOA standards. Highly accurate results were obtained for the GLC determinations. Typically, the standard deviation for three determinations did not exceed 0.04.

This procedure, unlike the others, is capable of determining the individual concentrations of 2(3)-benzoxazolinone analogs in plant materials. Data from all the determinations were obtained from less than 0.2 gm of fresh sample. Consequently, this method may be adapted as a non-destructive procedure for plant breeding.

Comparisons of BOA and MBOA concentrations in corn as determined by the GLC procedure with those obtained by other methods indicate that the concentrations are higher than those previously reported (Klun et al., 1967; Klun and Robinson, 1969). However, no direct comparisons could be made because fresh tissues were used here in contrast to the dry tissues used by Klun's group. Further, the etiolated seedlings used here may have an influence on the concentrations. The sensitivity of this procedure is further demonstrated by the determination of an MBOA concentration of 0.08 mg/g fresh weight in the mutant genotype, bxbx, a feat out of the range of the earlier colorimetric methods.

This powerful technique can aid in the further understanding of the role of the three known analogs in basic plant metabolism and the defense against pests and diseases. The previously neglected analog,

BOA, and the recently discovered dimethoxy-BOA can now be individually monitored and their toxicities studied.

Concentration gradients of MBOA in the various parts of the corn seedlings were successfully determined in one-week old, etiolated CI31A and WF9. MBOA concentration was greatest in the shoot, followed by the root. The third highest concentration was found in the remainder of the embryo. The lowest concentration was detected in the remainder of the endosperm (Table 65). The concentration of MBOA appeared to be correlated with tissue activity. High MBOA concentrations were found in tissues with active growth activity (shoot and root). Further work in this area may help explain the physiological implication of this phenomenon.

Teosinte, Zea mexicana (Shrader) Kuntze, and the Tripsacum complexes were assayed for the 2(3)-benzoxazolinones. Previously, this group of unique compounds were known to be present in coix, rye, wheat and corn. Using the GLC procedure, MBOA and dimethoxy-BOA were detected in the teosinte races from Balsas, Chalco and Jutiapa. BOA was not found. MBOA was found in Tripsacum dactyloides (2N), T. dactyloides (4N), T. floridanum and T. laxum (Table 66). Detailed examination of T. dactyloides (2N) showed that all three analogs were present.

Based on these results, it appears that the benzoxazolinones are well distributed in teosinte and the Tripsacum complexes. The findings here strengthens past evidence of the close taxonomic relationship between corn, teosinte and Tripsacum. Studies of the distribution and

concentration of these analogs in appropriate interspecific crosses may help further our understanding of the origin of corn.

APPENDIX

APPENDIX

Several field experiments were conducted at the Waimanalo Experiment Station without success. Despite efforts to increase aphid populations by growing of susceptible corn lines and also susceptible sorghum, successful infestation of the test plants was only sporadic.

The unusually favorable weather conditions and the low aphid predator and parasite populations may account for the good infestations observed in the AA8sh2 plantings of 1972 and 1973; and again in the 9 x 9 diallel planting in the latter part of 1973.

Two parasitic wasps, Aphelinus maidis Timberlake and Lysiphelus testaceipes (Cresson), are very effective in controlling the aphid population. Once parasitized, the aphid becomes bloated and eventually turns black (Aphelinus) or reddish-brown (Lysiphlebus). The parasites emerge from the abdomen of the aphid through a circular or crescent-shaped opening. Predators that feed on the aphid in Waimanalo are the ladybird beetle larvae, Coelophora inaequalis (Fab.), the syrphid fly larvae, Allographa obliqua (Say), the anthocorid predacious bug, Orius persequens (White), the lace wing larvae, Chrysopa sp., the aggravating grasshopper, Euconocephalus nasutus (Thunberg), and the long-horned grasshopper, Conocephalus saltator (Saussure).

LITERATURE CITED

- Auclair, J. L. 1959. Feeding and excretion by the pea aphid, Acyrtosiphon pisum (Harr.) (Homoptera:Aphididae), reared on different varieties of peas. Entomol. Exp. Appl. 2:279-286.
- Auclair, J. L. 1963. Aphid feeding and nutrition. Ann. Rev. Entomol. 8:439-490.
- Auclair, J. L., J. B. Maltais, and J. J. Cartier. 1957. Factors in resistance of peas to the pea aphid, Acyrtosiphon pisum (Harr.) (Homoptera:Aphididae). II. Amino Acids. Can. Entomol. 89:457-464.
- Beck, S. D. 1965. Resistance of plants to insects. Ann. Rev. Entomol. 10:207-232.
- Beck, S. D., E. T. Kaske, and E. E. Smissman. 1957. Quantitative estimation of the resistance factor, 6-methoxybenzoxazolinone, in corn plant tissue. J. Agric. Food Chem. 5:933-935.
- Beck, S. D., and J. F. Stauffer. 1957. The European corn borer, Pyraustra nubilalis (Hubn.) and its principal host plant. Entomol. Soc. Amer. 50:166-170.
- Bemiller, J. N., and A. J. Pappelis. 1965. 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside in corn. I. Relation of water-soluble, 1-butanol-soluble glycoside fraction content of pith cores and stalk rot resistance. Phytopathology 55:1237-1240.
- Bowman, M. C., M. Beroza, and J. A. Klun. 1968. Spectrophotofluorometric determination of 6-methoxy-2-benzoxazolinone, an indicator of resistance to European corn borer in Zea mays. J. Econ. Entomol. 61:120-123.
- Brewbaker, J. L., and S. H. Chang. 1974. Aphid resistance under apparent monogenic control. Maize Genetic Cooperation News Letter 48:37-38.
- Briggs, J. B. 1965a. Insect resistance in fruit plants. Ann. Appl. Biol. 56:325.
- Briggs, J. B. 1965b. The importance of the strains of Amphorophora rubi (Kalt.), the rubus aphid, in the problem of breeding for resistance in the raspberry. Proc. Int. Congr. Entomol., 12th, 1964. p532.
- Calub, H. G., G. M. Dunn, D. G. Routley, and R. M. Couture. 1974. Genetic and environmental effects on production of inhibitory compounds in corn resistant to Helminthosporium turcicum. Crop Sci. 14:359-361.

- Carnahan, H. L., R. N. Peaden, F. V. Lieberman, and R. K. Petersen. 1963. Differential reactions of alfalfa varieties and selections to the pea aphid. *Crop Sci.* 3:219-222.
- Cartier, J. J. 1959. Recognition of three biotypes of the pea aphid from Southern Quebec. *J. Econ. Entomol.* 52:293-294.
- Cartier, J. J. 1960. Growth, reproduction and longevity in one biotype of the pea aphid, Acyrtosiphon pisum (Harr.) (Homoptera:Aphididae). *Can. Entomol.* 92:762-764.
- Cartier, J. J., and R. H. Painter. 1956. Differential reactions of two biotypes of the corn leaf aphid to resistant and susceptible varieties, hybrids and selections of sorghums. *J. Econ. Entomol.* 49:498-508.
- Change, S. H., and J. L. Brewbaker. 1976. The genetics of resistance to the corn leaf aphid, Rhopalosiphum maidis (Fitch). *Maize Genetic Cooperation News Letter* 50:31-32.
- Coon, B. F. 1948. The influence of tassel type on aphid infestation in the field corn nursery. *Penn. Agric. Exp. Sta. Dept. Zool. and Entomol. Multilith Rpt.* 10p.
- Coon, B. F., R. C. Miller, and L. W. Aurand. 1948. Correlation between the carotene content of corn and infestation by the corn leaf aphid. *Penn. Agric. Exp. Sta. Dept. Zoo. and Entomol. Multilith Rpt.* 8p.
- Couture, R. M., D. G. Routley, and G. M. Dunn. 1971. Role of cyclic hydroxamic acids in monogenic resistance of maize to Helminthosporium turcicum. *Physiol. Plant Pathol.* 1:515-521.
- Curtis, B. C., A. M. Schlehner, and E. A. Wood, Jr. 1960. Genetics of greenbug (Toxoptera graminum Rond.) resistance in two strains of common wheat. *Agron. J.* 52:599-602.
- Daniels, N. E., and K. B. Porter. 1958. Greenbug resistance studies in winter wheat. *J. Econ. Entomol.* 51:702-704.
- Davis, J. J. 1909. Biological studies of three species of aphididae. *USDA Bur. Entomol. Tech. Serv.* 12(8):123-168.
- Day, P. R. 1974. The genetics of host-parasite interaction. *W. H. Freeman & Co.* 238p.
- Dishner, G. H., and R. T. Everly. 1961. Greenhouse studies on the resistance of corn and barley varieties to survival of the corn leaf aphid. *Proc. Indiana Acad. Sci.* 71:138-141.

- Elnaghy, M. A., and P. Linko. 1966. Correlation between resistance to stem rust and the concentration of glucoside in wheat. *Nature* 210: 417-418.
- van Emden, H. F. 1969. Plant resistance to Myzus persicae induced by a plant regulator and measured by aphid relative growth rate. *Entomol. Exp. & Appl.* 12:125-131.
- Everly, R. T. 1960. Loss in corn yield associated with the abundance of the corn leaf aphid, Rhopalosiphum maidis, in Indiana. *J. Econ. Entomol.* 53:924-932.
- Everly, R. T. 1965. Corn leaf aphid resistance in dent corn. *Proc. Indiana Acad. Sci.* 75:109.
- Everly, R. T. 1966. Review of factors affecting the abundance of the corn leaf aphid. *Proc. Indiana Acad. Sci.* 76:260-264.
- Forbes, S. A. 1905. The corn leaf louse Aphis maidis Fitch. *State Entomol. Ill. Rpt.* 23:123-133.
- Gentile, A. G., and A. K. Stoner. 1968. Resistance in Lycopersicon spp. to the tobacco flea beetle. *J. Econ. Entomol.* 61:1347-1349.
- Gernert, W. B. 1917. Aphis immunity of Teosinte-corn hybrids. *Science n.s.* 46:390-392.
- Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing system. *Aust. J. Biol. Sci.* 9:463-493.
- Guthrie, F. E., W. V. Campbell, and R. L. Baron. 1962. Feeding sites of the green peach aphid with respect to its adaptation to tobacco. *Ann. Entomol. Soc. Amer.* 55:42-46.
- Haber, E. S., and W. G. Gaessler. 1942. Sugar content of sweet corn pollen and kernels of inbred and hybrid strains susceptible to tassel infestation by aphid. *Amer. Soc. Hort. Sci. Proc.* 40:429-431.
- Hackerott, H. L., T. L. Harvey, and W. M. Ross. 1969. Greenbug resistance in sorghums. *Crop Sci.* 9:656-658.
- Hamilton, R. H. 1964. Tolerance of several grass species to 2-chloro-s-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. *J. Agric. Food Chem.* 12:14.
- Harvey, T. L., and H. L. Hackerott. 1967. Use of parasitized pea aphids to evaluate alfalfa for resistance. *J. Econ. Entomol.* 60: 573-575.

- Harvey, T. L., and H. L. Hackerott. 1969. Plant resistance to a greenbug biotype injurious to sorghum. *J. Econ. Entomol.* 62:1271-1274.
- Harvey, T. L., and H. L. Hackerott. 1974. Effects of greenbugs on resistant and susceptible sorghum seedlings in the field. *J. Econ. Entomol.* 67:377-380.
- Harvey, T. L., H. L. Hackerott, and E. L. Sorensen. 1971. Pea aphid injury to resistant and susceptible alfalfa in the field. *J. Econ. Entomol.* 64:513-517.
- Howe, W. L., and G. R. Pesho. 1960. Influence of plant age on the survival of alfalfa varieties differing in resistance to the spotted alfalfa aphid. *J. Econ. Entomol.* 53:142-144.
- Howe, W. L., and O. F. Smith. 1957. Resistance to the spotted alfalfa aphids in Lahontan alfalfa. *J. Econ. Entomol.* 50:320-324.
- Howitt, A. S., and R. H. Painter. 1956. Field and greenhouse studies regarding the sources and nature of sorghum (*Sorghum vulgare* Pers) resistance to the corn leaf aphid, *Rhopalosiphum maidis* (Fitch). *Kansas Agric. Exp. Sta. Bull.* 82. 38p.
- Huber, L. L., and G. H. Stringfield. 1940. Strain susceptibility to the European corn-borer and the corn-leaf aphid in maize. *Science* 92:172.
- Huber, L. L., and G. H. Stringfield. 1942. Aphid infestation of strains of corn as an index of their susceptibility to corn borer attack. *J. Agric. Res.* 64:283-291.
- Hughes, P. R., R. E. Hunter, and T. F. Leigh. 1966. A light-weight leaf cage for small arthropods. *J. Econ. Entomol.* 59:1024-1025.
- Kehr, W. R., G. R. Manglitz, and R. O. Ogden. 1968. Dawson alfalfa - a new variety resistant to aphids and bacterial wilt. *Nebr. Agric. Exp. Sta. Bull.* 497. p1-23.
- Kircher, H. W., R. L. Misiorowski, and F. V. Lieberman. 1970. Resistance of alfalfa to the spotted alfalfa aphid. *J. Econ. Entomol.* 63:964-969.
- Kishaba, A. N., and G. R. Manglitz. 1965. Non-preference as a mechanism of sweet clover and alfalfa resistance to the sweet clover aphid and the spotted alfalfa aphid. *J. Econ. Entomol.* 58:566-569.
- Klun, J. A., and T. A. Brindley. 1966. Role of 6-methoxybenzoxazolinone in inbred resistance of host plant (maize) to first-brood larvae of European corn borer. *J. Econ. Entomol.* 59:711-718.

- Klun, J. A., and J. F. Robinson. 1969. Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. *J. Econ. Entomol.* 62:214-220.
- Klun, J. A., C. L. Tipton, and T. A. Brindley. 1967. 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), an active agent in the resistance of maize to the European corn borer. *J. Econ. Entomol.* 60:1529-1533.
- Klun, J. A., C. L. Tipton, J. R. Robinson, D. L. Ostrem, and M. Benozza. 1970. Isolation and identification of 6,7-dimethoxy-2-benzoxazinone from dried tissues of Zea mays (L.) and evidence of its cyclic hydroxamic acid precursor. *J. Agric. Food Chem.* 18:633.
- Koyama, T., M. Yamato, and K. Kubota. 1955. Constituents of the root of Coix lachryma (jobi). *J. Pharm. Soc. Japan.* 76:1077.
- Lammerink, J. 1968. A new biotype of cabbage aphid (Brevicoryne brassicae (L.)) on aphid resistant rape (Brassica napus L.). *N.Z. J. Agric. Res.* 11:341-344.
- Leibbrand, R. J., and L. L. Dunham. 1973. Preparing high efficiency packed GC columns. *Res. Develop.* 24(9):32-39.
- Long, B. J., G. M. Dunn, J. S. Bowman, and D. G. Routley. 1976. Relation of hydroxamic acid concentration (DIMBOA) to resistance to the corn leaf aphid. *Maize Genetics Cooperation News Letter.* 50:91.
- Long, B. J., G. M. Dunn, and D. G. Routley. 1974. Rapid procedure for estimating cyclic hydroxamate (DIMBOA) concentration in maize. *Crop Sci.* 14:601-603.
- Loomis, R. S., S. D. Beck, and J. F. Stauffer. 1957. The European corn borer, Pyrausta nubilalis (Hubn.), and its principal host plant. V. A chemical study of host plant resistance. *Plant Physiol.* 32:379-385.
- McMurtry, J. A., and E. H. Stanford. 1960. Observations of feeding habits of the spotted alfalfa aphid on resistant and susceptible alfalfa plants. *J. Econ. Entomol.* 53:714-717.
- Maltais, J. B., and J. L. Auclair. 1957. Factors in resistance of peas to the pea aphid, Acyrtosiphon pisum (Harr.) (Homoptera: Aphididae). I. The sugar-nitrogen ratio. *Can. Entomol.* 89:365-370.
- Maxwell, F. G., J. N. Jenkins, and W. L. Parrott. 1972. Resistance of plants to insects. *Advances in Agronomy* 24:187-265.
- Melton, B. A. 1968. Mesilla alfalfa. *N. M. Agric. Exp. Sta. Bull.* 530. p1-8.

- Neiswander, C. R., and C. A. Triplehorn. 1961. Differential resistance of dent corn strains to the corn leaf aphid, Rhopalosiphum maidis (Fitch), in Ohio. Ohio Agric. Exp. Sta. Res. Bull. 898.
- Nielson, M. W., M. H. Schonhorst, H. Don, W. F. Lehman, and V. L. Marble. 1971. Resistance in alfalfa to four biotypes of the spotted alfalfa aphid. J. Econ. Entomol. 64:506-510.
- Ortman, E. E., and R. H. Painter. 1960. Quantitative measurements of damage by the greenbug, Toxoptera graminum, to four wheat varieties. J. Econ. Entomol. 53:798-802.
- Painter, R. H. 1951. Insect resistance in crop plants. The University Press of Kansas.
- Painter, R. H. 1958a. The study of resistance of aphids in crop plants. Proc. 10th Intern. Congr. Entomol., 1956. 3:451-458.
- Painter, R. H. 1958b. Resistance of plants to insects. Ann. Rev. Entomol. 3:267-290.
- Painter, R. H., and M. D. Pathak. 1960. The distinguishing features and significance of the four biotypes of the corn leaf aphid, Rhopalosiphum maidis (Fitch). Proc. Int. Congr. Entomol., 11th, 1960. 2:110-115.
- Patch, E. M. 1938. Food plant catalog of the aphids of the world, including the Phylloxeridae. Maine Exp. Sta. Bull. 393:35-431.
- Pathak, M. D., and R. H. Painter. 1958a. Effect of the feeding of the four biotypes of corn leaf aphid, Rhopalosiphum maidis (Fitch) on susceptible White Martin sorghum and Spartan barley plants. Kans. Entomol. Soc., J. 31:93-100.
- Pathak, M. D., and R. H. Painter. 1958b. Differential amounts of material taken up by four biotypes of corn leaf aphids from resistant and susceptible sorghums. Ann. Entomol. Soc. Amer. 51: 250-254.
- Pathak, M. D., and R. H. Painter. 1959. Geographical distribution of the four biotypes of corn leaf aphid, Rhopalosiphum maidis (Fitch), in Kansas. Trans. Kans. Acad. Sci. 62:1-8.
- Pesho, G. R., and F. V. Lieberman. 1960. A biotype of the spotted alfalfa aphid on alfalfa. J. Econ. Entomol. 53:146-150.
- Porter, K. B., and N. E. Daniels. 1963. Inheritance and heritability of greenbug resistance in a common wheat cross. Crop Sci. 3:116-118.

- Radcliffe, E. B., and R. K. Chapman. 1965a. Seasonal shifts in the relative resistance to insect attack of eight commercial cabbage varieties. *Ann. Entomol. Soc. Amer.* 58:892-897.
- Radcliffe, E. B., and R. K. Chapman. 1965b. The relative resistance to insect attack of three cabbage varieties at different stages of plant maturity. *Ann. Entomol. Soc. Amer.* 58:897-902.
- Radcliffe, E. B., and R. K. Chapman. 1966. Varietal resistance to insect attack in various cruciferous crops. *J. Econ. Entomol.* 59:120-124.
- Radcliffe, E. B., and F. I. Lauer. 1966. A survey of aphid resistance in the tuber-bearing Solanum (Tourn.) L. species. *Univ. Minn. Agric. Exp. Sta. Tech. Bull.* 253. 23p.
- Radcliffe, E. B., and F. I. Lauer. 1970. Further studies on resistance to green peach aphid and potato aphid in the wild tuber-bearing Solanum species. *J. Econ. Entomol.* 63:110-114.
- Rhodes, A. M., and W. H. Luckmann. 1967. Survival and reproduction of the corn leaf aphid on twelve maize genotypes. *J. Econ. Entomol.* 60:527-530.
- Sandmeyer, E. E., O. J. Hunt, W. H. Arnett, and C. R. Heisler. 1971. Relative resistance of six selected alfalfa clones to the pea aphid and spotted alfalfa aphid. *J. Econ. Entomol.* 64:155-162.
- Schuster, D. J., and K. J. Starks. 1973. Greenbugs: Components of host-plant resistance in sorghum. *J. Econ. Entomol.* 66:1131-1134.
- Singh, S. R., and R. H. Painter. 1965. Reactions of four biotypes of corn leaf aphid, Rhopalosiphum maidis (Fitch), to differences in host plant nutrition. *Proc. Int. Congr. Entomol.*, 12th, 1964. p543.
- Singh, S. R., and E. A. Wood. 1963. Effect of temperature on fecundity of two strains of the greenbug. *J. Econ. Entomol.* 56:109-110.
- Smith, R. F. 1959. The spread of the spotted alfalfa aphid, Therioaphis maculata (Buckton), in California. *Hilgardia* 28:647-685.
- Smith, O. F., and R. N. Peaden. 1960. A method of testing alfalfa plants for resistance to the pea aphid. *Agron. J.* 52:609-610.
- Snelling, R. O., R. A. Blanchard, and J. H. Bigger. 1940. Resistance of corn strains to the corn leaf aphid, Aphis maidis. *Amer. Soc. Agric. J.* 32:371-381.

- Stanford, E. H., and J. A. McMurtry. 1959. Indications of biotypes of the spotted alfalfa aphid. *Agron. J.* 51:430-431.
- Sullivan, S. L., V. E. Gracen, and A. Ortega. 1974. Resistance of exotic maize varieties to the European corn borer, Ostrinia nubilalis (Hubner). *Environ. Entomol.* 3:718-720.
- Teetes, G. L., C. A. Schaefer, and J. W. Johnson. 1974. Resistance in sorghum to the greenbug; Laboratory determination of mechanisms of resistance. *J. Econ. Entomol.* 67:393-396.
- Thurston, R. 1961. Resistance in Nicotiana to the green peach aphid and some other tobacco insect pests. *J. Econ. Entomol.* 54:946-949.
- Thurston, R., W. T. Smith, and B. P. Cooper. 1966. Alkaloid secretion by trichomes of Nicotiana species and resistance to aphids. *Entomol. Exp. Appl.* 9:428-432.
- Thurston, R., and J. A. Weber. 1962. Toxicity of Nicotiana gossei Domin to Myzus persicae (Sulzer). *Entomol. Exp. Appl.* 5:233-238.
- Triplehorn, C. A. 1959. The possible effect of weather on incidence of corn leaf aphid infestation and damage. *Proc. North Central Branch Entomol. Soc. Amer.* 14:28-29.
- Triplehorn, C. A. 1960. Corn leaf aphid - Ecology, life history and control. *Proc. North Central Branch Entomol. Soc. Amer.* 15:97-98.
- Viale, E. 1950. The biology of the corn leaf aphid, Aphis maidis Fitch, as affected by various strains of corn, Zea mays L., and certain other environmental factors. Ph.D. Thesis. Kansas State College.
- Virtanen, A. I., and P. K. Hietala. 1955. 2(3)-benzoxazolinone, an anti-Fusarium factor in rye seedlings. *Acta. Chem. Scand.* 9:1543-1544.
- Virtanen, A. I., and O. Wahlroos. 1958. On the anti-fungal activity of wheat and rye seedlings. *Soumen Kemistrilehti* B31, 402.
- Wahlroos, O., and A. I. Virtanen. 1959. The precursors of 6MBOA in maize and wheat plants: their isolation and some of their properties. *Acta. Chem. Scand.* 13:1906-1908.
- Walter, E. V., and A. M. Brunson. 1940. Differential susceptibility of corn hybrids to Aphis maidis. *J. Econ. Entomol.* 33:623-628.
- Walter, E. V., and A. M. Brunson. 1946. Selection for aphid resistance within inbred lines of maize. *Amer. Soc. Agron. J.* 38:974-977.

- Whitney, N. J., and C. G. Mortimore. 1959a. An anti-fungal substance in the corn plant and its effect on the growth of two stalk-rotting fungi. *Nature* 183:341.
- Whitney, N. J., and C. G. Mortimore. 1959b. Isolation of the anti-fungal substance, 6-methoxy-benzoxazolinone, from field corn (*Zea mays* L.) in Canada. *Nature* 184:1320.
- Whitney, N. J., and C. G. Mortimore. 1961. Effect of 6-methoxy-benzoxazolinone on the growth of *Xanthomonas stewartii* (Ern-Smith) Dowson and its presence in sweet corn (*Zea mays* var. *saccharata* Barley). *Nature* 189:596-597.
- Williams, I. H. 1971. Carbamate insecticide residues in plant material: Determination by gas chromatography. *Residue Rev.* 38: 1-20.
- Wood, E. A., Jr. 1961. Biological studies of a new greenbug biotype. *J. Econ. Entomol.* 54:1171-1173.
- Wood, E. A., Jr. 1971. Designation and reaction of three biotypes of the greenbug cultured on resistant and susceptible species of sorghum. *J. Econ. Entomol.* 64:183-185.