CORN LEAF APHID AND POLYSORA RUST RESISTANCE

IN TROPICAL MAIZE

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

HORTICULTURE

AUGUST 2003

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ACKNOWLEDGMENTS

I am grateful to my committee chairman Dr. James L. Brewbaker for his support. His knowledge and experiences were of great value throughout the entire process of this research project. I would also like to express my sincere appreciation to Drs. Richard M. Manshardt and Chifumi Nagai who served on my thesis committee.

I had a great help from all Waimanalo Research Station staff involving the station manager, Mr. Rogelio Corrales. Without their excellent field management and practices, this project would never have been successful. I would like to acknowledge all of them here; Francisco Pagay, Ronald Luning, Juanito Gorces, Olivio Arios, Curtis Kobashigawa, Wilson Kaahanui, Michael Parmacio, Thoran Evans. In addition, my appreciation extends to Lynnette Horiuchi and Susan Takahashi in the Department office who have been always kind, helpful and supportive to me.

I wish to express my sincere appreciation to Dr. Soon-Kwon Kim, my former advisor in South Korea, whose passion and fervor have led me to pursue my study in maize breeding. I'm also particularly indebted to Drs. Hee-Chung Ji and Hyung-Joo Jin for their time and effort for setting up the artificial inoculation technique and field evaluation. I thank Drs. Robert E. Paull, Chung-Shih Tang, Russell H. Messing, Ray Ming, Young-Soo Keum for their time and counsel on various aspects on the project for which I am extremely grateful.

Finally, special thanks are expressed to my parents, brother and sister-in-low for their patience, understanding and emotional assistance during entire period of the study in Hawaii.

ABSTRACT

This research includes two separate studies both of which incorporated generation mean analyses to interpret genetically the resistance to corn leaf aphid and polysora rust in tropical maize.

The first study focused on resistance to corn leaf aphid in tropical sweet corn inbred Hi38-71. An inoculation technique using hair-pin clip cages and infestation quantification method using digital image analysis were developed for this study. During the study, a heavy natural infestation of corn leaf aphids occurred in a seed production nursery. Yield loss by ranged from 38.9% to 98.8%, with an average loss of 71.7%. The clip-cage method was effective in distinguishing resistant and susceptible plants under field conditions. Resistance to corn leaf aphid from Hi38-71 appeared to be monogenic and recessive. Aphid reproduction and population growth were measured on four different genotypes of varying aphid tolerance. Aphids on Hi38-71 had poorest performance over all aspects of growth and reproduction examined. Difference in number of progenies produced and days to 50% mortality appeared to account for most of the difference observed in the genetic study.

The second study estimated genetic parameters for polysora rust resistance in Hi38-71. Hi38-71 exhibited moderately high resistance to polysora rust as well as resistance to corn leaf aphid. Generation mean analysis showed that epistatic interactions of [*aa*] and [*dd*] along with simple dominance and additive gene effects were involved in controlling resistance in Hi38-71 to polysora rust. It is concluded that polysora resistance

breeding cannot be based on selection of a single parent but a hybrid-breeding or reciprocal recurrent selection approach appears justified.

The tropical sweet corn inbred, Hi38-71 is a sib line of Hi38 which was bred from a *bt*-1 conversion of AA8sh2. AA8sh2 was studied for its resistance to corn leaf aphid in 1970's in Hawaii and was converted to common rust resistance, *Rd*1-D which broke down due to evolved racial variation of the pathogen. Hi38-71is thus of particular value in sweet corn breeding for tropical regions. This is due not only to its resistance to corn leaf aphid and polysora, but to its high sweet corn qualities and generally good combining abilities.

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CHAPTER ONE

LITERAURE REVIEW

1. 1. CORN LEAF APHID, *Rhopalosiphum maidis* (Fitch)

Corn leaf aphid is a tiny bluish-green, soft-bodied, piercing-sucking insect, that belongs to the family of Aphididae within the order of Homoptera. It was first named *Aphis maidis* by Fitch (1986) and was later given the scientific name *Rhopalosiphum maidis* Fitch. It is one of the most abundant species among the twelve species found on maize in the United States (Stoetzel and Miller, 2001).

1. 1. 1. Distribution

Corn leaf aphid is considered to have originated in Asia (Blackman and Eastop, 1984). It occurs throughout the world and is an economically important cereal aphid species in tropical climates (Blackman and Eastop, 1984). In North America, it migrates annually from south to north (Forbes, 1905; McColloch, 1921; Cartier, 1957; Kieckhefer et al., 1974). Foott (1977) also found no evidence in southwestern Ontario that corn leaf aphid can overwinter in that area. Dispersal of the aphid population is primarily by the winged form (Berry, 1969). The aphid species is not a strong flyer but is light enough to get into wind currents, being spread through the atmosphere as "aerial plankton" (Johnson, 1969). Thus, geographic distribution and spread are mainly by the winged aphid, while wingless form accounts for population increase within a host plant.

1. 1. 2. Host range

Corn leaf aphid has a wide host range in the Graminae, including more than 30 genera and most of the cereal crops, especially barley (*Hordeum vulgare* L.), maize, and sorghum (*Sorghum bicolor* (L.) Moench) (Blackman and Eastop, 1984). Among four crop plants tested including *Andropogon sorghum*, broad bean (*Vicia faba*), and corn, using 7-day old seedlings barley (*Hordeum vulgare*) was the most favorable host under laboratory conditions (El-Ibrashy et al., 1972).

1. 1. 3. Biology

Biology of corn leaf aphid has been studied under uncontrolled, field and controlled conditions (Davis, 1909; Wildermuth and Walter, 1932; Branson and Ortman, 1967). The aphid species undergoes 4 nymph stages to maturity. The average durations of the instars reared on barley under field (Wildermuth and Walter, 1932) and controlled conditions (Branson and Ortman, 1967) were: (1) first instar, 1.30 and 1.88 days; (2) second instar, 1.36 and 1.29 days; (3) third instar, 1.16 and 1.00 days; and (4) fourth instar, 1.41 and 1.29 days for totals 5.23 and 5.46 days, respectively. Lifespan averaged 23.83 days and the aphids gave birth to an average of 61.33 offspring. El-Ibrashy et al. (1972) observed about 50 generation per year on barley under laboratory condition.

The biology of corn leaf aphid is closely linked with environment. Temperature is probably the most important environmental variable influencing rates of aphid development and reproduction (Elliott et al., 1988). For winged aphids, temperature can be regarded as the effective releaser of takeoff in the morning and light as the inhibitor in

the evening (Johnson and Taylor, 1957). However, light is also required to initiate and maintain aphid flight, and to orient it (Kennedy and Booth, 1963).

1. 1. 4. Aphid feeding activity

When aphids land on host plants, they initiate a series of short tests using stylets called "probing" into epidermis. They distinguish host and non-host by this activity. Once they find host plants, epidermal penetration by stylets follows. In most aphid species, the penetration takes place intercellularly with exception of stomatal penetrations (Staniland, 1924; Sorin, 1966; Parry, 1971). Aphids secret saliva from the stylet during penetration and form a tube around the stylets. This tube or stylet sheath remains in the plant even after stylet is withdrawn. This stylet track provides evidence of the feeding pathway.

Bing et al. (1991) observed under a light microscope stylet penetration and feeding sites of corn leaf aphid on seedlings and late whorl stages of maize. Fifty-seven percent of corn leaf aphid stylets entered plants in the late whorl stage through stomata, whereas only 8% of the aphid penetrated seedling-stage plants through stomata. This indicated that stomatal penetration by the aphid was preferred in whorl-stage maize but not in seedling maize. They concluded that stomatal penetration by the aphid in seedling maize might be random events because of the tenderness of seedling leaves. The majority of the style penetrations (86%) occurred intercellularly between epidermal cells. In their study, pectinase was found in the saliva of corn leaf aphid. However, salivary pectinase has not been found in this species before (McAllan and Adams, 1961; Ma et al., 1990).

1. 1. 5. Reproduction

Corn leaf aphid is an anholocyclic cereal aphid, which means its reproduction is exclusively parthenogenetic (Brown and Blackman, 1988). During the parthenogenetic reproduction, corn leaf aphid produces morphologically different phenotypes, winged (or alate) and wingless (or apterous) morphs (Fig. 1). Dixon (1988) defined it "polyphenism", that is, the production of two or more alternative phenotypes by a single genotype. The alate morph is able to fly short distances and colonize new host plants, and in general has a longer developmental time and lower fecundity than apterous forms (Zera and Denno, 1997; Dixon, 1988). It was observed by Foott (1977) in a barley field that approximately 10% of the aphids were apterous females and 90% were nymphs. Only 6.7% of the nymphs had wing pads. Although there have been reports on the discovery of corn leaf aphid males in lab colonies (Wildermuth and Walter, 1932) and in the wild (Eastop, 1954), oviparous females have never been reported and all literature supports reproduction as being asexual (Lambers, 1966).

1. 1. 6. Biotype

Painter and Pathak (1962) described 4 biotypes on the basis of aphid reproduction on different plants and plant reaction to aphid feeding, and named them KS-1, KS-2, KS-3 and KS-4. Wilde and Feese (1973) found a population of corn leaf aphid which differed significantly from those 4 biotypes, based on ability to attack previously considered resistant plants and ability to reproduce well at higher temperatures. This population was designated KS-5. Recent development of molecular technique allowed Caballero et al. (2001) to obtain a total of 20 distinguishable polymorphic bands which revealed 23

Fig. 1. Corn leaf aphid population feeding on a corn leaf (upper) and close-up of a wingless (lower left) and a winged (lower right) form aphid.

different clones from Johnson grass (*Sorghum halepense*) in Chile using RAPD-PCR based on three primers. Steiner et al. (1985) employed isozyme or allozyme electromorphism analysis with 21 electrophoretic loci in 15 natural populations and revealed 8 loci useful for population comparisons. Karyotypes of different corn leaf aphid biotypes were found to be 2n = 8, 9 and 10. Differences in chromosome numbers explained the discrepancies in host plant preference (Brown and Blackman, 1988).

1. 1. 7. Corn leaf aphid in Hawaii

Corn leaf aphid is known to have introduced to Hawaii by commercial trade, and first reported on Oahu in 1906. Since then it spread to neighboring islands (Mau and Kessing, *http://www.extento.hawaii.edu/kbase/crop/type/rhopalos.htm*). There has been no males reported in Hawaii, thus population increase is entirely dependent upon parthenogenesis by females. The primary hosts in Hawaii are corn and sorghum. Secondary hosts include bermudagrass (*Cynodon* spp.), asparagus (*Asparagus officinalis*), sudangrass (*Sorghum sudanense*), job's tears, johnson grass, oats (*Avena* spp.), sugarcane (*Saccharum officinarum*), wheat (*Triticum* spp.), oxalis (*Oxalis* spp.), and many species of *Panicum* and other grasses.

Biological control of corn leaf aphid has been successful with several predators and parasites. Predators and parasitoids of corn leaf aphids in Hawaii are well documented (Bergquist, 1975). Parasitic wasps include *Aphelinus maidis* Timberlake and *Lysiphlebus testaceipes* Cresson. Predators in Waimanalo Research Station include ladybird beetle larvae (*Coelophora inaequalis* Fab.), syrphid fly larvae (*Allographa obliqua* Say), anthocorid predacious bug (*Orius persequens* White), long-horned

grasshopper (*Conocephalus saltator* Saussure), lace-wing larvae (*Chrysopa* spp.) and aggravating grasshopper (*Euconocephalus nasutus* Thunberg). Fungus diseases are also important in reducing the aphid species.

1. 2. APHID-PLANT RELATIONSHIP

1. 2. 1. Damage on plant by aphid attack

Yield loss in maize by direct feeding of corn leaf aphid is periodic and sporadic but considerable when it occurs. Everly (1960) reported that when plants were lightly infected with corn leaf aphid, about 10% of yield was reduced. However, significant reduction in yield by corn leaf aphid can be encountered when corn plants suffer from drought stress (Triplehorn, 1959). Foott and Timmins (1973) reported up to 91.8% yield reduction in heavily infected, drought-stressed maize.

Direct feeding by colonies of corn leaf aphid may cause the followings ; (a) injury to the central tassel spike resulting in failure to shed pollen; (b) gumming up of the lateral branches of the tassel with honeydew which prevents pollen shedding; (c) failure of tassel to emerge completely; (d) development of molds and rots on the upper portion of the plant which often extends down to the ears; (e) yellow and red discoloration of corn leaves especially under high level of infestation; (f) accelerated maturity with partially filled ears, an effect due to aphid feeding on kernels and silk; (g) a concomitant increase in the infestation of corn earworm (*Helicoperva zea* Boddie), which is attracted by the honeydew produced by corn leaf aphids (Everly, 1960). Bing et al. (1991) suggested a possibility that leaf gas exchange in the late whorl stage of maize plant could be disrupted

by direct feeding of corn leaf aphid, assuming that stomatal penetration of aphid stylet physically damages the stomata.

1. 2. 2. Vector of plant viruses

Besides mechanical injury, corn leaf aphid is able to transmit more than 15 plant viruses including barley yellow dwarf, guinea grass mosaic, maize leaf fleck, millet red leaf, abaca mosaic, maize dwarf mosaic, sugar cane mosaic, cucumber mosaic, onion yellow dwarf and papaya ring spot viruses in persistent and non-persistent manners (Chan et al., 1991). In the case of vectoring plant viruses, the alate form is more responsible than apterous form of aphids.

1. 2. 3. Development at different plant stages

It is generally believed that seedling maize is virtually immune to corn leaf aphid colonization. Dicke and Sehgal (1990) showed, however, that alate corn leaf aphids were attracted to, and might establish colonies, on seedling dent and sweet corn plants in Iowa and Jamaica. On barley, corn leaf aphid reproduced significantly more on the earlier growth stages than on the later stages (Kieckhefer and Gellner, 1988). In late growth stages, the enclosed whorl area of a corn plant provides a very favorable environment for aphid development. It is apparent that the moist, protected environment within the whorl has a greater influence on aphid development than the nutrition supplied by the tassels (Foott, 1977). When the tassels became exposed there was a movement of aphids down the plant to occupy positions on the leaves, beneath leaf sheaths, and on the ear shoot. The degree and rapidity of movement down and the percentages of alate adults and

nymphs with wing pads were usually directly related to the size of the infestations (Foott, 1997). After the tasseling stage, populations of corn leaf aphid persist on maize until the plants begin to dry. It is common to find that neighboring plants of the same variety exhibit a great range in levels of aphid infestations when tassels become exposed near pollination.

1. 3. HOST PLANT RESISTANCE TO INSECTS

1. 3. 1. Host plant resistance

Host plant resistance is the most effective, economical and environmentally sound management tactic to control insect pests in crop plants. Smith (1997) defined host-plant resistance as the inherited qualities that result in a plant of one variety or species being less damaged than a susceptible plant lacking these qualities. crop plants with insect resistance have increased agricultural productivity in the United States for over 200 years. Insect resistance in maize has been a subject of research from the early 1900's, starting with relation of corn earworm damage to husk tightness and thickness (Hinds, 1914) and corn leaf aphid resistance in teosinte x yellow dent corn hybrids (Gernert, 1917).

The use of insect-resistant cultivars has many economic and environmental advantages (Smith, 1997). It can reduce or eliminate the costs and use of insecticides, increase farming efficiency, improve the quality of the environment and the health of agricultural producers and consumers. Moreover, when compared to research on insecticide development, insect resistance research gives a substantially greater payoff for each research dollar invested.

1. 3. 2. Categories of resistance

According to Painter (1968), plant resistance mechanism can be categorized into three types; antibiosis, non-preference and tolerance. The term "categories" was proposed by Smith (1997) to refer to antibiosis, antixenosis and other undefined types of plant-insect interactions, observed as responses of insects to plant resistance mechanisms. In antibiosis, feeding on the plant affects adversely the biology of the pest insect, leading to death of the insect, abnormal life span, reduction in food reserves, unsuccessful hibernation, smaller size, decreased fecundity or abnormal behavior.

Non-preference relates to host plant selection by the insect. With non-preference resistance, insects treat resistant plants as poor hosts and then select an alternate host. Non-preference is now referred to as antixenosis by many researchers (Kogan and Ortman, 1978).

Painter (1968) defined tolerance as "a basis of resistance in which the plant shows an ability to grow and reproduce itself or repair injury to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host". Antibiosis and antixenosis are based on the reaction of an insect to a plant while tolerance is related with the reaction of a plant to insect infestation and damage. From an ecological and environmental standpoint, tolerance has several advantages over antibiosis and antixenosis in pest management programs (Smith, 1997; Reese et al., 1994). Unlike antibiosis and antixenosis, tolerance does not adversely affect beneficial insects and natural enemies or exert sufficient selection pressure to develop biotypes. Moreover, it also tends to delay expensive chemical treatments or reduce the number of treatments. However it is often difficult to separate tolerance from antibiosis and antixenosis (Reese

et al., 1994). Experiments must be precisely and accurately designed to delineate actual contributions of resistance factors into each category of resistance.

1. 3. 3. Mechanism of resistance

Smith (1997) proposed the term "mechanisms" to be used to describe the underlying chemical or morphological plant processes that are responsible for the negative reaction of insects to resistant plants. Both chemical and morphological defenses mediate resistance to insect pests. Chemically induced resistance to aphids may result from the presence of olfactory repellents, feeding or oviposition deterrents, or the absence of feeding or oviposition stimulants.

Among secondary metabolites in plants, hydroxamic acids have been reported to play a role in resistance of certain cereals including maize to several aphid species such as corn leaf aphid (Long et al., 1977; Beck, 1983), rose-grain aphid (*Metopolophium dirhodum*) (Argandona et al., 1980), greenbug (*Schizaphis graminum*) (Corcuera et al., 1985), grain aphid (*Sitobion avenae*) (Thackray et al., 1990), bird-cherry oat aphid (*Rhopalosiphum padi*) (Thackray et al., 1990) and Russian wheat aphid (*Diuraphis noxia*) (Mayoral et al., 1996). In maize, it has also been studied in relation to a chemical resistance factor to European corn borer, *Ostrinia nubilalis* (Wahlroos and Virtanen, 1959; Klun and Brindley, 1966; Klun et al., 1967; Klun and Robinson, 1969), stalk rot by *Diplodia maidis* (BeMiller and Pappelis, 1965) and northern corn leaf blight, *Exserohilum turcicum* (Long et al., 1975). High concentrations of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) were correlated with observed resistance to diseases and insects in those studies.

Hydroxamic acids begin to appear soon after germination in maize (Klun and Robinson, 1969). Levels increase with age and reach a maximum a few days after germination, followed by a subsequent decrease. The rate of decrease, however, is dependent upon genotypes (Klun and Robinson, 1969). The main hydroxamic acid in maize is 2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc), a glucoside form of DIMBOA (Tipton et al., 1967) (Fig. 2). When plants are injured, DIMBOA-Glc is converted into corresponding aglycones by β-glucosidase (Wahlroos and Virtanen, 1959).

However, there have been conflicting reports on the role of DIMBOA as primary resistance factor to aphids (Bing et al., 1990; Cambier et al., 2001). Cambier et al. (2001) studied DIMBOA derivatives such as DIMBOA-Glc and HDMBOA-Glc with *Metopolophium dirhodum*. They believed that DIMBOA is not the main resistance factor to the aphids, since DIMBOA was not detected in the phloem sap (Molyneux et al., 1990; Caillaud and Niemeyer, 1996). Phloem is the main nutrition site of aphids, and DIMBOA-Glc and the β-glucosidase are stored in extravacuolar space and vacuolar spaces, respectively (Massardo et al., 1994).

In some cases, nutritional condition of host plant may influence aphid resistance. Nitrogen level in diets is considered one of the most important factors influencing the performance of the aphid species on their host plants. Principal carbon and nitrogen sources used by most aphids are sugars (especially sucrose) and amino acids, respectively (Dadd, 1985). Despite the abundance of sugars in plant phloem sap, nitrogenous compounds are rare in phloem sap (Douglas, 1993). Thus aphids show a strong response

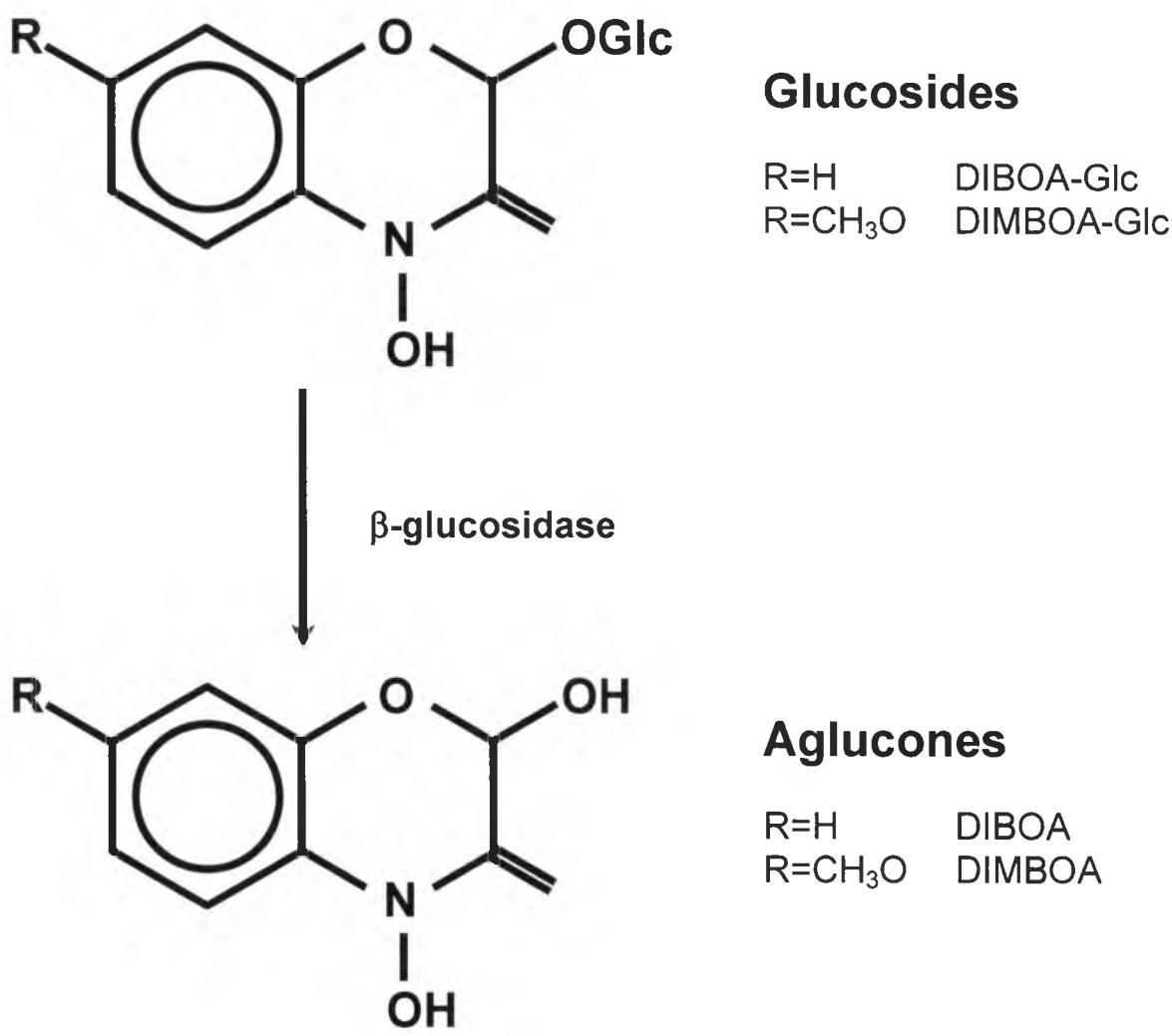


Fig. 2. Hydroxamic acids (Hx) structure from Gramineae.

to nitrogen level in their host plants (van Emden, 1996). Susceptibility to aphid feeding generally has been shown to increase with the level of nitrogenous compounds in plant tissue.

External or internal plant structural features may also alter aphid behavior or reduce aphid digestion. Plant morphology may provide a favorable habitat for aphid populations. Maize tassel type has been associated to aphid infestation by Coon (1945). Caillaud and Niemeyer (1996) suggested a possible mechanical mechanism of resistance in some lines of *Triticum monococcum* to *Sitobion avenae*. In their study, hydroxamic acids did not account for the resistance, but there was possible involvement of the phloem sealing system in aphid resistant lines.

1. 3. 4. Corn leaf aphid resistance in maize

Resistance in maize to corn leaf aphids was first reported in 1917 by Gernert (1917) in the F_1 hybrid between annual teosinte (*Zea mexicana*) and yellow dent maize. Resistance to corn leaf aphid has been reported to be caused by a combination of plant morphology, soil and climatic conditions, and physiochemical factors (Coon et al., 1948). Haber and Gaessler (1942) studied the chemical constituents, especially sugar content of tassels of sweet corn inbreds, but found no relationship between resistance and susceptibility to aphids on one hand and sugar content or other constituents on the other. Coon (1945) reported on the relation between the type of tassel and the aphid infestation of both inbreds and hybrids. He found that inbreds in which the tassel was exposed quickly and completely from the leaves tended to have the lowest aphid populations. On the basis of tassel type, he classified maize into five categories. The tassels that were

enclosed the longest time provide the most favorable habitat for the aphids (Painter, 1968). Although the correlations between tassel type and aphid population were significant, exceptions were noted for all categories. Coon et al. (1948) found significant correlation (r=0.5697) between the carotene content of corn grain and degree of aphid infestation in 44 hybrids.

Chang and Brewbaker (1976) reported that resistance to corn leaf aphid is conditioned by a single recessive gene. They also suggested a possible linkage of the resistance gene to the *Rp1* locus on chromosome 10 which controls resistance to maize rust caused by *Puccinia sorghi* Schw. The aphid resistance locus was later named *aph1*. Lu and Brewbaker (1999) also showed the recessive nature of aphid resistance using a generation mean analysis and recombinant inbred lines.

Long et al. (1977) reported that the cyclic hydroxamate, DIMBOA, was the antibiotic factor for resistance to aphids. Maize lines with high concentrations of DIMBOA in the leaves were found to suffer less damage from corn leaf aphid than those with low DIMBOA content. Bing et al. (1990), however, reported that DIMBOA was not the primary factor conditioning resistance to corn leaf aphid among inbred lines of maize. Generation mean analysis using inbreds Mo17 (resistant) and B96 (susceptible) by Bing and Guthrie (1991) revealed significance of both additive and dominant genetic effects, but showed greater importance of additive genetic effects in resistance of Mo17 to corn leaf aphid. Results of a diallel study involving 10 inbred lines by Bing et al. (1992) showed that general combining ability (GCA) effects were more important than specific combining ability (SCA). They found evidence for the involvement of multiple genes and

a greater influence of additive gene effects relative to non-additive gene effects in resistance to the corn leaf aphid.

1. 4. POLYSORA RUST IN MAIZE

Polysora rust or southern corn rust of maize caused by *Puccinia polysora* Underwood is common throughout the southern United States (Bailey et al., 1987) and West Africa (Rhind et al., 1952; Cammack, 1954; Robinson, 1996). Polysora rust was devastating in Africa in the 1950's, but it was considered to be a minor disease in the United States (Rodriguez-Ardon et al., 1980). Since the outbreak of epidemics in the Mississippi Valley during 1972 ~ 1974, however, the potential of rust to cause severe yield losses when it arrives early in the growing season has been recognized in the southern United States (Zummo, 1988). The disease has also been reported as far north as Wisconsin, but does not overwinter in temperate regions (Pavgi and Flangas, 1959). Occurrence of the disease in northern United States is usually late in the season and does not have significant impact on yield. Severity of the disease was attributed to high susceptibility of temperate U.S. corn hybrids to polysora rust and the increase of double cropping practices in southern areas (Futrell, 1975; Raid, 1988). Yield losses ranging from 4% to 50% have been observed (Rhind et al., 1952; Rodriguez-Ardon et al., 1980).

Polysora rust can be distinguished from common rust caused by *Puccinia sorghi* Schw. by its pustule size, shape and color (Fig. 3). But the most significant distinction is that polysora rust is more devastating and can eventually kill the plants, while common rust rarely does. Although there have been nine physiological races of southern corn rust identified so far (Ryland and Storey, 1955; Robert, 1962; Ullstrup, 1965), no information

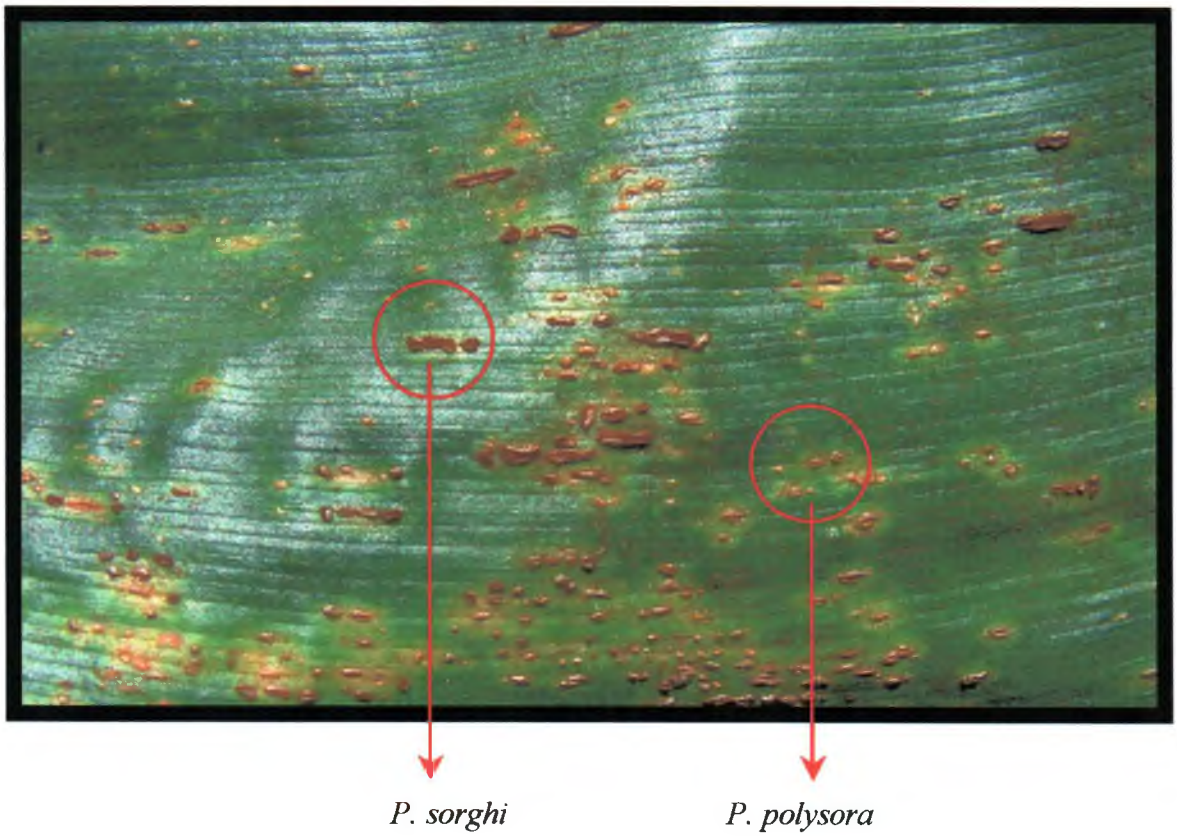


Fig. 3. Mixed infection of *P. sorghi* and *P. polysora* on a maize leaf. *P. polysora* can be distinguished by the size, shape and color of uredinia, which are generally smaller, more circular and lighter in color than those of *P. sorghi* as seen in this picture.

is available about races of polysora rust in Hawaii on relationships with previously identified races of the rust.

Monogenic resistant gene *Rpp*1 and *Rpp*2 were identified around 1950, but both soon proved useless in Africa, and other monogenes followed the same fate. A single dominant gene, *Rpp*9 has been identified by Ullstrup (1965) from a South American plant introduction (PI 186208). The gene conferred resistance to Indiana isolates of polysora rust race, PP.9. Later, Futrell et al. (1975) found another source of single gene resistance to the same race. Holland et al. (1998) suspected a relationship between those two single resistance genes since both were obtained from South African germplasm. Genes for race-specific resistance were not effective in controlling polysora rust in Africa (Robinson, 1996). The resistance gene, *Rpp*9, was introduced to South Africa where it broke down even before commercial release of hybrids with the gene. Robinson (1996) strongly suggested the use of horizontal (general) resistance for reducing polysora rust disease below economic level. General resistance in maize was then bred into many local open-pollinated populations in Africa in the 1950s and proved effective in controlling the disease.

General resistance to polysora rust is inherited quantitatively in maize. Bailey et al. (1987) identified maize inbreds and single crosses as "slow-rusting" (a general form of resistance) based on weekly assessments of pustule density to determine the area under the disease progress curve (AUDPC). Among inbreds tested, Tx601 was highly resistant, and the performance of Tx601 was consistent in south central Texas and Nigeria. Tx601 also exhibits high tolerance to southern corn rust in Hawaii. Zummo (1988) reported different responses of maize genotypes to southern corn rust fungus in its pustule

incidence, size, tumescence and sporulation. He used those characters to identify components contributing partial resistance to southern corn rust.

Moon (1995) evaluated a set of recombinant inbred lines (RILs) in Hawaii and the Philippines, which segregated approximately 50% tolerant and 50% susceptible. Ming (1995) further identified restriction fragment length polymorphism (RFLP) markers linked to quantitative trait loci (QTLs) in the same set of RILs and found five QTLs on chromosomes 2, 4, 6, 9 and 10 with emphasis on the possible important role of the QTL on chromosome 6.

Holland et al. (1998) studied the inheritance of resistance to polysora rust in $F_{2:3}$ populations. Broad-sense heritabilities estimated from two populations were 30% and 50% respectively. RFLP markers were also utilized to localize and estimate the effects of genes conferring resistance to polysora rust in the two populations. A single locus on the short arm of chromosome 10 was identified to contribute 82-83% of the variation among field resistance scores in the two populations. QTLs on chromosomes 3, 4, and 10 and their epistatic interactions explained 96-99% of the variation in the two populations. Those chromosomal regions were previously known to possess genes for resistance to either polysora rust or common rust.

1. 5. GENERATION MEAN ANALYSIS

Robinson (1996) is among authors who strongly advise the avoidance of gene-for gene resistance as opposed to horizontal resistance of disease and insect pests. Horizontal resistance differs in degree, with every grade of variation between a minimum and a maximum. Continuous variation is attributable in part to a heritable component, but also

to environmental components. These include external environments affecting both host and parasite, and/or vagaries in the internal development of the individuals. The heritable component may consist of genes at many loci throughout the genome which function together in a polygenic system. Individual gene effects may be small and similar to one another. These properties of individual genes along with their supplementary effects on the phenotype give rise to continuous variation. The individual gene effects cannot be uncovered unless we employ special and appropriate quantitative genetic techniques, such as generation mean analysis.

Generation mean analysis utilizes observed means and variances of various generations. The generations should be derived from a cross between two parents that are homozygous for differences in a trait of interest. Modes of gene action or effects which can be revealed by the analysis are additivity, dominance and three types of non-allelic interactions ("epistasis"). The theoretical foundation of generation mean analysis will be summarized here from a classic textbook on biometrical genetics by Mather and Jinks (1977), followed by techniques in the analytical procedure in the Materials and Methods in section of chapter 2 and 3.

1. 5. 1. Components of mean

When two loci are involved (disomic inheritance), there will be three genotypes, AA, Aa and aa in a segregating locus. There are two parameters required in order to measure the differences in phenotypic expression of these three genotypes. The mid-point between two homozygotes AA and aa is defined as *m*, mid-parent. A parameter *a* is defined to measure the departure of each homozygote AA and aa from the mid-parent

(often called mid-point), while the other parameter *d* measures the departure of heterozygote Aa from *m* (Fig. 4). Thus, parameters *a* and *d* represent additive and dominance effects, respectively.

Mather and Jinks (1977) used notations such as *d* for additivity and *h* for dominance, and they used *i*, *j*, and *l* to describe additive x additive, additive x dominance and dominance x dominance non-allelic interactions. Since the notation system was confusing many authors use a modification of Gamble's notation (1962). In this notation system, Gamble simply took the initial of each gene effect - *a* for additive, *d* for dominance, *aa* for additive x additive interaction, *ad* for additive x dominance interaction and *dd* for dominance x dominance interaction.

In Fig. 4, the genotype AA has an expression, $m + a$, while aa equals $m - a$ and Aa $m + d$. When dominance is absent, *d* will be zero and consequently the heterozygote's expression will equal *m*. In the case of complete dominance, *d* equals a. In the rare event that Aa falls outside the range between AA and aa, then it will display over-dominance. Single-gene over-dominance has not been verified.

Individual genes that contribute to gene effects normally cannot be distinguished. Considering two homozygous lines which differ at two loci, A-a and B-b, with no interaction or linkage between them, there will be two possible combination of genes in two lines. If one of them is AABB, then the other will be aabb. If the effect of these genes are simply additive, the first will depart from mid-point by $a_a + a_b$ and the second by $-(a_a + a_b)$. If the lines are AAbb and aaBB, they will depart from mid-point by $a_a - a_b$ and $-a_a + a_b$, respectively. When *k* loci are involved, $[a]$ symbolizes their pooled additive effects. Similarly, when two homozygous lines are crossed, the phenotypic expression of

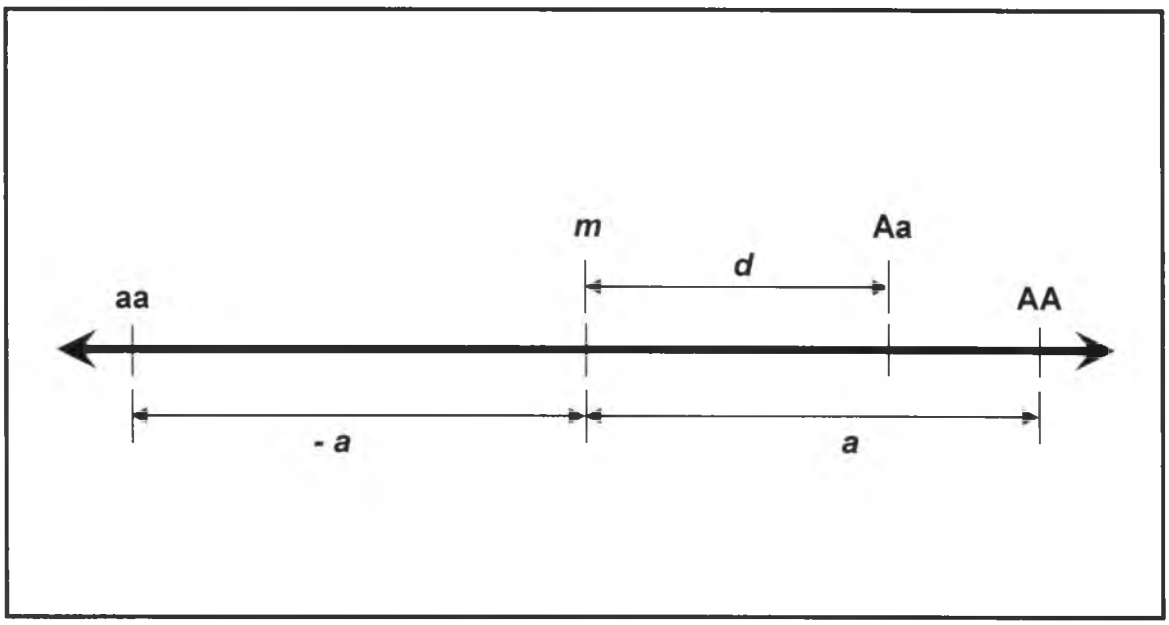


Fig. 4. The *a* and *d* increments of the gene difference A-a. Deviations are measured from the mid-parent, *m*, midway between the two homozygotes AA and aa. Aa may lie on either side of *m* and the sign of *d* will vary accordingly (Mather and Jinks, 1977). Notation for parameters *a* and *d* has been replaced with Gambles' one (1962).

heterozyotes will have pooled dominance effects represented by [*d*]. In each case, individual gene effects can be both positive and negative, and thus tend to balance out each others' effects.

There will be 1/4AA, 2/4Aa and 1/4aa when an F_2 is raised. Therefore, this gene will contribute $1/4a_a + 2/4d_a - 1/4a_a = 1/2d_a$ to the departure of average expression in F_2 from the mid-parent. When extended to *k* genes, the F_2 mean becomes 1/2[*d*] and the mean phenotype of the F_2 will be $\bar{F}_2 = m + 1/2[d]$. In the same way, we can generate $\bar{B}_1 = m + 1/2[a] + 1/2[d]$ and $\bar{B}_2 = m - 1/2[a] + 1/2[d]$. Components of means for generations that can be derived from two homozygous parents, P_1 and P_2, are summarized in Table 1.

Table 1. Components of means for different generations of a GMA on the six-parameter models (Mather and Jinks, 1977). Note that 3 parameter model involves only *m*, [*a*] and [*d*].

Generation	Six-parameter model					
	m	[*a*]	[*d*]	[*aa*]	[*ad*]	[*dd*]
P_1	1	1	0	1	0	0
P_2	1	-1	0	1	0	0
F_1	1	0	1	0	0	1
F_2	1	0	0.5	0	0	0.25
B_1	1	0.5	0.5	0.25	0.25	0.25
B_2	1	-0.5	0.5	0.25	-0.25	0.25

1. 5. 2. Scaling test for additive-dominance model

Now the following relations occur among observed generation means;

$\bar{B}_1 = 1/2(\bar{F}_1 + \bar{P}_1)$, $\bar{B}_2 = 1/2(\bar{F}_1 + \bar{P}_2)$, and $\bar{F}_2 = 1/4(2\bar{F}_1 + \bar{P}_1 + \bar{P}_2)$. The relationships can be rearranged to produce the following scales $A = 2\bar{B}_1 - \bar{F}_1 - \bar{P}_1$, $B = 2\bar{B}_2 - \bar{F}_1 - \bar{P}_2$, and $C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2$. The values of A, B and C must sum to zero within the limits of their standard error. These expected relationships can be used to test deviations from this model. The test of the deviations has been termed "scaling tests" by Mather (1949). Significance of deviations from zero for any one of these scales is an indication of the presence of non-allelic interaction. Singh and Chaudhary (1977) noted that A and B tests provide evidence on possible involvement of any of three interactions (*aa*, *ad* and *dd*), while C and D tests indicate the presence of *dd* and *aa* type interaction, respectively. Sets of such scaling tests can be devised to cover any combination of generations.

1. 5. 3. Joint scaling test and perfect fit

Cavalli (1952) proposed a procedure known as "joint scaling test" which combines the whole set of scaling tests into one, rather than testing the various expected relationships one at a time. This can also be applied to six-parameter model, in which three types of interactions are incorporated along with additive and dominance effects. The joint scaling test not only estimates the model's parameters, m, [*a*], and [*d*] but provides a test of goodness of fit of the model when number of families exceed that of parameters to be estimated. If the number of families available is same as number of

parameters estimated, no test can be made of the goodness of fit of the model. In such a case, "perfect fit" must be obtained.

1. 5. 4. Non-allelic interaction

There are two possible causes for departure from the additive-dominance model. These are non-allelic interaction and linkage. Two gene pairs, A-a and B-b are used here to explain the effects of non-allelic interaction without linkage. The two gene pairs can give rise to nine different genotypes with nine potential phenotypes (Table 2). The differences among these phenotypes can be described by eight parameters, which correspond to the 8 df among the nine types. Additive (a_a and a_b) and dominance (d_a and d_b) parameters were previously defined. The remaining four parameters represent epistatic interactions defined as aa_{ab}, ad_{ab}, ad_{ba}, and dd_{ab}.

Table 2. Phenotypes from nine genotypes comprising all combinations of A-a and B-b in the presence of non-allelic interaction without linkage (Mather and Jinks, 1977).

	AA	Aa	aa
BB	$a_a + a_b + aa_{ab}$	$d_a + a_b + ad_{ba}$	$-a_a + a_b + aa_{ab}$
Bb	$a_a + d_b + ad_{ab}$	$d_a + d_b + dd_{ab}$	$-a_a + d_b - ad_{ab}$
Bb	$a_a - a_b + aa_{ab}$	$d_a - a_b - ad_{ba}$	$-a_a - a_b + aa_{ab}$

If a_a and a_b are independent, a_a will be the same whether or not the difference AA-aa is measured in BB or bb background. Thus, AABB – aaBB will be equal to AAbb – aabb, that is, AABB – aaBB – AAbb – aabb = 0. However, in the presence of interaction, it is necessary to accommodate prospective interaction of a_a and a_b. Thus, phenotypes of AABB, AAbb, aaBB, and aabb involve either negative or positive interaction parameter aa_{ab}. The remaining interactions such as ad_{ab}, ad_{ba} and dd_{ab} incorporated into the corresponding genotypes similarly can provide contributions to the interaction phenotypes. In all cases, the coefficient of the interaction term is the product of the coefficients of the two main items by which the interaction takes place.

The definition of mid-point, *m,* as mid-way between two homozygous parents becomes no longer adequate when interactions occur. The average of a cross between AABB and aabb now has a mid-parent average of 1/2 $[(m + a_a + a_b + aa_{ab}) + (m - a_a - a_b + aa_{ab})] = m + aa_{ab}$. The alternative cross, AAbb x aaBB, gives a mid-parent of $m - aa_{ab}$. Therefore, there is a need to redefine the mid-parent as the mean of all the possible combinations which can be obtained from the two gene pairs, AABB, AAbb, aaBB and aabb.

The genotype AaBb in F_1 heterozygous can be a production of either of crosses between AABB and aabb or between AAbb and aaBB. Genes of each parent carrying the increasing allele of one gene and the decreasing allele of the other is said to be dispersed whereas genes of each parent carrying the increasing alleles together and the decreasing alleles in the other being associated. Association and dispersion of genes in F_1 and F_2 generations do not make difference in phenotypic expression. However, this relationship may need to be taken into account for generations such as the parental and backcross

generations. For example, with the association of genes, the parental phenotypes will have $m + a_a + a_b + aa_{ab}$ and $m - a_a - a_b + aa_{ab}$, while with dispersion, the phenotypes will be $m + a_a - a_b - aa_{ab}$ and $m - a_a + a_b - aa_{ab}$. This kind of difference will occur in backcross generations.

These theories are then generalized to cover the case of more than two gene loci. With dispersion, the a's of different genes tend to balance one another out, leading to define $[a]$ as the sum of the a's taking sign into account where some genes are associated in the parents while others are dispersed. This also can be applied to the definition of $[d]$ being the sum of the d's of the individual genes, although the sign of d does not depend on gene association nor dispersion but on the direction of the dominance itself (Table 3). Having taken into account the effect of association and dispersion as well as the direction of interaction, the following generalized formulations are produced.

$$\bar{P}_1 = m + [a] + [aa]$$
$$\bar{P}_2 = m - [a] + [aa]$$
$$\bar{F}_1 = m + [d] + [dd]$$
$$\bar{F}_2 = m + 1/2[d] + 1/4[dd]$$
$$\bar{B}_1 = m + 1/2[a] + 1/2[d] + 1/4[aa] + 1/4[ad] + 1/4[dd]$$
$$\bar{B}_2 = m - 1/2[a] + 1/2[d] + 1/4[aa] - 1/4[ad] + 1/4[dd]$$

Thus, genetic parameters can be estimated as follows;

$$m = 1/2\bar{P}_1 + 1/2\bar{P}_2 + 4\bar{F}_2 - 2\bar{B}_1 - 2\bar{B}_2$$
$$[a] = 1/2\bar{P}_1 - 1/2\bar{P}_2$$
$$[d] = 6\bar{B}_1 + 6\bar{B}_2 - 8\bar{F}_2 - \bar{F}_1 - 3/2\bar{P}_1 - 3/2\bar{P}_2$$
$$[aa] = 2\bar{B}_1 + 2\bar{B}_2 - 4\bar{F}_2$$
$$[ad] = 2\bar{B}_1 + \bar{P}_1 - 2\bar{B}_2 + \bar{P}_2$$
$$[dd] = \bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 + 4\bar{F}_2 - 4\bar{B}_1 - 4\bar{B}_2$$

Table 3. Interactions in the means of families of a digenic cross (Mather and Jinks, 1977).

	m	a_a	a_b	d_a	d_b	aa_{ab}	ad_{ab}	ad_{ba}	dd_{ab}
Associated	AABB x aabb								
$\bar{P}_1$	1	1	1			1			
$\bar{P}_2$	1	-1	-1			1			
$\bar{F}_1$	1			1	1				1
$\bar{F}_2$	1			1/2	1/2				1/4
$\bar{B}_1$	1	1/2	1/2	1/2	1/2	1/4	1/4	1/4	1/4
$\bar{B}_2$	1	-1/2	-1/2	1/2	1/2	1/4	-1/4	-1/4	1/4
Dispersed	AAbb x aaBB								
$\bar{P}_1$	1	1	-1			-1			
$\bar{P}_2$	1	-1	1			-1			
$\bar{B}_1$	1	1/2	-1/2	1/2	1/2	-1/4	1/4	-1/4	1/4
$\bar{B}_2$	1	-1/2	1/2	1/2	1/2	-1/4	-1/4	1/4	1/4

CHAPTER TWO

YIELD LOSS AND RESISTANCE TO CORN LEAF APHID

ABSTRACT

Corn leaf aphid causes occasional yield loss of maize in Hawaii. Severe aphid damage was experienced in a seed corn production nursery in late 2000. Yield loss by direct feeding ranged from 38.9% to 98.8% with average of 71.7%. To exploit sources of resistance, we developed an inoculation technique using a hair-pin clip cage and summarize our studies on genetics of resistance to corn leaf aphid through generation mean analysis. A total of 360 plants from two parents (Hi38-71; resistant and Hi27; susceptible), F_1, F_2 and backcrosses were artificially inoculated with three wingless aphids per cage. Aphid population increase was classified on a ten-point scale based on aphid density. Average ratings of resistant and susceptible parents were 2.89 and 7.25 respectively. The average F_1 rating (6.72) showed susceptibility to the aphid. Resistance began to show up in F_2 and backcross to Hi38-71, while backcross to Hi27 remained susceptible. Resistance to corn leaf aphid from Hi38-71 appeared to be monogenic and recessive. The clip-cage method seemed effective in separating resistant and susceptible plants in segregating populations. For future understanding of the underlying cause of resistance, various aspects of aphid growth and reproduction on different genotypes were also examined. Newly born aphids were fed on four different genotypes including two parents used in the genetic study. Aphids on Hi38-71 had poorest performance over all. Difference in days to 50% mortality might be the major cause of resistance in Hi38-71 against corn leaf aphid.

2. 1. INTRODUCTION

Corn leaf aphid is a cosmopolitan insect pest in the Graminae including barley, sorghum and maize. It occurs throughout the year in tropical climates and is considered an economically important cereal aphid (Blackman and Eastop, 1984). Aphid occurrence in temperate regions is dependent upon migration rather than overwintering of the aphid (Kieckhefer et al., 1974; Foott, 1977). Unlike in the tropics, the aphid species is considered a minor insect pest in maize in temperate regions. It appears to be linked to periodic and sporadic nature of aphid incidence.

Reduction in yield, however, is considerable, especially when population increase is accompanied by drought condition. Foott and Timmins (1973) reported up to 91.8% yield loss in heavily infected, drought-stressed maize. Resistance to corn leaf aphid is indeed present in maize in the form of single and multiple gene(s). Resistance in maize to corn leaf aphids was first reported in 1917 by Gernert (1917) in the F_1 hybrid between annual teosinte (*Euchlaena mexicana*) and yellow dent maize. Bing and Guthrie (1991) and Bing et al. (1992) found evidence for the involvement of multiple genes and a greater influence of additive gene effects relative to non-additive gene effects in resistance to the corn leaf aphid. Chang and Brewbaker (1976) and Lu and Brewbaker (1999), however, showed that resistance to corn leaf aphid was conditioned by a single recessive gene. Causes of resistance to corn leaf aphid have been reported to be a combination of plant morphology, soil and climatic conditions, and physiochemical factors (Coon et al., 1948). Despite the fact that resistance to the aphid species exists in maize, pesticide use has been a major practice for field control. This is in part due to lack of appropriate screening methods for identifying resistance genotypes.

The purposes of this study were to develop artificial inoculation techniques and quantification methods for screening aphid resistance in tropical maize and to apply them in field trials. Attempts were also made to study genetics of resistance in a sweet corn inbred, Hi38-71 through generation mean analysis and to investigate effect of the resistance on aphid performance. In addition, yield loss observed in a seed corn production nursery is reported.

2. 2. MATERIALS AND METHODS

2. 2. 1. Observation of yield loss

There was a severe aphid attack late in 2000 on a field corn seed production nursery at Waimanalo Research Station that provided an excellent chance to estimate yield loss by corn leaf aphid. The nursery was planted on 9th Sept. 2000 with two single cross female hybrids (H1012 and H1012Crf) and one male inbred (Hi26). The two female hybrids were isogenic lines produced by crosses between Hi34Crf and Hi34 as female parents and lCAL210 as a male parent. Hi34Crf is genetically identical to Hi34 except that Hi34Crf has C-cytoplasm which makes plants male sterile. Since the male parent in H1012 and H1012Crf lacks the fertility restorer gene for C-cytoplasm, H1012Crf becomes male-sterile while H1012 produces normal pollens. H1012Crf had been planted in an attempt to get rid of hand detasseling in seed production nursery.

Hybrids H1012 and Hi1012Crf were planted in 21 and 24 rows, respectively and the male parent Hi26 was planted every 4th row starting from the first row. Thus there were three rows of female hybrid (a block) in between male rows. Corn leaf aphid began

to colonize on male parents at late whorl stage. At tasseling, detasseling only took place on males of Hi1012. However, the last block of H1012Crf was detasseled by accident. At harvest, blocks were divided into three parts and a sample was taken from the center of each part and from second rows of each block. A sample consisted of ears from 10 adjacent plants. Sample ears were dried to 15% moisture content.

2. 2. 2. Generation mean analysis

A tropical super sweet corn inbred, Hi38-71, with high resistance to corn leaf aphid was crossed to a field corn inbred, Hi27, to produce F_1, F_2 and two backcross populations (BCr and BCs). Hi38-71 is one of sub-lines of a *brittle*-1- based commercial super sweet parental inbred, Hi38. Hi27 is a flint corn inbred and a parent of Near Isogenic Lines for more than 130 single maize mutants. Both inbreds have gone through numerous generations for years and were assumed to be highly homozygous.

Field evaluation for six generations (Pr, Ps, F_1, F_2, BCr and BCs) was carried out in spring and fall of 2002 at Waimanalo Agricultural Research Station of University of Hawaii. The experiments were designed as randomized complete blocks with three replications. Plots consisted of two 5-m rows for non-segregating populations (Pr, Ps and F_1) and four and six rows for the two backcrosses and F_2 populations, respectively. The two parents were paired during randomization and planted in adjacent plots to minimize competition from hybrid generations. Row and hill spacings were 0.75 and 0.2 m, respectively. Two untreated seeds were planted per hill and resulting plants were thinned to one per hill at around 3 weeks after plantings.

2. 2. 3. Hair-pin clip cage

In order to generate uniform infestations under field conditions and obtain better quantification of resistance, a clip-cage method has been employed. Prototypes of cages were obtained from a previous study (Chang, 1976) and other entomologists (Fig. 5). Chang's leaf cage had two nails embedded in opposing positions on one side of plexiglass tubing to pin down on a plywood floor support through a plant leaf. The tubing was covered with a removable lid. However, it is possible that this method of pinning down plant leaves induces biochemical changes affecting aphids. DIMBOA can occur as a result of wounds and may be responsible for increased corn leaf aphid resistance. Hence nails were replaced with a hair-pin clip which was used in a prototype from entomologists. However, the prototype from entomologists had closed top on the tubing so access to interior was impossible. The hair-pin clip cage used in this study was made to take advantage of two prototypes.

The cage (Fig. 5) consisted of three parts; (1) a bottom plate (3 x 3 cm), (2) a confining tube (2.2 cm i.d x 1.2 cm height) made from a transparent plexiglass and (3) a removable ventilation lid. The bottom plate was to support the tube. Plant leaves were placed between the tube and the plate. Foam sponge was attached to one side of the tubing which directly contacts leaf surface, in order to avoid wounding plants and to seal up the tube completely to leaf surface. The sponge also sucks away honeydews produced by aphids confined inside the tube. The lid top was covered with fine mesh cloth for ventilation and was easy to take off from the tube with a knife. The removable lid gave easy access to the cage interior and facilitated data collection when many cages were used.

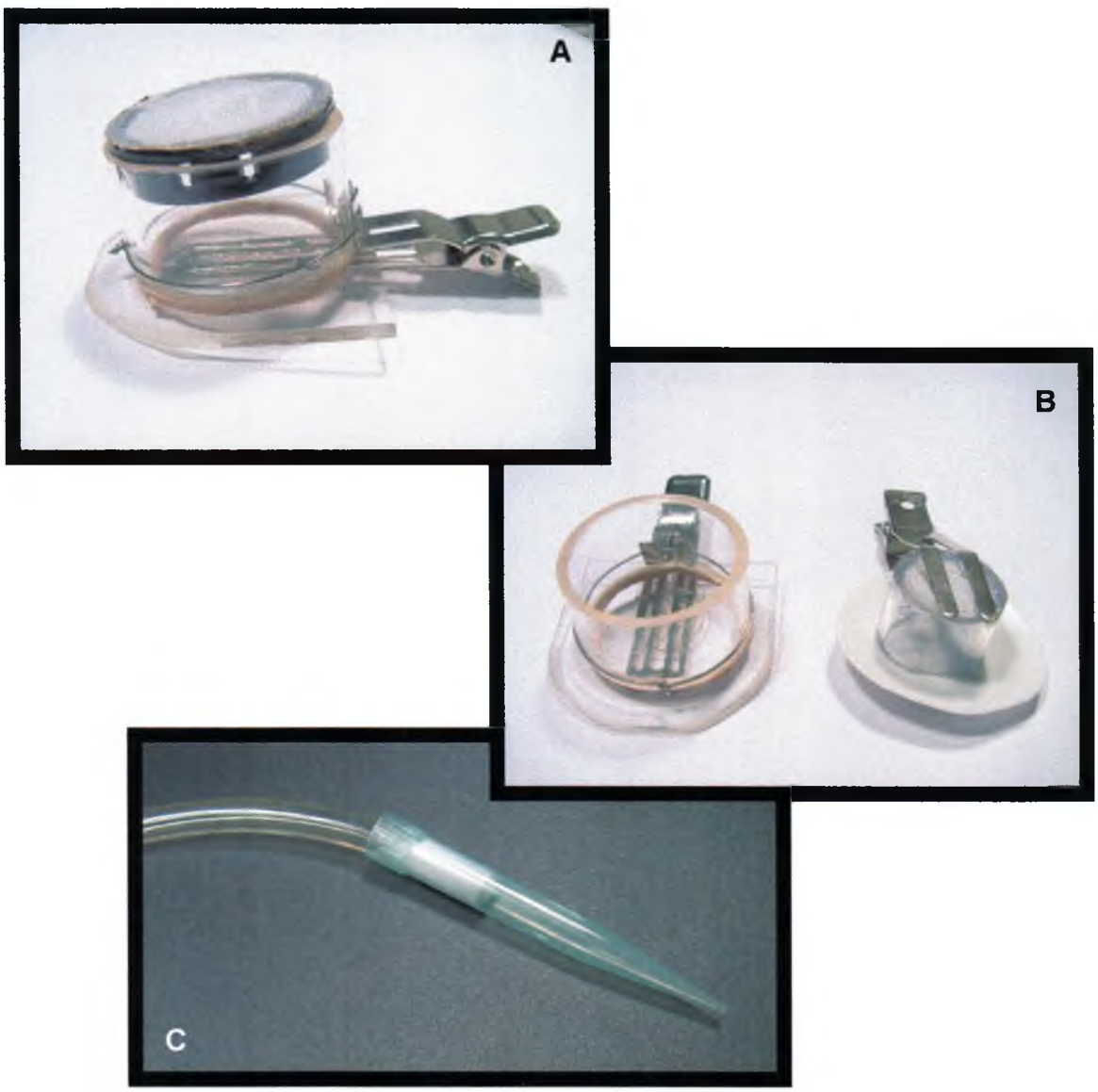


Fig. 5. Hair-pin clip cage and an aspirator. A. Hair-pin clip cage with a ventilation lid on top. B. prototype cage (right) in comparison to one used in this study (left). C. aspirator made and used for this study.

2. 2. 4. Inoculation

Natural population of corn leaf aphids was collected in maize breeding nurseries at the time of inoculation. Three wingless viviparous adults were carefully collected on maize tassels by an aspirator and were blown out into the cage. The aspirator (Fig. 5c) was made simply by plugging in mesh-cloth-covered one end of a 20cm flexible vacuum tubing (Tygon, R-3603 , 1/8" i.d. and 1/4" o.d.,) into a 1-ml micropipeting tip. The micropipeting tip was cut about 1mm from the tip to make the entrance hole big enough to accommodate an adult aphid.

Inoculation was made on three plants per row, giving total of 180 samples in each trial; 18 samples for Pr, Ps and F_1, 54 samples for F_2 and 36 samples for two backcross generations. The cage was clipped on to abaxial side of a fully expanded uppermost leaf of about 50 days old plant. The lower surface of corn leaves has more stomata (Kiesselbach, 1980) through which corn leaf aphid is found to be more frequently penetrating stylets during the late whorl stage (Bing et al., 1991). Leaves with cages were cut and brought into the laboratory 12 to 14 days after inoculation.

2. 2. 5. Quantification of resistance

Cages were removed from the leaf and close-up digital photographs of confined area covered with aphids were obtained by a Sony DSC-F505V digital camera (Sony Co., Japan) at high resolution of 2,240 x 1,680 pixels. The camera has a built-in macro mode which enables one to take a close-up digital picture of an object as close as 2mm from its lens. The images were transferred to a personal computer and were rated visually from 1 to 10 on the computer monitor based on the aphid density or coverage of the confined

area. Rating score 1 was considered to have aphids covered with 0 ~ 10% of the area. Similarly, aphid coverage at 91 ~ 100% was given a rating score 10. There were no samples rated 10, however, since cast skins were left as aphids morph, covering up approximately 10% of the area at maximum. Cast skins were not included in aphid coverage rating.

2. 2. 6. Statistical methods

Analysis of variance over seasons was conducted for the genetic study of corn leaf aphid prior to generation mean analysis. If the source of variation for seasons was significant, generation mean analyses were carried out for each season as well as across seasons. Data from all samples in all replications were pooled for the computation of generation means, while variances for each generation were calculated by averaging variance of each replication in same generation. Variance of mean for a generation was obtained by its variance divided by number of plants in the generation. Standard error of a generation was a square root of variance of mean for the generation.

The initial analyses were under the assumption that observed variation was due to additive and dominance effects with no epistasis or linkage (additive-dominance model, or often called 3 parameter model). The assumption was tested by simple scaling test and was confirmed by a joint scaling test with the 3-parameter model. Joint scaling test could not only be used to test the adequacy of 3- and 6-parameter model, but provided estimates of genetic parameters and expected means for each generation.

Each scale and its variance were computed by the following provide by Mather and Jinks (1977);

$$A = 2\bar{B}_1 - \bar{F}_1 - \bar{P}_1 \qquad V(A) = 4V(\bar{B}_1) + V(\bar{P}_1) + V(\bar{F}_1)$$

$$B = 2\bar{B}_2 - \bar{F}_1 - \bar{P}_2 \qquad V(B) = 4V(\bar{B}_2) + V(\bar{P}_2) + V(\bar{F}_1)$$

$$C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2 \qquad V(C) = 16V(\bar{F}_2) + 4V(\bar{F}_1) + V(\bar{P}_1) + V(\bar{P}_2)$$

The variance was calculated from variance of means and standard error was the square root of the variance. Significant difference of individual scale was accessed by a Student's *t*-test at the degree of freedom which was a sum of degree of freedom for the generations involved in each scale. Degrees of freedom for each generation was the number of plants in a generation minus numbers of replication (Singh and Chaudhary, 1985). If the scales are adequate, the values of A, B and C should be zero within the limits of their respective standard errors. On the other hand, significance of any one of these scales indicates the presence of non-allelic interaction.

The joint scaling test utilizes a weighted least square (multiple linear regression) with weights that are the reciprocals of the variance of mean of the generation means. It was indicated that the reason for weighting is that all of the genetic information is not estimated with the same precision (Mather and Jinks, 1977). Computational procedure was modified from Rowe and Alexander (1980);

N matrix

$$\begin{bmatrix} N(P_1) & & & & & \\ & N(P_2) & & & & \\ & & n(F_1) & & & \\ & & & n(F_2) & & \\ & & & & n(B_1) & \\ & & & & & n(B_2) \end{bmatrix}$$

S matrix

$$\begin{bmatrix} V(P_1) & & & & & \\ & V(P_2) & & & & \\ & & V(F_1) & & & \\ & & & V(F_2) & & \\ & & & & V(B_1) & \\ & & & & & V(B_2) \end{bmatrix}$$

Y matrix

$$\begin{bmatrix} \bar{P}_1 \\ \bar{P}_2 \\ \bar{F}_1 \\ \bar{F}_2 \\ \bar{B}_1 \\ \bar{B}_2 \end{bmatrix}$$

C matrix

$$\begin{bmatrix} 1 & 1 & 0 \\ 1 & -1 & 0 \\ 1 & 0 & 1 \\ 1 & 0 & 0.5 \\ 1 & 0.5 & 0.5 \\ 1 & 0.5 & 0.5 \end{bmatrix}$$

M matrix

$$\begin{bmatrix} m \\ [a] \\ [d] \end{bmatrix}$$

Matrices were defined as follows; the matrix N consisted of the number of plants as diagonal elements, while S matrix contained, as its diagonal elements, variance of generation, not variance of mean. For example with first parent, $V(P_1)$ was the variance of P_1 while $V(\bar{P}_1)$ was the variance of mean of P_1 obtained by $V(P_1) / n(P_1)$. Matrix Y was a column vector of the generation means. The C matrix depended upon the genetic model and here consisted of the genetic expectations of the six generations in terms of the three parameters, *m*, [*a*] and [*d*]. Beginning with three-parameter model, C matrix has been replaced with six parameters and then later with five parameters based on perfect fit result. Finally, M was the vector of the genetic parameters to be estimated by least squares.

Parameter estimates ($\hat{E}$) for the model applied to a joint scaling test were obtained by the following equation ; $\hat{E} = (C' \times N \times S^{-1} \times C)^{-1} \times (C' \times N \times S^{-1} \times Y)$, where ' indicates the transpose and $^{-1}$ the inverse. Computation of matrices was done in Quattro Pro 10, a spreadsheet computer program (COREL Co. Ltd., 2001). Built-in spreadsheet functions used were "@MINVERSE" for inverting a matrix, "@TRANSPOSE" for transposing a matrix and "@MMULT" for the product of two matrices. Variance of parameter

estimates ($V(\hat{E})$) was simply the diagonal elements of $(C' \times N \times S^{-1} \times C)^{-1}$ and standard errors were the square root of variance of parameter estimates. Student's *t*-test at (k-p) degree of freedom was used to test significance of parameter estimates.

Expected generation means ($\acute{Y}$) were derived by multiplying C matrix with the estimated genetic parameters ($\hat{E}$). Further, χ^2 value of a goodness of fit was obtained for the assessment of a genetic model by the following equation;

$\chi^2_{(k-p)} = (Y - \acute{Y})^{-1} \times (N \times S^{-1}) \times (Y - \acute{Y})$, where k is the number of generation means and p is the number of parameters estimated. Hence, the computed χ^2 value was compared at (k-p) degrees of freedom to tabular value.

2. 2. 7. Survival and reproduction of corn leaf aphid.

Ten plants from four genotypes, Hi38-71, G24, 190 and Hi27 were sampled in a breeding nursery during winter of 2002 for this study. One cage per plant was attached to a lower leaf surface in the manner described above. Three apterous aphids collected in field were transferred into the cages in the morning and were removed in late afternoon, leaving newly born 1st instars in the cages. All the apterous aphids reproduced a few offspring in each cages. First nymphs born on the day of inoculation (0th day) were considered at the same age. Instars were allowed to feed for 3 days to insure adaptation in the confined area. Three days after adult removal, all except one offspring were also removed, thus only one aphid was left, feeding in the confined area. This aphid was marked first generation and was observed everyday.

When the first generation began to reproduce, newly born nymphs per cage were counted and removed until there were no longer nymphs produced. First generations were

observed until death. Data obtained were (1) total number of progenies produced by ten aphids, (2) days to first winged form aphid emergence, (3) number of winged form emerged, (4) total number of progeny produced by wingless form, (5) days to first progeny emergence, (6) days to 50% mortality, (7) average life cycle (days) and average reproduction period (days).

2. 3. RESULTS

2. 3. 1. Yield loss trial

There was great difference in yield between H1012 and undetasseled H1012Crf female parent (Fig. 6). Both detasseled H1012 and H1012Crf female parents supported fewer aphids than undetasseled H1012Crf. There was exponential growth of aphid population on tassels of H1012Crf. Numerous aphids were observed over entire plants. Predators came in late after mid-silking and began to feed on corn leaf aphid, but initial number of predators was not sufficient to reduce the aphid population below economically damaging levels in a short period of time. Aphid feeding and honeydew production up to mid-silking, in the absence of predators resulted in many barren and poorly filled ears in undetasseled H1012Crf block.

Average sample weights of detasseled H1012 and undetasseled H1012Crf were 1402.7g and 457.8g, respectively. Range of sample weights from undetasseled H1012Crf was 19~987g. Despite great yield reduction observed in undetasseled H1012Crf block, one block of H1012Crf that was mistakenly detasseled produced the highest yield

Fig. 6. Yield loss by outbreak of corn leaf aphid in seed corn production nursery. Sample of ten adjacent plants from two isogenic single cross female parents (above – H1012 (male fertile, detasseled), below – H1012Crf (male sterile, undetasseled)). Five ears out of ten plants were barren in H1012Crf.

(1616.7g). When this was compared to individual samples from undetasseled H1012Crf, yield loss due to aphid feeding ranged from 38.9% to 98.8% with average of 71.7%.

2. 3. 2. Resistance in Hi38-71

Performance of corn leaf aphids in the hair-pin clip cages was good enough to establish initial populations in the confined cage area. The viviparous adult aphids transferred into the clip cage started reproduction and offspring were observed around their parents the day after inoculation. After 12 ~ 14 days, some of early nymphs became adults and produced offspring. Most of them became the winged form while a few morphed to wingless adults. Thus, two generations were coexisting in the form of nymphs, winged and wingless adults in one cage. In most cases, initial adults had died when cages were removed.

Fig. 7 shows a close-up view of confined area after the cages were removed. There was a great difference in population increase on resistant (Hi38-71) and susceptible (Hi27) plants. Rating scores for sample pictures in Fig. 7 were 2 for Hi38-71 and 8 for Hi27, with aphid counts of 61 and 270, respectively. Cast skins took up some spaces in the confined area, but were not considered in the coverage ratings.

Analysis of variance for mean aphid coverage ratings from two seasons revealed significant differences between two seasons and among six generations (Table 4). Significant differences among generations were expected, while the seasonal difference was a somewhat unexpected event. However, the interaction between generations and seasons was not significant. This indicates that overall performance of aphids was

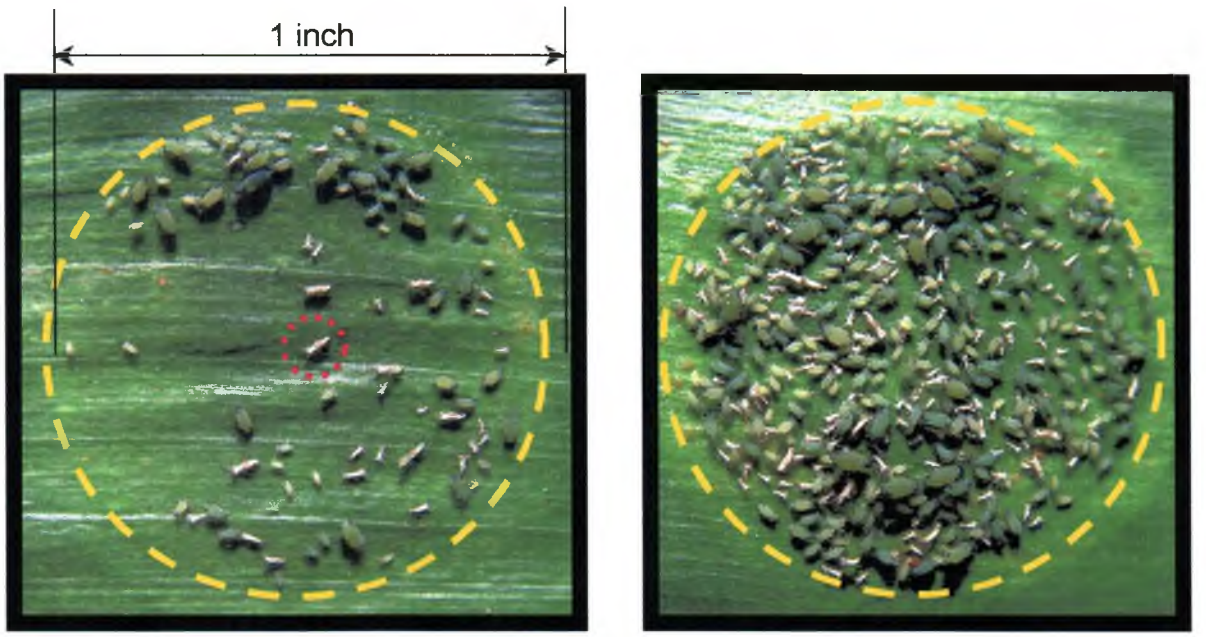


Fig. 7. Aphid population increase in hair-pin clip cage 12 days after inoculation. Rating score for Hi38-71 and Hi27 is 2 (11~20% coverage) with 61 aphids and 8 (71~80% coverage) with 270 aphids, respectively. A white substance in a red circle is a cast skin left over by an aphid. Area taken by cast skins are excluded from estimation of aphid coverage.

different in the two seasons, but remained consistent among generations within each season. The consistency was also reflected by correlation between mean rating scores of the generations in two seasons ($r = 0.96$). Despite use of highly homozygous parents, some variations in aphid performance within lines were observed.

Table 5 summarizes average rating scores and standard errors of aphid coverage for six generations in two seasons. Overall performance of corn leaf aphid in fall was better than that in spring. That is, means in fall for all generations were higher than those in spring. Average CVs' were as follows for the two seasons; Pr 35.9%, Ps 16.5%, F_1 17.1%, F_2 36.0%, BCr 36.9% and BCs 16.5%.

For the spring trial, mean rating scores for resistant (Hi38-71) and susceptible (Hi27) parents were 1.83 and 6.56 with mid-point of 4.19, respectively. F_1 hybrids supported slightly fewer aphids (6.06) than the susceptible parents, but this difference was not statistically significant. Resistance segregated in F_2 population (4.91). Backcrosses to the susceptible parent gave mean aphid rating of 7.00, high susceptibility, even exceeding that of susceptible parents. Mean of backcross to the resistant parent (4.53) was not significantly different from mid-parent value (4.19), with large variances.

Data taken in the fall showed higher mean aphid coverage ratings. The mean rating scores for resistant and susceptible parents were 3.94 and 7.94, respectively, with mid-point of 5.94. F_1 hybrid (7.39) also was susceptible, but less than the susceptible parent. Resistance segregated in F_2 population (6.83 ± 0.23). Two backcross generations also showed higher means than in summer (7.25 and 6.06 for backcross to susceptible and resistant parent, respectively). When two seasons were combined, mean aphid coverage ratings were 2.89 and 7.25 for resistant and susceptible parents, respectively,

Table 4. Analysis of variance for mean aphid coverage ratings for six generations of Hi38-71 (resistant) x Hi27 (susceptible).

Sources	df	Sum of squares	Mean squares	*F*
Total	35	126.74		
Seasons	1	18.22	18.22	13.57*
Reps in seasons	4	5.37	1.34	
Generations	5	80.56	16.11	16.60**
Generation x Seasons	5	3.18	0.64	0.66^{ns}
Error	20	19.41	0.97	

***, ** significant at 5% and 1% level of probability, respectively.**
^{ns} not significant.

Table 5. The corn leaf aphid coverage ratings for two parents, Hi38-71(Pr) and Hi27(Ps), F_1, F_2 and backcross (BCr & BCs) generations.

Generations	Corn leaf aphid coverage rating (1~10)†		
	Spring	Fall	Combined
Ps	6.56 ± 0.342	7.94 ± 0.206	7.25 ± 0.200
Pr	1.83 ± 0.224	3.94 ± 0.264	2.89 ± 0.173
Mid-parent	4.19	5.94	5.07
F_1	6.06 ± 0.331	7.39 ± 0.194	6.72 ± 0.192
F_2	4.91 ± 0.332	6.83 ± 0.234	5.87 ± 0.203
BCs	7.00 ± 0.185	7.25 ± 0.206	7.13 ± 0.139
BCr	4.53 ± 0.305	6.06 ± 0.345	5.29 ± 0.230

† Rating scale (1~10); 1 = 0~10% of confined area covered with aphids, 10 = 91~100% of confined area covered with aphids.

with mid-parent value of 5.07. Their F_1 hybrid mean of 6.72 was less than that of the susceptible parent. Mean rating for F_2 (5.87) and backcross to resistant parent (5.29) was lower than backcross to susceptible parent (7.13). There was no plant rated 10 because cast skins were not included in the estimation of aphid coverage.

Frequency distributions of combined aphid coverage ratings in the six generations clearly showed the genetic differences and segregation pattern of resistance in F_2 and backcrosses to resistance (Fig. 8). Parental distributions showed great differential response to aphid colonization. The F_1 hybrids had same response as the susceptible parent, indicating a recessive nature of resistance. However, these non-segregating populations showed a wide range in the data values. Segregating populations were distributed very widely, as expected. There were more susceptible than resistant individuals in F_2 population, while backcross to resistant parent had about equal numbers of susceptible and resistant individuals. There was no clear distinction between resistance and susceptibility among populations observed, with intermediates that made it difficult to define a classic 3:1 segregation. Nevertheless, the backcross to resistant parent appeared to provide strong evidence of 1:1 segregation. There were fewer intermediates in the backcross to resistant parent than in the F_2. Although the backcross to the susceptible parent had the same range (4 ~ 9) of rating scores as the susceptible parent, most data fell between 6 and 9, with mean of 7.13. The frequency distributions of segregating populations suggest that the resistance in Hi38-71 may be controlled by a single recessive locus.

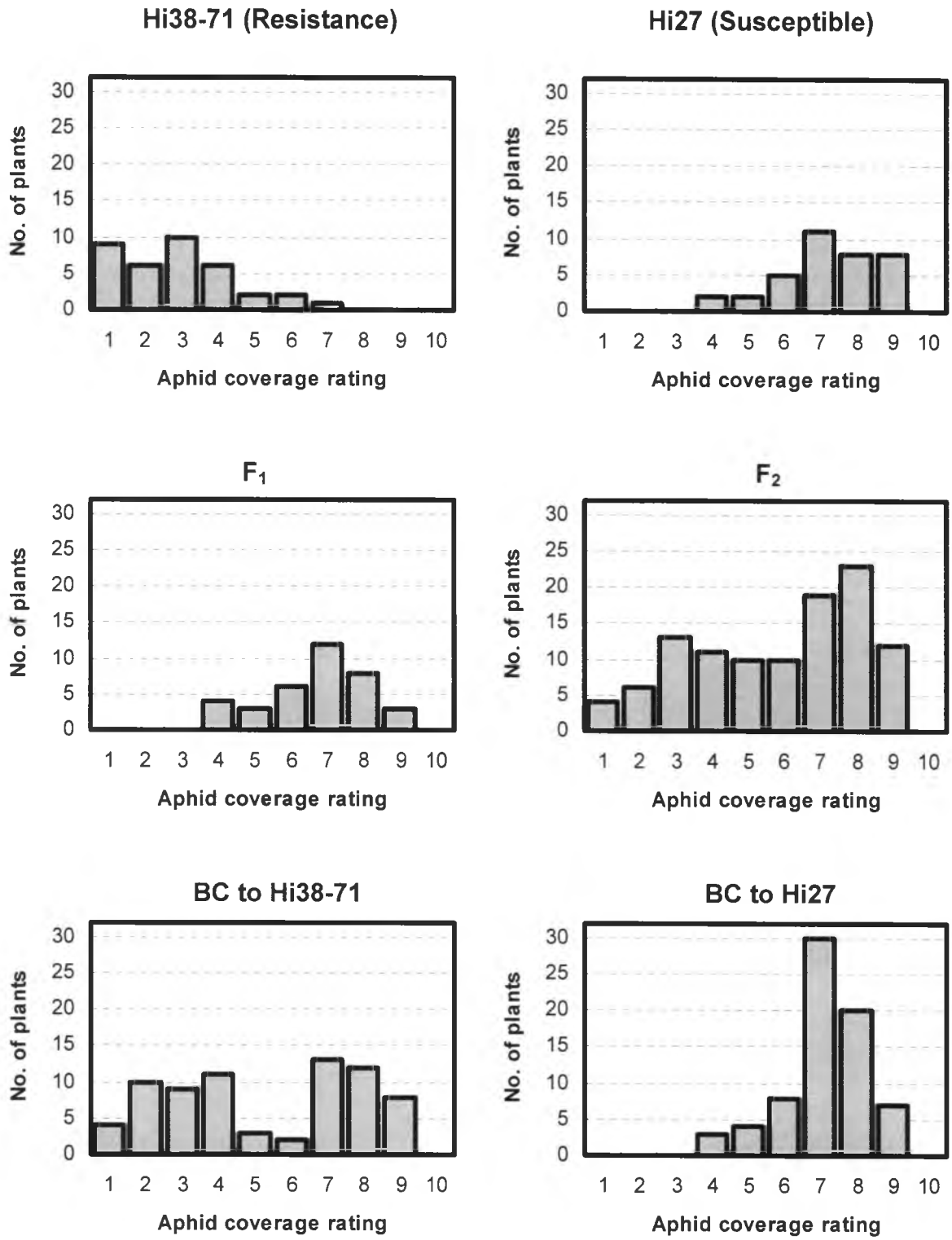


Fig. 8. Frequency distribution of aphid coverage rating in six generations of Hi38-71 x Hi27 family from combined data.

Generation mean analysis

Since analysis of variance showed significant seasonal effects, generation mean analyses (GMA) were conducted on each season and then two seasons were combined for the analysis. Individual scaling tests for each season and across seasons were performed as an initial investigation to uncover possible involvement of non-allelic interactions among or between genes contributing to resistance (Table 6). One of scaling tests in spring trial indicated a significant deviation from zero while no significant differences were found in the fall trial or from the combined data. It is concluded that a simple additive-dominance model explains the data, without consideration of epistatic variances.

Generation mean analyses with additive-dominance model on each season and across seasons were carried out by a joint scaling test using a weighted least square technique. Joint scaling test has advantages over individual scaling test that this test can not only confirm the individual scaling test result, but also provide estimates of genetic parameters (m, a & d) and expected means for each generations, which can then be used for goodness of fit for the model applied.

Table 6. Individual scaling tests on aphid coverage ratings from Hi38-71 x Hi27 family.

Test	Spring	Fall	Combined
$A = 2BCr - Pr - F_1$	1.17 ± 0.729 *	0.78 ± 0.764 ns	0.97 ± 0.528 ns
$B = 2BCs - Ps - F_1$	1.39 ± 0.603 ns	-0.83 ± 0.501 ns	0.28 ± 0.392 ns
$C = 4F_2 - 2F_1 - Pr - Ps$	-0.87 ± 1.540 ns	0.67 ± 1.066 ns	-0.10 ± 0.937 ns

*** significant deviation from zero, according to a *t*-test at the 5% level of probability. ns not significant.**

Generation means predicted from the 3-parameter additive-dominance model fit to the observed means from the fall trial and combined data. Goodness of fit of χ^2 test for the spring trial, however, turned out to be significant (Table 7 & 8). This is in agreement with individual scaling tests. For spring trial, individual scaling tests and generation mean analysis implied that there might be non-allelic interaction involved and a need of incorporating additional set of parameters. However, in combination with fall trial, the significant deviation of expected means from observed means became insignificant.

2. 3. 3. Survival and reproduction of aphids

Corn leaf aphids were evaluated on four different maize genotypes of varying aphid tolerance. Table 9 shows general trends of aphid performance during a complete cycle. In general, observations presented in Table 9 were in agreement with level of resistance/ susceptibility observed over years under natural conditions. Aphids on Hi38-71 had the poorest performance over all.

Total number of progenies produced by 10 aphids ranged from 65 for Hi38-71 to 213 for inbred 190. Aphids fed on Hi27 have produced slightly more offspring than Hi38-71, but far less than G24 and 190. There seemed to be no difference in juvenile period among genotypes. Instars took about 7 days to become adults. All aphids morphed into winged forms except two on 190. Those two wingless form adults produced 100 progenies that constituted half the population on 190.

Aphids started to give birth 7 to 9 days after being born. Aphids on the most susceptible inbred 190 took the shortest time (7 days) to have the first progeny emerge,

Table 7. Joint scaling test of the additive-dominance (3 parameter) model on Hi38-71(Pr) x Hi27(Ps) family for aphid coverage rating from spring and fall trials.

Gene-ration	Season	No. of Plant	Variance of mean	Weight†	Model			Mean		Difference O – E
					m	[*a*]	[*d*]	Observed	Expected	
Ps	Spring	18	0.117	8.526	1	1	0	6.56	6.93	-0.370
	Fall	18	0.043	23.478				7.94	7.81	0.132
Pr	Spring	18	0.050	20.000	1	-1	0	1.83	1.87	-0.035
	Fall	18	0.070	14.336				3.94	4.10	-0.153
F_1	Spring	18	0.109	9.153	1	0	1	6.06	6.48	-0.422
	Fall	18	0.038	26.557				7.39	7.35	0.034
F_2	Spring	54	0.110	9.051	1	0	0.5	4.91	5.44	-0.530
	Fall	54	0.055	18.313				6.83	6.65	0.178
BCs	Spring	36	0.034	29.094	1	0.5	0.5	7.00	6.70	0.299
	Fall	36	0.043	23.486				7.25	7.58	-0.334
BCr	Spring	36	0.093	10.743	1	-0.5	0.5	4.53	4.17	0.355
	Fall	36	0.119	8.386				6.06	5.73	0.329
χ^2 (3)	Spring					9.31*				
	Fall					4.88 ns				

† weight = 1 / Variance of mean
*** significant deviation at the 5% level of probability.**
ns not significant

Table 8. Joint scaling test of the additive-dominance (3 parameter) model on the Hi38-71(Pr) x Hi27(Ps) family for aphid coverage rating from combined data.

Generation	No. of plant	Variance of mean	Weight†	Model			Mean		Difference O - E
				m	[*a*]	[*d*]	Observed	Expected	
Ps	36	0.040	25.019	1	1	0	7.25	7.28	-0.031
Pr	36	0.030	33.402	1	-1	0	2.89	2.97	-0.085
F_1	36	0.037	27.227	1	0	1	6.72	6.85	-0.132
F_2	108	0.041	24.229	1	0	0.5	5.87	5.99	-0.120
BCs	72	0.019	51.982	1	0.5	0.5	7.13	7.07	0.058
BCr	72	0.053	18.838	1	-0.5	0.5	5.29	4.91	0.378
χ^2 (3) = 3.95 ns									

† weight = 1 / Variance of mean
ns not significant

whereas those on the resistant inbred Hi38-71 took longer (9 days) than others. Reproductive period lasted only 6.1 days for Hi38-71, while that for others lasted longer (8.1 days for Hi27, 8.5 days for G24 and 11.3 days for 190). However, there was great difference in days to 50% mortality between resistant inbred Hi38-71 and others. Half of the aphids on Hi38-71 died by the 12th day. Other inbreds had 50% mortality at 23rd (G24) and 25th day (190 and Hi27). When compared to Hi27, which was the susceptible inbred used in the genetic study, difference in days to 50% mortality may be the major cause of difference of aphid coverage ratings seen in the previous genetic study.

Table 9. Performance of corn leaf aphid on different maize genotypes[†]

	Hi38-71	Hi27	G24	190
Level of aphid tolerance	High	Inter-mediate	Low	Very low
Total no. of progenies produced	65	74	128	213
Days to first winged form aphid emergence	7	7	7	7
Numbers of wingless form emerged	0	0	0	2
Number of progenies produced by wingless form	-	-	-	100
Days to first progeny emergence	9	8	8	7
Days to 50% mortality	12	25	23	25
Average life cycle (days)	17.7	21.4	19.8	23.9
Average reproduction period (days)	6.1	8.1	8.5	11.3

[†] **Ten samples per genotype.**

2. 4. DISCUSSION

2. 4. 1. Yield loss

Yield losses due to corn leaf aphid infestations were observed in this study to range widely and occasionally very high. Previous studies indicate that significant yield reduction by aphids may occur when a dry spell occurs during the growing season (Triplehorn, 1959; Foott and Timmins, 1973). Our observation, however, clearly shows that heavy infestation of corn leaf aphid itself can cause great yield reduction without drought stress in maize. This may be particularly true in tropical areas where corn leaf aphid is present throughout the year. Corn leaf aphids have no trouble finding their host plants even when there is no maize growing near them.

Population dynamics of corn leaf aphids in Hawaii correlates with population of their predators and parasites. Potential for aphid outbreaks is present throughout the year except during the summer growing season in Hawaii. It is unlikely that aphids can survive during hot and dry summer of Hawaii. During summer, aphids were rarely observed in the field. When temperatures and day-length began to drop, aphid populations started building up and reached a peak around October and November in fields, where tassels were about to emerge. It appears that it takes some time for predators and parasites to reach population levels at which biological control takes effect. If aphids successfully increase their population in absence of predators and parasites, yield could be greatly reduced. This exactly happened in a hybrid seed corn production nursery during the winter of 2000, where an average of 72% reduction in yield was observed. It seemed that arrival of predators and parasites after mid-silking was too late to prevent yield reduction in the field. This pattern of aphid population increase and biological

control repeated in early spring (late February to middle of March) when populations of predators and parasites had disappeared in the field. It must be emphasized that biological control has of great value in controlling aphid pest where no chemical treatment is needed. The use of resistant cultivars also plays a key role to prevent potential yield loss, particularly during the period where predators and parasites are absent from the field. Predators and parasites found in the Waimanalo Research Station are illustrated in Appendix A. It appeared that ladybird beetles and their larvae were the most prevalent species followed by syrphid fly larvae. Lacewing larvae were also found occasionally.

Major causes of yield loss seem to be related to barren ears and poor set of kernels by heavy infestation during tassel development and emergence. Foott and Timmins (1973) suggested that the most critical and vulnerable time for plant damage is two weeks prior to pollen shed during the late whorl stage. Excessive number of corn leaf aphids on emerging tassel consumes ample amount of phloem sap and disrupts pollen shed. Poor kernel set on ears of heavily infested plants does not only seem to be caused by the disruption of pollen shed but also by excessive honeydew produced by the aphids that land on silks.

The emerging tassel and whorl of maize were major feeding sites of corn leaf aphid. Aphids were rarely observed on exposed areas of plants, such as the leaf surfaces and stalks, due both to predators and parasites and to unfavorable environmental conditions like direct sunlight. Corn leaf aphids in the seed production nursery, however, were found all over the H1012Crf plants, literally covering entire maize plants when the tassels were not detached from H1012Crf.

There is no evidence that C-cytoplasmic male sterility is linked to corn leaf aphid susceptibility. Male florets provide a protective hiding place for aphids. One, however, should be aware of the possibility of linkage of certain type of cytoplasm to disease or insect susceptibility. Historical yield loss on seed corn production in early 1970's by southern corn leaf blight (*Bipolaris maidis*) is a great example of this kind.

2. 4. 2. Inoculation and quantification

Artificial inoculation and quantification methods developed in this study provided reasonable results. Although aphids responded differently in two seasons, there was no interaction between aphid performance and seasons. Thus, field evaluation with artificial inoculation still has some environmental effects on aphids. It is doubtful whether this kind of environmental effect could be eliminated under screenhouse or growth chamber. Crops like maize and sorghum are difficult to evaluate in large numbers under such conditions.

Some predators were able to crawl in and attack aphids confined in the cages. Lacewing larvae and, most frequently, syrphid fly larvae were occasionally found in some of cages, especially those in the spring trial. Readings from attacked cages might have biased the generation mean analysis of spring data, resulting in significant deviation from additive-dominance model. As the experiment was repeated and population size became larger, observed generation means became adequately explained by three-parameter model.

Aphids used in the artificial inoculation were collected from a natural population. There was no study done on the genetic structure nor biotypes of corn leaf aphid

populations in Hawaii. Genetic diversity does exist in corn leaf aphid populations in nature (Caballero et al., 2001; Steiner et al., 1985; Brown and Blackman, 1988) despite their parthenogenetic reproduction system. Aphids collected in the same field were assumed to be genetically identical, having come from a single clone of aphids.

An attempt was also made to utilize Digital Image Analysis (DIA) to measure the exact area covered by aphids in confined area. Since there were aphids at all different growth stages, sizes and forms, counting the number of aphids per cage alone may not accurately represent the population. DIA uses digital image softwares to analyzes a color property in a digital image and effectively separates two distinctive colors. DIA was demonstrated to effectively quantify turfgrass cover on the ground (Richardson et al., 2001). DIA in this study, however, failed to separate aphids from their cast skins and from leaf areas since aphids were more or less similar to maize leaves in terms of color. DIA may be useful with different species of aphids which are distinctive in color from leaves of their host crops.

2. 4. 3. Resistance to corn leaf aphid

The hair-pin clip-cage approach was effective in distinguishing resistant and susceptible maize plants and intermediates. Hi38-71 supported fewer aphids than the susceptible parent, Hi 27. Our data clearly shows that the resistance to corn leaf aphid in Hi 38-71 is controlled by a single recessive factor. This agrees with the previous findings of Lu and Brewbaker (1999) who evaluated Hi38-71 under natural corn leaf aphids infestation. Chang and Brewbaker (1974, 1976) also reported that resistance to corn leaf aphid in a maize inbred, AA8sh2, was under monogenic control. It is likely that the gene

discovered by Chang and Brewbaker (1974, 1976) may be the same since Hi38-71 has its origin from sweet corn inbred AA8sh2. Unfortunately, AA8sh2 has lost viability over the intervening time and there is no way to confirm this hypothesis. In addition to the monogenic resistance, resistance to corn leaf aphid in maize is also present in the form of multiple genes (Bing et al., 1992). Resistance to corn leaf aphid was also reported in barley and sorghum (Ram, 1983; Fisk, 1978).

The resistance described in this work was not completely toxic to the point of lethality to the aphids, but feeding on resistant plants may have altered somehow the growth or reproduction of the aphids, resulting in fewer aphids on resistant plants than on susceptible ones. Chang (1976) examined the resistance mechanism in AA8sh2 and found a strong antibiotic effect on corn leaf aphid, based on the number of days to reproduction, the number of nymphs produced and the rate of reproduction. In his study, corn leaf aphid was not completely eliminated by feeding on resistant parents. His observation also indirectly supports the hypothesis that the resistance in Hi38-71 may have been derived from the sweet corn inbred AA8sh2 by chance.

Plant secondary metabolites such as hydroxamic acid have been widely studied in relation to antibiotic effects on corn leaf aphids. Early studies demonstrated that DIMBOA concentrations had significant positive correlation with corn leaf aphid populations in maize (Chang, 1976; Long et al., 1977; Beck et al., 1983). This result was further supported by the fact generally accepted factor, that the maize seedling was almost immune to corn leaf aphid, and maize seedlings contained high concentration of DIMBOA. Recent studies, however, produced a conflicting result in which DIMBOA in maize may have no direct effects on corn leaf aphid (Bing et al., 1990). Cambier et al.

(2001) perceived that DIMBOA is not present in phloem sap when plants were not injured. Thus, rather than DIMBOA itself, they studied antibiotic effects of DIMBOA derivatives such as DIMBOA-Glc and HDMBOA-Glc present in phloem sap in the absence of wounding. The authors reported a negative correlation between high levels of these compounds and performance of *Metopolophium dirhodum* on artificial diet. It may help to understand the underlying mechanism of resistance in Hi38-71 to examine high dosage effect of those compounds on corn leaf aphid. Cry1Ab protein produced by Bt gene did not have significant effect on corn leaf aphid fed on artificial diets (Head et al., 2001).

Plant morphology may make some contribution to corn leaf aphid response in addition to chemical factors. Maize genotypes in which the tassel emerges quickly and completely from the upper leaves tend to reduce the aphid population (Coon, 1945). In contract those that enclose the tassel longer in the whorl provide a favorable habitat for aphids (Painter, 1968) and protection from their predators and parasites. Inbred Mo17 is known to exhibit moderately high resistance to corn leaf aphids. Tassels of this inbred emerge out of whorl quickly and completely. In contract, susceptible inbred 217 in the aphid performance test here has broad and tender leaves with its tassel very close to the uppermost leaf and often not completely emerged. Morphological traits such as leaf color, glossiness of leaf, days to maturity and number of tillers per row were not found to contribute towards resistance or susceptibility to corn leaf aphid in barley (Narang et al., 1997).

Breeding for resistance against corn leaf aphid appears to have received little attention so far due mainly to the periodic and sporadic nature of its occurrence in nature.

It is very difficult to attain uniform infestation under field conditions and there has been no effective inoculation technique for maize. Since corn leaf aphids are present year-round and hold potential threat to yield in this study, the use of resistant cultivars would be of benefit to small-scale farmers who hardly can afford the cost of insecticides.

Breeding for resistance to aphids could be achieved through backcrossing and selfing with the resistance gene of Hi38-71 and the clip-cage method under field conditions. Backcross procedure will be straightforward and will include hybridization to recurrent parents, followed by self-pollination and screening of BCF_2 for resistant individuals. The use of DNA markers associated with the resistance gene can replace the tedious artificial inoculation procedure and will improve the efficiency of backcross breeding.

CHAPTER THREE

ESTIMATION OF GENETIC PARAMETERS FOR POLYSORA RUST RESISTANCE

ABSTRACT

Polysora rust of maize (*Puccinia polysora* Underw.) is found primarily in the tropics and occurs throughout the year in Hawaii. Most temperate maize varieties are highly susceptible and must be sprayed regularly to minimize yield loss. In contrast, tropical maize varieties often are highly resistant to the rust. However, little genetic information has been published on this resistance. This information would be of special value in Hawaii, where hybrids of temperate x tropical maize are exploited. The genetic parameters for the rust resistance were estimated by evaluating six generations following the hybridization of inbreds G24 and Hi38-71 under uniform epiphytotics of the rust at Waimanalo Research Station. A visual rust rating score of 1 to 9 was adopted 15 to 20 days after mid-silking. Mean rating scores for Hi38-71 and G24 were 3.22 and 6.90, respectively. The F_1 hybrid showed high resistance to the rust (2.93), while F_2 had a higher mean (4.20) but showed a wide range of variation about the mean. A simple 3-parameter model (m, [*a*] & [*d*]) did not fully explain the data. However, the 5-parameter model that incorporated epistasis adequately explained the variation in resistance. Epistatic interactions aa and dd were highly significant, while the ad interaction was not. The high significance of non-additive and epistatic effects makes clear that polysora resistance breeding cannot be based on selection of a single parent. A hybrid-breeding or reciprocal-recurrent selection approach appears justified.

3. 1. INTRODUCTION

Polysora rust or southern corn rust is caused by *Puccinia polysora* Underwood. Host range of polysora rust is confined to maize and its relatives such as teosinte and *Tripsacum* speices (Ullstrup, 1977). Polysora rust is favored by high temperatures (27°C) and high relative humidity (Shurtleff, 1980). It mainly occurs in the tropics at elevations below 4,000 ft (1,220 m) and is present throughout the year in Hawaii. In the continental United States, the disease is principally seen in the southeastern states but has been reported as far north as Wisconsin (Pavgi and Flangas, 1959).

Polysora rust can be distinguished from common rust caused by *Puccinia sorghi* Sche. by its pustule size, shape and color (Ullstrup, 1977). However, it is considerably more devastating and has ability to kill the plants which common rust rarely does (Scott et al., 1984).

Severe polysora epiphytotics were observed in Africa during early 1950's (Robinson, 1996) and the Mississippi Valley during 1972~74 (Zummo, 1998). Observed yield losses by polysora rust range from 4% to 50% (Rhind et al., 1952; Rodriguez-Ardon et al., 1980).

Although several monogenic resistant genes have been identified so far (Ullstrup, 1965), these race-specific resistances were not durable and have been overcome by racial variation of the pathogen (Robinson, 1996). The resistance gene, *Rpp*9, was introduced to South Africa but soon broke down before commercial release of hybrids with the gene. General resistance has been identified in field corn (Bailey et al., 1987; Zummo, 1988; Moon, 1995, Holland et al., 1998). This type of resistance is also available in sweet corn

gemplasm and is particularly important in sweet corn breeding program for tropical regions.

In the winter cropping season of 2002, a uniform natural infection of the disease occurred at Waimanalo Research Station. Genetic variation was observed in F_2 and backcross families from the hybrid of Hi38-71 x G24, and attempts have been made to determine mode of gene action and to estimate genetic parameters through generation mean analysis.

3. 2. MATERIALS AND METHODS

3. 2. 1. Parent inbreds for generation mean analysis

Tropical super sweet corn inbred Hi38-71 (resistant) and a semi-dent maize inbred G24 (susceptible) were used to produce F_1, F_2 and two backcross populations (BCr and BCs). Hi38-71 was intermediate in mature-plant resistance and many tropical inbreds show greater resistance. The G24 inbred derived its susceptibility from corn belt dent inbred B68. G24 is one of the G Set Recombinant Inbred Lines (RILs) from the cross of Ki14 (a Thailand inbred) and B68 (as Hawaii conversion, Hi31). This G Set of RILs segregated approximately 50% susceptible: 50% resistant (Moon, 1995).

3. 2. 2. Disease evaluation

The experiment design and plot size were similar to those in studies of corn leaf aphid resistance. The epiphytotic of polysora rust in winter of 2002 was uniformly severe, and genotype evaluation relied on the natural infection. About two weeks after mid-

silking, ten individual plants per row were visually rated using 1 to 9 scale based on the plant appearance in relation to percent of leaf surface with rust infection, chlorosis and necrosis beyond the lesions. The rating scale was modified from a rating scale used in common rust evaluation by Kim et al. (1980):

1 = No symptom or less than 1% area on the lower leaves infected and considered monogenic resistance

2 ~ 3 = Resistant ; 2 - 20% area on the lower leaves and an ear leaf covered by the pustules.

4 = Moderately resistant ; 21 – 35 % area on the lower leaves and an ear leaf covered by the pustules.

5 = Intermediate ; 36 ~ 50% area of the lower leaves and an ear leaf covered by the pustules and ambiguous for classification into resistant or susceptible. Light infection on stalks.

6 = Moderately susceptible ; 51 – 65% area on the lower leaves, an ear leaf and upper leaves covered by pustules. Intermediate infection on stalks.

7 ~ 8 = Susceptible ; 66-80% are on the lower leaves, an ear leaf and upper leaves covered by pustules. Heavy infection on stalks.

9 = Highly susceptible ; More than 80% of the area on entire plant covered by pustules and premature death of plants.

3. 2. 3. Statistical methods

Initial analysis began with a three-parameter model that excludes non-allelic gene interactions. The model was tested by simple scaling tests followed by a joint scaling test, also used in the genetic study of resistance to corn leaf aphid. When the simple and joint scaling tests with three-parameter model were significant, the simple additive-dominance model (monogenic model) was judged inadequate, and non-allelic interactions were considered. The genetic model thus became digenic involving the six parameters *m*, [*a*], [*d*], [*aa*], [*ad*] and [*dd*]. No test of goodness of fit was possible with the six generation means, since no degree of freedom would be left for a new six-parameter model. In other words, a joint scaling test with six generations cannot test the six-parameter model.

Estimates of the six parameters and their variances for the test of significance were obtained from the following formulas of Mather and Jinks (1977) ;

$m = 1/2\bar{P}_1+1/2\bar{P}_2+4\bar{F}_2-2\bar{B}_1-2\bar{B}_2$ $\quad V(m) = 1/4\bar{P}_1+1/4\bar{P}_2+16\bar{F}_2+4\bar{B}_1+4\bar{B}_2$

$[a] = 1/2\bar{P}_1-1/2\bar{P}_2$ $\quad V([a]) = 1/4\bar{P}_1+1/4\bar{P}_2$

$[d] = 6\bar{B}_1+6\bar{B}_2-8\bar{F}_2-\bar{F}_1-3/2\bar{P}_1-3/2\bar{P}_2$ $\quad V([d]) = 36\bar{B}_1+36\bar{B}_2+64\bar{F}_2+\bar{F}_1+9/4\bar{P}_1+9/4\bar{P}_2$

$[aa] = 2\bar{B}_1+2\bar{B}_2-4\bar{F}_2$ $\quad V([aa]) = 4\bar{B}_1+4\bar{B}_2+16\bar{F}_2$

$[ad] = 2\bar{B}_1-\bar{P}_1-2\bar{B}_2+\bar{P}_2$ $\quad V([ad]) = 4\bar{B}_1+\bar{P}_1+4\bar{B}_2+\bar{P}_2$

$[dd] = \bar{P}_1+\bar{P}_2+2\bar{F}_1+4\bar{F}_2-4\bar{B}_1-4\bar{B}_2$ $\quad V([dd]) = \bar{P}_1+\bar{P}_2+4\bar{F}_1+16\bar{F}_2+16\bar{B}_1+16\bar{B}_2$

Standard errors of estimates were attained as the square root of variance and the test of significance for parameter estimates was equivalent to finding significant deviations from zero in the simple scaling tests.

Any non-significant gene effects were then eliminated resulting in a new genetic parameter model. For the computation, matrices C and M were modified to accommodate genetic parameters with significant difference. With a reduced number of genetic parameters, it became possible to have a test of goodness of fit for the new model in the joint scaling test.

3. 3. RESULTS

Epiphytotics of polysora rust started approximately one week before tasseling and became so uniform as to clearly distinguish individuals with different degree in resistance and susceptibility. Common rust (sorghi) infection is often observed in Waimanalo Research Station to follow after polysora rust, and can make it difficult to separate those two rusts for visual rating purpose. However, there was no common rust infection in these nurseries.

Generation means and standard errors are summarized in Table 10. Mean rating scores for resistant (Hi38-71) and susceptible (G24) were 3.22 and 6.90 with mid-point of 5.06, respectively. Hi38-71 showed high uniformity in expression of resistance to polysora rust. The F_1 hybrid showed uniformly high resistance to the rust (2.93), while F_2 had a higher mean (4.20) but showed a wide range of variation about the mean.

F_1 hybrid resistance was a bit higher than the resistant parents. Heterosis evidently played a role in contributing to resistance of the hybrids. Mean ratings for backcrosses to resistant and susceptible parents tended to lean toward its parental mean with a wider range. Standard errors for non-segregating generations were generally lower than those for segregating generations except the susceptible parent.

Table 10. Estimates of mean, variance, number of plant, variance of mean and standard error of polysora rust rating for the Hi38-71 x G24 family.

Generations	Mean†	Variance	C.V. (%)	No. of Plant	Variance of mean	Standard Error
Pr	3.22	0.176	13.0	60	0.0029	0.0542
Ps	6.90	0.377	8.9	60	0.0063	0.0793
Mid-parent	5.06					
F_1	2.93	0.195	15.1	60	0.0033	0.0570
F_2	4.20	0.922	22.9	180	0.0051	0.0716
BCr	3.82	0.452	17.6	120	0.0038	0.0614
BCs	5.70	0.706	14.7	120	0.0059	0.0767

† **Rating scale (1~9); 1 = higly resistant, 9=higly susceptible**

Genetic differences in response to polysora rust became clear in frequency distributions (Fig. 9). Parental distributions differed greatly in response to polysora rust infection. Rating scores for Hi38-71 had a very narrow range, reflecting high uniformity in resistance to polysora resistance. A somewhat wider range in polysora rust ratings was observed in G24, largely a function of scale. G24 was produced by six successive self-pollinations from a cross between Ki14 and B68 followed by a few sib-matings for seed production while Hi38-71 results from years of self- and sib-pollinations and is considered highly homozygous.

The F_1 hybrid showed a lower mean and wider range than the resistant parents. Segregation in F_2 population resulted in a very wide range of response to polysora rust, but the mean (4.2) remained toward resistance. None of the F_2 plants exceeded parental ranges. The distribution of polysora rust ratings in backcross to resistant and susceptible parents showed wider ranges, with their means biased toward those of the parents.

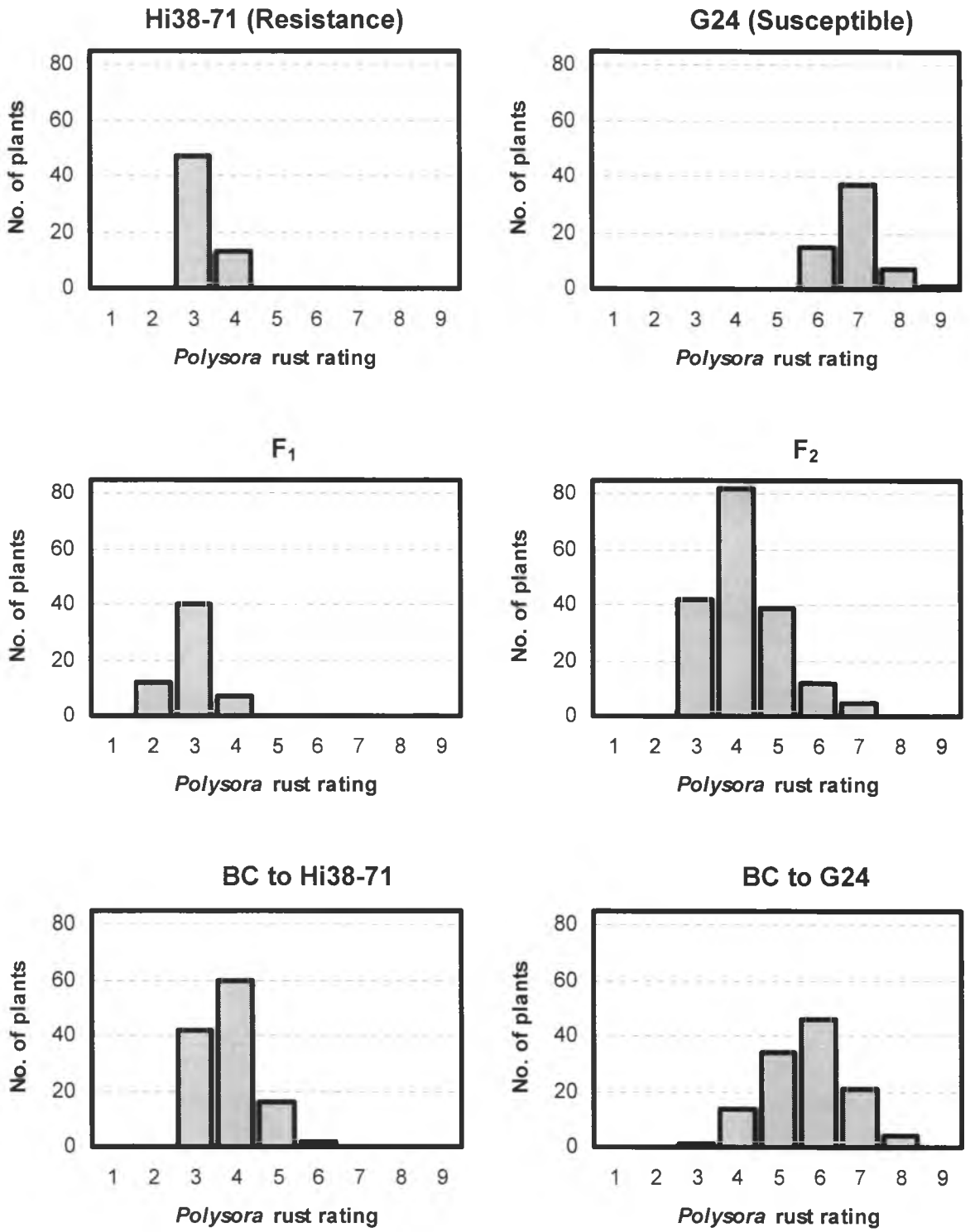


Fig. 9. Frequency distributions of polysora rust rating in the six generation of the Hi38-71 x G24 family.

The possible involvement of non-allelic interaction was initially investigated with individual scaling tests (Table 11). All scale tests were found to be significant, implying that non-allelic interactions were involved in the observed genetic variation. The joint scaling test for additive-dominance model (Table 12) was also employed. It confirmed the individual scaling test with a goodness of fit value for additive-dominance model that was highly significant. Thus it can be concluded from the individual and joint scaling tests that observed variation among six generations can not be explained with three genetic parameters.

Three epistatic interactions, [*aa*], [*ad*] and [*dd*] were then included for analysis. All six parameters were computed by the perfect fit method proposed by Mather and Jinks (1977). The test revealed that all but the additive x dominance interaction were highly significant (Table 13). No test of the adequacy of the six-parameter model was possible because the number of estimated components was equal to that of observed means, leaving no degree of freedom for the test of goodness of fit. However, since there was no significant [*ad*] interaction, the non-significant component was omitted to fit a five-parameter model. This provided a mean to test the goodness of fit of the five-parameter model, with one degree of freedom. At the same time, it improved the precision with which the remaining parameters were estimated.

The joint scaling test for the five-parameter model produced a χ^2 value of 0.13, indicating no significance departure from expectation (Table 13) and validating the model. Moreover, all five parameters remained significant after fitting the five-parameter model.

Table 11. Individual scaling tests on polysora rust rating from the Hi38-71 x G24 family.

Test		
$A = 2BCr - Pr - F_1$	=	$1.49 \pm 0.146^{**}$
$B = 2BCs - Ps - F_1$	=	$1.57 \pm 0.182^{**}$
$C = 4F_2 - 2F_1 - Pr - Ps$	=	$0.82 \pm 0.323^{*}$

***, ** significant deviation from zero, according to a *t*-test at the 5% and 1% level of probability, respectively.**

Table 12. Joint scaling test of the additive-dominance (3 parameter) model on a cross between Hi38-71 and G24 for polysora rust rating.

Gene-ration	No. of plant	Variance	Weight†	Model			Mean		Difference O - E
				m	a	d	Observed	Expected	
Pr	60	0.176	5.6818	1	1	0	3.22	3.40	-0.1814
Ps	60	0.377	2.6525	1	-1	0	6.90	7.15	-0.2458
F_1	60	0.195	5.1282	1	0	1	2.93	3.26	-0.3281
F_2	180	0.922	1.0846	1	0	0.5	4.20	4.27	-0.0659
BCr	120	0.452	2.2124	1	0.5	0.5	3.82	3.33	0.4902
BCs	120	0.706	1.4164	1	-0.5	0.5	5.70	5.20	0.4980
			$\chi^2(3) = 160.77^{**}$						

† weight = 1 / Variance of mean
**** significant deviation at the 1% level of probability.**

Thus, observed genetic variations can be fully explained by the five parameters including two types of epistatic interactions, [*aa*] and [*dd*]. Trigenic interactions and similar complex factors appear not to be making a significant contribution to the difference among the generation means. There was also a marginal improvement in the precision, reflected in slightly lower standard errors.

Table 14 shows estimates of expected generation means using the three- and five-parameter models. As previously observed, the three-parameter model produced expected means that differed significantly from observed means (χ^2=160.77). Expected means by the five-parameter model were essentially identical to observed means, with χ^2 value of 0.13.

Table 13. Estimates of the components of the generation means for the cross Hi38-71 x G24 fitting a six-parameter model by perfect fit estimation and a five-parameter model by the weighted least square.

	Six-parameter model	Five-parameter model
m	2.82 ± 0.351 **	2.84 ± 0.347 **
[*a*]	-1.84 ± 0.048 **	-1.85 ± 0.043 **
[*d*]	5.41 ± 0.836 **	5.36 ± 0.825 *
[*aa*]	2.24 ± 0.347 **	2.23 ± 0.345 *
[*ad*]	-0.08 ± 0.219 ns	—
[*dd*]	-5.30 ± 0.509 **	-5.27 ± 0.500 **
χ^2 (1)	—	0.13 ns

*, ** significant at 5% and 1% levels, respectively: ns, not significant.
— gene effect identified as non-significant from perfect fit estimation and, therefore, excluded in the weighted least square analysis.

Table 14. Estimates of expected generation means of 3- and 5 parameter model by the weighted least square.

Generation	Observed mean	Expected mean	
		3 parameter	5 parameter
Pr	3.22	3.401	3.215
Ps	6.90	7.146	6.911
F_1	2.93	3.258	2.930
F_2	4.20	4.266	4.200
BCr	3.82	3.330	3.833
BCs	5.70	5.202	5.680
$\chi^{2\dagger}$	—	160.77 **	0.13 ns

† χ^2 test at 3 df and 1 df for expected generation means by 3- and 5-parameter model.
** significant at 1%: ns not significant.

3. 4. DISCUSSION

This study demonstrates conclusively the involvement of epistasis in the resistance to polysora rust. A digenic model with additive x additive and dominance x dominance type epistasis adequately explained the observed genetic variation. Therefore, higher order interactions such as trigenic epistasis and similar complex factors do not make significant contributions to the differences in polysora rust resistance among the generations studied. The involvement of epistatic interactions for polysora rust resistance in field corn was also reported by Holland et al. (1998).

When epistasis is significant in such a study, some bias in estimating additive and dominance effects is expected (Hallauer and Miranda, 1981). This observation should be considered to provide a better understanding of maize-polysora rust association rather than to obtain precise estimates of gene effects.

Gene effects of quantitative traits often show interaction with environment (Gonzalez-Morezo and Dudley, 1981). No information was gained on gene effects in different genetic backgrounds or environments in this study. Further study is recommended to evaluate genotype x environment interactions of polysora resistance.

The results of this study provide direction for the breeding of polysora-resistant maize cultivars. A negative sign for [*dd*] suggests interaction between increasing and decreasing alleles. This gives evidence for some level of dispersion in the inbred, Hi38-71, and indicates that further improvement in the level of resistance in Hi38-71 is possible. Significance of [*a*] and [*aa*] also implies that part of resistance can be fixed in inbred lines of maize. Due to simultaneous significance of [*d*] and [*dd*], a reciprocal recurrent selection scheme seems most appropriate. Genetic gains through reciprocal

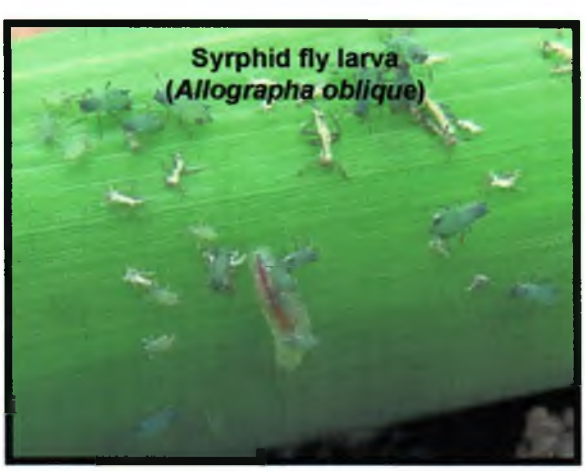
recurrent selection, however, are slow and time-consuming. If gene effect x environment interaction is present, testing in more than one location is required to detect favorable gene effects and for rapid breeding progress. In terms of selection sites in Hawaii, Waimanalo Research Station provides appropriate levels of natural infection in most years, although severity is somewhat higher during the winter. Development of artificial inoculation technique under field conditions and quantification of this kind of foliar disease might provide better understanding of plant-pathogen relationships.

Polysora rust resistance is more important in field corn, as sweet corns are often harvested before leaf diseases become serious. High levels of polysora resistance exist in tropical field corns, but using them in sweet corn improvement may result in having undesired traits such as thick pericarp along with the resistance.

Hi38-71 is a sib line of Hi38 which was bred from a *bt*-1 conversion (6 backcrosses) of AA8sh2. It was studied for its resistance to corn leaf aphid in 1970's in Hawai'i and was converted to common rust resistance, *Rp*1-D which broke down due to racial variation of the pathogen. *Rp*1-D is located on a short arm of chromosome 10 of maize which carries a cluster of complex resistance gene loci to common rust as well as polysora rust (Hulbert et al., 2001; Holland et al., 1998). Chang (1976) observed a possible linkage of corn leaf aphid resistance to the common rust resistance in AA8sh2. Resistance to corn leaf aphid in Hi38-71 probably originated from AA8sh2. Thus, it might be possible that the resistance to corn leaf aphid is somehow linked to the polysora rust resistance in Hi38-71 on a short arm of chromosome 10 in maize. Mapping with molecular markers will help determining the relationship between two resistances in Hi38-71.

Hi38-71 is particularly of value in sweet corn breeding for tropical regions not only due to its dual resistance to corn leaf aphid and polysora resistance, but to erectness, high sweet corn qualities, and generally good combining abilities (Nourse, 2000).

Appendix A. Predators of corn leaf aphid in Waimanalo Agricultural Research Station



Appendix B. Rating scores of corn leaf aphid coverage among Hi38-71 x Hi27 family.

Gene-ration	Aphid coverage rating score (1-9)								
	Spring				Fall				Grand mean
	R1	R2	R3	AVG	R1	R2	R3	AVG	
H27	6	9	6	6.56	9	8	7	7.94	7.25
	6	4	7		7	9	8		
	7	7	6		5	9	8		
	7	7	8		8	9	8		
	7	4	5		7	9	7		
	6	7	9		8	9	8		
Hi38-71	1	1	1	1.83	3	4	6	3.94	2.89
	3	3	1		4	4	6		
	3	1	1		3	4	7		
	2	3	1		2	4	5		
	1	2	2		2	3	3		
	1	4	2		3	5	3		
F_1	6	6	7	6.06	8	8	8	7.39	6.72
	6	5	9		9	8	7		
	4	9	7		7	8	7		
	7	5	7		6	6	8		
	4	4	6		7	7	7		
	4	5	8		8	7	7		
F_2	7	2	8	4.91	9	8	4	6.83	5.87
	4	1	8		8	7	7		
	6	4	7		7	5	7		
	3	5	3		6	5	8		
	1	8	4		7	7	8		
	6	3	3		7	9	9		
	2	7	3		8	4	4		
	6	4	2		9	7	5		
	7	3	1		8	8	7		
	4	3	8		8	8	8		
	6	7	3		9	9	4		
	5	9	2		9	8	7		
	3	8	3		8	5	5		
	2	1	7		9	4	6		
	2	8	6		7	3	6		
	6	8	8		5	7	3		
	5	9	8		4	9	8		
	3	7	6		9	8	5		

Appendix B. (continued)

Gene-ration	Aphid coverage rating score (1-9)								
	Spring				Fall				Grand mean
	R1	R2	R3	AVG	R1	R2	R3	AVG	
BCr	1	4	8	4.53	4	7	9	6.06	5.29
	3	2	7		8	8	3		
	2	2	6		4	4	3		
	2	9	9		9	4	8		
	4	9	7		8	4	4		
	3	7	7		8	7	8		
	1	2	8		8	5	7		
	1	1	8		7	4	7		
	2	5	7		5	9	4		
	3	2	7		8	3	9		
	2	2	9		7	4	3		
	2	3	6		8	3	7		
BCs	4	7	8	7.00	1	4	8	4.53	5.76
	7	6	7		3	2	7		
	6	8	7		2	2	6		
	7	9	8		2	9	9		
	7	5	8		4	9	7		
	8	8	7		3	7	7		
	7	9	4		1	2	8		
	7	7	7		1	1	8		
	7	8	6		2	5	7		
	8	7	7		3	2	7		
	6	8	7		2	2	9		
	7	7	6		2	3	6		

Appendix C. Rating scores of polysora rust among Hi38-71 x G24 family.

Generation	Replication						Mean
	Rep 1		Rep 2		Rep 3		
Hi38-71	3	4	4	3	3	3	3.22
	3	3	3	3	3	3	
	3	3	3	3	3	3	
	3	3	3	3	3	3	
	4	3	3	4	3	4	
	3	3	3	3	4	3	
	3	3	3	3	3	4	
	4	4	3	3	4	3	
	3	3	3	4	3	4	
	3	4	3	3	3	3	
G24	7	7	7	6	7	7	6.90
	8	7	7	6	6	6	
	7	7	8	6	7	7	
	7	7	8	6	7	6	
	7	7	8	7	7	7	
	7	7	7	6	6	6	
	7	7	7	8	6	7	
	6	7	8	8	7	7	
	7	7	7	7	6	6	
	7	7	9	7	7	6	
F_1	3	2	3	3	3	3	2.93
	2	3	3	3	3	4	
	2	3	3	3	3	3	
	3	2	3	3	3	4	
	2	2	4	3	3	3	
	2	2	4	3	3	3	
	2	3	3	4	4	3	
	2	3	3	3	3	3	
	2	3	3	4	3	3	
	2	3	3	4	3	3	
BCr	6	4	3	4	3	5	3.82
	4	4	3	3	3	3	
	4	4	5	5	3	3	
	3	4	3	3	3	3	
	3	4	3	5	3	4	
	4	4	4	4	3	4	
	4	5	4	4	3	4	
	4	5	3	6	4	3	
	4	4	4	4	4	5	
	4	5	3	3	3	3	
	4	4	4	3	4	4	
	4	4	4	3	3	3	
	4	4	4	3	3	3	
	4	5	4	4	4	3	
	3	4	4	4	3	3	
	4	4	4	5	3	4	
	5	4	4	4	3	5	
	4	4	4	4	3	4	
	4	4	5	4	3	3	
	5	5	3	3	5	4	

Generation	Replication						Mean
	Rep 1		Rep 2		Rep 3		
F_2	4	3	3	4	3	7	4.20
	3	3	3	4	3	5	
	5	5	6	4	4	3	
	4	5	5	4	4	4	
	4	4	3	4	5	4	
	3	3	4	5	3	4	
	3	4	6	4	6	3	
	3	4	5	5	3	3	
	3	6	3	4	4	4	
	6	4	5	6	3	5	
	3	4	3	5	4	6	
	5	4	4	5	6	5	
	5	3	3	4	3	4	
	4	5	3	3	4	4	
	4	4	4	4	4	5	
	7	5	4	5	4	4	
	4	4	5	4	5	4	
	4	5	6	4	3	4	
	5	4	5	4	4	5	
	4	5	3	3	3	4	
	6	4	3	4	4	4	
	5	6	4	4	3	3	
	4	4	5	3	4	4	
	4	4	3	4	4	5	
	4	5	4	4	4	4	
	4	4	3	5	3	4	
	5	7	3	5	5	5	
	5	4	3	4	4	6	
	4	7	4	4	4	3	
	4	7	5	3	5	4	
BCs	7	6	7	7	6	4	5.70
	7	6	6	5	5	5	
	7	6	6	6	6	5	
	7	6	6	6	5	4	
	7	7	7	6	5	6	
	7	6	7	5	6	6	
	5	6	6	5	6	4	
	6	6	5	6	5	4	
	7	7	5	5	5	4	
	7	7	7	5	4	6	
	8	8	6	6	5	4	
	8	6	6	5	5	5	
	5	8	6	6	4	5	
	5	5	6	6	4	6	
	7	4	6	6	4	6	
	6	5	5	7	6	6	
	6	5	5	6	4	5	
	7	6	5	5	4	5	
	6	7	5	6	4	6	
	6	7	5	6	3	5	

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