Corn leaf aphid feeding behavior, injury to seedling maize, and colony development on inbred lines differing in DIMBOA concentrations

James Wayne Bing
Iowa State University

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Corn leaf aphid feeding behavior, injury to seedling maize, and colony development on inbred lines differing in DIMBOA concentrations

Bing, James Wayne, Ph.D.
Iowa State University, 1990
Corn leaf aphid feeding behavior, injury to seedling maize,
and colony development on inbred lines
differing in DIMBOA concentrations

by

James Wayne Bing

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1990
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INTRODUCTION

Distribution and Life Cycle

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae), is an occasional pest, occurring on numerous species of Gramineae; maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L. Moench) and barley (*Hordeum vulgare* L.) are preferred hosts (Wildermuth and Walter 1932). Corn leaf aphids are a cosmopolitan species found between the latitudes of 40° N. and 40° S. (Wildermuth and Walter 1932). In North America, it is generally accepted that corn leaf aphids are unable to overwinter at latitudes north of northern Texas; thus, populations in the midwest originate from southern alate migrants (Irwin and Thresh 1988). In South Dakota, a high proportion of cereal aphids, including corn leaf aphid, found in small grain fields arrived in coincidence with low level jet winds indicating that migration was occurring (Kieckheffer et al. 1974).

Corn leaf aphids reproduce entirely by parthenogenetic and viviparous means. No sexual female forms are known, and only a few reports of sexual alate males have been recorded (Wildermuth and Walter 1932, Cartier 1957). Corn leaf aphid populations are comprised of both apterous and alate parthenogenetic females; alates are less fecund than apterous females. In Illinois, corn leaf aphids were found to complete 17 generations from 26 June until 21 December (Davis 1909). In the southern United States, the maximum number of generations was 35 to 40 per year, with the minimum number of generations varying from 13 to 20 (Wildermuth and Walter 1932).
Corn leaf aphids have four nymphal stages and an adult prereproductive period, reproductive period, and post reproductive period. Corn leaf aphids have an average lifespan of 23.8 days in a growth chamber with temperatures ranging from 24° to 28°C. During the reproductive period, apterous females produced an average of 61 progeny, with a maximum of 12 per day and an average of 6 (Branson and Ortman 1967).

Factors Influencing Populations of Corn Leaf Aphids

Populations of corn leaf aphid are influenced by abiotic factors (e.g., temperature and rainfall), as well as biotic factors (e.g., host quality, crowding, and natural enemies). The optimal temperature for reproduction of four corn leaf aphid biotypes on Reno and Spartan barley was 24°C. Fecundity rates were low for all biotypes at 29.3° and 32.1°C, and at 32.1°C only one biotype survived on barley (Singh and Painter 1964). Observations of corn leaf aphids on water stressed wheat grown in a growth chamber showed a significant decrease in total corn leaf aphid fecundity, longevity, and length of the reproduction period (Sumner et al. 1986). Field studies in Ontario have indicated that moisture stress may not adversely affect corn leaf aphids on maize; corn leaf aphid populations were found to continue to increase during adversely high temperatures and low rainfall periods in July and August (Foott and Timmins 1973). These authors concluded that the protective environment of the whorl prior to anthesis provided a favorable microclimate for corn leaf aphid development and protection against adverse environmental conditions.
The number of corn leaf aphids that a host plant is able to support is directly related to the quantity and quality of plant biomass. Sorghum plants grown in a high nitrogen soil were observed to have twice as many corn leaf aphids as opposed to plants grown in a low nitrogen soil (Branson and Simpson 1966). Crowding on nitrogen deficient plants caused a reduction in fecundity; however, reproduction occurred at a fixed rate and did not vary on plants grown in a high nitrogen soil (Branson and Simpson 1966).

Corn leaf aphid populations, in addition to being influenced by temperature and host quality, are affected by a number of natural enemies. Eighty-two predators and parasitoids have been recorded worldwide to attack the corn leaf aphid (Table 1).

Formation of Alates

Host quality and crowding are generally recognized as being the most important factors influencing the development and dispersal of corn leaf aphid alates. A higher ratio of alate to apterous morphs were observed to develop on nutrient deficient plants when crowded conditions prevailed (Branson and Simpson 1966). A study in Wisconsin documented peak numbers of alate corn leaf aphids in August and September when maize was at anthesis and again when the maize began to senesce (Evans and Medler 1967).

Factors influencing dispersal of alates from the host plants are complicated and involve both environmental and physiological components. Work with other aphid species indicated that two forms of alates existed,
flitters and long distance dispersers (Walters and Dixon 1983). Flitters were differentiated from long distance dispersers by their tendency to move from plant to plant, having large gonads, being sluggish in flight, and take-off at a low angle. Long distance dispersers, in contrast, have small gonads, resist starvation for longer periods, and takeoff at steeper angles. Nymphal crowding influences the frequency of flitters and dispersers in the colony, and dispersal is a function of continued crowding (Walters and Dixon 1983).

Dispersal of corn leaf aphids is influenced by light intensity and temperature. The take-off temperature range of corn leaf aphid is between 14° and 42° C, with increased flight activity above 20° C (Berry 1969). Corn leaf aphid flight activity increased when light intensity was above 100 ft.C, and decreased when light intensity dropped below 100 ft.C. Flight activity never completely stopped, but continued at a low rate in total darkness (Berry 1969).

Aphid Feeding Behavior

Feeding behavior in aphids involves mechanical and chemical disturbance of plant tissues during the penetration and feeding process. Aphids penetrate plant tissue with bristle-like mandibular and maxillary stylets (Miles 1987). The maxillary stylets rest within the mandibular stylets and contain inner groves that form the food canal and salivary canal (Miles 1987). Two types of saliva are emitted from the stylets during the feeding process. One gels as it is emitted forming a tube around the stylets called the "stylet sheath". The other is a watery
substance which is thought to be involved in digestion (Miles 1987). This "watery saliva" is secreted into the plant and then sucked back into the food canal allowing the insect to ingest substances from the plant (Miles 1987).

Aphid feeding activity is typically initiated by a series of short test probes into the epidermis, to assess the quality of a potential feeding site (Miles 1987). Once a feeding site is chosen, penetration of the epidermis can occur inter- or intra-cellularly, or through stomata. Intercellular penetration is the most common method deployed by aphids, but is seldom the sole method (Pollard 1973). Corn leaf aphids typically penetrate the epidermis of whorl-stage maize through a stomatal guard cell (Brandes 1923).

Stylet pathways through the mesophyll to feeding sites occur either inter- or intra-cellularly (Pollard 1973). McAllan and Adams (1961) suggested that aphid species with pectinase in their salivary secretions penetrate plants inter- or intra-cellularly, whereas, those species without pectinase penetrate only intracellularly. Although corn leaf aphids do not have pectinase (McAllan and Adams 1961), they can penetrate plants inter- or intra-cellularly (Brandes 1923). This contradicts McAllan and Adams (1961), indicating that corn leaf aphids may not require pectinase for intercellular penetration of plant tissue.

The majority of aphid species use phloem as their primary feeding site (Pollard 1973). Other feeding sites (e.g., epidermis, mesophyll, and xylem) are primary or alternative feeding sites for selected species (Pollard 1973). Corn leaf aphids were observed to use phloem as their preferred feeding site (Brandes 1923).
Plant Responses to Aphid Feeding

Mechanical damage from aphid feeding and direct entry or diffusion of salivary components into cells may result in localized swellings, transient increases in cytoplasmic streaming, increases in cell permeability, accumulation of cytoplasm near the origin of the disruption, enlargement of the nucleus and nucleoli, nuclear disorganization, increases in mitochondria, degenerative changes in size and number of plastids, and change in cell wall thickness (Miles 1989). Aphid saliva, containing amino acids, also affects water uptake and transpiration (Miles 1989). Aphids act as a "sink" and in large numbers may reduce photosynthates by causing the plant to move assimilates away from plant tissues toward this sink. This causes a breakdown of insoluble reserves that mobilizes free amino acids and amide nitrogen (Miles 1989).

While aphid feeding causes considerable internal changes at the cellular level, aphid feeding damage is often not readily observed but is expressed as plant growth reductions (Miles 1989). Aphid feeding may also result in chlorotic spots surrounding feeding areas. These spots may be the result of hypersensitivity to aphid feeding, the removal of epidermal and mesophyll cell contents, or the injection of a localized toxin. Toxins injected by aphids may also have systemic effects and in perennials can carry over to the next season (Miles 1989). Most economic aphid damage is due to vectoring of viruses or by large colonies that drain plant nutrients (Miles 1989). The loss of large quantities of photosynthates has been associated with reduced growth, flower abortion, and yield loss (Miles 1989).
Corn leaf aphids are most commonly found feeding in maize whorls before anthesis. Following anthesis, corn leaf aphids move down the plant attacking the leaves, stalks, and ears (Foott 1977). Damage resulting from a heavy infestation is characterized by a yellow or red leaf color followed by shriveling and death of leaves. Leaves and tassels become coated with honeydew preventing pollen shed and are blackened by mold growth (Wildermuth and Walter 1932). Damage to tassels is characterized by: 1) emerged tassels may be so heavily infested that they fail to function, 2) tassel dessication reduces pollen production and honey dew restricts the spread of pollen, 3) the tassel may be delayed or only partially emerged from the boot. When poor tassel emergence occurs, the top leaf axil collects large amounts of pollen forming an ideal medium for development of fungi and bacteria, which may result in the loss of the plant top. Occasionally populations of corn leaf aphids will infest ears, causing quickened maturity of small, poorly filled ears (McColloch 1921).

Yield losses associated with infestations of corn leaf aphids are usually the result of barren stalks and poorly filled ears. In fields of heavily infested maize in Indiana, 44% of the plants produced barren stalks; the difference in yield between uninfested and heavily infested plants was 38 bushels per acre (Everly 1960).

In years when maize plants are stressed by high temperatures and low moisture, yield reductions can be correlated with heavy aphid infestations. Even though yield reductions occur in years with adequate moisture, they are not as significant as in dry years (Triplehorn 1959,
Everly et al. 1965). In Ontario, yield was reduced by 91.8% in moisture stressed maize severely infested with corn leaf aphids (Foott and Timmins 1973). The severely infested plants were barren or had ears with just a few kernels, regardless of the available moisture. Most corn leaf aphid-induced injury occurred before the end of pollination, and was characterized by stunted plants, whorl leaves too desiccated to unfold, short ear shoots, and failure of ear shoots to develop (Foott and Timmins 1973). Plants with severe infestations were up to 18 inches shorter than healthy plants. These authors suggested that the 2-week period before pollination was the critical time for plant damage. When ears did form, yield reductions were due to fewer kernels rather than light kernels (Foott and Timmins 1973). This study also suggested that corn leaf aphid feeding on maize causes physiological changes in the plant due to removal of nutrients and moisture.

Large colonies of corn leaf aphids, found in maize whorls before anthesis, have been implicated as being the most detrimental to maize yields (Bigger 1958, Triplehorn 1959, Everly 1960, Everly et al. 1965, Foott and Timmins 1973). However, a study conducted in Jamaica and Iowa found that alates were attracted to and established colonies in maize from the time the first leaf emerged (Dicke 1989, Dicke and Sehgal 1990). These authors speculated that corn leaf aphid feeding on seedling maize could lead to increased levels of virus infection and yield loss.

Changes in plant development caused by insects with piercing sucking mouthparts have been documented in seedling maize, sorghum, and wheat (Triticum aestivum L.). For example, feeding by Schizaphis graminum (Rondani) in seedling wheat reduced plant height and root and shoot
weights (Burton 1986). Feeding damage by stink bugs *Euschistus servus* (Say), *E. variolarius* (Palisot de Beauvois), and *Acrosternum hilare* (Say)] and chinch bug [*Blissus leucopterus leucopterus* (Say)] causes temporary stunting in seedling maize plants, and stink bugs have been shown to reduce yields (Negron and Riley 1985, Annan and Bergman 1988, Apriyanto et al. 1989). Chinch bug feeding causes temporary stunting and yield reductions in sorghum (Ahmad et al. 1984).

The corn leaf aphid is a vector of two major viral pathogens, maize dwarf mosaic virus (MDMV) and barley yellow dwarf virus; the most damaging in maize is MDMV (Dicke and Sehgal 1990). Johnson grass, along with other grasses, serves as the major reservoir for MDMV. Corn leaf aphids are able to transmit MDMV from Johnson grass to young maize early in the spring (Williams et al. 1964, Hilty and Josephson 1966).

Maize Resistance to Corn Leaf Aphids

Resistance in maize to corn leaf aphids was first reported in 1917 for an F1 hybrid of annual teosinte crossed with yellow dent maize (Gernert 1917). Since this report, resistance in maize lines and hybrids to corn leaf aphids has been documented several times (McColloch 1921, Snelling et al. 1940, Walter and Brunson 1940, Huber and Stringfield 1942, Everly 1960, Dishner and Everly 1961, Neiswander and Triplehorn 1961, Everly 1967).

These studies did not identify the chemical resistance factors involved. They did observe, however, that these factors were heritable and subject to genetic manipulation (Huber and Stringfield 1942, Everly...
1967, Walter and Brunson 1940). The cyclic hydroxamate, 2,4-dihydroxy-7-methoxyl, 4-benzoxazin-3-one (DIMBOA), a resistance factor in maize to the European corn borer, has been suggested as a resistance factor in maize to corn leaf aphids (Long et al. 1977). Artificial diet tests indicated that 86.6% of the corn leaf aphids survived on a control diet with no DIMBOA while 68.8% survived on a high DIMBOA diet. Field tests also indicated that inbreds and hybrids high in DIMBOA generally had a lower infestation index. Further studies showed that lines with low DIMBOA levels in the tassel were more heavily infested than lines with large amounts of DIMBOA in the tassel (Beck et al. 1983).

Study Objectives

The objectives of Part 1 of this study were to evaluate the relationship between DIMBOA concentration and corn leaf aphid colony development on different maize growth stages, and to determine if DIMBOA acts as a resistance factor in maize to the corn leaf aphid.

The objectives of Part 2 of this study were to document the effects of corn leaf aphid feeding on the growth and development of seedling maize plants when infested at three different growth stages and to determine the relationship between seedling feeding and yield loss.

The objectives of Part 3 of this study were to document corn leaf aphid feeding behavior on seedling maize, to compare corn leaf aphid feeding behavior on whorl-stage and seedling-stage maize, and to determine entrance sites preferences into maize leaves.
Explanation of Dissertation Format

This dissertation is comprised of three manuscripts which have been submitted to entomological journals for publication. Research was conceived and conducted by myself as a member of the Department of Entomology at Iowa State University from 1987 through 1990. Coauthorship was shared with Dr. W.D. Guthrie, Mr. F.F. Dicke, Dr. J.J. Obrycki, and Mr. M.G. Novak.
Table 1. Parasitoids and predators recorded for the corn leaf aphid (Hetting and Simmonds 1972, Thompson 1944, Thompson and Simmonds 1964)

**Predators - Dermaptera**

*FORFICULIDAE*

*Deru lineare* Esch.

**Predator - Hemiptera**

*NABIDAE*

*Nabis alternatus* Parshley

**ANTHOCORIDAE**

*Orius albidipennis* Reuter

*Q. laevigatus* Fieber

*Q. insidiosus* (Say) (Wagner and Ruesink 1982)

*Q. tristicolor* White

**Predator - Neuroptera**

*CHRYSOPIdae*

*Chrysopa brevilata* Banks

*G. carnea* Stephens

*G. clavell* Navas

*G. plorabunda* Fitch

*G. silvana* Navas

**Predators - Coleoptera**

*COCCINELLIDAE*

*Adalia bipunctata* L.

*A. rufescens* Mulsant

*Adonia variegata* Goeze
Table 1. Continued

Cheilomenes lunatus F.
G. propinqua Mulsant
G. vicina Mulsant
Coccinella arcuata F.
G. septempunctata L.
G. undecimpunctata L.
Coleomegilla maculata DeGeer
Coelophora inaequalis F.
Cyconeda sanguinea L.
Exochomus flavipes Thunberg
Hippodamia convergens Guer.
H. tridecimpunctata (Say) (Foot 1974)
Hyperaspis delicatula Mulsant
H. festiva Mulsant
H. senegalensis Mulsant
H. usambarica Weise
Leis conformis Boisd.
Micraspis striata F.
Pharoscymnus varius Kirsch.
Psyllobora nana Mulsant
Scymnus castaneus Sicard
S. interruptus Goeze
S. moreleti Mulsant
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Predators - Diptera

CECIDOMYIIDAE

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<td>Lygocerus aphidum Kieff.</td>
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<td>Aphidencyrtus aphidivorus (Mayr)</td>
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<td>EULOPHIDAE</td>
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<td>Pachyneuron aphidias Bche. (Etchegaray 1975)</td>
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<td>P. siphonophorae (Ashmead) (Jackson et al. 1970)</td>
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Relation of Corn Leaf Aphid (Homoptera: Aphididae) Colonization to DIMBOA Content in Maize Inbred Lines

J. W. BING, W. D. GUTHRIE, F. F. DICKE, and J. J. OBRYCKI
Department of Entomology, Iowa State University, Ames, IA
USDA-Agriculture Research Service-MWA, Ankeny, IA

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Abstract

Corn leaf aphid \textit{[Rhopalosiphum maidis] (Fitch)} colonization was evaluated on five inbred maize (\textit{Zea mays} L.) lines (B37, B73, C103, Mol7, and 41:2504B) from emergence to the eight-leaf stage. Concentration of 2,4-dihydroxy-7-methoxyl-1, 4-benzoxazin-3-one (DIMBOA) in these inbreds was analyzed from emergence to anthesis to determine its effect on corn leaf aphid colonization. Inbred 41:2504B, which was colonized by corn leaf aphids as it emerged from the soil, had the highest DIMBOA concentration. Inbreds B73 and B37 were low in DIMBOA and were colonized in the three- and six-leaf stages, respectively. Inbreds C103 and Mol7 also were low in DIMBOA and supported small colonies after the six- and seven-leaf stage, respectively. Contrary to findings of previous studies, DIMBOA does not appear to be the primary factor conditioning resistance to the corn leaf aphid in these inbreds.
The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is an occasional pest which occurs on numerous species of Gramineae, of which maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L. Moench), and barley (*Hordeum vulgare* L.) are preferred hosts. In the Midwest, corn leaf aphid-induced yield losses in maize occur periodically; however, when dry weather is coupled with large infestations, yield losses significantly increase (Triplehorn 1959). Everly (1960) found a 10% yield loss in lightly infested plants, and Foott and Timmins (1973) reported yield reductions of up to 91.8% in heavily infested maize under low moisture and high temperatures.

Resistance in maize to corn leaf aphids was first reported by Gemert (1917) in an F₁ hybrid of annual teosinte crossed with yellow dent maize. Several other studies have reported on the variation in resistance to corn leaf aphids found in maize (McCulloch 1921, Snelling et al. 1940, Everly 1960, Dishner and Everly 1961, Neiswander and Triplehorn 1961, Everly 1967, Walter and Brunson 1940).

The first studies to identify the chemical factors involved in maize resistance to the corn leaf aphid were by Long et al. (1977) and Beck et al. (1983). These studies implicated the cyclic hydroxamate 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) as the antibiotic resistance factor in maize. Long et al. (1977) measured maize stem DIMBOA content and aphid colonies on 12 inbred lines and found a significant correlation ($r = -0.72$) between these variables. Not all inbreds, however, followed this trend. Inbred Oh45 contained relatively low concentrations
of DIMBOA while supporting small aphid populations. This suggests that there may be other resistance factors involved. Two other inbreds, Bx and R101, were intermediate in DIMBOA concentration but supported large aphid populations. Beck et al. (1983) found a significant correlation ($r=-0.63$) between tassel DIMBOA content and corn leaf aphid populations. Although this was true for the majority of the inbreds used in their study, one inbred line, A632 was low in DIMBOA but highly resistant. This also suggested that other resistant factors were involved.

DIMBOA is a secondary plant chemical that is known to condition resistance in maize to leaf-feeding by the European corn borer, *Ostrinia nubilalis* Hübner (Wahlroos and Virtanen 1959, Klun and Brindley 1966, Klun et al. 1967, Klun and Robinson 1969). Selection for resistance to leaf feeding by European corn borer was equally effective when plants were selected by the corn borer or by chemical analysis for high DIMBOA concentrations (Russell et al. 1975). DIMBOA also has been implicated in wheat (*Triticum aestivum* L.) as a resistance factor to stem rust (*Puccinia graminis* var. *Ztriticci*) (Elnaghy and Linko 1962), and in maize to stalk rot [*Diplodia maydis* (Berk) Sacc] (BeMiller and Pappelis 1965) and northern corn leaf blight (*Helminthosporium turcicum* Pass) (Long et al. 1978). These studies indicate that resistance to several organisms may be obtained by selecting for high DIMBOA concentrations.

With the exception of the European corn borer, data supporting DIMBOA as a resistance factor to numerous organisms have been collected for only a small sample of genotypes. Guthrie et al. (1985) evaluated 7,537 genotypes of maize for resistance to northern corn leaf blight and first-generation European corn borer and found no correlation for resistance
between these two organisms ($r=0.003$).

The objectives of this study were to evaluate the relationship between DIMBOA concentration and the ability of the corn leaf aphid to colonize at different maize growth stages, and to determine if DIMBOA acts as a resistance factor in maize.

**Materials and Methods**

Five inbred maize lines (B37, B73, C103, Mol7, and 41:2504B) were selected for use in this study because of their resistance or susceptibility to the European corn borer (Guthrie and Dicke 1972) and corn leaf aphid (F.F. Dicke unpublished data). Inbreds B37 and B73 are susceptible to both insects, whereas C103 and Mol7 are susceptible to corn borer but resistant to aphids, and 41:2504B is resistant to corn borer and susceptible to aphids.

In 1988 and 1989, a greenhouse study was conducted to evaluate the ability of the corn leaf aphid to colonize at different growth stages of these five inbred maize lines. The study was a completely randomized design with two replications each year. The experimental unit was a cage containing 10 plants in each of nine growth stages (coleoptile, one- to eight-leaf stages) of each maize line. Leaf stages were designated by the method described by Ritchie et al. (1986). Plants were grown outside the cages in 15.2 cm plastic pots and fertilized (Peters Fertilizer Products, Fogelsville, Pa., 15:30:15) once a week until they were in the three-leaf stage, after which they were fertilized two times a week until time of infestation. Ten randomly selected plants were placed in a randomly
chosen cage, and each plant was infested with approximately 10 alate corn leaf aphids. Seven days after infestation, plants were dissected, and the total numbers of first, second, third, and fourth instars and apterous and alate adults were recorded. On susceptible plants, this was sufficient time for fourth-instar aphids to molt to adults, but very little F$_1$ reproduction occurred. Greenhouse temperatures averaged 25.9±2°C during the day (16 h) and 18.6±3°C at night. Sodium lamps (Energy Technics, York, Pa.) were used as supplemental lighting in the experiment and for growing plants.

Corn leaf aphid colonies were maintained in a separate greenhouse under the same temperature and lighting conditions as used in the experiment. Aphids were reared on barley (cv. Robust) planted in 7.6 cm plastic pots. Twelve pots were placed in a cage and infested with aphids. Barley plants were fertilized (Peters 15:30:15) once a week until the aphids caused the plants to decline. During this period, large numbers of alates were produced for use in the experiment.

Plants that exhibited an antibiotic effect caused high mortality in the first and second instars and contained very few third-instar or older aphids. Susceptible plants, however, usually possessed a range of aphid stages, with most individuals in the second and third instars. All analyses were conducted on third-instar aphids because they were the best measure of potential corn leaf aphid colonization. Third-instars were identified by their bottle-green head and first thoracic segment, dark red eyes, antennae five segmented and pale green with black tips, tips of legs black, tip of abdomen dark chromium-green, and black cornicles surrounded at the base by a dark-green ring (Davis 1909).
Plants analyzed for DIMBOA content were planted in the field in a randomized complete-block design with three replications. The whorls (with 10.2 cm of the tips removed) of 10 plants of each inbred were removed from each replication when plants reached the two-, four-, six-, eight-, 10-, and 14-leaf stages as well as at anthesis. In addition, 400 seeds of each line were grown until they emerged in flats placed in the field. Upon plant emergence, flats were taken to the laboratory, and three replications of 100 plants of each line were dissected to separate the coleoptile from the plumule. These lines were also grown in 15.2 cm plastic pots in the greenhouse in a completely randomized design with three replications. Sampling procedures for DIMBOA analyses in the greenhouse at the three-, five- and seven-leaf stages were the same as those in the field. The greenhouse samples were used as checks for the field samples.

All whorl material analyzed for DIMBOA was frozen at -23°C. After thawing, the plant material was dried at 45°C for 4 days and ground into a fine powder. Because DIMBOA is unstable and decomposes stoichiometrically to MBOA (Brendenberg et al. 1962), DIMBOA concentrations were determined by analyzing dried plant material for mg MBOA/g of plant tissue (Klun and Robinson 1969, Klun et al. 1970, Klun 1969). Samples used in the analysis were 0.5 g, except for some coleoptile and plumule samples which weighed less; these growth stages contained a large amount of DIMBOA. MBOA extraction procedures were a modification of those reported by Klun and Robinson (1969); details of the procedure were reported by Tseng (1984).

The relationship between DIMBOA and corn leaf aphid colonization was analyzed with partial correlations and regression analysis (Snedecor and
Partial correlations were determined on these five inbreds to adjust for leaf-stage effects. This procedure was used because both DIMBOA concentration and corn leaf aphid colonization are closely tied to the growth stages of maize.

Results and Discussion

Inbred 41:2504B (an Argentine flint) contained the largest number of third instars on all leaf stages. All inbreds, with the exception of B37, were colonized when infested at the coleoptile stage, but with the exception of 41:2504B, they became resistant at the one-leaf stage. This suggests that there may have been a time period between emergence and the one-leaf stage when a possible antibiotic factor in maize was not present in sufficient amounts to affect aphid colonization on some genotypes (Figure 1). Inbreds B73 and B37 were resistant to colonization until the three- and six-leaf stages, respectively. Inbreds C103 and Mol7 had low numbers of third instars after the six- and seven-leaf stages, respectively. This result was not unexpected because C103 and Mol7 are related (Mol7 is an extraction of the cross C103 X CI187-2).

In the field, seedling maize plants separated into coleoptile and plumule components shortly after emergence showed that the plumule had considerably more DIMBOA than the coleoptile. Inbred C103 had the lowest DIMBOA concentration (7.6 mg MBOA/g dry tissue) in the plumule; all other lines had DIMBOA concentrations of 13.5 mg MBOA/g dry plumule tissue or greater. DIMBOA concentration was inversely related to growth stage. Inbred 41:2504B remained high in DIMBOA through the 10-leaf stage (3.07 mg
MBOA/g dry tissue) and had more DIMBOA through the 14-leaf stage than did the other four inbreds. Inbreds B37, B73, C103, and Mol7 showed a large drop in DIMBOA concentration from the plumule to the two-leaf stage.

DIMBOA concentration in these four lines showed similar trends from the two-leaf stage through anthesis, with the exception of Mol7, which remained high in DIMBOA through the four-leaf stage. By the six-leaf stage, DIMBOA concentration was below 1.93 mg MBOA/g dry tissue in all four inbreds (Figure 2).

Similarly in the greenhouse, all five lines showed reductions in DIMBOA concentration with increasing leaf stage (Figure 3). Compared to the field, DIMBOA concentrations were lower in the greenhouse than those found in the field. Concentrations in 41:2504B were still relatively high, 3.3 mg MBOA/g dry tissue in the three-leaf stage and 2.2 mg in the seven-leaf stage. Mol7 was high in DIMBOA in the three-leaf stage (2.8 mg MBOA/g dry tissue), but decreased rapidly from the three- to seven-leaf stage as was observed in the field. Inbreds B37, B73, and C103 were low in DIMBOA in these leaf stages (Figure 3); field data showed similar trends.

Comparisons of data in Figure 1 and Figure 2 by maize line give an indication that DIMBOA content of field-grown plants is not involved in conditioning resistance to corn leaf aphid colonization. DIMBOA content in greenhouse-grown plants showed the same trends as did field-grown plants (Figure 3). A relationship of decreasing DIMBOA concentrations with increasing ability of aphids to colonize the plant was observed in inbreds B73 and B37. Aphids, however, were able to start colonizing B73 at the much higher DIMBOA level of 1.5 mg MBOA/g dry tissue in greenhouse
(three-leaf stage) and 5.40 mg MBOA/g dry tissue in field-grown plants (two-leaf stage), than B37 at 0.67 mg MBOA/g dry tissue in greenhouse (five-leaf stage) and 1.03 mg MBOA/g dry tissue in field-grown plants (six-leaf stage). Inbred lines C103 and Mol7 contained low levels of DIMBOA from the five-leaf stage through anthesis in both field-grown and greenhouse-grown plants, but very few colonies developed on these genotypes through the eight-leaf stage. Both these genotypes were resistant to the corn leaf aphid even though they are low in DIMBOA.

Inbred 41:2504B was highly susceptible, and colonization occurred from emergence through the eight-leaf stage. This line also had a high concentration of DIMBOA through the ten-leaf stage in field-grown plants and through the seven-leaf stage in greenhouse-grown plants. There was no consistent relationship between DIMBOA and corn leaf aphid colonization in the five inbreds.

Regression analysis was used to determine if a straight-line relationship existed between DIMBOA concentrations and number of third-instar corn leaf aphids. Regression lines for both the four- and eight-leaf stages in field grown maize showed no linear relationship, and the slopes of the lines were not significantly different from zero (Figure 4). Regression analysis at the three- and seven-leaf stages in greenhouse-grown plants also showed no linear relationship, and the slopes were not significantly different from zero (Figure 5). In all leaf stages tested, 41:2504B was high in DIMBOA and had large colonies, whereas, C103 had low DIMBOA concentrations and supported small or no colonies. Inbred Mol7 was low in DIMBOA in all growth stages tested (except in the three-leaf stage in greenhouse grown plants) and had small or no colonies. Inbred B37 was
low in DIMBOA in all leaf stages tested but had no aphid colonies in the three- and four-leaf stages. Inbred B73, however, showed an increase in colony size with decreasing DIMBOA concentrations in both field-grown and greenhouse-grown plants. This was the only genotype in the study in which a relationship between DIMBOA and aphid colonization seemed to exist. From these studies we conclude that DIMBOA is not the limiting antibiotic factor in maize affecting the corn leaf aphid and that other resistance factors are undoubtedly present.

The partial correlation coefficient for these five inbreds grown in the field ($r=-0.415$) was significant ($P<0.019$), and the partial correlation coefficient for greenhouse-grown plants ($r=-0.604$) was significant ($P<0.008$). Because this correlation (adjusted for leaf stage) was positive, it seems that, as DIMBOA increases, so does colonization. This positive correlation may be explained partly by the large number of aphids found on early growth stages of 41:2504B and the ability of corn leaf aphids to colonize some maize genotypes as they emerge from the soil when DIMBOA concentrations are high. Although there probably would not be a positive relationship between these variables if a large number of genotypes were evaluated, it does indicate that there is little relationship between DIMBOA and corn leaf aphid colonization in these inbreds. We conclude that DIMBOA is not the primary resistance factor in maize to the corn leaf aphid contrary to reports by Long et al. (1977) and Beck et al. (1983).
Figure 1. Number of third-instar corn leaf aphids/plant on nine maize growth stages of five inbred lines.
Figure 2. Mg MBOA/g dry tissue in nine maize growth stages of five inbred lines
Figure 3. Mg MBOA/g dry tissue in three maize growth stages of five inbred lines grown in the greenhouse.
mg MBOA/g Dry Tissue

Maize Growth Stages

- C103
- B37
- B73
- Mo17
- 41:2504B
Figure 4. Relationship between field-grown maize DIMBOA concentration and third instar corn leaf aphids on five maize inbreds at two plant growth stages
mg MBOA/g Dry Tissue

Number of Corn Leaf Aphids/Plant

- Fourth-Leaf Stage
- Eight-Leaf Stage
Figure 5. Relationship between greenhouse-grown maize DIMBOA concentration and third instar corn leaf aphids on five maize inbreds at two plant growth stages.
Number of Corn Leaf Aphids/Plant

- Three-Leaf Stage
- Seven-Leaf Stage

mg MBOA/g Dry Tissue

- 41:2504B
- Mo17
- B37
- C103
- B73
- 873
Corn Leaf Aphid (Homoptera: Aphididae)

Feeding on Seedling-Stage Maize:
Influence on Plant Development

J. W. BING, W. D. GUTHRIE, F. F. DICKE, and J. J. OBRYCKI
Department of Entomology, Iowa State University, Ames, IA
USDA-Agriculture Research Service-MWA, Ankeny, IA

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Five maize (*Zea mays* L.) inbred lines (B37, B73, C103, Mol7, and 41:25045) were infested with alate corn leaf aphids, *Rhopalosiphum maidis* (Fitch), in the greenhouse and then transplanted to the field to quantify the effects of seedling feeding. Feeding by corn leaf aphids on coleoptile and two-leaf stage plants delayed plant development by reducing plant height and delaying pollen shed and silking. Grain fill was also reduced in inbreds B37, C103, and 41:25045 when infested in the coleoptile, two-leaf, and four-leaf stages. Aphid feeding on seedling plants did not reduce grain fill in B73 and Mol7. Feeding on seedling maize plants by alate corn leaf aphids can have a large impact on plant development and grain fill later in the season.
Introduction

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), occurs in the Midwest as an occasional pest of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L. Moench), barley (*Hordeum vulgare* L.), and numerous common weeds. Corn leaf aphids are unable to overwinter in the Midwest; thus, early-season populations depend on the coincidence of low-level jet winds containing large numbers of alates from southern states (Wildermuth and Walter 1932, Kieckheffer et al. 1974, Foott 1977, Irwin and Thresh 1988).

Corn leaf aphid-induced yield losses occur periodically in the Midwest, particularly when large populations occur during dry years. For example, Everly (1960) found that 44% of heavily infested maize plants were barren, whereas Foott and Timmins (1973) reported yield reductions of up to 91.8% in heavily infested moisture-stressed maize. These yield reductions have historically been associated with large whorl-stage infestations that damage leaves and cover tassels with honeydew, preventing pollen production and dispersal (McColloch 1921, Wildermuth and Walter 1932). Everly et al. (1965) suggested that, in addition to visible damage, corn leaf aphids caused physiological changes in the plant by removing nutrients and moisture.

Corn leaf aphids not only attack late whorl-stage maize, but Dicke and Sehgal (1989) reported that alates are highly attracted to seedling maize at the time of emergence from the coleoptile. They speculated that in early growth stages maize plants may be highly susceptible to physiological damage and viral infection.

The objectives of this study were to evaluate the effect of corn leaf...
aphid feeding on the growth and development of seedling maize plants when
infested at three different growth stages and to determine the
relationship between seedling feeding and yield loss.

Materials and Methods

Five inbred maize lines (B37, B73, C103, Mol7, and 41:2504B) were
selected for use in this study because of their resistance or
susceptibility to late-whorl stage damage by corn leaf aphids (F.F. Dicke
personal communication, Corn Insects Research Laboratory, USDA-ARS,
Ankeny). Inbreds B37, B73, and 41:2504B are aphid susceptible, whereas
C103 and Mol7 are aphid resistant. Inbred C103 was tested only in 1989

Experiment I

In 1987 and 1988, 1200 maize seeds (300 of each inbred) were planted
in individual 10-cm circular peat pots in the greenhouse. Upon emergence
of the coleoptile, second leaf, and fourth leaf, 80 plants of each inbred
were randomly selected and infested with ca. 15 alate corn leaf aphids.
Leaf stages were designated by the method described by Ritchie et al.
(1986). All plants were infested on an individual basis. Plants in the
coleoptile and two-leaf stage were caged individually with a 2 liter
plastic bottle with the bottom removed. A 1 liter plastic cup with the
base cut out was taped to the bottom of the plastic bottle, and the top of
the plastic bottle was covered with organdy cloth to allow for
ventilation. Plants in the four-leaf stage were too large to be caged
individually, so they were separately infested inside large organdy covered cages. Control plants were also caged but not infested. Three days after placing aphids on the plants, the infested plants and 20 uninfested plants in the same growth stage were transplanted to the field.

In the field, a 15.2-cm deep by 15.2-cm wide trench was dug with a mechanical trencher. Fertilizer, 8-32-16, was applied into the bottom of the trench before planting. Young maize plants in jiffy pots were set into the trench ca. 15.2 cm apart, and soil was hoed around them. These plants were watered three times a week until their root systems became established. Border rows (100 cm wide) of sweet maize were planted along each side of the experiment.

The experiment was designed as a split plot with four replications, and analyzed for each of three growth stages (coleoptile, two-leaf, and four-leaf). The whole plot consisted of the five inbreds. The split plot consisted of five treatments of five plants and was designed to show the effects of removing corn leaf aphids permanently from plants at different growth stages. Uninfested controls were maintained aphid free for the duration of the experiment by applications of Furadan\textsuperscript{R} 15G directly to each whorl. The second treatment consisted of chemically removing corn leaf aphid infestations with Furadan\textsuperscript{R} 15G from the time maize plants were transplanted until anthesis. This treatment was designed to show the effect of aphid feeding on seedling plants. The third and fourth treatments consisted of chemically removing aphids with Furadan\textsuperscript{R} 15G when plants were in the 10- to 12- and 14- to 15-leaf stages, respectively; treatments were maintained infestation free until anthesis. In the fifth treatment, corn leaf aphids were allowed to feed and reproduce on the
inbreds throughout the growing season. The third, fourth, and fifth treatments were designed to evaluate both seedling feeding damage and the effect of natural infestations on whorl stage corn.

The influence of corn leaf aphid feeding on maize development and yield loss was examined by measuring plant heights at mid-whorl and tasseling, pollen shedding date, silking date, nicking (silking date - pollen shedding date), corn leaf aphid numbers, and grain fill rating differences within and among inbred lines. Extended leaf measurements were taken in the midwhorl stage to determine if height differences existed between the treatments. A final measurement from the base of the plant to the top of the tassel was taken to determine if mid-whorl height differences were permanent. Pollen shedding date and silking date were a measurement of the number of days from July 1 until these events occurred in each plant. Corn leaf aphid populations were estimated shortly before anthesis when aphid numbers were at their peak. A rating scale that reflects the exponential growth rate of corn leaf aphid colonies was used (e.g., 0 = 0 aphids, 1 = 1-5 aphids, 2 = 6-10 aphids, 3 = 11-20 aphids, 4 = 21-40 aphids, and 5 = 41-80 aphids, 6 = 81-160 aphids, 7 = 161-320 aphids, and 8 = 321-640 aphids).

Inherent yield differences among inbred lines make yield comparisons meaningless across inbred lines. To overcome this problem, a scale was used to rate grain fill (1 = a perfect ear, 2 = 90-99% grain fill, 3 = 75-89% grain fill, 4 = 60-74% grain fill, 5 = 45-59% grain fill, 6 = 30-44% grain fill, 7 = 15-29% grain fill, 8 = 1-14% grain fill, and 9 = barren ear). Pearson correlation coefficients were used to determine how well ear weight and grain weight correlated with the rating scale (SAS
Institute 1985).

The two years of data were combined and analyzed from plot means. Total sums of squares for the parameters were partitioned into components for environments (years with 1 df), replication in environments (6 df), inbreds (3 df), environments by inbreds (3 df), error A (18 df), treatments (4 df), inbreds by treatments (12 df), environments by treatments (4 df), environments by inbreds by treatments (12 df), and error B (96 df). Least significant differences (P<0.05) values were calculated to determine the level of significant differences between means (Cochran and Cox 1957).

Experiment II

In 1989, the experiment was modified to examine more closely differences in grain fill across infestation growth stages. Three thousand six hundred plants (720 of each of the five inbred lines) were planted in the greenhouse; the same infestation and transplanting procedures were used as in Experiment I.

The 1989 experiment was planted in the field as a modified split-split-plot design with infestation growth stage (whole plot) replicated three times. The inbreds (first split) and treatments (second split) were replicated (blocks) an additional four times in each whole plot. The infestation growth stages consisted of plants infested in the coleoptile, two-leaf and four-leaf stages. The inbreds used in 1989 were B37, B73, C103, Mol7, and 41:2504B. Treatments were aphid-infested plants and uninfested plants (controls) with 10 plants in each plot. Furadan\textsuperscript{R} 15G was applied to both treatment plots at transplanting to insure that
subsequent aphid feeding did not confound seedling damage. Measurements were the same as Experiment I, except aphid density ratings, because weekly insecticide applications were used to remove aphid infestations.

The data from 1989 were analyzed on an individual-plant basis. Total sums of squares for the parameters of extended leaf height, tassel height, pollen shedding date, silking date, and nicking were partitioned by infestation growth stage into components for blocks (11 df), inbreds (4 df), error A (44 df), treatments (1 df), treatments by inbreds (4 df), and error B (df varied with number of plants missing in the experiment from 1119 to 1029). Total sum of squares for ear ratings were partitioned into components for replication (2 df), infestation growth stages (2 df), error A (4 df), blocks in replications (9 df), inbreds (4 df), infestation growth stages by inbreds (8 df), error B (150 df), treatments (1 df), infestation growth stages by treatments (2 df), inbreds by treatments (4 df), infestation growth stages by inbreds by treatments (8 df), and error C (3171 df). LSD (P<0.05) values were calculated to determine the level of significant differences between means (Cochran and Cox 1957).

Results and Discussion

Experiment I

Uninfested controls were significantly taller than all plants infested at the coleoptile stage (Table 1). This effect, however, was not reflected in tassel height (no significant differences), which indicates that corn leaf aphid feeding caused a delay in plant development but did not permanently stunt the plant. In addition, a three-way interaction,
environments by treatments by inbreds, was significant ($F = 2.85; \text{df} = 12, 96; P = 0.0022$). This interaction occurred because reactions of inbreds differed by treatments in respective years. In 1987, B37, B73, Mo17, and 41:2504B had taller extended leaf heights in uninfested plots than in infested plots; however, in 1988, only B37 and 41:2504B showed this trend. In 1988, poor growing conditions caused a reduction in tassel height of 45 to 66 cm less than was observed in 1987. This reduction in height due to the drought also was present in extended leaf height measurements, and may have masked any damage caused by early aphid feeding in B73 and Mo17.

Inbred effects and the environments by treatments interaction were also significant at the $P = 0.05$ level which was expected. Plants infested in the two-leaf and four-leaf stages were not significantly different from uninfested plants in extended leaf height or tassel height.

In addition to temporarily stunting plant growth, plants infested in the coleoptile stage showed delayed pollen shed and silking (Table 1). In these treatments, uninfested plants shed pollen and silked significantly earlier (ca. 2 days) than infested plants. These differences in flowering also indicated that there was a delay in growth resulting from aphids feeding on coleoptile-infested plants.

The three-way interaction, treatments by inbreds by environments, was significant for pollen shedding date ($F = 2.40; \text{df} = 12, 96; P = 0.0091$). This interaction showed similar trends and supported the interpretation of the same interaction for extended leaf height also indicating the poor growing conditions in 1988 may have prevented B73 and Mo17 from expressing aphid damage. The effects of inbreds and the interaction, treatments by environments, were significant at the $P = 0.05$ level, as was expected.
There was no significant differences in pollen shedding date or silking date in the treatments of two- and four-leaf stage infested maize, indicating that aphid damage on seedling maize was limited to the coleoptile stage. The nicking interval (silking date - pollen shedding date) did not differ significantly in plants infested at the coleoptile, two-, or four-leaf stages.

Grain fill ratings were highly correlated with ear weight ($r = -0.9445$, $P < 0.0001$) and kernel weight ($r = -0.9428$, $P < 0.0001$) indicating that the rating system was an adequate indicator of these yield components. Significant differences in grain fill ratings occurred only in plants infested at the coleoptile stage (Table 1). Uninfested treatments had higher rating than infested treatments. Inbreds B37 and 41:2504B showed a significant decrease in grain fill due to corn leaf aphid feeding. Grain fill reduction, however, may be dependent upon the genotype because Mo17 and B73 showed an inconsistent trend (some infested plots were not significantly different from the uninfested plots, LSD = 0.59), which suggests that aphid damage was not the main factor involved in grain fill reduction in these inbreds (Figure 1).

Plants infested in the coleoptile stage had less grain fill than plants infested in the two- and four-leaf stage, and plants infested in the two-leaf stage had less grain fill than plants infested in the four-leaf stage. Grain-fill ratings for control treatments in coleoptile and two-leaf infested plants were the same (3.7) while plants infested in the four-leaf stage had higher grain fill ratings (2.9). It appears that the control treatments in plants infested in the coleoptile and two-leaf stage had similar grain fill ratings, and the difference between plants infested
in the four-leaf stage and plants infested in the two-leaf and coleoptile stage in control plots may represent a transplanting effect (Table 1).

Ratings of corn leaf aphid populations showed that, for both years, natural corn leaf aphid populations attacking late whorl-stage maize were low, with inbreds B37 and 41:2504B having the highest ratings (3.4 and 3.2, respectively). The lack of differences in height, flowering, and grain fill ratings taken from infested plots (Table 1) probably was attributable to these low natural populations.

**Experiment II**

Maize plants infested in the coleoptile and two-leaf stage behaved differently in 1989 than in 1988 and 1987. Plants infested in the coleoptile stage showed no significant differences in extended leaf heights, pollen shedding date, and silking date (Table 2), but tassel heights of uninfested plants were significantly taller than those of infested plants. Corn leaf aphid feeding may have caused permanent stunting.

Maize plants infested at the two-leaf stage showed the most damage from aphid feeding (Table 2). Extended leaf and tassel heights were significantly greater for uninfested plants than for infested plants, indicating that these plants were also permanently stunted by aphid feeding. Pollen shedding and silking both occurred significantly earlier in uninfested plants than in infested plants, suggesting that early infestations of aphids delayed flowering.

The two-way interaction, inbreds by treatments, was significant in plants infested in the two-leaf stage for extended leaf height ($F = 36.70$;
df = 4, 1105; $P = 0.0001$), tassel height ($F = 5.98; df = 4, 1060; P = 0.0001$), pollen shedding date ($F = 35.02; df = 4, 1066; P = 0.0001$), and silking date ($F = 6.21; df = 4, 1061; P = 0.0001$). This was primarily because inbred Mol7 had smaller extended leaf heights in uninfested plants (88.0-cm) than in infested plants (94.5-cm) and had very little difference in tassel height between uninfested (191.1-cm) and infested plants (190.3-cm). The uninfested Mol7 plants also shed pollen and silked ca. 2 days later (18.5 and 24.2, respectively) than infested plants (16.5 and 22.1, respectively). Inbred B73 contributed to this interaction also because tassel height of the uninfested plants was lower (199.9-cm) than those of infested plants (203.1-cm). These data supported the 1988 results in which both B73 and Mol7 did not seem adversely affected by early aphid infestations.

Plants infested in the four-leaf stage did not show significant differences in either plant height measurements. They did, however, have significantly earlier pollen shedding and silking dates in the uninfested plants than in the infested plants. This indicates that early infestations of aphids under some environmental conditions may affect flowering when feeding occurs as late as the four-leaf stage. The nicking period was not significantly different in any infestation growth stage and was not affected by early aphid feeding.

Grain-fill ratings indicated that plants infested in the coleoptile, two-leaf, and four-leaf stage did not differ significantly, despite a trend for plants infested in the four-leaf stage to rate better (2.5) than plants infested in the two-leaf (3.2) and coleoptile (3.0) stage. Grain fill in infested plants (3.0) was significantly less than in
uninfested plants (2.7) ($F = 32.6$; $df = 1, 3171$; $P = 0.0001$). Although there was no significant treatment by infestation growth stages interaction, the infested plants had significantly lower grain-fill ratings ($LSD = 0.12$) than uninfested plants in all three infestation growth stages (Figure 2). This indicates that early aphid infestations may affect grain fill in the coleoptile through the four-leaf stage. The greatest differences in grain fill and height measurements occurred in plants infested in the two-leaf stage. This differed from Experiment I when plants infested in the coleoptile growth stage had the largest differences in grain fill and height. These differences in years may reflect on variations in spring weather. The accumulated degree-days, base 50°F, by the 15 June were higher than normal by 359 (1987), 223 (1988), and 56 (1989) degree days. Thus, the cooler spring weather in 1989 may have suppressed effects of early aphid feeding in plants infested in the coleoptile growth stage.

The 1989 results were similar to Experiment I, in that grain-fill ratings in coleoptile uninfested controls (2.9) and two-leaf uninfested controls (3.0) were not significantly different. However, the grain-fill rating in four-leaf uninfested plants (2.4) was different from the other two infestation growth stages ($LSD = 0.48$). This suggests that transplanting affected grain-fill ratings in plants infested in the coleoptile or two-leaf stage similarly. Grain-fill ratings were significantly lower in uninfested four-leaf stage plants than uninfested coleoptile and two-leaf stage plants. This was indicated by the significant three-way interaction, infestation growth stages by inbreds by treatments, ($F = 33.64$; $df = 8, 3171$; $P = 0.05$). Coleoptile (4.5) and
two-leaf stage plants (4.6) had less grain fill than did four-leaf stage plants (2.2) of 41:2504B. This suggests that 41:2504B was the most sensitive to transplanting effects.

Inbred differences similar to those found in Experiment I were observed in 1989. The interaction, inbreds by treatments, was significant. Inbreds C103, B37, and 41:2504B had significantly less grain fill in infested plants than in uninfested plants (LSD = 0.18). Inbreds B73 and Mol7 did not have significantly different grain-fill ratings in infested and uninfested plants (Figure 3). This indicates that some genotypes may be resistant to early feeding damage by corn leaf aphids.

In conclusion, seedling feeding on maize by corn leaf aphids caused temporary stunting, and in 1989 permanent stunting of maize occurred. This response to aphid feeding was observed in reduced height, delayed pollen shed, and silking. Plants infested in the coleoptile and two-leaf stage were the most sensitive to aphid feeding; however, the expression of this damage may be modified by conditions that stress maize. All five inbred maize lines showed temporary or permanent stunting in one or more of the three years of the study, with B37 and 41:2504B the most sensitive inbreds.

Aphid feeding also resulted in lower grain-fill ratings in infested plants than in uninfested plants. This was observed from plants infested at the coleoptile, two-leaf, and four-leaf stage. The plants infested in the coleoptile and two-leaf stage had larger reductions in grain fill than did plants infested in the four-leaf stage. Inbreds B73 and Mol7 were observed not to have reduced grain fill in infested plants when compared with uninfested plants, suggesting that these plants may be resistant.
Inbreds B37, C103, and 41:25048 all showed reductions in grain fill in infested plants.

Temporary stunting followed by reduced yields is a common result of damage to seedling plants by piercing-sucking insects. Both chinch bugs and green stink bugs cause temporary stunting in seedling maize plants, and green stink bugs have been shown to reduce yields (Negron and Riley 1985, Annan and Bergman 1988, Apriyanto et al. 1989). Chinch bugs also cause temporary stunting and yield reductions in sorghum (Ahmad et al. 1984). Burton (1986) found that greenbug damage to seedling wheat plants reduced height growth and root and shoot weights. The evidence from these studies and the evidence presented in this study on the corn leaf aphid indicate that piercing-sucking insects, feeding on seedling maize plants, cause significant reductions in yield. Corn leaf aphid damage to seedling maize may be most significant in seed production fields where planting is delayed for susceptible genotypes to insure proper pollination. Genotypes, such as 41:2504B, that have poor seedling vigor may also be more sensitive to early aphid feeding because they can not withstand the removal of assimilates as well as more vigorous genotypes. Corn leaf aphid feeding on seedling maize plants will be more of a problem in areas that have aphid populations year round. This type of damage has been generally overlooked and is poorly understood. Future studies are needed to investigate the physiological changes in plants caused by these insects and how these changes affect yield.
Table 1. Effects of corn leaf aphid infestations in 1987 and 1988 on extended leaf height, pollen shedding, silking, and grain fill in maize when infested at three growth stages

<table>
<thead>
<tr>
<th>Treatments within growth stages</th>
<th>Measurements*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extended leaf (cm)</td>
</tr>
<tr>
<td>Coleoptile infested</td>
<td></td>
</tr>
<tr>
<td>Control (no aphids)</td>
<td>140.8±2.9</td>
</tr>
<tr>
<td>Removal at planting</td>
<td>133.4±2.7</td>
</tr>
<tr>
<td>Removal at 10-12 leaf</td>
<td>131.4±2.5</td>
</tr>
<tr>
<td>Removal at 14-15 leaf</td>
<td>132.6±2.3</td>
</tr>
<tr>
<td>No removal</td>
<td>133.8±2.7</td>
</tr>
<tr>
<td>Two-leaf infested</td>
<td></td>
</tr>
<tr>
<td>Control (no aphids)</td>
<td>132.1±2.5</td>
</tr>
<tr>
<td>Removal at planting</td>
<td>134.7±2.5</td>
</tr>
<tr>
<td>Infestation Level</td>
<td>LSDs</td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Coleoptile infested</strong></td>
<td></td>
</tr>
<tr>
<td>Control (no aphids)</td>
<td>136.1±2.3</td>
</tr>
<tr>
<td>Removal at planting</td>
<td>135.9±2.2</td>
</tr>
<tr>
<td>Removal at 10-12 leaf</td>
<td>135.9±2.3</td>
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<tr>
<td>Removal at 14-15 leaf</td>
<td>132.1±2.4</td>
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<tr>
<td>No removal</td>
<td>135.0±2.0</td>
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<tr>
<td><strong>Two-leaf infested</strong></td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Four-leaf infested</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LSDs, F, df, and P values</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Measurements are Means ± SEM.</td>
<td></td>
</tr>
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Table 2. Effects of corn leaf aphid infestations in 1989 on extended leaf height, tassel height, pollen shedding, and silking in maize when infested at three growth stages

<table>
<thead>
<tr>
<th>Treatments within growth stages</th>
<th>Measurementsa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extended leaf</td>
</tr>
<tr>
<td>Coleoptile infested</td>
<td></td>
</tr>
<tr>
<td>No aphids</td>
<td>84.7±0.7</td>
</tr>
<tr>
<td>Infested</td>
<td>83.8±0.7</td>
</tr>
<tr>
<td>Two-leaf infested</td>
<td></td>
</tr>
<tr>
<td>No aphids</td>
<td>88.4±0.9</td>
</tr>
<tr>
<td>Infested</td>
<td>83.5±0.9</td>
</tr>
<tr>
<td>Four-leaf infested</td>
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<tr>
<td>No aphids</td>
<td>88.3±0.5</td>
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<tr>
<td>Infested</td>
<td>88.0±0.5</td>
</tr>
<tr>
<td></td>
<td>LSDs</td>
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<td>------------------</td>
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</tr>
<tr>
<td>Coleoptile infested</td>
<td></td>
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<tr>
<td></td>
<td>NS</td>
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<tr>
<td></td>
<td>NS</td>
</tr>
<tr>
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</tr>
<tr>
<td>Two-leaf infested</td>
<td>1.1</td>
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<tr>
<td></td>
<td>F=82.06</td>
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<tr>
<td></td>
<td>df=1,105</td>
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<tr>
<td></td>
<td>P=0.0001</td>
</tr>
<tr>
<td>Four-leaf infested</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>F=37.72</td>
</tr>
<tr>
<td></td>
<td>df=1,1060</td>
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<tr>
<td></td>
<td>P=0.0001</td>
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</table>

*Measurements are Means ± SEM.*
Figure 1. Mean (±SEM) grain fill ratings in plants infested with corn leaf aphids at the coleoptile growth stage for inbreds B37 (solid bar), B73 (crosshatch bar), Mol7 (diagonal line bar), and 41:2504B (white bar) at five treatments (1 = uninfested, 2 = aphid removal at transplanting, 3 = aphid removal at the 10 to 12-leaf stage, 4 = aphid removal at 14 to 15-leaf stage, and no removal) in 1987 and 1988.
Figure 2. Mean (+SEM) grain fill ratings for 1989 corn leaf aphid infested (White bar) and uninfested (solid bar) maize plants at the coleoptile, two-leaf, and four-leaf growth stage.
Maize Growth Stages

- Coleoptile
- 2-Leaf
- 4-Leaf

Grain Fill Rating
Figure 3. Mean (+SEM) grain fill ratings for maize inbreds B37, B73, C103, Mo17, and 41:2504B in corn leaf aphid infested (white bar) and uninfested (solid bar) plants ($F=6.57$; df=4, 3171; $P=0.0001$)
Feeding Behavior of Corn Leaf Aphid

(Homoptera: Aphididae) on Two Growth Stages of Maize

J. W. BING, M. G. NOVAK, J. J. OBRYCKI, AND W. D. GUTHRIE

Department of Entomology, Iowa State University, Ames, IA
USDA-Agriculture Research Service-MWA, Ankeny, IA

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50011, and Ankeny, IA 50021, Project No. 2513.
PART 3: FEEDING BEHAVIOR OF CORN LEAF APHID (HOMOPTERA: APHIDIDAE) ON TWO GROWTH STAGES OF MAIZE

Abstract

Differences in *Rhopalosiphum maidis* (Fitch) feeding behavior were observed on late whorl-stage (V15) and seedling (plumule to V1) maize inbred 41:2504B (*Zea mays* L.). Fifty-seven percent of the corn leaf aphid stylet tracks initially penetrated late whorl-stage plants through the stomata, whereas only 8% of the corn leaf aphids penetrated seedling-stage plants through stomata, and 86% penetrated intercellularly through epidermal cells. As indicated by stylet track termination, phloem was the preferred feeding site in late-whorl (57%) and seedling-stage plants (79%). In seedling maize, 13% of all stylet tracks had multiple branches, whereas, 22% of the stylet tracks in late whorl-stage maize had multiple branches. These data suggest that corn leaf aphids were most successful in locating the phloem in seedling plants.
Introduction

Aphid feeding activity is initiated by a series of short test probes into epidermis, followed by epidermal penetration. Most aphid species penetrate the epidermis intercellularly, but intracellular and stomatal penetrations occur (Miles 1987). Stylet pathways through the mesophyll to feeding sites occur inter- or intra-cellularly (Pollard 1973, Miles 1987). Phloem is the primary feeding site, but other feeding sites (e.g., epidermis, mesophyll, and xylem) can be utilized (Pollard 1973, Miles 1987).

During penetration, saliva emitted from the stylets gels forming a tube around the stylets called the "stylet sheath." This tube or "stylet track" remains inside the plant after the stylets are retracted forming visible evidence of the feeding pathway. Aphid species with pectinase in their salivary secretions penetrate plants inter- or intra-cellularly, whereas, those species without pectinase use only intracellular penetration (McAllan and Adams 1961, Miles 1987).

Penetration of the epidermis by corn leaf aphids, *Rhopalosipnum maidis* (Fitch), in whorl-stage maize (*Zea mays* L.) occurs primarily through stomatal guard cells (Brandes 1923). Inter- and intra-cellular penetration of mesophyll cells has been observed, and the primary feeding site was phloem (Brandes 1923).

The objectives of this study were to document corn leaf aphid feeding behavior on seedling maize, to compare corn leaf aphid feeding behavior on whorl-stage and seedling-stage maize, and to determine entrance site preferences into maize leaves.
Materials and Methods

Maize inbred 41:2504B (corn leaf aphid susceptible) was used for both late whorl-stage (V15) and seedling-stage (plumule to V1) observations (Ritchie et al. 1986). Forty maize plants were grown in three 38 by 53 cm flats for seedling observations; 20 plants were grown individually in 19 one liter pots for late whorl-stage observations. As coleoptiles emerged from the soil, plants in flats were caged and infested with approximately 300 alate corn leaf aphids. Late whorl-stage plants were infested in the mid-whorl stage (V10) by placing six corn leaf aphid infested sorghum seedlings (approximately 200 corn leaf aphids) into the whorl of each plant.

Seedling plants were collected for observation in the late-plumule or V1 stage, following five to six days of corn leaf aphid feeding. Infested leaves from late whorl-stage plants (V15 stage) were collected and dissected into strips (about 4 by 10 cm) which contained large numbers of aphids. These leaf strips and seedling plants were dipped into 60°C distilled water containing a 2% solution of insecticidal soap (Safer Agrochemicals, Memphis, TN) to kill corn leaf aphids in situ (Pollard 1971). Leaf strips were then cut into smaller sections (about 1 by 2 cm), fixed in formalin-acetic acid-alcohol, embedded in paraplast II Wax (Monoject Scientific, St. Louis, MO) in a vacuum oven at 55°C, and sectioned at 12 μm. Sections were stained with safranin O and counterstained with fast green (Berlyn and Miksche 1976). The seedling and whorl-stage maize sections containing attached corn leaf aphids were examined under a light microscope to determine the epidermal penetration points, stylet track
pathway through cells, and points of stylet termination.

Percentage of stomatal area on late-whorl and plumule-stage leaves was calculated to determine if corn leaf aphids preferred the stomata as an epidermal entrance site. Sections (about 1 by 1 cm) were dissected from greenhouse-grown maize, and placed in 95% alcohol at 60°C to remove the chlorophyll from the cells. Sections were then passed through solutions of 5% sodium hydroxide (NaOH), saturated chloral hydrate (Cl₃CCH(OH)₂), and stained in fast green or safranin O (Berlyn and Mikscke 1976). Photographs of these leaf sections were taken and the percentage of stomatal area was determined with a dot grid (Paine 1981). Differences between percentage of stomatal area and percentage stomatal corn leaf aphid stylet penetration for both plumule and late whorl-stage plants were compared by calculating an approximate t value (using between section variation as the error term) (Snedecor and Cochran 1967). These data were not transformed because the mean was ≥ 2 standard deviations from 0, and arcsin transformations did not improve the fit to a normal distribution.

Results and Discussion

Epidermal penetrations

Four hundred and eleven stylet tracks, of which 21 contained stylets, were examined in late whorl-stage maize. Fifty-seven percent of all stylet tracks penetrated the epidermis through the stomata. Twenty-nine percent penetrated intercellularly between epidermal cells (Figure 1A, F, G) or between epidermal cells and bulliform cells, 8% penetrated intracellularly through epidermal cells, and 6% intracellularly through
bulliform cells (Figure 1C). Stylet penetrations through stomata occurred intercellularly through stomatal openings, intracellularly through guard cells (Figure 1E), and intercellularly between epidermal cells and guard cells.

Observations of plumule-stage 41:25048 revealed 166 stylet tracks, with 81 stylets still in plant tissue. The most frequent point of epidermal penetration was intercellularly between epidermal cells (86%) (Figure 2A, B, C), whereas 6% of the penetrations involved intracellular penetration of epidermal cells. Stomatal penetrations (8%) occurred intercellularly through stomatal openings and intracellularly through guard cells.

A $t$ test between percentage of stomatal area (7.3%) and percentage aphid stomatal penetration for late whorl-stage plants was highly significant ($t = 4.163, \text{df} = 8, \text{P} = 0.002$), indicating stomatal penetration by corn leaf aphids was not occurring by random chance. One possible advantage for stomatal penetration by corn leaf aphids would be lower cellular resistance because of the large gas filled substomatal chamber and the small width of the stomatal apparatus (Figure 1E). In seedling plants, there were no significant differences between stomatal area (5.1%) and percentage of aphid stomatal penetration. This suggests that corn leaf aphids did not actively search for stomata as an entrance site into seedling maize plant; stomatal penetrations were probably random events.
Stylet track pathways

Ninety-nine percent of the stylet tracks in late whorl-stage maize followed an intercellular pathway (Figure 1D, E). Evidence of both inter- and intra-cellular routes was common (48%) in a single stylet track. Intracellular pathways were often around vascular bundles (Figure 1A). Intercellular stylet tracks and penetrations through free intercellular spaces provide unrestricted passage for stylets and allow stylet sheaths to develop unconfined forming lobular shaped stylet tracks (Pollard 1971). This type of lobular track was observed for all intracellular penetrations and for penetrations through intercellular cavities (Figure 1F). In addition, lobular stylet tracks also occurred along intercellular stylet tracks. This indicates that intercellular penetrations were damaging cell walls and allowing for movement of stylet sheath material into cells. Evidence from other aphids using electrical penetration studies indicate that brief intracellular stylet penetrations occur while stylets follow an intercellular route forming lobular tracks (Tjallingii 1985).

Stylet tracks through plumule-stage maize were primarily intercellular (99%), but mixed inter- and intra-cellular pathways (33%) were common (Figure 2A). Intracellular tracks frequently occurred in vascular bundle constituents (Figure 2B). These results were similar to those observed in late whorl-stage maize.

Stylet track termination

Phloem is generally considered to be the primary feeding site for most aphids, and the source of the highest quality food (Miles 1987). Fifty-seven percent of the stylet tracks in late whorl-stage maize
terminated in the phloem, indicating that phloem was the primary feeding site in maize (Figure 1B, C, E, F). Termination of stylet tracks also occurred intra- and inter-cellularly in the mesophyll (21%), in the bundle sheath cells (14%) (Figure 1G), in the xylem (6%), and in differentiating tissues (2%). Observations of a large proportion of the stylet tracks terminating in the phloem in late whorl-stage maize are similar to that reported by Brandes (1923).

Fifty-two percent of the stylet tracks in late whorl-stage maize reached the phloem when penetration occurred in the stomata, compared to 59% when penetration occurred by other means. The ability of corn leaf aphids to locate the phloem was not dependent upon the site of plant penetration.

Phloem was also the primary point of stylet termination (79%) in seedling maize and the primary feeding site (Figure 2A). Stylet tracks were occasionally observed to terminate in the mesophyll (12%) (Figure 2B), bundle sheath cells (6%) (Figure 2B), xylem (1%) (Figure 2C), and differentiating tissue (2%). Intracellular termination of stylet tracks indicated that feeding was most likely occurring at these sites (Figure 2B). Seventy-nine percent of all stylet tracks reached the phloem of seedling maize compared to 57% in late whorl-stage maize, indicating that corn leaf aphids were more successful at finding the phloem in seedling plants. In addition, 13% of the stylet tracks in seedling maize contained multiple-branched tracks (Figure 2C), but 22% of the stylet tracks in late whorl-stage maize contained two or more branches (Figure 1G). In most multiple-branched stylet tracks, only one branch reached the phloem, if it was reached at all. This was another indication that corn leaf aphids
were more successful in reaching the phloem of seedling plants.

Mechanical cell damage from aphid feeding can result in localized swellings, transient increases in cytoplasmic streaming, and increased cell permeability (Miles 1989). Large numbers of aphids may also act as a "sink", pulling assimilates from other plant tissues and causing localized reductions in photosynthesis (Miles 1989). While aphid feeding can cause internal changes at the cellular level, damage is often not readily apparent but is expressed as a reduction in plant growth (Miles 1989).

Physiological damage caused by corn leaf aphid feeding on late whorl-stage maize has been rarely studied and is poorly understood, but appears to cause growth reductions and yield losses (Triplehorn 1959, Everly 1960, Foott and Timmins 1973). Large corn leaf aphid infestations on late whorl-stage maize, coinciding with moisture stress, resulted in higher yield reductions than similar heavy infestations on plots with adequate moisture (Foott and Timmins 1973). The high incidence of corn leaf aphids using stomata as the principle penetration point into late whorl-stage maize provides a possible basis to explain this observation. Assuming that stomatal penetration of late whorl-stage maize by corn leaf aphids physically damages the stomata, then feeding would not only cause yield reductions by removing plant assimilates, but may also reduce yields by disrupting leaf gas exchange. In a combined greenhouse and field study, we observed reduced growth and yield resulting from corn leaf aphid feeding on seedling maize (Bing et al. 1990). Since we observed little physiological damage to cells in the present study, these reductions in growth and yield may be attributed to the removal of assimilates during seedling stage feeding.
Figure 1. Corn leaf aphid stylet penetration and pathway in whorl-stage maize. (A) (1) Intercellular epidermal stylet penetration. (2) Intracellular stylet pathway through bundle sheath cell. (B) (1) Tip of stylet shown in A embedded in phloem. (C) (1) Intracellular penetration through a bulliform cell. (2) Tip of stylet embedded in phloem. (D) (1) Section of stylet shown in C showing an intercellular stylet route around a mesophyll and bundle sheath cell. (E) (1) Epidermal stylet penetration through a stomatal guard cell. (2) Stylet passing through substomatal chamber. (3) Intercellular stylet pathway around mesophyll and bundle sheath cells. (4) Tip of stylet embedded in phloem. (F) (1) Intercellular stylet track pathway around mesophyll cells showing saliva ballooning out into a mesophyll cell. (2) Tip of stylet track embedded in phloem. (G) (1) Tip of stylet imbedded between two bundle sheath cells. (2) Second branch of stylet track ending in bundle sheath cell. RS, rostrum; EP, epidermal cell; M, Mesophyll cell; X, xylem; P, phloem; BS, bundle sheath cell; BF, bulliform cell
Figure 2. Corn leaf aphid stylet penetration and pathway in seedling-stage maize. (A) (1) Intercellular epidermal stylet penetration. (2) Intercellular stylet pathway around mesophyll and bundle sheath cells. (3) Tip of stylet embedded in phloem. (B) (1) Stylet and stylet track embedded in bundle sheath cell. (2) Stylet tracks embedded in mesophyll cell. (C) (1) Stylet embedded in xylem. (2) Three branches of a stylet track. RS, rostrum; EP, epidermal cell; M, Mesophyll cell; X, xylem; P, phloem; BS, bundle sheath cell; BF, bulliform cell
Corn leaf aphids, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae), are an occasional pest of maize (*Zea mays* L.), causing poor grain fill and barrenness. Previous research on corn leaf aphid damage has focused on whorl-stage infestations; no information was known about damage resulting from seedling feeding. These studies evaluated the effect of the secondary plant chemical DIMBOA (2,4-dihydroxy-7-methoxyl-1,4-benzoxazin-3-one) on corn leaf aphid feeding and colony development, determined the severity of plant damage resulting from seedling feeding, and documented differences in feeding behavior between seedling and late whorl-stage maize.

Five inbred maize lines (B37, B73, C103, Mol7, 41:2504B) were evaluated from emergence through anthesis for DIMBOA concentration, and from emergence to the eight-leaf stage for corn leaf aphid colony development. Inbred 41:2504B had the highest [DIMBOA], and was colonized by corn leaf aphids as it emerged from the soil. Inbreds B73 and B37 had low [DIMBOA] and were colonized in the third and six-leaf stages, respectively. Inbreds C103 and Mol7 also had low [DIMBOA] but were not colonized until the six- and seven-leaf stage, respectively. Regression analysis showed no linear relationship between DIMBOA and corn leaf colonization. A positive correlation existed between corn leaf aphid colonization in these five inbreds and DIMBOA concentration (partial correlation analysis), indicating that as DIMBOA concentration increases, so does corn leaf aphid colony development. These findings indicated that DIMBOA is not a resistance factor to the corn leaf aphid in these inbreds.
In a three-year combined greenhouse and field study, corn leaf aphid feeding on seedling maize reduced plant height and delayed pollen shedding and silking. Plants infested in the coleoptile and two-leaf stage were the most sensitive to aphid feeding; however, the expression of this damage may have been modified by abiotic factors. Aphid feeding also resulted in lower grain-fill ratings on infested plants. Maize infested in the coleoptile and two-leaf stages had larger reductions in grain fill than did plants infested in the four-leaf stage. Inbreds B37, C103, and 41:2504B had the largest reductions in grain fill, whereas inbreds B73 and Hol7 did not show grain fill reductions.

Observations of corn leaf aphid feeding behavior was conducted on late whorl-stage and seedling stage inbred 41:2504B. Fifty-seven percent of the aphids penetrated late whorl-stage plants through the stomata, whereas in seedling plants, penetration occurred primarily intercellularly around epidermal cells (86%) and only 8% penetrated through the stomata. Corn leaf aphids preferred stomatal penetration to other entrance sites into late whorl-stage maize, but did not prefer stomatal entrances in seedling maize. The preferred feeding site was phloem in late whorl- and seedling-stage plants with 57% and 79% of the stylets terminating there, respectively. Thirteen percent of the stylet tracks in seedling maize contained multiple-branched tracks, but 22% of the stylet tracks in late whorl-stage maize contained two or more branches. In most multiple-branched stylet tracks, only one branch reached the phloem, if the phloem was reached at all. These data indicated that corn leaf aphids were more successful at locating the phloem in seedling-stage plants.

Corn leaf aphid feeding on seedling maize delays maturity and reduces
yield; however, this damage is probably not due to mechanical damage because little physical damage was observed in cells around stylet tracks. This suggests that damage in seedling plants may be due to the removal of assimilates. Feeding on seedling-stage maize by chinch bugs and green stink bugs causes growth reductions in maize similar to those observed for corn leaf aphid feeding (Negron and Riley 1985, Annan and Bergman 1988, Apriyanto et al. 1989). Future research is needed to investigate the physiological changes in seedling maize fed on by sucking insects.


Berry, R. E. 1969. Effects of temperature and light on takeoff of
*Rhopalosiphum maidis* and *Schizaphis graminum* in the field (Homoptera:

Bigger, J. H. 1958. Damage to the corn crop by the corn leaf aphid.

Seedling stage feeding by corn leaf aphid (Homoptera: Aphididae):
Influence on plant development in maize. J. Econ. Entomol. (in
press).

Brandes, E. W. 1923. Mechanics of inoculation with sugar-cane mosaic

host and crowding on the corn leaf aphid. J. Econ. Entomol. 59: 290-
293.

Entomol. Soc. Am. 60: 1118-1119.

Brendenberg, J. B-Son, E. Honkanen, and A. I. Virtanen. 1962. The
kinetics and mechanism of decomposition of 2,4-dihydroxy-1,4-

Burton, R. L. 1986. Effect of greenbug (Homoptera: Aphididae) damage
on root and shoot biomass of wheat seedlings. J. Econ. Entomol. 79:
633-636.

Entomol. 50: 110-112.


Elnaghy, M. A. and P. Linko. 1962. The role of 4-O-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. Physiol. Plant. 15: 764-771.


Everly, R. T. 1960. Loss in corn yield associated with the abundance of the corn leaf aphid, Rhopalosiphum maidis, in Indiana. J. Econ. Entomol. 53: 924-932.


Klun, J. A. and J. F. Robinson. 1969. Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. J. Econ. Entomol. 59: 711-718.
Klun, J. A., C. L. Tifton, and T. A. Brindley. 1967. 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), an active agent in resistance of maize to the European corn borer. J. Econ. Entomol. 60: 1529-1533.


Long, B. J., G. M. Dunn, and D. G. Routley. 1978. Relationship of hydroxamate concentration in maize and field resistance to 


(Heteroptera: Lygaeidae) feeding in seedling field corn. J. Econ.
Entomol. 78: 1370-1372.

of dent corn strains to the corn leaf aphid, *Rhopalosiphum maidis*

Paine, D. P. 1981. Aerial photography and image interpretation for

Pollard, D. G. 1971. Some aspects of plant penetration by *Myzus*
61: 315-324.

Pollard, D. G. 1973. Plant penetration by feeding aphids (Hemiptera,

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1986. How a corn plant

Selection for resistance in maize to first-brood European corn borer
leaf feeding damage by the insect and chemical analysis for DIMBOA in
the plant. J. Econ. Entomol. 68: 31-34.

SAS Institute, Cary, NC.

plants on progeny production of four biotypes of corn leaf aphid,

Iowa State University Press, Ames.


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Data for parts 1 through 3 are presented in the enclosed computer disk:

Part 1: Relation of Corn leaf Aphid (Homoptera: Aphididae) Colonization to DIMBOA Content in Maize Inbred Lines

<table>
<thead>
<tr>
<th>File</th>
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<tbody>
<tr>
<td>Dimboa^1</td>
<td>DIMBOA content in field grown maize</td>
</tr>
<tr>
<td>Dimhos^2</td>
<td>DIMBOA content in greenhouse grown maize</td>
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<tr>
<td>Greenhos^3</td>
<td>Corn leaf aphid colony development data</td>
</tr>
</tbody>
</table>

^Column headings on data set read as follows: Line: 1=C103, 2=B37, 3=B73, 4=Mol7, 5=41:2504B; Leaf: 1-coleoptile, 2-plumule, 3-2-leaf, 4-4-leaf, 5-6-leaf, 6-8-leaf, 7-10-leaf, 8-14-leaf, 9-anthesis; Rep: replication number; Dimboa: DIMBOA concentration.

^Column headings on data set read as follows: Line: 1=C103, 2=B37, 3=B73, 4=Mol7, 5=41:2504B; Leaf: 1-3-leaf, 2-5-leaf, 3-7-leaf; Rep: replication number; Dim boa: DIMBOA concentration.

^Column headings on data set read as follows: Rep: replication number; Line: 1=C103, 2=B37, 3=B73, 4=Mol7, 5=41:2504B; Leaf: 1-coleoptile, 2-1-leaf, 3-2-leaf, 4-3-leaf, 5-4-leaf, 6-5-leaf, 7-6-leaf, 8-7-leaf, 9-8-leaf; Plant No: number of plants in a cage; First Instar; Second Instar; Third Instar; Fourth Instar; Apterous; Alate.
Part 2: Corn Leaf Aphid (Homoptera: Aphididae) Feeding on Seedling Maize: Influence on Plant Development

<table>
<thead>
<tr>
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<th>Explanation</th>
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<tbody>
<tr>
<td>Aphdat89^1</td>
<td>1989 data</td>
</tr>
<tr>
<td>Aphdat88^2</td>
<td>1988 data</td>
</tr>
<tr>
<td>Aphdat87^3</td>
<td>1987 data</td>
</tr>
</tbody>
</table>

^1 Column headings on data set read as follows: Block: replication number; Infest: 1-infested in the coleoptile stage, 2-infested in the 2-leaf stage, 3-infested in the 4-leaf stage; Line: 1-C103, 2-B37, 3-B73, 4-M017, 5-41:2504B; Treatment: 1-no aphids, 2-greenhouse infested with aphids; Leaf Height: extended leaf measurements; Tassel Height: tassel measurements; Pollen Date: day of first pollen shed; Silk Date: day of silk emergence; Ear Rating: grain fill rating.

^2 Aphdat88 column headings are the same as Aphdat89 columns except: Treatment: 1-control, 2-4-different aphid removal periods, 5-aphids never removed; Leaf Hght1: first extended leaf height measurement; Leaf Hght2: second extended leaf height measurement; Aphid Rating: aphid rating of size of aphid colony at time of anthesis.

^3 Aphdat87 column headings are the same as aphdat88 columns.
Part 3: Feeding Behavior of Corn Leaf Aphid (Homoptera: Aphididae) on Two Growth Stages of Maize

<table>
<thead>
<tr>
<th>File Name</th>
<th>Explanation</th>
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<tbody>
<tr>
<td>Stomplum$^1$</td>
<td>Plant penetration data for plumule stage maize</td>
</tr>
<tr>
<td>Stompen$^2$</td>
<td>Plant penetration data for late whorl-stage maize</td>
</tr>
<tr>
<td>Stomarea$^3$</td>
<td>Percent leaf area represented by stomata in plumule and late whorl-stage maize</td>
</tr>
</tbody>
</table>

$^1$Column headings on data set read as follows: Section: leaf section; Stomata: number of stomatal penetrations; Other: number of penetrations in epidermal tissues other than stomata.

$^2$Column headings in stompen are the same as those in stomplum.

$^3$Column headings on data set read as follows: Rep-replication number; Whorl Area-percent of late whorl-stage leaf represented by stomata; Section1-leaf section number in late whorl-stage maize; Plumule Area-percent of plumule-stage leaf represented by stomata; Section2-leaf section number in plumule-stage maize.