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Developmental and overwintering biology, ecology, and ant interactions of deltocephaline leafhoppers (Homoptera:Cicadellidae)

Larsen, Kirk Jon, Ph.D.
The Ohio State University, 1991
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For my wife, Shirley
ACKNOWLEDGEMENTS

As I arrive at the completion of this last major goal of my graduate education, I'd like to acknowledge some of those who have helped me accomplish this task. First, I'd like to thank Dr. Lowell R. Nault, my major professor, for giving me the opportunity to study with him at the OARDC, and for his friendship, guidance, insight, and time throughout my graduate program. I am most thankful for his willingness to allow me to switch to the Dalbulus project mid-stream, and encouraging me to get further sidetracked in the wonderful world of ant-leafhopper mutualistic interactions! Being able to travel to Mexico and participate in international cooperative research was the opportunity of a lifetime, one I will never forget.

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PUBLICATIONS


**FIELDS OF STUDY**

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INTRODUCTION

Leafhoppers (Homoptera: Cicadellidae) are small insects which can damage crops by direct feeding or as vectors of plant pathogenic organisms (DeLong 1971). Pest species represent only a small fraction of the approximately 15,000 described species of leafhoppers (Nielson 1985). Among the leafhoppers, the largest subfamily is the Deltocephalinae, which also includes the greatest number of vector species (Nielson 1979).

Two economically important pests of maize (Zea mays L.) in North America are the subject of this dissertation and both are deltocephaline leafhoppers. The first of these is the blackfaced leafhopper, Graminella nigrifrons (Forbes), a member of the tribe Deltocephalini. This leafhopper is a temperate species, distributed throughout the eastern United States (Kramer 1967). The second is the corn leafhopper, Dalbulus maidis (DeLong & Wolcott), a member of the tribe Macrostellini. D. maidis is a widely distributed neotropical species found from the southern United States into South America (Nault 1990).

The blackfaced leafhopper, G. nigrifrons, is a major pest of maize as a vector of maize chlorotic dwarf virus (MCDV), an important stunting disease of maize in the United...

The neotropical leafhopper genus *Dalbulus* is composed of 11 species, all of which utilize maize or its wild relatives, the teosintes (*Zea* spp.), or the gamagrasses (*Tripsacum* spp.) as hosts (Nault & DeLong 1980, Nault 1985). Within this genus, only the corn leafhopper, *D. maidis*, is considered a significant pest of maize, primarily as a vector of three maize stunting pathogens (Nault 1980, Nault et al. 1980). Numerous field and laboratory studies conducted on the distribution, behavior, ecology, population dynamics, and vector relations (see references in Nault 1990) have expanded our understanding of why *D. maidis* is a pest and how it differs from its non-pest congeners.
Information on non-pest relatives of pest species provides important clues on how pests may have evolved. Two areas that remain to be explored involve apparent mutualistic interactions between Dalbulus leafhoppers and ants (Hymnoptera: Formicidae), and most importantly, how Dalbulus leafhoppers overwinter.

The fivespotted gamagrass leafhopper, *D. quinquenotatus* DeLong & Nault, is the only deltocephaline leafhopper known to be associated with and probably attended by ants (Nault et al. 1983, Triplehorn & Nault 1985). The behavior of *D. quinquenotatus* is distinctly different from the behaviors of other Dalbulus species (Nault et al. 1983, Heady & Nault 1985), but typical of myrmecophiles from other homopteran families (Way 1963). In Chapter II of this dissertation, I report on ants associated with *D. quinquenotatus* on *Tripsacum* in the field, and some behavioral observations of interactions between ants and *D. quinquenotatus*. In Chapter III, I examine differences in honeydew production among several Dalbulus species and the interactions between ants and these leafhoppers.

Mexico is the hypothesized center of origin for the Dalbulus genus (Nault & DeLong 1980). Mexico has a two-season climate, a dry winter and a wet summer. The dry winter occurs from October to May (Mosino Aleman & Garcia 1974, Moya-Raygoza et al. 1990) and is characterized by relatively low rainfall and low temperatures, and short days
compared to the wet season. In Mexico, maize is present only during the wet season from June to October (Barnes 1954) except in a few small irrigated areas. How D. maidis survives the dry winter in the absence of its primary host, maize, is not known.

Chapters IV-VI address the overwintering biology of Dalbulus leafhoppers, with the primary purpose of determining how D. maidis overwinters. What happens to populations of Dalbulus in the field during the dry season is unknown. Chapter IV examines the field population dynamics of Dalbulus leafhoppers and how the drought tolerance of D. maidis and its congeners varies seasonally. This seasonal fluctuation in drought tolerance appears to influence the field populations during the dry season. A second factor important during the dry season is cold temperatures, and cold-hardiness of Dalbulus leafhoppers is examined in Chapter V. Finally, Chapter VI examines the observed seasonal variation in the size and color of Dalbulus leafhoppers, and discusses how this seasonal polyphenism relates to cold-hardiness and dry season survival.
CHAPTER I

EFFECT OF TEMPERATURE AND HOST PLANT ON THE DEVELOPMENT OF
THE BLACKFACED LEAFHOPPER, GRAMINELLA NIGRIFRONS

Introduction

The blackfaced leafhopper, *Graminella nigrifrons* (Forbes), is one of the most abundant and ubiquitous leafhoppers found in the principle maize (*Zea mays* L.) producing areas in the eastern United States (Kramer 1967, Stoner & Gustin 1967). *G. nigrifrons* is the most common (Rosenkranz 1969) and efficient vector (Nault et al. 1973, Nault & Madden 1988) of maize chlorotic dwarf virus (MCDV), which causes an important stunting disease of maize in the United States (Gordon & Nault 1977).

Previous research has demonstrated that a wide variety of grasses are capable of serving as hosts for *G. nigrifrons* (Boyd & Pitre 1968, Boyd & Pitre 1969). Studies involving oats (*Avena sativa* L.), maize, and johnsongrass (*Sorghum halepense* (L.) Pers.) have resulted in conflicting interpretations regarding their suitability to serve as oviposition, developmental and feeding hosts. Oats serves as a field and laboratory host, while johnsongrass is a
perennial which serves as the overwintering host of MCDV, but probably not the leafhopper vector. Stoner & Gustin (1967) determined that maize is a better oviposition host than oats, and field tests confirmed maize is used as a feeding host early in the growing season (Pitre & Hepner 1967, Pitre 1968). However, laboratory tests on the survival and fecundity of \textit{G. nigrifrons} showed oats to be the superior host, followed by johnsongrass, with maize least preferred (Boyd & Pitre 1969). In another study, maize was considered a poor host for either development or survival of the leafhopper as compared with johnsongrass (Sedlacek et al. 1986). Nevertheless, in our laboratory, oats is used as the principal oviposition host, and maize as the developmental and feeding host for \textit{G. nigrifrons} (Nault 1977). Hunt & Nault (1990) have shown no significant difference in the suitability of these three grasses as developmental hosts for immatures at ca. 26°C in the laboratory.

\textit{G. nigrifrons} is normally bivoltine in Ohio (Stoner & Gustin 1967, Knoke & Louie 1981, Knoke et al. 1983), but has been reported as having three generations in northern Ohio near Lake Erie (Teraguchi 1986). It is still unclear whether \textit{G. nigrifrons} migrates into Ohio (Taylor 1985, Sedlacek & Freytag 1986), overwinters in Ohio in the egg and/or adult stage (Stoner & Gustin 1967), or combines migration and overwintering strategies (Teraguchi 1986).
The influence of temperature on the blackfaced leafhopper has an important bearing on whether this vector is capable of overwintering, is a migrant, or both, and on the number of generations possible in Ohio and other Corn Belt States during the growing season. However, incomplete information is available on the influence of temperature on the population dynamics of *G. nigrifrons*. This paper provides information on the effects of temperature and host plant on *G. nigrifrons* population dynamics. The objectives of this research were to 1) examine the effects of temperature and host plant on egg to adult development, including the duration of adult emergence, mean developmental time, and fecundity, 2) examine the weight and size of resultant adults, and 3) determine the statistical relationship between mean development rate and temperature.

**Materials and Methods**

The leafhoppers used in this study were reared on a mixture of oats and maize (sweet corn; cv. Aristogold Bantam Evergreen). Laboratory colonies were originally started with adults collected from grasses on the Ohio Agricultural Research and Development Center campus, Wooster, Ohio. Leafhoppers were reared in 19.5 x 38.5 x 39 cm cages (D’Arcy & Nault 1982) in a rearing room maintained at ca. 26°C, relative humidity of 60-90%, and with a light:dark regime of L16:D8.
To test for developmental time from oviposition to adult eclosion at different temperatures and on separate host plants, 100 adults (50 males and 50 females) were placed in rearing cages for oviposition. Comparable amounts of plant material (by weight) in three 10.2 cm dia plastic pots were used in each cage. Host plants tested included oats at ca. 3-4 leaf stage, maize (sweet corn; cv. Aristogold Bantam Evergreen) at ca. 2-3 leaf stage, and johnsongrass at ca. 3-4 leaf stage. Cages were kept in an environmental growth chamber at 26°C under L16:D8 while leafhoppers were given a 48 h period for mating and oviposition. Following this period, all adults were removed from the cages. Three cages, each with a different species of egg-bearing test plant were then put into environmental growth chambers (Percival E-30B; Boone, IA) with a L16:D8 cycle set at either 18, 21, 24, 27 or 30°C. The experiment was replicated three times. Lighting was provided by a combination of 34-W incandescent and VHO fluorescent lamps (ca. 31.1 µE/m²/s inside cages).

Cages were checked daily for the appearance of newly emerged adults. Once adults began to emerge, they were removed daily until all leafhoppers had been taken from the cages. Adults were then held for 24 h on maize plants to allow complete sclerotization of the exoskeleton. They were then frozen and held at -20°C for later observations. After thawing, leafhoppers were sexed and counted, and then
placed in a convection oven to dry at 80°C for 3 days. Males and females were separated into groups of 10 and weighed on an electric balance to the nearest 10⁻⁵g. Measurements of head capsule width as measured by the distance between the compound eyes and length of the second medial cell of the right elytra were made on randomly selected individuals of each sex reared at all temperatures (Fig. 1).

Analysis of variance (ANOVA) of untransformed data was used to determine the effect of temperature, host plant, sex, and replication on the mean development time, the total number of eclosed adults, and morphological differences. Experimental design consisted of a factorial with main effects of temperature, host plant, and sex. Sex, however, was treated as a subplot in a split-plot design. Orthogonal polynomials were used to determine if the response to temperature was linear, quadratic, or of a higher order (Hicks 1973). To evaluate the relationship between the mean development rate (100/mean development time) and temperature, regression analysis was utilized. The biophysical model of Sharpe & DeMichele (1977) was fitted to the data using the nonlinear program of BMDP (Dixon et al. 1988). Proper form of the model was chosen using the techniques described in Wagner et al. (1984). For comparison purposes, a linear model was also fitted to the development rate data.
Figure 1. Morphological measurements taken of adult leafhoppers: $A =$ distance between the compound eyes (head width), and $B =$ length of the right forewing second medial-cell.
Results

The number of emerging adults ranged from an average of 1.39 adults/female/day on johnsongrass at 30°C to 6.47 adults/female/day on maize at 30°C (Fig. 2). The mean numbers of adults which emerged at each temperature on each host plant were influenced significantly by the interaction of temperature and host plant (F=3.65; df=8,30; P<0.05), therefore host plant effects depended on the temperature. Analysis with orthogonal polynomials indicated that there was a quadratic change in numbers with temperature (F=11.3; df=1,30; P<0.01). There also was an interaction of host plant and the linear temperature component (F=9.19; df=2,30; P<0.01), indicating the number-temperature curves for the three hosts were not parallel. The mean numbers of adults emerging at 18°C were significantly less on the annuals, maize and oats, than on the perennial, johnsongrass (Fisher's Protected Least Significant Difference=1.91; df=30; p=0.10). At 30°C, the reverse was true, with significantly fewer adults emerging on johnsongrass than on the annuals (LSD=2.30; df=30; p=0.05). The low numbers on johnsongrass at 30°C were influenced by observed high mortality of 5th instar nymphs.

The sex of emerging adults was not significantly effected by host plant (F=1.0; df=1,30; P>0.05). The sex ratio averaged 2.29 males/female/day to 2.25 females/female/day and did not differ from 1:1 for any temperature.
Figure 2. Average number of adult offspring per female per day of *G. nigrifrons* emerging on each host plant at each temperature (n=3). Means at 18°C with different letters are significantly different (P=0.10), and those means at 30°C with different letters are significantly different (P=0.05).
The duration from first to last adult emergence did not vary among host plants, but did vary with temperature (Fig. 3). As expected, there was an inverse relationship between temperature and the duration of this emergence period (Table 1). The interaction of host and temperature was not significant \( F=0.52; \ df=8,30; \ P>0.05 \).

Host plant type had no significant effect on the mean development time \( F=0.52; \ df=2,30; \ P>0.05 \). Only temperature had a significant effect on the mean development time \( F=357.76; \ df=4,30; \ P<0.01 \). There was both a linear \( F=1297.2; \ df=1,30; \ P<0.01 \) and quadratic \( F=126.3; \ df=1,30; \ P<0.01 \) change in development time over temperature. Overall, males developed into adults significantly faster than females by an average of 1.2 days \( F=221.48; \ df=1,30; \ P<0.05 \). There was a significant interaction of temperature and sex on development time \( F=10.4; \ df=4,30; \ P<0.05 \). The interaction of sex and the linear and quadratic temperature components were both significant \( F=1285 \) and \( F=124; \ df=1,30; \ P<0.01 \). However, this interaction, although significant, only indicated a difference between sexes that somewhat depended on temperature since at 21-30°C, development time for males was ca. 1 day faster than females, but at 18°C, male development was ca. 2 days faster.

There was an approximately linear relationship between mean development rate and temperature (Table 1; Fig. 4).
Figure 3. Daily mean number of emerging adults and cumulative percent of emerging *G. nigrifrons* adults at five temperatures on maize, oats, and johnsongrass.
Table 1. Development of *G. nigrifrons* as measured by the mean development time from egg to adult, the mean duration from first to last adult emergence, and mean development rate, combining host plants and sexes.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mean egg to adult development time (days)</th>
<th>Mean duration of adult emergence (days)</th>
<th>Mean development rate (mean %/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>73.2</td>
<td>43.4</td>
<td>1.37</td>
</tr>
<tr>
<td>21</td>
<td>46.6</td>
<td>25.7</td>
<td>2.15</td>
</tr>
<tr>
<td>24</td>
<td>35.9</td>
<td>21.7</td>
<td>2.79</td>
</tr>
<tr>
<td>27</td>
<td>24.2</td>
<td>15.3</td>
<td>4.13</td>
</tr>
<tr>
<td>30</td>
<td>21.3</td>
<td>11.7</td>
<td>4.69</td>
</tr>
</tbody>
</table>
Figure 4. Mean developmental rate per day for *G. nigrifrons* (both sexes) from egg to adult ($\frac{100}{\text{mean developmental time}}$) on seedling maize, oats, and johnsongrass in relation to temperature. Each point represents the mean for one replication by sex and host plant at each temperature ($n=18$).
The inhibitory effect of low and high temperature on development rate was not observed with the temperatures studied. This was confirmed by a linear Arrhenius plot, i.e., log(rate) versus 1/temperature (not shown). The simplified two-parameter biophysical model of Sharpe & DeMichele (1977) was, thus, fit to the mean development rate data. This model resulted in a precise representation of the data ($R^2=0.96$) (Fig. 4) with the following equation for the two parameter model:

$$r(T) = \frac{RH025}{298.15} \exp \left[ \frac{HA}{R} \left( \frac{1}{298.15} - \frac{1}{T} \right) \right]$$

where $r(T) =$ mean development rate at temperature $T \,(°K)$. For an explanation of model parameters, RH025 and HA, see Wagner et al. (1984). Here the two parameters were: $RH025 = 0.031 \,(s.e.=0.0005)$, and $HA = 15897.6 \,(s.e.=692.5)$.

Moreover, a simple linear model fitted to the data equally well ($R^2=0.96; r(T)=0.298*(T)-3.89; \, s.d.=0.254$), where $T =$ temperature ($°C$) (Fig. 4). Separate regressions for each sex resulted in nearly identical results. A developmental threshold or temperature at which a rate controlling enzyme is half active (see Wagner et al. 1984) could not be precisely estimated from our data, but extrapolation showed that it likely was in the range of 12-15°C.

The dry weight, head capsule width, and wing length measurements of leafhopper size were significantly affected by temperature, host plant, and sex in all instances.
(P<0.05) (Fig. 5). There was a significant linear and quadratic change in weight and wing length over temperature (F=13 and F=14 27; df=1,135; P<0.001). Head width declined over temperature in a linear fashion (F=601.7; df=1,135; P<0.001). Females always weighed more (304 µg) than males (226 µg), and also were longer and wider (P<0.05) (Fig. 5). Slightly, although significantly heavier adults (P<0.05) were produced on maize (278 µg), as compared to johnsongrass (259 µg), and oats (258 µg).

**Discussion**

Temperature plays an important role in determining host plant suitability as a developmental host of the blackfaced leafhopper. Significantly greater numbers of adults emerged at 30°C from the annuals, oats and maize, than emerged from the perennial, johnsongrass. This is consistent with the hypothesis by Whitcomb et al. (1987) that annual grasses are more suitable than perennials for generalist leafhoppers as feeding hosts during the logarithmic phase of plant growth. Our study examined developmental hosts rather than feeding hosts, and a direct comparison between annuals and perennials of different genera is not always possible (Hunt & Nault 1990). A recent report on Dalbulus leafhoppers indicates that developmental host studies using seedlings may not give a complete picture of host suitability (Todd et al. 1991). As maize matures, the morphological and physiological changes the plant undergoes may influence
Figure 5. Dry body weight, head capsule width, and wing cell length measurements (mean ± standard error) of G. nigrifrons by sex and temperature.
negatively its ability to serve as a host to phytophagous insects such as *G. nigrifrons*.

Past studies which evaluated developmental host suitability for the blackfaced leafhopper have not considered the effect of temperature. Maize, oats, and johnsongrass were all shown to be suitable developmental hosts at 21-27°C in this study. This confirms earlier work on the suitability of maize (Pitre & Hepner 1967, Stoner & Gustin 1967, Hunt & Nault 1990) and johnsongrass (Chandler 1966, Hunt & Nault 1990) as developmental hosts for *G. nigrifrons*. It is possible that the investigators who concluded that maize (Sedlacek et al. 1986, Boyd & Pitre 1969) and johnsongrass (Boyd & Pitre 1969) were not good hosts for blackfaced leafhopper development used methods which significantly reduced the suitability of these host plants (see later discussion on the use of restrictive leaf and tube cages).

An observation of high 5th instar mortality in the 30°C johnsongrass treatment agree with similar conclusions reached by Sedlacek et al. (1986) that johnsongrass is not a suitable host at high temperatures for nymphal development. Alternatively, johnsongrass appears to be a better developmental host than either oats or maize at 18°C for *G. nigrifrons*. This may indicate that perennials serve as better developmental hosts in the spring when cooler temperatures prevail. However, our study did not test for
this relationship and further research is necessary to see if temperature-mediated host switching by *G. nigrifrons* is occurring.

There is an indication that a temperature-dependent functional response to a host may be widespread among insects (Mack et al. 1981), yet few insect-host plant interaction studies have taken temperature into account. In the Colorado potato beetle, *Leptinotarsa decemlineata* Say, temperature appears to determine the food preference of adult beetles (Bongers 1965). Temperature also plays a role in enabling the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), to overcome resistant alfalfa cultivars (Hackerott & Harvey 1959, Schalk et al. 1969). Our study is the first we know of to indicate temperature-mediated host utilization by leafhoppers.

The fecundity of the blackfaced leafhopper consistently has been underestimated in previous reports. A conservative minimum can be estimated in this study from the numbers of emerging adults. We estimate a daily fecundity for ca. 2 wk old *G. nigrifrons* females to be 4.59 on *Z. mays*, 4.42 on *A. sativa*, and 4.61 on *S. halepense*. These numbers are higher than estimates from previous reports (Stoner & Gustin 1967, Boyd & Pitre 1969, Sedlacek et al. 1986, Hunt & Nault 1990).

Procedures used in lifetable studies on leafhoppers (eg. Simonet & Pienkowski 1980, Sedlacek et al. 1986) that
require handling individual insects in confined cages may interfere with normal insect behavior and result in reduced acceptability of and performance on certain hosts. Small clip-on leaf cages (used by Sedlacek et al. 1990) may prevent leafhoppers from reaching preferred sites of oviposition or feeding, and both clip-on cages and small tube cages often result in the accumulation of honeydew on feeding surfaces that may interfere with leafhopper development (Nault, unpublished).

Nault (1977) previously determined that the developmental threshold for G. nigrifrons is between 15-20°C. However, with results from this study, we now estimate a minimum developmental threshold of 12-15°C, which is comparable to those determined by Sedlacek et al. (1990).

Our results confirm earlier work by Nault (1977) and Sedlacek et al. (1990) that the duration of egg to adult development increases with decreasing temperature. Greater numbers of leafhoppers emerged on a daily basis during shorter developmental periods, resulting in a fairly even total number of adults produced over the range of temperatures. DeLong (1971) observed that in many leafhopper species, adult males appear in the field a short time before females. The results of this study that males develop 1-2 days faster than females confirm DeLong's observations. The emergence of males before females may encourage mate-seeking males to disperse from developmental
hosts, thereby reducing the likelihood that they will mate with siblings.

The increase in overall size and weight of leafhopper adults with decreasing temperature may be due to the longer developmental periods before emergence (Simonet & Pienkowski 1980). Colder temperatures result in longer developmental periods, allowing greater feeding and subsequent growth. Slightly, but significantly larger adults were produced on maize and oats, as compared to johnsongrass. Although this confirms work by Hunt & Nault (1990) which found annuals produced larger adults than perennials, our study did not compare annuals and perennials within the same genera.

In summary, temperature has been shown to significantly alter the suitability of developmental hosts for *G. nigrifrons*. The perennial, johnsongrass, was the better developmental host at 18°C, while the annuals, oats and maize, were better developmental hosts at 30°C. However, all three hosts were found suitable for blackfaced leafhopper development. The developmental rate of the leafhoppers did not differ significantly between the three host plants, although males developed into adults an average of 1.2 days faster than females. Also, larger and heavier adults developed at low rather than high temperatures on all three hosts.
CHAPTER II

ANTS (HYMENOPTERA: FORMICIDAE) ASSOCIATED WITH THE FIVESPOTTED GAMAGRASS LEAFHOPPER, DALBULUS QUINQUENOTATUS ON MEXICAN GAMAGRASSES

Introduction

Despite extensive studies that report on the relationships between the Homoptera and ants, there are few references dealing with ant-leafhopper (Homoptera: Cicadellidae) interactions (Dietrich & McKamey 1990, and references therein). Previous studies of the maize pest, the corn leafhopper, Dalbulus maidis (DeLong & Wolcott), and its nonpest congeners in Mexico have revealed that the fivespotted gamagrass leafhopper, Dalbulus quinquenotatus DeLong & Nault, is associated with ants (Nault et al. 1983, Triplehorn & Nault 1985, Nault 1990). This would be the first known instance of a deltocephaline leafhopper being associated with ants.

The leafhopper genus Dalbulus is composed of 11 neotropical species found in Latin America. Members of the genus use maize (Zea mays L.), the teosintes (Zea spp.), or the closely related gamagrasses (Tripsacum spp.) as their
primary hosts (Nault 1990). Dalbulus are small, slender, leafhoppers with adults 3.0 - 4.4 mm in length (DeLong 1950, Nault & DeLong 1980, Nault et al. 1983, Triplehorn & Nault 1985). Adults are pale yellow or stramineous for species that specialize on Zea, while those that utilize Tripsacum are bright yellow, orange, or brown (Nault 1990).

*D. quinquenotatus* is a specialist on gamagrasses, and is dark brownish-orange in color. On gamagrass, colonies of aggregating nymphs and adults occur at the bases of its hosts. This colonial habit, observed in the field with attendant ants and in greenhouse populations without ants, distinguishes this species from other members of the genus. *D. quinquenotatus* is an experimental vector of corn stunt spiroplasma and maize bushy stunt mycoplasma and therefore a potential pest of maize (Madden & Nault 1983). In the laboratory, large populations develop on seedling maize (Nault & Madden 1985), but on maturing maize it lacks the behavioral and biological attributes necessary to sustain viable populations, especially to track the younger, nutrient-rich leaves of the whorl as the plant matures (Todd et al. 1991).

This paper documents 18 ant species found associated with the apparent myrmecophile, *D. quinquenotatus*, describes the behavioral interactions between some of these ant
species and this leafhopper, and reports that ants are not associated with other Dalbulus leafhopper species found on gamagrasses.

**Materials and Methods**

Three trips were made to Jalisco, Mexico in May 1989, October 1989, and March 1990 to study the field biology of Dalbulus leafhoppers. Additional collecting in Mexico of attendant ants was performed by the third author (G.M.) throughout this period. Prior to the March 1990 trip, the behavior of D. quinquetoratus and tending ants was observed at nine sites on ca. 100 Tripsacum plants before the insects were collected with an aspirator and preserved in 70% ethanol for later identification. Leafhoppers were identified in the laboratory at Wooster, while the ants were sorted and sent to the Systematic Entomology Laboratory, U.S.D.A., for identification.

During the March 1990 trip, gamagrass plants were visually surveyed in detail at 13 different sites in Jalisco to test the hypothesis that D. quinquetoratus is always associated with ants. A total of 195 randomly selected Tripsacum plants was carefully examined for Dalbulus leafhopper adults and nymphs, tending ants, and ant-leafhopper interactions. Ants found on the plants were classified as "tending" only if they were observed soliciting honeydew from the leafhoppers.
Results

Ants were often observed in small groups of several workers tending each leafhopper. Often one or several workers would be positioned at the head or perpendicular to the side of the leafhopper and have antennae and/or forelegs in contact with the dorsum of the leafhopper. The honeydew collecting ant would antennate the posterior end of the leafhopper, and ingest droplets as they were excreted from the tip of the abdomen.

Eighteen species of ants, representing four phylogenetically separate subfamilies and five tribes (Table 2), were collected associated with D. quinouenotatus. Identifications were limited to genus for nine ants because for these species only minor workers were collected (other castes or males are needed for species identification), or because a generic group is in need of revision (D.R. Smith, personal communication).

A small ant (1.5-2 mm long), Brachymyrmex obscurior Forel, was the most common species associated with D. quinouenotatus. In the detailed field survey, this ant was collected at 12 of the 13 Jalisco sites and on 88 of the 121 (72.7%) surveyed plants colonized by both D. quinouenotatus and attendant ants. When disturbed, this ant became very agitated and vigorously attacked the authors. Normally, several hundred ants were on the infested plants, often in clusters of 5-10 tending each
Table 2. Ants (Hymenoptera: Formicidae) associated with the leafhopper *Dalbulus quinquenotatus* (Homoptera: Cicadellidae) and the number of sites each ant species was found at from 13 sites in Jalisco, Mexico.

<table>
<thead>
<tr>
<th>Ant Identification</th>
<th>Number of sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subfamily: <em>Pseudomyrmecinae</em></strong></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomyrmex</em> sp. <em>gracilis</em> group</td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomyrmex</em> <em>gracilis</em> (F.)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Subfamily: <em>Dolichoderinae</em></strong></td>
<td></td>
</tr>
<tr>
<td>Tribe: Tapinomini</td>
<td></td>
</tr>
<tr>
<td><em>Azteca</em> sp.</td>
<td>1</td>
</tr>
<tr>
<td><em>Iridomyrmex</em> <em>pruinosus</em> (Roger)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Subfamily: <em>Formicinae</em></strong></td>
<td></td>
</tr>
<tr>
<td>Tribe: Formicina</td>
<td></td>
</tr>
<tr>
<td><em>Brachymyrmex</em> sp.</td>
<td>3</td>
</tr>
<tr>
<td><em>Brachymyrmex</em> <em>obscurior</em> Forel</td>
<td>12</td>
</tr>
<tr>
<td><em>Paratrechina</em> sp.</td>
<td>2</td>
</tr>
<tr>
<td>Tribe: Camponotini</td>
<td></td>
</tr>
<tr>
<td><em>Camponotus</em> sp.</td>
<td>2</td>
</tr>
</tbody>
</table>

(continued)
Table 2. (continued)

<table>
<thead>
<tr>
<th>Ant Identification</th>
<th>Number of sites</th>
</tr>
</thead>
</table>

**Subfamily: Myrmicinae**

**Tribe: Myrmicini**

- *Monomorium ebeninum* Forel 1
- *Pheidole* sp. (4 distinct species) 6
- *Pogonomyrmex barbatus* (F. Smith) 1
- *Solenopsis aurea* Wheeler 1
- *Solenopsis geminata* (F.) 7
- *Tetramorium spinosum* (Pergande) 4

**Tribe: Crematogastrini**

- *Crematogaster* sp. 3
leafhopper. On six of the surveyed plants, *B. obscurior* and low numbers of a much larger ant, *Tetramorium spinosum* (Pergande) (3.75-4.25 mm long) were found tending the *D. quinquenotatus* colonies. Nevertheless, *B. obscurior* was the most numerous ant on these six plants. It was capable of reaching leafhoppers feeding deep within tightly curled *Tripsacum* leaves that were inaccessible to larger ants.

*Solenopsis geminata* (F.) is a small to moderately sized ant (2.75-3.25 mm long) which was found at 7 of the 13 Jalisco sites on various *Tripsacum* species. Commonly known as the "fire ant", *S. geminata* is very aggressive and will attack when disturbed. This species was always abundant on plants where they were found tending *D. quinquenotatus*. Usually, only 1-2 workers were observed tending a leafhopper at any one time.

The red harvester ant, *Pogonomyrmex barbatus* (F. Smith), is a large (7.5-8 mm long), robust, dark red ant. This ant clears all vegetation from an area several meters in diameter from around the nest entrance. At one site near Tequila, we found a single *Tripsacum dactyloides* L. plant growing within this cleared area (ca. 4.5 m dia), only 1 m from the nest entrance. This plant harbored a large colony of *D. quinquenotatus* adults and nymphs that were tended by *P. barbatus* workers.

In the detailed survey of 195 *Tripsacum* plants, all 121 plants with colonies of *D. quinquenotatus* had attendant ants
associated with them (Table 3). Ants were not present on four plants with only a few scattered adults on the upper leaf canopy. The association between *D. quinquenotatus* and ants, and the lack of association between other *Dalbulus* species and ants was significant ($X^2=150.6; \text{df}=4; P<0.001$). None of the plants colonized with *D. quinquenotatus* had other *Dalbulus* leafhoppers present. Other *Dalbulus* species were found on the upper canopy of 43 of the 195 surveyed *Tripsacum* plants. No *Dalbulus* leafhoppers were found on the remaining 27 plants, although 9 had ants present.

**Discussion**

The aggregating and quiescent behaviors of most myrmecophilous homopterans make them well suited for attendance by ants (Buckley 1987). These behaviors enhance the effectiveness of ant defenses (McEvoy 1979). Because myrmecophilous homopterans rely upon ants to defend them from their natural enemies, they show a reduction or loss of intrinsic defense mechanisms (Nault et al. 1976b, Messina 1981). Ants also provide a sanitary service by removing honeydew, thus maintaining host plant quality (Way 1963, Messina 1981, Buckley 1987). In return, ants obtain large amounts of food from a localized, immediately renewable food source (Flanders 1951, Carroll & Janzen 1973). This food is comprised of sugars and amino acids in the form of honeydew (Klingauf 1987), and occasionally proteins and other nutrients in the form of homopteran prey (Buckley 1987).
Table 3. Numbers of Tripsacum plants surveyed with *Dalbulus* leafhoppers (Homoptera: Cicadellidae) and ants (Hymenoptera: Formicidae) at 13 sites in Jalisco, Mexico.

<table>
<thead>
<tr>
<th>LEAFHOPPERS</th>
<th>ANTS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Dalbulus quinquenotatus</td>
<td>121</td>
<td>4</td>
</tr>
<tr>
<td>D. gelbus</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>D. tripsacoides</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>D. elimatus</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>no Dalbulus found</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>
The leafhopper *D. quinquenotatus* exhibits many of the characteristics typical of a myrmecophilous homopteran not expressed by other *Dalbulus* species. It releases large amounts of honeydew (Chapter III), and is gregarious, with groups congregating within the bases of unfolded leaves of its gamagrass hosts (Nault et al. 1983, Triplehorn & Nault 1985). Compared to other *Dalbulus* leafhoppers, *D. quinquenotatus* is relatively unresponsive to a mechanical stimulus, often exhibiting no escape behavior when disturbed (Heady & Nault 1985). Thus, this species, unlike other *Dalbulus* leafhoppers, seems well suited for a mutualistic relationship with ants.

The niche occupied by *D. quinquenotatus* at the base of partially unfolded leaves, where honeydew accumulates, creates an environment where removal of honeydew by ants would be of great benefit to the leafhopper. Although *D. quinquenotatus* seems to be relatively well protected from natural enemies in these leaves, it is a host to two parasitoid species: *Gonatopus moyaraygoza* n. sp. and *Anteon ciudadi* Olmi (Hymenoptera: Dryinidae) (Moya-Raygoza 1990). It is widely assumed that a major role for ants is to protect their hosts from natural enemies (Way 1963, Carroll & Janzen 1973, Buckley 1987). The role of ants in protecting cicadellids from predators and parasitoids is unknown, however, this role has been reported for protection of aphids (Banks 1962, Banks & Macaulay 1967, Bristow 1984),
lycaenid butterfly larvae (Pierce & Mead 1981), treehoppers (Bristow 1984, Cushman & Whitham 1989), and scale insects (Bartlett 1961, Bradley 1973).

Although many ant-homopteran interactions are highly species specific (Way 1963, Buckley 1987), it appears that this is not the case with the ants tending *D. quinquenotatus*. Eighteen species of ants from 12 genera and four subfamilies were found associated with *D. quinquenotatus* (Table 2). This wide variety of ant species found in association with *D. quinquenotatus* indicates that the ants are facultative, as opposed to obligatory members of this mutualistic relationship (Fritz 1982).

Despite the widespread distribution and abundance of several other *Tripsacum* specializing *Dalbulus* leafhoppers in Jalisco (Nault 1990), our survey did not reveal congeners occurring on plants infested with *D. quinquenotatus*. Although other *Dalbulus* leafhoppers utilize primarily the upper canopy of the host plant (Todd et al. 1991), and would therefore not compete directly with *D. quinquenotatus* for host sites, it is possible that attendant ants exclude congeneric competitors. In greenhouse studies, ants were observed to attack and drive away *D. gelbus* and *D. maidis* from the plants (authors, unpublished).

In several cases, ant attendance of Homoptera has been shown to benefit the host plant (Way 1953, Room 1972, Jutsum
et al. 1981, Messina 1981). Our one observation of the red harvester ant leaving a *T. dactyloides* plant within an area cleared of other plants leads us to believe that *D. quinquenotatus* protected the plant from removal by this ant. Additionally, protection of *Tripsacum* may occur during the dry season when the plants are under severe drought stress. The "shrinking" region of living leaf tissue as the dry season progresses would serve to concentrate ants drawn to the plants by the food source provided by *D. quinquenotatus*. This concentration of ants tending *D. quinquenotatus* could indirectly protect the meristematic tissue of the plant from other, more damaging, herbivores.
CHAPTER III

HONEYDEW PRODUCTION AND ANT INTERACTION BEHAVIORS
OF DALBULUS LEAFHOPPERS

Introduction

The fivespotted gamagrass leafhopper, Dalbulus quinquenotatus DeLong & Nault is the only deltocephaline leafhopper (Homoptera: Cicadellidae) known to be associated with ants (Hymenoptera: Formicidae) (Larsen et al. 1991). D. quinquenotatus is a close relative of the corn leafhopper, D. maidis (DeLong & Wolcott), a major pest of maize (Zea mays L.) in Latin America (Nault 1990). Adults of the corn leafhopper and most other Dalbulus species are highly mobile (Heady & Nault 1985), and live on the exposed upper leaf canopy of their host plants (Nault et al. 1983, Todd et al. 1991). D. quinquenotatus differs from other Dalbulus leafhoppers in that it is sedentary (Heady & Nault 1985) and gregarious. Nymphs and adults aggregate within partially unfolded leaves at the bases of their gamagrass (Tripsacum spp.) hosts (Nault et al. 1983, Triplehorn & Nault 1985). Sedentary and gregarious behaviors are unusual
among cicadellids, but typical of myrmecophiles from other homopteran families (Way 1963).

If *D. quinquenotatus* is a myrmecophile, then this species should display behavioral modifications such as a high honeydew output, ant-altered excretory behavior, and the absence of an escape response when approached by ants. To test these hypotheses, in this paper I compare 1) honeydew excretion rates, 2) behaviors associated with the formation and release of honeydew droplets, and 3) behavioral interactions between leafhoppers and the pavement ant, *Tetramorium caespitum* (L.), for *D. quinquenotatus* and four of its non-ant attended congeners: *D. maidis*, *D. gelbus* DeLong, *D. guzmani* DeLong & Nault, and *D. chiapensis* Triplehorn & Nault. Furthermore, I present a detailed analysis, with a kinematic diagram, of the behavioral interactions between *D. quinquenotatus* and *T. caespitum.*

**Materials and Methods**

All experiments were performed using laboratory and greenhouse reared leafhoppers originally collected in Mexico and Guatemala (Table 4). *Dalbulus* leafhoppers studied included three species found exclusively on *Tripsacum* in the field, *D. quinquenotatus*, *D. guzmani*, and *D. chiapensis*, and two other species, *D. gelbus*, a *Tripsacum* specialist that also utilizes maize, and the corn leafhopper, *D. maidis*, found primarily on maize and the teosintes (other *Zea* spp.)
Table 4. Populations of *Dalbulus* leafhopper species used in laboratory and greenhouse experiments for the study of honeydew excretion and ant-leafhopper interactions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Host Plant</th>
<th>Collection Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. quinquenotatus</em></td>
<td>Puerto Los Mazos, Jalisco, Mexico</td>
<td><em>T. pilosum</em></td>
<td>7 October 1989</td>
</tr>
<tr>
<td><em>D. gelbus</em></td>
<td>Instituto de Botanica, Zapopan, Jalisco, Mexico</td>
<td><em>T. pilosum</em></td>
<td>10 October 1989</td>
</tr>
<tr>
<td><em>D. maidis</em></td>
<td>El Grullo, Jalisco, Mexico</td>
<td><em>Z. mays</em></td>
<td>4 May 1989</td>
</tr>
<tr>
<td><em>D. chiapensis</em></td>
<td>El Progresso, Guatemala</td>
<td><em>T. latifolium</em></td>
<td>17 October 1984</td>
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<td><em>D. guzmanii</em></td>
<td>Instituto de Botanica, Zapopan, Jalisco, Mexico</td>
<td><em>T. pilosum</em></td>
<td>10 October 1989</td>
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in the field. *D. maidis* and *D. gelbus* colonies were maintained on sweet corn, (cv. Aristogold Bantam Evergreen) in 19.5 x 38.5 x 39 cm rearing cages, while *D. chiapensis* was reared on *T. latifolium* Hitchcock in 29.5 x 38.5 x 51.5 cm rearing cages (*D'Arcy & Nault 1982*). *D. quinquenotatus* and *D. guzmani* were maintained on *T. pilosum* Scribner & Merrill in 30.5 x 38.5 x 102 cm tall rearing cages. All colonies were kept in an environmental growth chamber set for a light:dark regime of 16h:8h LD, 26°C and RH of 60-90%.

The pavement ant, *T. caespitum*, was the attendant ant used throughout this study. Although *T. caespitum* is known to tend other myrmecophilous homopterans (Smith 1965), this species has not been found naturally in association with *D. quinquenotatus*. However, this species is closely related to *Tetramorium spinosum* (Pergande), one of 18 species of ants found in association with *D. quinquenotatus* in Mexico (Larsen et al. 1991). *T. caespitum* has been observed in association with *D. quinquenotatus* in previous greenhouse studies (Todd et al. 1991), and I took advantage of these naturally occurring greenhouse populations.

**VIDEO RECORDING**

Honeydew excretion and ant-leafhopper interaction behaviors were recorded on videotape using a Panasonic color video camera (model WV-3230) fitted with a lens mount adaptor (WV-AD16), 14 cm extension tube, and Unitron 1:6.5 zoom lens. Images were recorded by a Panasonic VCR tape
recorder (NV-8420) onto 1/2" video cassette tapes. A ruler with 1 mm markings and a digital stopwatch, operating at 0.01 sec intervals, were video recorded as controls for later slow speed measurement of honeydew droplet size and formation times.

HONEYDEW EXCRETION

Honeydew excretion was observed using adult leafhoppers reared on host plants in the greenhouse within 2 x 2 x 2 m screen cages. Video recording allowed close observation of honeydew droplet formation and excretion rates. Honeydew droplet diameter (mm), interval between droplet excretions (sec), and droplet formation and retention times (sec) were recorded on video tape from greenhouse populations of *D. quinquenotatus* (n=23 leafhoppers), *D. gelbus* (n=6) and *D. maidis* (n=13). Honeydew droplet formation time was the period from the first external appearance of a droplet on the anal tube until the droplet reached maximum size. The effect of attendant ants on honeydew excretion behavior by *D. quinquenotatus* was recorded with and without *T. caespitum* workers.

Two to 14 droplets (mean=4.48 drops/leafhopper) were measured for individual leafhoppers, with individual leafhopper means used for statistical analysis. Honeydew excretion rate for each leafhopper was calculated by multiplying the average droplet volume times the number of droplets excreted per hour.
ANT-LEAFHOPPER INTERACTIONS

Interactions between *T. caespitum* ants and *D. quinquenotatus* adults were observed on *Tripsacum* plants growing in greenhouse cages and recorded on videotape. Video recordings were played back in the laboratory, and behaviors of ants and leafhoppers were verbally recorded on an audio cassette tape for 85 *D. quinquenotatus* adults. Behavioral sequences were recorded for each leafhopper from the initial approach by an ant until the ant departed. Behavioral sequence data were organized into a 9 x 9 transition matrix with 10 behavioral categories. First-order transitions were tested for nonrandomness with a G test for goodness of fit (Sokal & Rohlf 1981). Significant transitions occurring at a frequency greater than .10 were included in a kinematic diagram.

Behavioral interactions between ants and adults of the five *Dalbulus* leafhopper species were recorded on video tape. Observations were performed in a small behavioral arena. The arena consisted of a 100 x 15 mm petri dish with an open 2 cm dia access hole centered in the bottom half of the dish. A corked hole was located near the edge of the top half of the dish to introduce leafhoppers and ants. Insect behaviors could be observed and recorded with the video camera through the top of the dish. The access hole was placed in contact with a host plant leaf and four to five adult leafhoppers were placed in the arena. Adult
leafhoppers were given 24 hr to settle down and begin feeding prior to introduction of five T. caespitum workers. Observations of interactions between leafhoppers and ants continued for 5 min following the first contact between an ant and leafhopper.

Results

Honeydew droplet size (F=15.29; df=3,61; p<0.01) and time interval between droplet excretions (F=24.29; df=3,61; p<0.01) were significantly different among D. quinquenotatus, D. gelbus and D. maidis. Larger honeydew droplets were produced by ant-attended and non-attended D. quinquenotatus than were produced by D. gelbus or D. maidis (Fig. 6a). There was no significant difference in droplet size produced by ant-attended and non-attended D. quinquenotatus, or between D. gelbus and D. maidis (Fisher's Protected LSD's for uneven replications; p=0.05).

Time interval between droplet excretions was significantly shorter for D. quinquenotatus than D. gelbus or D. maidis (Fig. 6b). There were significant differences among the species but not between ant-attended and non-attended D. quinquenotatus (Fisher's protected LSD's for uneven replications; p=0.05).

Ant-attended and non-attended D. quinquenotatus produced honeydew at a significantly faster rate than D. gelbus and D. maidis (F=10.31; df=3,61; p<0.01; Fig. 7). Honeydew excretion rates did not vary significantly for
Figure 6. a. Droplet diameter, and b. time interval between droplets (mean ± S.E.) for honeydew droplets produced by Dalbulus leafhoppers, with and without attendant ants.
Figure 7. Honeydew excretion rate (ml/hr) for *Dalbulus* leafhoppers (mean ± S.E.) as determined from droplet volume and time interval between droplet production.
D. quinquenotatus with and without ants, or between
D. gelbus and D. maidis (Fisher’s protected LSD for uneven
replications; p=0.05).

There was no significant difference (F=0.47; df=3,61;
p>0.05) in the time it took each species to form a droplet
(overall mean=0.147 sec; Fig. 8). However, D. maidis and
D. gelbus would immediately flick the droplet away, whereas
non-attended D. quinquenotatus would hold the droplet an
average of 0.28 sec prior to flicking the droplet away.
Ant-attended D. quinquenotatus would hold the droplet 1.31
sec before it was removed by ants or flicked away. The
duration of droplet formation and retention (Fig. 8), was
significantly different among the various species and
between ant-attended and non-attended D. quinquenotatus
(F=66.88; df=3,61; p<0.01). Although the duration of
droplet formation and retention were not significantly
different between D. gelbus and D. maidis, they were
significantly less than for D. quinquenotatus without ants,
which in turn was significantly less than D. quinquenotatus
with attendant ants (Fisher’s Protected LSD’s for uneven
replications; p=0.05).

When the five Dalbulus species were observed
interacting with ants, there were significant differences in
how each species responded to contact with ants and how the
ants responded to different Dalbulus leafhoppers (Fig. 9).
To quantify these interactions, specific behaviors of ants
Figure 8. Mean honeydew droplet formation and retention times for Dalbulus leafhoppers prior to shooting the droplet away or removal by attendant ants.
Figure 9. Behavioral responses to interactions with ants by five Dalbulus leafhoppers. $n =$ number of recorded contacts between Tetramorium caespitum ants and leafhoppers of each species; number in parentheses indicates actual number of leafhoppers observed.
and leafhoppers were categorized and are listed below. The letter preceding each behavior title is the code used in Table 5.

**Ant Behaviors**

- **A** - **Approach.** Approach by the ant into contact with the target leafhopper.

- **G** - **Grooming.** Grooming includes the ant pausing from its other behaviors to rest momentarily or groom itself (antennae, legs, etc.).

- **O** - **Trophallaxis.** Sharing of a recently harvested droplet with another ant.

- **R** - **Harvesting.** Harvesting of honeydew droplets by the ants involved the ant first steadying the droplet with its antennae, then grasping it with its mandibles, followed by the removal of the droplet from the tip of the leafhopper abdomen. The ant would then imbibe the droplet while its antennae continued to slowly massage the leafhopper abdomen.

- **S** - **Solicitation.** Solicitation involves a rapid, alternate stroking of the posterior end of the leafhopper abdomen by the ants’ antennae. This antennation would often result in the excretion of a honeydew droplet by the leafhopper.

- **T** - **Touch.** Touch was any physical contact between the ant and leafhopper other than solicitation that did not result in an obvious response by the leafhopper. Ants would touch the leafhopper head, thorax or side of the abdomen.
with antennae or legs, often in a slow caressing motion. This category included single touches, followed by the ant moving away, or occasionally the ant crawling across the back of the leafhopper.

Z - Leaves. Ant breaks off contact with the leafhopper and departs the location.

**Leafhopper Behaviors**

D - Droplet. Honeydew droplet is excreted and held externally on the tip of the leafhopper abdomen.

F - Discharge. Discharge is the flicking or shooting of a honeydew droplet away from the leafhopper.

K - Wing Flick. This possibly defensive response by the leafhopper was when it would attempt to knock away the ant by a flick of its wings or with a kick with a leg.

W - Walk Away. Walk away was when a leafhopper rapidly walked away in response to an approaching ant.

J - Jump/Fly. Jump/fly was an escape behavior by a leafhopper in response to an approaching ant, and involved an immediate jump or flight away from its resting or feeding site when approached by an ant.

*D. quinquenotatus* was the only *Dalbulus* species solicited for honeydew by ants, with 34% of their interaction behaviors in this category. However, when contacts between ants and leafhoppers were limited to five behavioral categories, solicitation, touch, wing flick, walk away, and jump/fly (Fig. 9), over 80% of the behavioral
Table 5. Transition matrix with number of occurrences and transitional probabilities of behavioral interactions between *T. caespitum* ants and *D. quinquenotatus* leafhoppers. Key to behavior categories and description may be found in text. (n=85 leafhoppers observed).

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contacts between ants and *D. quinquenotatus* were solicitations for honeydew, which were rewarded with droplets 44% of the time. *D. gelbus*, *D. maidis* and *D. guzmanii* responded by escaping by walking, jumping or flying away in over 80% of their contacts with ants, and were never solicited for honeydew. *D. chiapensis* had an escape response to ants 55% of the time and no response to ant touching the rest of the time. Ants were never observed soliciting *D. chiapensis*, and rarely caressed this leafhopper. Most of the contacts between ants and *D. chiapensis* in the touch category were single leg bumps and antennations, or the ant walking over the leafhopper.

Detailed behavioral sequence analysis of interactions between ants and *D. quinquenotatus* documented a more complex series of behaviors than occur between ants and other *Dalbulus* species. A transition matrix with the ten behavioral categories (Table 5) was used to construct a kinematic diagram (Fig. 10). This diagram shows only those behavioral transitions which were both statistically significantly different from those transitions randomly expected (G>3.84; df=1; p=0.05) and probabilities equal to or greater than .10 of one behavior being followed by another.

When most ants approached *D. quinquenotatus*, they immediately began to solicit the leafhopper for honeydew (Fig. 10). A droplet was excreted 44% of the time and then
Figure 10. Kinematic diagram of transition probabilities for behavioral interactions between *Tetramorium caespitum* ants and *Dalbulus quinquenotatus* leafhoppers. Ant behaviors are represented by circles; leafhopper behaviors by squares. Number inside circle or square are frequencies of occurrence of each behavior. Arrows represent transitions; numbers beside arrows are transition probabilities. Behavioral categories representing less than 1% of behaviors are not included, and transition probabilities less than or equal to 0.10 are not drawn.
harvested by the soliciting ant 94% of the time. Ants which harvested a honeydew droplet or spent time grooming were more likely to solicit for additional droplets. However, if an ant contacted the leafhopper at a location other than the posterior end of the abdomen and did not move to the leafhoppers posterior and solicit honeydew, the chances of the ant harvesting a honeydew droplet before departing were low.

Discussion

Most leafhopper adults and nymphs are active and easily can take evasive action to escape predators (Beamer & Michener 1950). However, there are 19 known leafhopper species associated with ants (Larsen, unpublished). These leafhoppers typically are gregarious (Bergevin 1910, Evans 1931) and sedentary (Heady & Nault 1985, Dietrich & McKamey 1990) and do not flee when approached by ants. Moreover, many of these ant-attended leafhopper species are found at the base of their host, protected within folded leaves (Bergevin 1910, Lavigne 1966, Leech 1966). Aggregation of leafhoppers and other homopterans enhances the effectiveness of the protection from predators and parasites by ants (McEvoy 1979). Aggregation also enables ants to readily obtain large amounts of food from a localized source (Flanders 1951, Carroll & Janzen 1973). Because of the protection offered by ants, attended homopteran species normally show a loss or reduction of intrinsic defense
mechanisms (Nault et al. 1976b, Messina 1981), including their ability to take evasive action (Buckley 1987). D. quinquenotatus behaves similarly to other myrmecophilous leafhoppers, i.e. it is sedentary and aggregates in groups of mixed age individuals at the base of its hosts in partially unfolded leaves (Nault et al. 1983).

An increase in feeding rate and honeydew production is not unusual among myrmecophilous homopterans (Klingauf 1987), as has been shown for ant-attended bean aphids, *Aphis fabae* Scopoli (Banks & Nixon 1958). *D. quinquenotatus* increased its honeydew output when attended by ants, but not significantly compared to when ants were absent. Differences in honeydew excretion rates between *D. quinquenotatus* and its congeners were considerable (Fig. 7), with *D. quinquenotatus* excreting significantly more honeydew than *D. maidis* and *D. gelbus*. The low honeydew output by *D. gelbus* and *D. maidis* may be the result of several factors, including body size and feeding behavior. *D. quinquenotatus* is larger than *D. maidis* or *D. gelbus* (Chapter VI), and this may explain why it produces larger honeydew droplets (Fig. 6a).

Myrmecophiles excrete honeydew in a way that facilitates droplet removal by attendant ants. Most leafhoppers quickly shoot or flick honeydew droplets away (Beamer & Michener 1950, Crane 1970). *Dalbulus* leafhoppers, such as *D. maidis* and *D. gelbus* have a similar behavior.
However, *D. quinquenotatus* is unique among *Dalbulus* leafhoppers in that it holds the droplet after formation, thus allowing attendant ants time to detect and remove it. This droplet retention by *D. quinquenotatus* was observed three times to last over 3.5 seconds when attendant ants were present.

The pavement ant did not solicit honeydew from the other *Dalbulus* species. The behaviors of *D. gelbus*, *D. maidis* and *D. guzmani* indicate they do not tolerate attendant ants, but react as they would to any predator, by escaping. Ants also respond differently to these species, walking rapidly and often holding their mandibles wide open when approaching these leafhoppers. Ants have been observed to attack and kill *D. maidis* and *D. gelbus* within small cages from which they cannot escape (Larsen, unpublished).

*D. chiapensis* did not react to contact with ants as did the other three non-attended *Dalbulus* species. Fewer than half of *D. chiapensis* tried to escape when contacted by *T. caespitum* (Fig. 9), yet ants never tried to solicit honeydew from this species. Among *Dalbulus* leafhoppers, *D. chiapensis* is phylogenetically most closely related to *D. quinquenotatus* (Triplehorn & Nault 1985). Recent work has shown the *D. quinquenotatus* line is likely ancestral to *D. chiapensis* (Heady et al. 1989). This would suggest that either *D. chiapensis* may have secondarily lost its myrmecophilous relationship with ants, or *D. quinquenotatus*
could have become a myrmecophile after the *D. chiapensis* line diverged. Like other non-attended *Dalbulus* leafhoppers, *D. chiapensis* utilizes the upper foliage of its *Tripsacum* hosts and is easily disturbed (Triplehorn & Nault 1985). However, its apparent tolerance of ants and the absence of aggressive behavior by the ants suggest that *D. chiapensis* may not have lost all myrmecophilous traits common to its *D. quinquenotatus* ancestor.

Mutualistic relationships between ants and homopterans have the potential to evolve rapidly. Ants exploit any source of sugars, including honeydew which is often readily available on plants inhabited by homopterans. Honeydew producers must adapt by changing how they excrete honeydew so it can be easily harvested by attendant ants. They must also tolerate physical contact with ants. *D. quinquenotatus* was never observed to walk away, jump away or take flight in response to physical contact with *T. caespitum*, although *D. quinquenotatus* would occasionally respond with a flick of its wing or kick of its legs when antennated by an ant along the head or thorax.

There is some evidence ants must learn that myrmecophilous homopterans are a tendable source of honeydew (Paul 1974, Sudd 1987). Most ants that solicit honeydew from *D. quinquenotatus* go directly to the posterior end of the abdomen. Even though solicitation is rewarded by excretion of a droplet only 44% of the time, there does seem
to be a feedback mechanism between ants and
*D. quinquenotatus* (Fig. 10). This behavioral sequence, in
which solicitation results in the production of a honeydew
droplet by the leafhopper, triggers repeated solicitation by
the ant. When ants touch *D. quinquenotatus* somewhere other
than at the posterior end of the abdomen, their chances of
harvesting a droplet were reduced greatly.

To understand how mutualistic relationships between
ants and myrmecophiles may have evolved, we need to know
more about how ants recognize and discriminate between
myrmecophiles and non-myrmecophiles. Virtually nothing is
known about myrmecophile recognition by ants. Ants
communicate using a wide variety of chemical, visual,
tactile, mechanical and acoustic mechanisms (Hölldobler &
Wilson 1990), and probably utilize a variety of cues when
recognizing myrmecophiles. An example of chemical
communication between a myrmecophile and its attendant ants
are the alarm pheromones released by injured aphids. This
pheromone triggers an aggressive response by ants towards
It is unknown if myrmecophiles produce some characteristic
identification chemical that plays a role in myrmecophile
recognition.

Visual signals such as color, shape, or movement may
play a role in close range myrmecophile recognition. The
aphid *Lachnus roboris* (L.) attracts ants by resembling the
shape and movement of the head of an ant worker offering food to other ants. *L. roboris* imitates the ant head by lifting its abdomen and waving its hind legs as antennae at approaching ants (Kloft 1959, 1960). Although *D. quinquenotatus* does not resort to this type of deception, its sedentary behavior may play a secondary role. Sudden movement by the non-myrmecophilous *Dalbulus* leafhoppers attempting to escape may trigger an aggressive response by the ants. By remaining still and not startling nearby ants, *D. quinquenotatus* and *D. chiapensis* may not be stimulating the ant’s aggressive response.

Ants communicate via substrate-borne acoustic signals (Hölldobler & Wilson 1990). A myrmecophilous riodinid butterfly caterpillar emits acoustic signals which attract attendant ants. These ants receive food and provide the caterpillar with protection from predators (DeVries 1990). Substrate-borne acoustic signals are the principle means of species recognition and communication among the Auchenorrhyncha (Claridge 1985), which includes leafhoppers and planthoppers. It is possible that certain acoustic signals issued by leafhoppers attract or are intercepted by ants and are important in the recognition of myrmecophiles. It is known that *D. quinquenotatus* produces a variety of acoustic calls (Heady et al. 1986), and if acoustic signals are important in myrmecophile recognition by ants, they may
play a significant role in attracting ants to

d. quinquenotatus.

This study is the first investigation to compare the
interactions of ants with myrmecophiles and their non-
attended congeners. I have shown that the myrmecophile

d. quinquenotatus, in comparison to other non-attended
Dalbulus species, excretes more honeydew, holds honeydew
droplets to allow droplet removal by attendant ants, and has
extensive physical contact with ants. Understanding these
differences in behavior between myrmecophiles and non-
attended sister species is crucial to understanding the
development of ant-homopteran mutualistic interactions.
CHAPTER IV

FIELD POPULATION DYNAMICS AND DROUGHT HARDINESS
OF DALBULUS LEAFHOPPERS

Introduction

The corn leafhopper, Dalbulus maidis (DeLong & Wolcott), is the most important pest of maize (Zea mays L.) in Latin America. Although *D. maidis* causes feeding damage to maize (Bushing & Burton 1974), it is more important as a vector of three maize disease pathogens, the corn stunt spiroplasma (CSS) (Kunkel 1946, Davis 1974, Nault 1980), maize bushy stunt mycoplasmalike organism (MBSM) (Bradfute & Robertson 1977, Nault 1980), and maize rayado fino virus (MRFV) (Gamez 1969, 1973, Nault et al. 1980). In addition to being the principal field vector, the corn leafhopper is also the only known overwintering host for these maize stunting pathogens. Surprisingly little is known about the field population dynamics and overwintering biology of the corn leafhopper.

To learn more about the overwintering biology of *D. maidis* I compared its biology with that of its non-pest relatives. The leafhopper genus Dalbulus is comprised of 11 neotropical species found in Latin America. Previous
biological, ecological and behavioral studies indicate three ecological groups of *Dalbulus* leafhoppers based on host plant relationships (Fig. 11). The three groups are composed of maize and teosinte (*Zea* spp.) specialists, those which utilize the perennial gamagrasses (*Tripsacum* spp.) as primary hosts but are found on maize, and exclusive *Tripsacum* specialists (Nault 1985).

In Mexico, the hypothesized center of origin of *Dalbulus* (Nault & DeLong 1980), the climate has two seasons, a dry winter and wet summer. The dry season occurs from October to May (Mosino Aleman & Garcia 1974, Moya-Raygoza et al. 1990), and is characterized by low rainfall, lower temperatures, and shorter days compared to the wet season (Fig. 12).

What is known about *Dalbulus* overwintering is limited. During the dry season, *D. eliminatus* (Ball) feeds and reproduces on winter wheat, *Bromus* spp., and other irrigated herbaceous plants in the Valley of Mexico (Barnes 1954, Delgadillo Sanchez 1984, Nault 1985). *D. eliminatus* adults have also been collected from *Tripsacum* (Nault & DeLong 1980), and *Zea perennis* (Hitchcock) Reeves and Mangelsdorf (Nault et al. 1983). Adults of other *Dalbulus* species which have been detected in low numbers during the winter on irrigated maize include the corn leafhopper, *D. maidis*, and two *Tripsacum* specialists, *D. quevarai* DeLong and *D. longulus* DeLong (Nault 1990).
Figure 11. Ecological cladogram of Dalbulus leafhoppers, with ecological traits verified by previous studies to the left and the hypothesized outcome of studies of ecological traits related to overwintering on the right. The cladogram arranges species along a continuum, with maize specialists on the top and Tripsacum specialists on the bottom.
Figure 12. Thirty year average (1931-1960) of a. temperature (mean, mean maximum high, mean minimum low), b. rainfall, and c. daylength for Guadalajara, Jalisco, Mexico. Data from Mosino Aleman & Garcia (1974).
The corn leafhopper, *D. maidis*, reaches high populations on maize by late summer (Gordon et al. 1985, Nault 1985). At the beginning of the dry season, *D. maidis* disappears from mature maize fields, apparently either moving to overwintering sites nearby or migrating to lower elevations to establish populations on irrigated maize (Nault 1990). During the dry season, low populations have been found on the perennial teosintes and irrigated maize (Triplehorn & Nault 1985, Nault 1990). However, because of the restricted distribution of perennial teosintes in southern Jalisco, Mexico (Doebley 1983), and the relatively small areas of irrigated maize, these low numbers are not believed sufficient to account for the populations of immigrating adults observed on maize seedlings at the beginning of the rainy season in Mexico and Central America (Gamez & Leon 1985; A. Power, personal communication).

Very little is known about the overwintering of *Tripsacum* specializing *Dalbulus* leafhoppers in Mexico. At the beginning of the dry season, after plants have flowered, leafhopper populations are high on mature green leaves (Nault et al. 1983, Triplehorn & Nault 1985). *Baldulus tripsaci* Kramer & Whitcomb, a *Tripsacum* specialist and a close relative of *Dalbulus* (Kramer & Whitcomb 1968), overwinters as eggs in the dead leaves of its host in eastern North America (Nault 1990), but it is not known if this overwintering strategy occurs for some of the *Dalbulus*
Tripsacum specialists in Mexico. During the course of studies designed to look at mutualistic interactions between ants and the fivespotted gamagrass leafhopper, D. quinquenotatus DeLong & Nault (Chapter II), both nymphs and adults of two Tripsacum specialists, D. gelbus DeLong and D. quinquenotatus were found on still green leaves at the middle and end of the dry season.

Temperate leafhoppers usually overwinter as adults or eggs (DeLong 1971). In North America, the potato leafhopper, Empoasca fabae (Harris), and the aster leafhopper, Macrosteltes fascifrons (Stal), migrate to warmer climates in the southern U.S. to survive the winter (DeLong & Caldwell 1935, Decker & Maddox 1967, Saini 1967). Many leafhopper species are known to overwinter as hibernating adults in protected sites within leaf litter and other debris (see DeLong & Caldwell 1935). Other temperate leafhoppers, including some Empoasca species (DeLong & Caldwell 1935), the geminate leafhopper, Colladonus geminatus (VanDuzee) (Kaloostian 1956), and Scaphytopius acutus (Say) (Palmiter et al. 1960), overwinter as eggs in plant tissues (DeLong 1971).

Most tropical phytophagous insects, including leafhoppers, are active during the food-rich wet season (Janzen 1983), and likely enter some state of dormancy to survive the environmental stress which occurs during the dry season (Denlinger 1986). If a seasonal return of suitable
conditions is predictable, entering a dormant stage within the host habitat may be a safer strategy than leaving it (Southwood 1962, Hamilton & May 1977). Because gamagrasses are perennial, *Tripsacum* specializing *Dalbulus* leafhoppers are likely to overwinter in their host habitat. The same may be true for leafhoppers that live on the perennial teosintes. However, cultivated maize is a much less stable habitat since the same ground may not be planted to maize every year. Therefore, I hypothesize that *D. maidis* overwinters as mobile adults and may not overwinter in the maize habitat.

Here I report on studies of the overwintering biology of several *Zea* and *Tripsacum* specializing *Dalbulus* leafhopper species. In field studies in Mexico I observed populations of *Dalbulus* during the dry season in October, March and May. *Dalbulus* populations were monitored weekly at three field sites in Jalisco, Mexico, for 18 months with yellow sticky card traps. Dried host plants were collected and examined for the presence of eggs. Finally, in the laboratory I tested adults for their ability to survive long periods in the absence of their hosts.

**Materials and Methods**

**Field Populations**

Field populations of *Dalbulus* leafhoppers were monitored at ca. weekly intervals beginning May or June 1989 until the end of October 1990 at three sites in Jalisco,
Mexico. The three sites were as follows. Site #1: Las Joyas - Laboratorio Natural las Joyas, Universidad de Guadalajara, ca. elev. 1950m, located ca. 25km SE of Autlan from May 3, 1989 to November 2, 1990. The dominant Dalbulus host plant at this site is Zea diploperennis Iltis, Doebley & Guzman. Site #2: Corralitos - a farm ca. elev. 1850m, located ca. 19km SE of Autlan from June 15, 1989 to October 31, 1990. The dominant host plant is maize. Site #3: Zapopan - an arroyo 1 km S of the Instituto de Botanica, Universidad de Guadalajara, Zapopan, ca. elev. 1675m from May 1, 1989 to May 18, 1990 (Fig. 13). The dominant host plants are several species of Tripsacum, with maize fields planted within 200m.

Dalbulus populations were monitored at each site with 12.5 x 20.5 cm yellow sticky card traps (Olson Products, Medina, Ohio). At each site, six traps were hung 0.75 m above the ground and at least 10 m apart among Dalbulus host plants. Traps were changed every one to two weeks by Mexican collaborators, then sent to OARDC, Wooster, Ohio for identification and counting of captured leafhoppers. Because of the variability in the time traps were exposed in the field and the occasional missing traps, for comparison purposes, total counts per trap were converted to the mean number of leafhoppers captured per trap per day for each site.
Figure 13. Zapopan field site, an arroyo full of *Tripsacum* located 1 km S of Instituto de Botanica campus, Universidad de Guadalajara, Zapopan, Jalisco, Mexico.
At each of the three field sites during the first week of May, 1989, two 2 x 2 x 2 m screen cages were placed over clumps of the endemic *Dalbulus* host plants for observations of restricted populations of *Dalbulus* leafhoppers. At Corralitos, the cages were placed in maize fields, and maize was hand planted within the cages on June 6, 1989, the same day as in the surrounding fields. Periodically throughout the wet season, Mexican collaborators collected *Dalbulus* leafhoppers from nearby foliage with a sweep net and aspirator. Leafhoppers were released in the cages to initiate high populations in each cage. Visual observations were made of leafhopper populations within the cages. Then, beginning in September, 1989, at ca. monthly intervals, 10 to 15 nymphs and adults were collected with aspirators from within each cage and placed in vials with 70% EtOH for later identification.

**Egg Overwintering**

To test for the presence of *Dalbulus* leafhoppers overwintering as eggs in their host plants, we collected host plant materials that could potentially harbor overwintering eggs. Eighteen samples (Table 6) of dried roots, culms and leaves were collected from six sites in Jalisco, Mexico, known to be heavily infested with one or more species of *Dalbulus* (Nault et al. 1983, Triplehorn & Nault 1985). Each sample consisted of two or three entire maize plants or one complete *Tripsacum* or *Z. diploperennis*
Table 6. List of date, location, and species of plant sampled to test for the presence of overwintering eggs of Dalbulus leafhoppers.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Plant Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 4/30/89</td>
<td>El Colli, Jalisco, Mexico</td>
<td>T. pilosum</td>
</tr>
<tr>
<td>2 4/30/89</td>
<td>El Colli, Jalisco, Mexico</td>
<td>T. pilosum</td>
</tr>
<tr>
<td>3 4/30/89</td>
<td>El Colli, Jalisco, Mexico</td>
<td>T. pilosum</td>
</tr>
<tr>
<td>4 5/03/89</td>
<td>Las Joyas, Jalisco, Mexico</td>
<td>Z. diploperennis</td>
</tr>
<tr>
<td>5 5/03/89</td>
<td>Las Joyas, Jalisco, Mexico</td>
<td>Z. diploperennis</td>
</tr>
<tr>
<td>6 5/03/89</td>
<td>Las Joyas, Jalisco, Mexico</td>
<td>Z. diploperennis</td>
</tr>
<tr>
<td>7 5/03/89</td>
<td>Las Joyas, Jalisco, Mexico</td>
<td>Z. diploperennis</td>
</tr>
<tr>
<td>8 5/03/89</td>
<td>Las Joyas, Jalisco, Mexico</td>
<td>Z. mays</td>
</tr>
<tr>
<td>9 5/03/89</td>
<td>Las Joyas, Jalisco, Mexico</td>
<td>Z. mays</td>
</tr>
<tr>
<td>10 5/03/89</td>
<td>Corralitos, Jalisco, Mexico</td>
<td>Z. mays</td>
</tr>
<tr>
<td>11 5/03/89</td>
<td>Corralitos, Jalisco, Mexico</td>
<td>Z. mays</td>
</tr>
<tr>
<td>12 5/04/89</td>
<td>Puerto los Mazos, Jalisco, Mex</td>
<td>T. maizar</td>
</tr>
<tr>
<td>13 5/05/89</td>
<td>Puerto los Mazos, Jalisco, Mex</td>
<td>T. maizar</td>
</tr>
<tr>
<td>14 5/05/89</td>
<td>Ojo de Agua, Jalisco, Mexico</td>
<td>T. pilosum</td>
</tr>
<tr>
<td>15 5/05/89</td>
<td>Zapopan, Jalisco, Mexico</td>
<td>T. pilosum</td>
</tr>
<tr>
<td>16 5/05/89</td>
<td>Zapopan, Jalisco, Mexico</td>
<td>T. pilosum</td>
</tr>
<tr>
<td>17 5/05/89</td>
<td>Zapopan, Jalisco, Mexico</td>
<td>Z. mays</td>
</tr>
<tr>
<td>18 5/05/89</td>
<td>Zapopan, Jalisco, Mexico</td>
<td>Z. mays</td>
</tr>
<tr>
<td>19 5/10/89</td>
<td>OARDC, Ohio</td>
<td>T. dactyloides</td>
</tr>
<tr>
<td>20 5/10/89</td>
<td>OARDC, Ohio</td>
<td>T. dactyloides</td>
</tr>
<tr>
<td>21</td>
<td>young control</td>
<td>Z. mays</td>
</tr>
<tr>
<td>22</td>
<td>young control</td>
<td>Z. mays</td>
</tr>
<tr>
<td>23</td>
<td>young control</td>
<td>T. dactyloides</td>
</tr>
<tr>
<td>24</td>
<td>young control</td>
<td>T. maizar</td>
</tr>
</tbody>
</table>
plant. Plants were collected between April 29 and May 6, 1989, near the end of the dry season, and brought back to the laboratory in Ohio. Two additional samples from OARDC field plots in Wooster of dried leaves of *T. dactyloides* that were infested with *B. tripsaci* the previous season were brought into the lab on May 10, 1989.

Plant samples were placed under conditions simulating the beginning of the wet season to trigger egg hatch. Materials were placed into 29.5 x 38.5 x 102 cm tall rearing cages with young healthy potted host plants as a food source. Two pots of maize seedlings, planted three per pot in 10 cm dia pots, and one plant each of *T. dactyloides* and *T. maizar* Hernandez & Randolf growing in 15.5 cm dia pots were used as food source plants in each cage. Four additional cages with no dried plant samples, two containing four pots of maize seedlings and one each with two pots of *T. dactyloides* or *T. maizar* were set up as controls. The cages were placed in the greenhouse under conditions of 14h:10h L:D at 27°C ± 8°C. Cages were gently watered daily to simulate the onset of the wet season. Close inspections of plants were made twice weekly for leafhopper nymphs or adults. The experiment was terminated after eight weeks.

**Laboratory-reared Leafhoppers**

The abilities of *Dalbulus* leafhoppers reared under simulated Mexican conditions to tolerate drought conditions were examined in the greenhouse and laboratory. Leafhoppers
studied included two maize specialists, the corn leafhopper, *D. maidis* and the Mexican corn leafhopper, *D. elimatus*. Three *Tripsacum* specialists were also examined; *D. gelbus*, which is also found on maize, and two exclusive *Tripsacum* specialists, the fivespotted gamagrass leafhopper, *D. quinquenotatus* (which can be reared on maize seedlings in the laboratory; Nault & Madden 1985) and *B. tripsaci*. Laboratory-reared populations were the same as those described in Triplehorn et al. (1990) and Todd et al. (1991). *Dalbulus* colonies were maintained on sweet corn (cv. Aristogold Bantam Evergreen) in 19.5 x 38.5 x 39 cm rearing cages. *B. tripsaci* was reared on eastern gamagrass, *T. dactyloides*, in larger 29.5 x 38.5 x 51.5 cm rearing cages (D'Arcy & Nault 1982). Colonies were maintained at ca. 26±2°C, RH of 60-90%, and with a light:dark regime (L:D) of 16h:8h.

Experimental leafhoppers were reared under two sets of conditions designed to simulate those occurring in Jalisco, Mexico at the beginning of the wet season in June ("June-reared") and at the beginning of the dry season in October ("October-reared") (Fig. 12).

**June Conditions.** June-reared leafhoppers were reared in cages filled with seedling sweet corn (ca. 4-5 leaf stage) in 10 cm dia pots. June-reared *B. tripsaci* was reared on young *T. dactyloides*. Adult leafhoppers from the laboratory colonies (n=150 females, 50 males) were given a 48 hr
oviposition access period (OAP) on the host plants. Eggs hatched and nymphs developed in an environmental chamber set for 14h:10h L:D with a 28°C/20°C temperature cycle, RH of 70-90%, with plants watered daily and replaced with fresh young seedlings when needed.

**October Conditions.** October-reared leafhoppers were reared in 30.5 x 38.5 x 102 cm rearing cages filled with tassled sweet corn or *Tripsacum* in 15.5 cm dia pots. Adult leafhoppers (n=150 females, 50 males) were given a 48 hr OAP. Egg hatch and nymphal development occurred under environmental conditions of 12h:12h L:D with a 23°C/17°C temperature cycle, with water stressed plants watered only once/week and replaced by mature plants only as needed.

**Dry Season Simulation**

*D. maidis, D. gelbus* and *D. quinquenotatus* populations were monitored under environmental conditions that simulated the Mexican dry season in the OARDC greenhouse (Table 7). Mature maize (*Z. mays*) and *Tripsacum* plants (*T. dactyloides, T. maizar*, and *T. latifolium* Hitchcock) were grown in 24 x 28 cm dia pots. Eight pots each (3 maize and 5 *Tripsacum*) were placed in two 2 x 2 x 2 m screen cages in the greenhouse. Plants were watered daily and fertilized weekly for four weeks prior to the introduction of the leafhoppers. On day 0, the date the simulation began, 200 October-reared females and 200 October-reared males each of *D. maidis, D. gelbus, and D. quinquenotatus* were released into each
Table 7. Schedule of environmental conditions in the greenhouse to simulate the Mexican climate based on daylength, mean daily temperatures, and occurrence of measurable precipitation data for Guadalajara, Jalisco, Mexico (Mosino Aleman & Garcia 1974).

<table>
<thead>
<tr>
<th>Month</th>
<th>Running Days</th>
<th>Date</th>
<th>Daylength Light:Dark (hour)</th>
<th>Mean Daily Temperatures (°C)</th>
<th>Measurable Precipitation Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>0-14</td>
<td>0</td>
<td>12:12</td>
<td>20</td>
<td>4X/wk</td>
</tr>
<tr>
<td>October</td>
<td>15-42</td>
<td>21</td>
<td>11.5:12.5</td>
<td>19</td>
<td>2X/wk</td>
</tr>
<tr>
<td>&quot;</td>
<td>42</td>
<td></td>
<td>11:13</td>
<td>18</td>
<td>2X/wk</td>
</tr>
<tr>
<td>November</td>
<td>43-70</td>
<td>63</td>
<td>10.5:13.5</td>
<td>17</td>
<td>1X/wk</td>
</tr>
<tr>
<td>December</td>
<td>71-98</td>
<td>84</td>
<td>10:14</td>
<td>16</td>
<td>1X/2 wks</td>
</tr>
<tr>
<td>January</td>
<td>99-126</td>
<td>105</td>
<td>10.5:13.5</td>
<td>15</td>
<td>1X/2 wks</td>
</tr>
<tr>
<td>&quot;</td>
<td>126</td>
<td></td>
<td>11:13</td>
<td>16</td>
<td>1X/2 wks</td>
</tr>
<tr>
<td>February</td>
<td>127-154</td>
<td>147</td>
<td>11.5:12.5</td>
<td>17</td>
<td>1X/4 wks</td>
</tr>
<tr>
<td>March</td>
<td>155-182</td>
<td>168</td>
<td>12:12</td>
<td>18</td>
<td>1X/4 wks</td>
</tr>
<tr>
<td>April</td>
<td>183-210</td>
<td>189</td>
<td>12.5:11.5</td>
<td>20</td>
<td>1X/4 wks</td>
</tr>
<tr>
<td>&quot;</td>
<td>210</td>
<td></td>
<td>13:11</td>
<td>21</td>
<td>1X/4 wks</td>
</tr>
<tr>
<td>May</td>
<td>211-238</td>
<td>231</td>
<td>13.5:10.5</td>
<td>23</td>
<td>1X/wk</td>
</tr>
<tr>
<td>June</td>
<td>239-266</td>
<td>252</td>
<td>14:10</td>
<td>24</td>
<td>4X/wk</td>
</tr>
<tr>
<td>July</td>
<td>267-294</td>
<td>273</td>
<td>13.5:10.5</td>
<td>22</td>
<td>6X/wk</td>
</tr>
<tr>
<td>&quot;</td>
<td>294</td>
<td></td>
<td>13:11</td>
<td>21</td>
<td>6X/wk</td>
</tr>
<tr>
<td>August</td>
<td>295-322</td>
<td>315</td>
<td>12.5:11.5</td>
<td>20</td>
<td>5X/wk</td>
</tr>
<tr>
<td>September</td>
<td>323-350</td>
<td>336</td>
<td>12:12</td>
<td>20</td>
<td>4X/wk</td>
</tr>
</tbody>
</table>
cage to simulate the high *Dalbulus* adult populations observed in Mexico at the end of the wet season.

Populations of *D. maidis*, *D. quinquevittatus*, and *D. gelbus* in the cages were monitored for one year, starting with environmental conditions similar to those at the end of the wet season in September. Beginning day 7, one yellow sticky card trap (12.5 x 20 cm) was suspended in the center of each cage and changed weekly to monitor adult leafhopper population levels.

Simulation conditions covered a one year climatic cycle, with a schedule based on months of 27 days. Lighting, temperature and watering schedules followed are listed in Table 7. These conditions approximated daylength, mean daily temperatures and precipitation in Guadalajara, Jalisco, Mexico (Mosino Aleman & Garcia 1974). Plants were allowed to die back as would happen during the dry season in the field. Maize seed was planted in the same pots as before on day 245, during the first week of June at the beginning of the wet season. Simulation and population monitoring continued until day 350, the equivalent of September at the end of the wet season.

**Drought Tolerance**

**Absolute Drought.** The ability of June-reared and October-reared adults to survive severe drought was estimated by measuring their survival in the absence of any source of food and water. Each of the five species was
tested by placing groups of ca. two wk old adult leafhoppers reared under the June or October conditions into small "exposure" cages, 3.3 x 3.3 x 2.5 cm plastic snap pill boxes, with a corked hole for introduction of leafhoppers and screened ventilation holes. Each cage had one waffled piece of 9 x 2 cm brown paper towel set vertically as a substrate. Five replicate cages of each species and rearing treatment (June or October) were placed in an environmental growth chamber under constant light (ca. 31.1 μE/m²/s inside cages) at 20°C, 65 ± 15% RH. Leafhopper survival within each exposure cage was recorded at 3, 6, 9, 12, 24, 48 hr and additional 24 hr intervals until 100% mortality.

**Moisture Only.** In a second test of drought tolerance, *D. maidis* adults were given access to water but no food. Two wk old adult *D. maidis* were given access to moist sand ("water only"). Controls were leafhoppers given access to dry sand or healthy maize seedlings. There were three replicates of 15 males and 15 females for each treatment of June-reared and October-reared *D. maidis* adults. Survival observation chambers were assembled for the water only and no food and water treatments. The base of these chambers consisted of one half of a 100 x 15 mm petri dish, open side up, with one half of a smaller 35 x 10 mm petri dish open side up in the center of the larger dish. Both dishes were filled to the rim with sterilized white sugar sand. A 20.5 x 7.5 cm dia butyrate tube cage with a corked introduction
hole and screened ventilation holes was placed over the small petri dish into the sand in the larger dish. In one treatment, deionized/distilled water was added daily with an eyedropper to keep the sand moist in the smaller dish within each tube cage. Water was not added in the other treatment. These survival observation chambers were placed in an environmental growth chamber under constant low light (ca. 4.4 µE/m²/s) at 18°C and RH of 50-80%.

In the third treatment, healthy maize controls were 2-3 leaf stage sweet corn seedlings three per pot in 10 cm dia pots. The soil surface was covered by ca. 5 mm of white sugar sand. The plants were contained within butyrate tube cages as above and placed in an environmental growth chamber under constant high light (ca. 31.1 µE/m²/s) at 18°C and RH of 60-90%.

The number of dead leafhoppers in each cage was recorded daily for two weeks, then weekly until 100% mortality was obtained for all treatments.

Statistical Analysis

To determine the mean time to 50% mortality of the population [(LT₅₀)], probit analysis was used to measure mean survival times as has been done with Diabrotica spp. (Elsey 1989). To obtain LT₅₀’s, I used a probit analysis macro procedure (PROBIT; developed by J.R. Sedcole and revised by L.V. Madden) for use with MINITAB. Survival data were analyzed both as mean time to 50% mortality (LT₅₀’s) as
described below, and by separating survival into quartiles, i.e. time to 75% \((t_{75})\), 50% \((t_{50})\) and 25% \((t_{25})\) survival, calculated by linear interpolation. Analysis of variance (ANOVA) determined the effect of leafhopper species, rearing conditions, and replication on the mean survival time and survival quartiles. MINITAB was used for all analyses (Anonymous 1989).

**Results**

**Field Populations**

Seven *Dalbulus* species were collected by yellow sticky card traps among the three field sites in Jalisco, Mexico (Table 8). A total of 10,504 *Dalbulus* leafhoppers were collected, of which 58% were collected at the Zapopan site.

The corn leafhopper, *D. maidis*, was the most abundant *Dalbulus* species collected at Las Joyas and Corralitos, but was ranked fourth in abundance of seven *Dalbulus* species at Zapopan (Table 8). Over time, there were two peaks of *D. maidis* at both Las Joyas (Fig. 14a) and Corralitos (Fig. 14b), but only one at Zapopan (Fig. 14c). Over 60% of all *D. maidis* collected by yellow sticky card traps were trapped between October and February at the Corralitos site.

Separation of *D. maidis* captured at Corralitos into males and females (Fig. 15) shows the highest peak of females occurred one week after the highest peak of males in late January. However, overall there was no apparent difference
Table 8. Total number of *Dalbulus* leafhoppers captured by yellow sticky card traps, and relative % abundance within each site at three field sites in Jalisco, Mexico between May 1, 1989 and November 1, 1990.

<table>
<thead>
<tr>
<th>Species</th>
<th>Las Joyas count (%)</th>
<th>Corralitos count (%)</th>
<th>Zapopan count (%)</th>
<th>Totals count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(count)</td>
<td>(count)</td>
<td>(count)</td>
<td>(count)</td>
</tr>
<tr>
<td><strong>D. maidis</strong></td>
<td>1290 (27.2)</td>
<td>2882 (60.7)</td>
<td>576 (12.1)</td>
<td>4748 (96.3)</td>
</tr>
<tr>
<td></td>
<td>(96.3)</td>
<td>(94.0)</td>
<td>(9.4)</td>
<td>(45.2)</td>
</tr>
<tr>
<td><strong>D. elimatus</strong></td>
<td>46 (4.8)</td>
<td>185 (19.5)</td>
<td>720 (75.7)</td>
<td>951 (3.4)</td>
</tr>
<tr>
<td></td>
<td>(3.4)</td>
<td>(6.0)</td>
<td>(11.8)</td>
<td>(9.1)</td>
</tr>
<tr>
<td><strong>D. gelbus</strong></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3780 (100.0)</td>
<td>3780 (0.0)</td>
</tr>
<tr>
<td></td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(62.0)</td>
<td>(36.0)</td>
</tr>
<tr>
<td><strong>D. quinquenotatus</strong></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>650 (100.0)</td>
<td>650 (0.0)</td>
</tr>
<tr>
<td></td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(10.7)</td>
<td>(6.2)</td>
</tr>
<tr>
<td><strong>D. guzmani</strong></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>350 (100.0)</td>
<td>350 (0.0)</td>
</tr>
<tr>
<td></td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(5.7)</td>
<td>(3.2)</td>
</tr>
<tr>
<td><strong>D. quevarai</strong></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>19 (100.0)</td>
<td>19 (0.0)</td>
</tr>
<tr>
<td></td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.3)</td>
<td>(0.2)</td>
</tr>
<tr>
<td><strong>D. tripsacoides</strong></td>
<td>4 (66.7)</td>
<td>0 (0.0)</td>
<td>2 (33.3)</td>
<td>6 (&lt;0.1)</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.0)</td>
<td>(&lt;0.1)</td>
<td>(&lt;0.1)</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>1340 (12.8)</td>
<td>3067 (29.2)</td>
<td>6097 (58.0)</td>
<td>10504</td>
</tr>
</tbody>
</table>
Figure 14. Number of *Dalbulus maidis* leafhoppers captured per trap per day by yellow sticky card traps at three sites in Jalisco, Mexico: a. Las Joyas, b. Corralitos, and c. Zapopan.
Figure 15. Number of male and female *D. maida* adults captured per trap per day by yellow sticky card traps in maize at the Corralitos site in Jalisco, Mexico.
in the numbers of males and females captured throughout the year.

The Mexican corn leafhopper, *D. elimatus*, was present at all three sites, but accounted for only 9.1% of all *Dalbulus* leafhoppers captured. At Las Joyas, *D. elimatus* was present throughout the year, but at low numbers (Fig. 16a). At Corralitos (Fig. 16b) and Zapopan (Fig. 16c), *D. elimatus* had only one generation peak captured by the yellow sticky card traps. Over 75% of all *D. elimatus* collected were trapped at Zapopan (Table 8).

The most abundant *Dalbulus* leafhopper at Zapopan was *D. gelbus*, representing 62% of all *Dalbulus* leafhoppers collected at this site. *D. gelbus* adults were collected year-round, but most were trapped between early November and early March (Fig. 17). No *D. gelbus* were collected at Las Joyas or Corralitos.

*D. quinquenotatus*, which maintained the most consistent population levels throughout the year, was trapped only at Zapopan (Fig. 18). This leafhopper apparently breeds continuously throughout the year.

The other *Dalbulus* leafhopper trapped in significant numbers at Zapopan was *D. guzmani* DeLong & Nault, representing almost 6% of the *Dalbulus* collected at this site (Table 8). Like *D. gelbus*, *D. guzmani* was collected primarily between November and March (Fig. 19). Two other rarely trapped *Dalbulus* species included *D. tripsacoides*
Figure 16. Number of *Dalbulus elimatus* leafhoppers captured per trap per day by yellow sticky card traps at three sites in Jalisco, Mexico: a. Las Joyas, b. Corralitos, and c. Zapopan.
Figure 17. Number of *Dalbulus gelbus* leafhoppers captured per trap per day by yellow sticky card traps at Zapopan, Jalisco, Mexico.
Figure 18. Number of *Dalbulus quinquenotatus* leafhoppers captured per trap per day by yellow sticky card traps at Zapopan, Jalisco, Mexico.
Figure 19. Number of D. guzmani leafhoppers captured per trap per day by yellow sticky card traps at Zapopan, Jalisco, Mexico.
DeLong & Nault at both Las Joyas and Zapopan, and
D. quevarai DeLong at Zapopan.

Observed trends of the Dalbulus populations contained
within the screen cages at Zapopan and Las Joyas were
consistent with the yellow sticky card trap captures,
although quantitative data were not available. The cages
over Tripsacum at Zapopan contained high numbers of
D. gelbus, moderate numbers of D. quinquenotatus, and low
numbers of D. guzmani throughout the dry season. At Las
Joyas, the cages over Z. diploperennis contained D. maidis
adults from September until November, nymphs in January and
February, followed by the reappearance of adults beginning
in late February on the small green shoots at the base of
the drying plants. In the cages at Corralitos, there were
high populations of D. maidis on maize during October and
early November which then declined as the maize dried up.
Careful searches after December in these cages did not
reveal any D. maidis adults in the dry corn stubble or on
herbaceous plants still growing within the cages.

Diversity of the Dalbulus fauna collected on yellow
sticky card traps for each site was defined by the Shannon-
Weaver diversity index $H'$, where $H'=-\sum p_i \log e p_i$ and $p_i$ is
the proportion of each species in the total sample (Price
1984). Zapopan was the most diverse site ($H'=1.20$), with
seven Dalbulus species present. Of the 6097 Dalbulus
leafhoppers collected at this site, D. gelbus was very
abundant, four species were common, and two were rare. The 3067 Dalbulus leafhoppers collected at Corralitos represented only two species, D. maidis (94%) and D. elimatus (6%), and their abundance made this site less diverse (H' = 0.23). Las Joyas was the least diverse site (H' = 0.17), despite the presence of three species, as D. maidis represented over 96% of the 1340 Dalbulus leafhoppers collected.

Egg Overwintering

Large populations of B. tripsaci developed in the two cages containing dried T. dactyloides plant materials collected in Ohio. Thirty days after attempting to initiate egg hatch with water applications, 523 and 667 B. tripsaci adults were removed from these cages. Both cages contained T. dactyloides plant materials collected from a field site infested the previous summer with B. tripsaci. No leafhopper nymphs or adults developed from the 18 Mexican field samples or the four young plant controls (Table 6). When the experiment was terminated after 56 days, the two B. tripsaci populations were well into the mid to late instar nymphal stages of a second generation. After 56 days there was still no evidence of any leafhopper nymphs or adults present in any of the other cages, and the experiment was terminated.
Dry Season Simulation

The simulation of the Mexican climate in the greenhouse was most like the Zapopan site, with both maize and Tripsacum host plants present. Mature maize plants dried up quite quickly with the lack of daily watering, and were completely dead by simulated December 1 on day 70. Tripsacum declined more slowly, remaining green much longer and with leaves gradually dying back except near the base of the plant during the dry season. When increased watering started on day 211 (May 1), new growth rapidly increased overall plant size.

The corn leafhopper, D. maidis, began the simulation period with a rapidly declining adult population which paralleled the decline of the maize as adults died or were trapped out (Fig. 20). Two or three female D. maidis were trapped as late as day 155, early in March, but a population of D. maidis never reappeared, even when maize was planted at day 245 (June 1).

The most abundant leafhopper was D. gelbus, which also began the simulation with a rapid population decline. However, this decline was followed by two large increases of adults, preceded by large numbers of developing nymphs. The first peak occurred from mid-November to March, and the second began in late July until the experiment was terminated in September (Fig. 21). Similarly, D. quinquenotatus also had two large peaks, the first from
Figure 20. Mean number of *Dalbulus maidis* adults captured per trap per day by yellow sticky card traps, and condition of maize host plants in greenhouse simulation of a one year cycle of environmental conditions occurring in Jalisco, Mexico.
Figure 21. Mean number of *Dalbulus gelbus* adults captured per trap per day by yellow sticky card traps, and condition of *Tripsacum* host plants in greenhouse simulation of a one year cycle of environmental conditions occurring in Jalisco, Mexico.
Figure 22. Mean number of *Dalbulus quinquenotatus* adults captured per trap per day by yellow sticky card traps, and condition of *Tripsacum* host plants in greenhouse simulation of a one year cycle of environmental conditions occurring in Jalisco, Mexico.
December until the end of March and the second beginning in August (Fig. 22).

**Drought Tolerance**

**Absolute drought.** Tolerance of five leafhopper species to survive absolute drought was measured as the ability to survive a lack of food and water (Figs. 23-27). There were significant differences in the ability of the various species to survive ($F=187.36; \text{df}=4,40; p<0.01$). Among June-reared leafhoppers, *D. gelbus* and *B. tripsaci* were the shortest lived. *D. maidis* and *D. quinquenotatus* were moderately tolerant, while *D. elimatus* was the most tolerant (Table 9). Among October-reared adults, *B. tripsaci* and *D. gelbus* were the shortest lived, whereas *D. maidis*, *D. elimatus* and *D. quinquenotatus* were the longest lived. There was a significant increase in the drought tolerance of October-reared leafhoppers compared to the June-reared leafhoppers for all species tested except *B. tripsaci*. *D. elimatus* had an increase of 1.28X, followed by *D. maidis* (1.51X), *D. gelbus* (1.53X), with *D. quinquenotatus* (2.13X) exhibiting the greatest overall increase in drought tolerance.

**Moisture Only.** Adult *D. maidis* which had access to moist sand as a water source had significantly improved survival over those denied food and water, based on $t_{75}$, $t_{50}$ and $t_{25}$ quartiles. However, their survival was significantly less than for *D. maidis* supplied with maize.
Figure 23. Absolute drought tolerance when denied food and water by *Dalbulus maidis* adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 24. Absolute drought tolerance when denied food and water by *Dalbulus elimatus* adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 25. Absolute drought tolerance when denied food and water by *Dalbulus gelbus* adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 26. Absolute drought tolerance when denied food and water by *Dalbulus quinquenotatus* adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 27. Absolute drought tolerance when denied food and water by Baldulus tripsaci adults as measured by the percent survival of the population over time (mean ± standard error).
Table 9. Absolute drought tolerance when denied food and water as measured by time to 50% mortality (LT$_{50}$) in hours reared under environmental conditions similar to those in June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) in Jalisco, Mexico, for adults of five leafhopper species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Rearing Regime</th>
<th>no food or water LT$_{50}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalbulus maidis</td>
<td>June</td>
<td>36.52 c</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>55.29 e</td>
</tr>
<tr>
<td>D. elimatus</td>
<td>June</td>
<td>47.29 d</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>60.37 f</td>
</tr>
<tr>
<td>D. gelbus</td>
<td>June</td>
<td>15.90 a</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>24.40 b</td>
</tr>
<tr>
<td>D. quinquetatus</td>
<td>June</td>
<td>33.63 c</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>71.68 g</td>
</tr>
<tr>
<td>Baldulus tripsaci</td>
<td>June</td>
<td>21.63 b</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>22.62 b</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different (Fisher's Protected LSD=4.88; df=40; p=0.05).
seedlings ($F=227.91; df=2,12; \ p<0.001$). October-reared adults survived significantly longer than June-reared adults for all treatments ($F=33.07; df=1,12; \ p<0.05$); however, the water only treatment showed the greatest improvement in survival (Fig. 28). For the water only treatment, October-reared leafhoppers survived almost 3X longer than the June-reared adults, while the absolute drought and maize controls had only 1.54X and 1.69X improvements in survival (Table 10). Separation into survival quartiles over time for the water only treatment revealed a 6.27X increase in the $t_{25}$ of October-reared ($t_{25}=613.2 \ hr$) over June-reared ($t_{25}=97.8 \ hr$) adults.

Sex of adults was not a factor in survival of June-reared leafhoppers ($F=0.08; df=1,173; \ p>0.05$), but was significant for October-reared adults ($F=17.29; df=1,202; \ df<0.05$). Among the three treatments of October-reared insects, the water only treatment was the only treatment in which sex was a significant factor ($F=109.35; df=1,80; \ p<0.05$). Further separation of the data (Fig. 29) show that much of the improvement in survival of moisture treated, October-reared adults was due to improved survival of females.

**Discussion**

These studies were the first extended monitoring of field populations of *Tripsacum*-specializing *Dalbulus* leafhoppers, and only the second study to quantitatively
Figure 28. Survival (mean ± standard error) of a. June-reared and b. October-reared Dalbulus maidis adults when limited to no water or food, moist sand (water only), or maize seedlings.
Table 10. Quartiles, time to 75% ($t_{75}$), 50% ($t_{50}$), and 25% ($t_{25}$) survival and LT$_{50}$'s (hrs) of June-reared and October-reared *D. maidis* adults (mean ± S.E.) when allowed access to no food or water, water only, or maize seedlings.

<table>
<thead>
<tr>
<th>Host</th>
<th>Rearing Regime</th>
<th>Time to % Survival (hrs)</th>
<th>LT$_{50}$'s mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$t_{75}$</td>
<td>$t_{50}$</td>
</tr>
<tr>
<td>no food or water</td>
<td>June</td>
<td>17.28</td>
<td>33.36</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>49.20</td>
<td>58.32</td>
</tr>
<tr>
<td>water only</td>
<td>June</td>
<td>57.62</td>
<td>84.78</td>
</tr>
<tr>
<td></td>
<td>*Oct.</td>
<td>76.06</td>
<td>149.79</td>
</tr>
<tr>
<td>maize plant</td>
<td>June</td>
<td>420.00</td>
<td>672.00</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>690.67</td>
<td>1053.82</td>
</tr>
<tr>
<td>*October-reared</td>
<td>*October-reared water only</td>
<td>67.54</td>
<td>138.79</td>
</tr>
<tr>
<td></td>
<td>males</td>
<td>83.30</td>
<td>180.00</td>
</tr>
</tbody>
</table>

*October-reared water only*
Figure 29. Survival (mean ± standard error) of male and female October-reared Dalbulus maidis adults on moist sterile sand (water only) or maize seedlings.
monitor field populations of *D. maidis* and *D. elimatus*. Population trends of *D. maidis* and *D. elimatus*, particularly at Corralitos and Zapopan (Figs. 14, 16), were similar to those populations detected on maize at Chapingo and several other sites in the central valley of Mexico by Barnes (1954). It is likely that the absence of maize following harvest and deteriorating conditions of the perennial teosinte and *Tripsacum* in the field early in the dry season are responsible for increased movement by *Dalbulus* leafhoppers as they search for alternate feeding hosts or overwintering sites. This movement may account for the increased yellow sticky card trap captures of all *Dalbulus* species in the early to mid part of the dry season from November to January.

There were significant differences in the diversity of *Dalbulus* fauna at the various field sites. This diversity was influenced by host plants present. The Las Joyas site was composed of primarily the perennial *Z. diploperennis*, with several small maize plots within 1 km. This site, high in the Sierra de Manantlan, was isolated from large maize fields or *Tripsacum* populations, and as a result had the lowest *Dalbulus* diversity of the three sites. Corralitos was almost as isolated. The site was high in the Sierra de Manantlan on a farm with several large fields, totalling several hundred acres of maize. The Zapopan site had the most diverse flora, with several species of *Tripsacum*,
surrounded nearby with large maize fields. It was this site in the Valle de Guadalajara which had the greatest diversity of *Dalbulus* leafhoppers.

The *Tripsacum* specialists, *D. gelbus* and *D. guzmani*, were captured in the highest numbers between November and March (Figs. 17, 19). *D. quinquenotatus*, however, was trapped consistently throughout the year, but at relatively low numbers (Fig. 18). *D. quinquenotatus* is a myrmecophile and utilizes the base of its *Tripsacum* host (Larsen et al. 1991). The base (or crown) is the only part of most *Tripsacum* plants still green at the end of the dry season. The utilization of this site allows continuous development of *D. quinquenotatus* populations throughout the year.

Eggs of many insect species require water absorption for embryonic development (Ando 1972, Denlinger 1986). The onset of rains at the beginning of the wet season may cue the break in diapause of overwintering eggs to coincide with new growth of host plants. I conclude that Mexican *Tripsacum* and *Zea* leaves did not harbor overwintering eggs of *Dalbulus* since no leafhoppers emerged from dead leaves and culms wetted in emergence cages. This was in contrast to leaves and culms collected from plants infested with *B. tripsaci*. This temperate leafhopper clearly overwinters in the egg stage on winter-killed host leaves. The geminate leafhopper, *C. geminatus*, a temperate species, also overwinters in the egg stage. However, no cold period or
obligate dormancy is necessary for hatching of overwintering eggs of *C. geminatus* from dead leaves. Cold temperatures evidently merely delay development in this leafhopper (Kaloostian 1956). It is possible that *B. tripsaci*, like *C. geminatus*, has egg development delayed by cold temperatures and an absence of living hosts, and the eggs of this leafhopper do not enter an obligate winter egg diapause.

Simulation of the maize and *Tripsacum* habitats in the greenhouse and the monitoring of *D. maidis*, *D. gelbus* and *D. quinquenotatus* populations may provide the best clues to what is happening in the field. The two *Tripsacum* specialists, *D. gelbus* (Fig. 21) and the fivespotted gamagrass leafhopper, *D. quinquenotatus* (Fig. 22), continued to reproduce on deteriorating *Tripsacum* throughout the simulated dry season. Additionally, I observed both nymphs and adults of these *Tripsacum*-specializing *Dalbulus* leafhoppers throughout the dry season in the field. *Dalbulus* species which specialize on *Tripsacum* therefore do not overwinter in the egg stage as does their temperate cousin, *B. tripsaci*.

In the field, the *Tripsacum* habitat is under severe drought stress during the dry season. The remaining areas of living tissue during the gradual leaf die-back provides a readily available, albeit shrinking food source throughout the dry season for *Tripsacum*-specializing *Dalbulus*
leafhoppers. Because of this resource, these leafhoppers are less susceptible to hazardous conditions associated with the dry season. Since Tripsacum specialists have a stable resource, they also exhibit reduced changes in their ability to survive drought conditions when compared with their maize specialist congener.

The only previously published long-term study (greater than 12 weeks) to monitor quantitatively Dalbulus leafhopper populations in the field was by Barnes (1954). He counted D. maidis and D. elimatus populations in an emergence trap on irrigated maize plots planted at two week intervals for one year. He reported high populations of D. maidis and D. elimatus from October to February. Populations were low from March until the end of the wet season in September.

Power (1987) also has observed populations of D. maidis in small irrigated maize field plots in Nicaragua to be highest on maize after the wet season in November. This would suggest most leafhopper population development occurs late in the wet season on mature maize plants.

There are several reports of populations of immigrating D. maidis adults appearing in young maize fields at the rate of three to four per plant at the beginning of the wet season in Costa Rica and Nicaragua (Gamez & Leon 1985; A. Power, personal communication). These immigrant populations are thought to be extremely important in the epidemiology of maize pathogens transmitted by Dalbulus.
leafhoppers. Neither Barnes (1954) nor I detected populations of *D. maidis* between mid-May and early July that could be construed as spring migrants.

Barnes (1954) used an absolute sampling technique to trap and count all *D. maidis* and *D. elimatus* adults on single maize plants twice per week for one year. I used yellow sticky card traps to monitor *Dalbulus* populations. Yellow sticky card trap captures have been shown to be reliable indicators of population generations occurring in field populations of other leafhopper species (Alverson et al. 1977, Larsen & Whalon 1988, Whitney & Meyer 1988). Although it is possible this immigrant population was not attracted to yellow sticky card traps, *D. maidis* adults are highly responsive to yellow (Todd et al. 1990). One explanation why Barnes and I did not observe the reported immigrant populations of *D. maidis* in maize early in the wet season is that we monitored during years of low *D. maidis* populations. Only a long-term study monitoring field populations over a wide area in Latin America will be able to answer this question. This project should use an absolute census, such as maize whorl counts, as well as relative trapping techniques, such as reported here, to measure quantitatively *D. maidis* populations.

In the simulated maize and *Tripsacum* habitats in the greenhouse, populations of *D. maidis* declined rapidly with the decline of maize (Fig. 20). Once maize was completely
dried, *Tripsacum* was available as an alternative host. Eastern gamagrass, *T. dactyloides*, has been shown to be an experimental feeding and developmental host for *D. maidis* in the greenhouse (Pitre et al. 1966, Pitre 1967), yet *D. maidis* did not survive on this host in my studies. It is unlikely this disappearance of *D. maidis* was due to interspecific competition with *D. quinquenotatus* or *D. gelbus* since green leaves and culms of *Tripsacum* were abundant throughout the simulated dry season.

Photoperiod and temperature are reliable cues in temperate regions to indicate periods of oncoming inclimate weather (Danks 1978), but cyclic changes within these cues are minimized in the tropics (Denlinger 1986). In addition, some cyclic cues, such as photoperiod, are not always reliable indicators of the often highly unpredictable beginning of the tropical rainy season (Janzen 1983). The availability of food, however, is often the most reliable indicator of seasonal conditions (Masaki 1980). In some insects, particularly those which undergo an aestival diapause, the physiological age of the host plant has been shown to induce dormancy (Masaki 1980, Tauber et al. 1984). The condition of the host plant can also increase the effectiveness of other stimuli such as photoperiod and/or temperature (Tauber et al. 1984). Thus, host plant condition may be playing a significant role in combination
with photoperiod and temperature in increasing the drought tolerance of these leafhoppers.

The ability to tolerate drought would be important in surviving the dry season for insects which do not enter a dormant state. For insects such as leafhoppers which live entirely on liquid diets, available moisture is extremely important (DeLong 1971). When drought occurs, water stress on host plants affects the plant sap supply and microclimate humidity, significantly influencing the survival and development of leafhoppers which depend on plants for food and development (DeLong 1965, Hoffman et al. 1990).

Among Dalbulus leafhoppers, October-reared adults exhibited better tolerance for lack of food and water than June-reared adults (Figs. 23-27; Table 9). This suggests that October-reared but not June-reared leafhoppers are adapted physiologically to tolerate drought. The maize specialists, D. maidis and D. elimatus, exhibited the greatest change in drought tolerance from June-reared to October-reared adults, indicating that these species are prepared best to deal with the significant decline of favorable conditions associated with the onset of the dry season. This significant change in drought tolerance supports the hypothesis that D. maidis overwinters as active or dormant adults.
When given moisture but no food, the survival of *D. maidis* adults was greater than when denied food and water (Fig. 28). Although males and females were equally intolerant of absolute drought, October-reared females survived significantly longer than October-reared males when maintained with moisture (Fig. 29; Table 10). In Mexico, many stream beds and ditches remain moist throughout the dry season (personal observation). In addition, scattered rainfall occurs at periodic intervals throughout this period (Mosino Aleman & Garcia 1974, Fig. 12b), and these rains would supply additional moisture. If mating occurs prior to the dry season, only females would need to endure the dry season, utilizing these limited moisture sources to survive. The field and greenhouse simulation data suggest this is precisely what is happening. In the field at Zapopan and Corralitos, a total of 25 adult *D. maidis* were collected between May 1 and July 1, of which 18 were females. In the greenhouse, the eight *D. maidis* adults trapped after day 77 were all females. These data, coupled with the longer survival of October-reared females when provided with moisture, suggest *D. maidis* females are best equipped to overwinter.

The known host range of *D. maidis* in the field is limited to maize and the teosintes (Triplehorn & Nault 1985, Nault 1990). Adults have been found at the beginning of the dry season on *Tripsacum* (Nault & DeLong 1980), but only
temporarily, even though T. dactyloides has been shown to be a potential oviposition and feeding host for D. maeidis in the greenhouse and field (Pitre et al. 1966, Pitre 1967, Nault & Madden 1985). Tripsacum, however, cannot be considered an important host during the dry season, as it is rare in much of Latin America (eg. Costa Rica, Nicaragua) whereas D. maeidis and the plant pathogens this species transmits are prevalent (Gamez 1983). Similarly, the limited distribution of annual and perennial teosintes in the central and southern Mexican highlands, Guatemala and northern Honduras (Mangelsdorf 1974, Iltis & Doebley 1980, Doebley 1983) prevent their consideration as important overwintering hosts. Irrigated maize, which serves as an overwintering host for low populations of D. maeidis, probably does not serve as a source of significant numbers of D. maeidis unless the irrigated areas are large.

In addition to maize and the teosintes, D. maeidis adults have been collected in the field from wheat, celery, grasses, and Solanum marginatum L. (Tsai & DeLong 1989), and potato, Solanum tuberosum L. (Stoner 1965). A wide variety of herbaceous plants have been tested as experimental hosts for D. maeidis, and many were utilized for oviposition and feeding, but were not preferred for development (Stoner 1965, Pitre 1967). Under special conditions, D. maeidis utilizes aster (Callistephus chinensis Nees) for feeding and survival, but not reproduction, if it first feeds on asters
infected with aster yellows mycoplasmalike organism (AY-MLO) (Maramorosch 1958, Purcell 1988).

Overwintering adult *D. maidis* may use unknown alternate hosts during the dry season, or may be able to tolerate the dry season in some facultative, semi-dormant state. One possibility is that *D. maidis* switches to another, as yet undetermined, host for feeding and reproduction. The potato leafhopper, *E. fabae*, utilizes pine trees (*Pinus* spp.) as a feeding host in the southern United States to survive the winter (P.S. Taylor, personal communication). Similarly, it is possible that *D. maidis* also overwinters on a woody host.

Accumulating evidence suggests the corn leafhopper is a local migrant. This conclusion is based on the instability and relative impermanence of the maize habitat, coupled with the reported immigration into young maize fields at the beginning of the wet season. In addition, the observed extinctions of *D. maidis* populations within the cages over maize at Corralitos and in the greenhouse simulation suggest they do not overwinter in the maize stubble. No *Dalbulus* leafhoppers have been found within protected overwintering sites such as leaf litter, plant stubble, and other debris as has been found for other leafhoppers (DeLong & Caldwell 1935). *D. maidis* may exhibit a migration pattern similar to the green rice leafhopper, *Nephotettix cincticeps* Uhler. *N. cincticeps* migrates from rice to hibernating sites.
adjacent to the rice patty fields after the growing season, then returns the following year (Miyashita et al. 1964). *D. maidis* also probably migrates to local overwintering sites outside maize fields, only to return the next year. The question remains, where is the overwintering site? The importance of finding this overwintering site is the most important task left in developing a comprehensive management plan to control this major maize pest.
CHAPTER V

INFLUENCE OF DEVELOPMENT CONDITIONS ON COLD-HARDINESS OF
ADULT DALBULUS LEAFHOPPERS: IMPLICATIONS FOR OVERWINTERING

Introduction

The corn leafhopper, Dalbulus maidis (DeLong & Wolcott), a vector of several maize pathogens, is probably the most important pest of maize (Zea mays L.) in Latin America (Nault 1990). D. maidis is a specialist on the genus Zea, comprised of maize and the teosintes (Nault 1990). This leafhopper overwinters as active adults (Chapter IV). The location of the majority of these overwintering adults is unknown, although low numbers are found on irrigated maize and perennial teosintes (Barnes 1954, Power 1987, Nault 1990).

Apparently, all other Dalbulus species also overwinter as adults (Chapter IV). With the exception of the Mexican corn leafhopper, D. elimatus (Ball), which is found at high elevations (Nault 1990) and uses winter wheat, Bromus spp. and other herbaceous plants as overwintering hosts (Barnes 1954), most of these Dalbulus species remain on their gamagrass (Tripsacum spp.) hosts during the Mexican winter.
These *Tripsacum* specialist *Dalbulus* continue to mate, lay eggs, and develop throughout the dry season.

The dry season in Mexico occurs from October to May (Mosino Aleman & Garcia 1974, Moya-Raygoza et al. 1990), and is characterized by low rainfall, low temperatures and short days as compared to the wet season (Fig. 12). During the dry season, temperatures can drop as low as -5°C, especially at night in the higher elevations. The ability of overwintering adult *Dalbulus* leafhoppers to tolerate low temperatures is therefore important to their survival during the coldest months of the dry season.

Those *Dalbulus* leafhoppers which specialize on *Zea* are likely tolerant of cold temperatures during the dry winter. In the absence of maize during the dry season (Barnes 1954), the distribution of perennial teosintes, their only other *Zea* host, is limited to higher elevations (Doebley 1983). *Tripsacum* specialists likely are less tolerant of cold temperatures as they are found primarily at lower elevations (Nault 1990), and utilize a fairly stable and protected resource. I hypothesized that the cold-hardiness of the two maize specialists, *D. maidis* and *D. elimatus*, would be greater than that of the *D. gelbus* DeLong, a *Tripsacum* specialist which also is found on maize, and the fivespotted gamagrass leafhopper, *D. quinquenotatus* DeLong & Nault, an exclusive *Tripsacum* specialist.
In this paper I discuss the influence of developmental conditions on the cold-hardiness of adult *Dalbulus* leafhoppers and how this seasonal variation in cold-hardiness improves leafhopper ability to survive the harsh dry season. This study examines the cold-hardiness of *D. maidis*, *D. elimatus*, *D. gelbus*, *D. quinquenotatus*, and a close relative, *Balduulus tripsaci* Kramer & Whitcomb. *B. tripsaci* is also a *Tripsacum* specialist, feeding on eastern gamagrass, *T. dactyloides* (L.) L. (Kramer & Whitcomb 1968). However, *B. tripsaci* is a temperate species which overwinters in the egg stage (Nault 1990, Chapter IV). The objectives of this study were to 1) measure the tolerance of each species reared under June and October environmental conditions to subfreezing temperatures (-5°C), 2) examine the effect of preconditioning at non-lethal, near-freezing temperatures prior to exposure to subfreezing temperatures on the tolerance of -5°C in *D. maidis*, and 3) measure supercooling points and glycerol levels for each species reared under June and October conditions.

**Materials and Methods**

Leafhoppers were used from the same laboratory colonies reported by Triplehorn et al. (1990) and Todd et al. (1991). *Dalbulus* colonies, including *D. quinquenotatus* which can utilize maize seedlings for feeding, oviposition and development (Nault & Madden 1985, Todd et al. 1991), were maintained on sweet corn (cv. Aristogold Bantam Evergreen)
in 19.5 x 38.5 x 39 cm rearing cages. B. tripsaci was reared on T. dactyloides in 29.5 x 38.5 x 51.5 cm rearing cages (D'Arcy & Nault 1982). Colonies were maintained at ca. 26°C, RH of 60-90%, with a 16h:8h light:dark regime.

Experimental leafhoppers were reared under two sets of conditions designed to simulate those occurring in Jalisco, Mexico at the beginning of the wet season in June and at the beginning of the dry season in October.

**June Conditions.** June-reared Dalbulus leafhoppers were reared in cages filled with seedling sweet corn (ca. 4-5 leaf stage) in 10 cm dia pots. June-reared B. tripsaci was reared on young T. dactyloides. Adult leafhoppers from the laboratory colonies (n=150 females, 50 males) were given a 48 hr oviposition access period (OAP) on hosts. Eggs hatched and nymphs developed in an environmental chamber set for 14h:10h L:D with a 28°C/20°C temperature cycle, RH of 70-90%, with plants watered daily and replaced with fresh young seedlings when needed.

**October Conditions.** October-reared leafhoppers were maintained in larger 30.5 x 38.5 x 102 cm rearing cages filled with mature sweet corn or Tripsacum in 15.5 cm dia pots. Adult leafhoppers (n=150 females, 50 males) were given a 48 hr OAP. Egg hatch and nymphal development was at 12h:12h L:D with a 23°C/17°C temperature cycle, with plants watered only once per week and replaced by mature plants only as needed.
Cold Tolerance. Effects of rearing conditions on cold tolerance at -5°C of all five leafhopper species were tested by placing groups of 25-35 two wk old adults (50:50 male to female ratio) into small "exposure" cages, 3.3 x 3.3 x 2.5 cm plastic snap pill boxes, with a corked hole for introduction of leafhoppers and screened ventilation holes. Leafhoppers from each treatment were given a 1 hr preconditioning period at +5°C in an environmental growth chamber under constant light. Groups of three cages were exposed to -5°C for 0, 1.5, 3, 6, 12, 24, or 36 hrs for June-reared and October-reared D. maidis and D. elimatus. The other three species were exposed to -5°C for 0, 1, 2, 4, 8 or 24 hrs, with D. gelbus receiving an additional 36 hr treatment. Following the -5°C temperature treatment, leafhoppers were given a postconditioning period of 1 hr at +5°C. Leafhoppers then were placed on 2-3 leaf stage maize seedlings in 15 x 3.8 cm dia plexiglass tubes at ca. 25°C under constant light. Following a recovery period of 48 to 72 hr on the maize plants after the freezing exposure, the number of surviving leafhoppers were counted.

Preconditioning period. To analyze the importance of a "preconditioning" period on cold tolerance, I used D. maidis adults and the same methods as described above in determining freezing tolerance. Groups of 20-30 two wk old D. maidis adults from each of the two rearing regimes were placed into the small exposure cages. For each rearing
regime, three cages for each freezing treatment were given a 1 hr preconditioning and 1 hr postconditioning period at +5°C prior to and after exposures at -5°C. Three other cages were not given a preconditioning or postconditioning period but placed directly to -5°C. Freezing exposure treatments at -5°C were 0, 1.5, 3, 6, 12, 24, or 48 hr in duration. Following treatment, leafhoppers were placed on maize plants in tube cages to recover and survivors counted 48 to 72 hr later.

Supercooling points. The mean supercooling point (SCP) was determined for each of the five species using both June-reared and October-reared adults. The SCP of a minimum of 8 individuals (4 females, 4 males) for each species and rearing treatment was measured by positioning a 36 gauge copper-constantan thermocouple in contact with the insect cuticle. A cooling rate of ca. 2°C/min was maintained using a foam insulated cooler placed in a -80°C freezer. Insect temperature was monitored every 5 seconds by a multi-channel data logger (Model OM500, Omega Engineering, Inc., Stamford, CT). The SCP was determined as the lowest temperature recorded prior to the release of the latent heat of fusion when body water freezes (Lee 1989).

Glycerol determination. Glycerol levels of June-reared and October-reared adults were determined for each of the five species. Two wk old adults were removed from their respective rearing cages and frozen at -70°C prior to
analysis. Because of the small size of Dalbulus leafhoppers (Chapter VI), about 500 individuals were required for each extraction sample. Glycerol was analyzed using high performance liquid chromatography (Waters Associates) as described by Lee et al. (1983) and Chen et al. (1987a).

Statistical Analysis. To measure the mean time to 50% mortality of the population (LT$_{50}$), probit analysis was used as it was to measure cold-hardiness in Diabrotica spp. (Elsey 1989). To determine LT$_{50}$'s, a probit analysis macro procedure (PROBIT; developed by J.R. Sedcole and revised by L.V. Madden) was used with MINITAB for calculations. Analysis of variance (ANOVA) from MINITAB (Anonymous 1989), was used to assess the effect of leafhopper species, rearing conditions, and replication on survival time.

Results

Freezing tolerance. Cold-hardiness of the five leafhopper species varied considerably as measured by the percent survival of adult leafhoppers when exposed to -5°C temperatures (Figs. 30-34). There were significant differences among species in the ability to tolerate subfreezing temperatures based on an analysis of LT$_{50}$'s (F=439.99; df=4,20; p<0.01). In general, the Tripsacum specializing leafhoppers D. quinquenotatus and B. tripsaci were the least tolerant of freezing temperatures, D. gelbus and D. maidis were moderately tolerant of -5°C temperatures,
Figure 30. Tolerance to -5°C by Dalbulus maidis adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 31. Tolerance to -5°C by *Dalbulus elimatus* adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 32. Tolerance to -5°C by *Dalbulus gelbus* adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 33. Tolerance to -5°C by Dalbulus quinquenotatus adults as measured by the percent survival of the population over time (mean ± standard error).
Figure 34. Tolerance to \(-5^\circ C\) by *Baldulus tripsaci* adults as measured by the percent survival of the population over time (mean \(\pm\) standard error).
whereas *D. elimatus* was the most tolerant of subfreezing temperatures (Table 11).

The LT$_{50}$ of October-reared adults was significantly longer (*F*=174.13; df=1,20; *p*<0.01) than for June-reared adults (Table 11), except for *B. tripsaci* (Fisher’s Protected LSD=2.49; df=20; *p*=0.05). *D. maidis* had a significant LT$_{50}$ increase of 2.16X from June-reared to October-reared adults (Fig. 30). The most cold tolerant leafhopper tested, *D. elimatus*, had a significant LT$_{50}$ increase of 1.80X from June-reared to October-reared adults (Fig. 31). *D. gelbus* had the greatest increase in its freezing tolerance among Dalbulus LT$_{50}$’s with a 2.50X increase (Fig. 32). *D. quinquenotatus*, despite having a 2.32X LT$_{50}$ increase for October-reared over June-reared adults, was the least tolerant of subfreezing temperatures among the four Dalbulus species (Fig 33); with an October-reared LT$_{50}$ of only 6.2 hr.

*B. tripsaci* (Fig. 34), with LT$_{50}$’s between 1.7 and 2.6 hr (Table 11) was the least tolerant of subfreezing temperatures among the five species tested. *B. tripsaci* had a relative increase of its freezing tolerance of 1.44X by rearing it under October conditions, less than the lowest freezing tolerance LT$_{50}$ increase among the Dalbulus species.

**Preconditioning period.** There was a significant increase in the capacity of *D. maidis* adults to survive freezing temperatures if they were first exposed for a short
Table 11. Tolerance to -5°C as measured by time to 50% mortality ($LT_{50}$) in hours, supercooling points and glycerol levels (mean ± S.E.) for five leafhopper species reared under June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) conditions typical of those in Jalisco, Mexico.

<table>
<thead>
<tr>
<th>Species</th>
<th>Rearing Regime</th>
<th>-5°C $LT_{50}$ (hr)</th>
<th>Supercooling Point (°C)</th>
<th>Glycerol (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dalbulus maidis</em></td>
<td>June</td>
<td>4.15 ab</td>
<td>-20.9 ± 0.3</td>
<td>141.0 ± 23.0</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>8.96 c</td>
<td>-20.1 ± 0.6</td>
<td>78.0 ± 9.0</td>
</tr>
<tr>
<td><em>D. elimatus</em></td>
<td>June</td>
<td>23.51 e</td>
<td>-20.0 ± 0.5</td>
<td>18.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>42.34 f</td>
<td>-21.9 ± 0.5</td>
<td>25.3 ± 1.3</td>
</tr>
<tr>
<td><em>D. gelbus</em></td>
<td>June</td>
<td>4.84 b</td>
<td>-21.6 ± 0.7</td>
<td>48.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>12.11 d</td>
<td>-20.8 ± 0.6</td>
<td>163.7 ± 17.3</td>
</tr>
<tr>
<td><em>D. quinquenotatus</em></td>
<td>June</td>
<td>2.67 a</td>
<td>-20.4 ± 0.2</td>
<td>54.0 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>6.20 b</td>
<td>-22.2 ± 0.6</td>
<td>48.0 ± 8.0</td>
</tr>
<tr>
<td><em>Baldulus tripsaci</em></td>
<td>June</td>
<td>1.78 a</td>
<td>-22.7 ± 0.5</td>
<td>155.0 ± 75.0</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>2.57 a</td>
<td>-22.4 ± 0.5</td>
<td>74.7 ± 7.3</td>
</tr>
</tbody>
</table>

$LT_{50}$ means within a column followed by different letters are significantly different (Fisher’s Protected LSD=2.49; df=20; p=0.05).
Figure 35. Tolerance by June-reared and October-reared *Dalbulus maidis* adults to -5°C when given 1 hr pre-conditioning and post-conditioning periods at +5°C versus no conditioning period. Tolerance was measured by the percent survival of the population over time (mean ± standard error).
preconditioning period to chilling, but not freezing, temperatures (Fig. 35). This increase was significant for June-reared and October-reared adults ($F=217.87; df=1,8; p<0.001$). When placed directly into $-5^\circ C$ conditions, *D. maidis* adult survival as measured by LT$_{50}$'s was 1.1 and 1.9 hr for June-reared and October-reared adults. However, when given a 1 hr preconditioning period at $+5^\circ C$ prior to freezing at $-5^\circ C$, survival LT$_{50}$'s lengthened to 4.6 and 8.7 hr respectively.

**Supercooling points and Glycerol determination.** There were no significant differences in the SCP's among the five species tested. For both June-reared and October-reared adults, SCP's ranged between $-20.0$ and $-22.7^\circ C$ (Table 11), more than $15^\circ C$ below the $-5^\circ C$ at which the freezing tolerance tests took place.

Glycerol levels among the five species varied considerably and were not consistently higher in October-reared adults (Table 11). There were lower glycerol levels in October-reared *D. maidis*, *D. quinquenotatus*, and *B. tripsaci* adults, whereas *D. elimatus* and *D. gelbus* showed higher glycerol levels in October-reared adults.

**Discussion**

The SCP is that subfreezing temperature at which spontaneous tissue freezing occurs (Sømmen 1982). In freeze susceptible insects, mortality at this temperature occurs due to irreversible tissue damage. Although SCPs have been
shown to fluctuate seasonally in some insects (e.g. Knight & Bale 1986, Lee 1991), SCPs did not vary significantly between June-reared and October-reared Dalbulus adults (Table 11). I feel that the SCP does not provide an accurate measure of cold-hardiness in Dalbulus leafhoppers, since mortality occurred at -5°C, temperatures much higher than the SCP’s. Mortality in the absence of tissue freezing which occurs at the SCP is most likely due to cold shock injury (Lee et al. 1988), which is thought to be the result of damage to the lipid membrane on the cellular level (Lee 1989). It is likely that this cold shock injury is responsible for the observed mortality of Dalbulus leafhoppers at -5°C.

During the dry season in Mexico, the temperatures drop below 0°C, particularly at night at higher elevations (Mosino Aleman & Garcia 1974). To survive cold temperatures, an insect must develop a cold-hardiness which often varies on a seasonal basis (Lee et al. 1988). Cold-hardiness is the capacity of an organism to survive exposure to low temperature, and is often greatly influenced by environmental conditions present during development (Salt 1953, Chen et al. 1987b). Environmental conditions characteristic of the late summer and early fall in Mexico include shorter photoperiods, lower temperatures, and decreased host plant quality, all of which are important

Results clearly show that environmental conditions present during development have a significant effect on the cold-hardiness of adult Dalbulus leafhoppers. Shorter photoperiods, cooler temperatures and mature host plants produced adult leafhoppers which were significantly more tolerant of subfreezing temperatures than those that developed under longer photoperiods, warmer temperatures and young host plants (Figs. 30-34). Results also support the hypothesis that Tripsacum-specializing Dalbulus species found at lower elevations are less tolerant of subfreezing temperatures than Dalbulus species which are found at higher elevations on maize and the teosintes.

In the field, temperatures drop gradually during the day to subfreezing levels. To partially simulate this gradual drop, we preconditioned leafhoppers for 1 hr at +5°C prior to treatment at -5°C. This preconditioning by a brief, non-lethal exposure to chilling temperatures before exposure to subfreezing temperatures has been shown to increase the length of time an insect can tolerate low temperature (Salt 1961; Casagrande & Haynes 1976; Tauber et al. 1984; Chen et al. 1987a; Elsey 1989). Enhanced survival of subfreezing temperatures after the preconditioning period at cool temperatures is likely due, at least in part, to a rapid increase in glycerol synthesis (Lee et al. 1987). Our
results with *D. maidis* also showed a significant improvement in the freezing tolerance of both June-reared and October-reared adults when the leafhoppers were preconditioned before freezing exposure (Fig. 35).

Many insects possess glycerol or an equivalent, such as sorbitol, as a cryoprotectant (Salt 1961, Sømme 1965, 1982, Danks 1978). In non-diapausing, freeze susceptible insects like *Dalbulus* leafhoppers, low temperatures during development often stimulate the gradual accumulation of significant amounts of cryoprotective substances (Baust & Lee 1981, Sømme 1982, Chen et al. 1987b).

Dehydration or drought, which occurs as plants mature and begin to senesce, and the subsequent deterioration of host plants as a food source can also trigger an accumulation of cryoprotectants in insects (Salt 1953; Baust 1982, Young & Block 1980, Rojas et al. 1986). Drought can effect the survival of insects, such as leafhoppers, which live entirely on liquid diets (DeLong 1971), and would be a significant factor in dry season survival. I previously showed that October-reared *Dalbulus* adults are more tolerant of drought (Chapter IV), and that drought tolerance may be related to the accumulation of cryoprotective substances.

Because of the cooler temperatures and drought associated with October rearing conditions, higher levels of glycerol were expected in these adults. Our results were not consistent (Table 11), as *D. elimatus* and *D. gelbus* had
higher glycerol levels in October-reared adults, while glycerol levels were lower in October-reared adults of the other three species. *D. elimatus* and *D. gelbus* are the two *Dalbulus* species with the highest elevational distribution in Mexico (Nault 1990). Perhaps they also are the only species tested to have evolved a mechanism of glycerol synthesis triggered by developmental conditions to protect against the freezing temperatures common at these elevations. The other three species had lower glycerol levels in October-reared adults. It is possible that these lower glycerol levels are a result of freeze drying that took place following specimen collection during storage at -70°C. Freeze drying would have lowered the moisture content in those samples, resulting in inflated glycerol level determinations.

An understanding of the implications of seasonal variation in cold-hardiness on overwintering among the leafhopper species requires an examination of the host plant utilization patterns, distribution and biology of each leafhopper species. The few previous leafhopper studies on cold-hardiness have shown a relatedness between distribution and overwintering biology. Both the potato leafhopper, *Empoasca fabae* (Harris) (Decker & Maddox 1967) and the aster leafhopper, *Macrosteles fascifrons* (Stal) (Saini 1967) are migrants which overwinter in the southern U.S., and these species cannot tolerate subfreezing temperatures as adults.
The corn leafhopper, *D. maidis*, is endemic to lower (<750m), more tropical elevations (Barnes 1954, Triplehorn & Nault 1985), but is often found in significant numbers at higher elevations by the end of the growing season (Chapter IV). Its presence at higher elevations would require a better tolerance of cold temperatures than species limited to lower elevations. The intermediate freezing tolerance LT50’s of this leafhopper indicate it can tolerate colder temperatures at high elevations, yet is not adapted to tolerate these conditions for long periods of time.

The Mexican corn leafhopper, *D. elimatus*, was the most tolerant of -5°C among the tested *Dalbulus* leafhoppers (Table 11). *D. elimatus* is endemic to elevations up to 2400m, higher than for other *Dalbulus* species (Barnes 1954, Triplehorn & Nault 1985, Nault 1990). It is found primarily on maize and the teosintes during the summer, but during the dry season is regularly found on winter wheat, *Bromus* spp. and other herbaceous plants (Barnes 1954). This leafhopper would be the *Dalbulus* species exposed most often to subfreezing temperatures during the dry winter. Therefore, its greater cold tolerance ensures survival during extended freezing conditions it would likely encounter between November and March at higher elevations.

*D. gelbus*, which is found on both *Tripsacum* and maize, had the greatest increase from June-reared to October-reared adults in ability to tolerate freezing temperatures. This
leafhopper is found primarily on *Tripsacum* during the dry season. Its distribution of 735 to 2350 m above sea level (Triplehorn & Nault 1985; Nault 1990) exposes it to colder temperatures more frequently than *D. maidis*. The relatively high tolerance of *D. gelbus* to freezing temperatures as compared to *D. maidis* is needed for survival at these higher elevations. In addition, it is possible that in utilizing *Tripsacum*, which grows as thick clumps, *D. gelbus* obtains temporary protection from colder temperatures.

*D. quinquenotatus*, with the lowest cold tolerance of *Dalbulus* leafhoppers, did have a significant increase in cold-hardiness of October-reared adults. This leafhopper is present in mixed-age breeding populations (both nymphs and adults) throughout the year (Chapter II) and an increase in cold tolerance would be both necessary and advantageous during the dry season. However, *D. quinquenotatus* is found at elevations of 125 to 1975 m (Nault 1990), and is protected within the leaf sheathes of the plant (Nault et al. 1983). *D. quinquenotatus* would not be exposed to freezing temperatures as often as its congeners, which utilize the more exposed upper leaf canopy of their hosts or are restricted to higher elevations. Because of its distribution at lower elevations and protected location on its host plant, *D. quinquenotatus* would not need the cold-hardiness required by the other three tested *Dalbulus* species.
B. tripsaci is a temperate species which specializes on T. dactyloides (Kramer & Whitcomb 1968). This study found that June-reared and October-reared B. tripsaci adults are relatively intolerant of cold. This suggests B. tripsaci adults do not need to tolerate subfreezing temperatures that occur during the winter in temperate regions. We know B. tripsaci overwinters as eggs in leaves of its host, T. dactyloides (Nault 1990, Chapter IV). The low tolerance of freezing temperatures by B. tripsaci adults would be expected of a species which overwinters in the egg stage.

In conclusion, the variability of the cold-hardiness among Dalbulus species can be explained by the various biological and ecological strategies of these leafhoppers. It is evident that D. maidis and its congeners overwinter during the dry season as active adults (Chapters II, IV). Despite the decline and/or absence of their host plants, Dalbulus adults tolerate drought (Chapter IV) and subfreezing temperatures, hazardous conditions which are characteristic of the dry season.
CHAPTER VI

SEASONAL POLYPHENISM OF LABORATORY-READED AND FIELD-COLLECTED ADULT DALBULUS LEAFHOPPERS

Introduction

The leafhopper genus *Dalbulus* is comprised of 11 neotropical species. Members of the genus use maize (*Zea mays* L.), the teosintes (*Zea* spp.), or the closely related gamagrasses (*Tripsacum* spp.) as their primary hosts (Nault 1990). *Dalbulus* are small, slender, leafhoppers with adults 3.0 - 4.4 mm long (DeLong 1950, Nault & DeLong 1980, Nault et al. 1983, Triplehorn & Nault 1985). Adults are pale yellow or stramineous for the two species that specialize on *Zea*, while those that utilize *Tripsacum* are bright yellow, orange, or brown (Nault 1990). However, among some *Dalbulus* species, size and color are highly variable and this variability appears to be environmentally influenced (Triplehorn & Nault 1985).

Seasonal polyphenism is reportedly a widespread occurrence in insects, especially in the Lepidoptera (Shapiro 1978). In leafhoppers, different rates of growth and development under variable temperature and photoperiod have been shown to influence the size and coloration
(pigmentation) of resultant adults (Müller 1979). Some temperate leafhoppers, such as *Euscelis lineolatus* Brulle and *Stirellus bicolor* (Van Duzee), have environmentally determined summer and winter forms which are so different that the two forms of each species were initially described as different species (Müller 1957, Whitcomb et al. 1972).

The goal of this study was to examine the effect of seasonal developmental conditions on adult size, weight, color and lipid content of laboratory reared and field collected leafhoppers. Body size, in conjunction with lipid levels, may be important in the ability of an insect to disperse or survive long periods of inclement conditions (Chapter IV). I did not attempt to separate and determine which individual environmental factors (temperature, photoperiod or host plant age) contributed to observed phenotypic differences. Seasonal polyphenism is discussed as it relates to the overwintering biology of *Dalbulus* leafhoppers.

**Materials and Methods**

Seasonal polyphenism and lipid levels were studied for five leafhopper species. These species included two maize specialists, the corn leafhopper, *D. maidis* (DeLong & Wolcott) and the Mexican corn leafhopper, *D. elimatus* (Ball). I also studied three *Tripsacum* specialists, *D. gelbus* DeLong, a species that also feeds on maize, and two exclusive *Tripsacum* specialists, the fivespotted
gamagrass leafhopper, *D. quinquenotatus* DeLong & Nault, which can be reared in the laboratory on maize seedlings (Nault & Madden 1985), and a close temperate relative, *Baldulus tripsaci* Kramer & Whitcomb, which overwinters in the egg stage (Chapter IV). Laboratory-reared populations were the same as those reported by Triplehorn et al. (1990) and Todd et al. (1991). *Dalbulus* colonies were maintained on sweet corn (cv. Aristogold Bantam Evergreen) in 19.5 x 38.5 x 39 cm rearing cages, while *B. tripsaci* was reared on eastern gamagrass (*T. dactyloides*) in larger 29.5 x 38.5 x 51.5 cm rearing cages (D’Arcy & Nault 1982). Colonies were maintained in a room maintained at ca. 26°C, RH of 60-90%, and with a light:dark regime (L:D) of 16h:8h.

Leafhoppers were reared under two contrasting regimes designed to simulate environmental conditions occurring in Jalisco, Mexico, at the beginning of the wet season in June and at the beginning of the dry season in October.

**June-reared.** June-reared leafhoppers were reared in cages filled with seedling sweet corn (ca. 4-5 leaf stage) in 10 cm dia pots. June-reared *B. tripsaci* was reared on young *T. dactyloides*. Adult leafhoppers from the laboratory colonies (n=150 females, 50 males) were given a 48 hr oviposition access period (OAP) on the host plants. Eggs hatched and nymphs developed in an environmental chamber set for 14h:10h L:D with a 28°C/20°C temperature cycle, RH of
70-90%, with plants watered daily and replaced with fresh young seedlings when needed.

October-reared. October-reared leafhoppers were reared in larger 30.5 x 38.5 x 102 cm rearing cages filled with mature sweet corn or Tripsacum in 15.5 cm dia pots. Adult leafhoppers (n=150 females, 50 males) were given a 48 hr OAP. Egg hatch and nymphal development occurred under environmental conditions of 12h:12h L:D with a 23°C/17°C temperature cycle, with water stressed plants watered only once per week and replaced by mature plants only as needed.

Morphological differences. To examine morphological differences, two week old adult leafhoppers from June or October conditions were frozen and stored at -20°C for later measurement. After thawing, leafhoppers were separated by sex, and placed in a convection oven to dry at 80°C for three days. Male and female adults of each species were then weighed separately on an electric balance to the nearest 10^-5g in 10 replicate groups of 10 leafhoppers each. Measurements of body size were: 1) the distance between the compound eyes for head capsule width, and 2) length of the second medial cell of the right elytra for wing size (Fig. 1). Measurements were made on 10 randomly selected individuals of each sex and species.

Color variation. Color is a combination of three components, intensity, hue and saturation (Judd & Wyszecki 1975). Intensity (brightness, value) is the total amount of
light reflected over a range of wavelengths, hue (commonly understood as color) is determined by dominant wavelengths, and saturation (tint, chroma) is the spectral purity of reflected light (Todd et al. 1990). In this study, we examined the color intensity of the pigmentation on the leafhopper abdomen as a measure of overall body color.

Differences in color intensity between June-reared and October-reared adults (Plate Ia) were measured by the intensity of light transmitted through color transparencies of photographic images of leafhoppers. Isolated pairs of two week old adults of the same species and sex, reared under June and October conditions were killed in the freezer at -70°C. Wings were removed exposing pigmentation on the dorsal surface of the 3rd-5th segments of the abdomen (Plate Ib). Pairs of adults were attached to a white index card with clear double-sided tape. Closeup photographs were taken of each pair with Kodak Ektachrome 100 slide film (Eastman Kodak Co., Rochester, NY). Slides were projected onto a white viewing screen from 2m by a slide projector (Kodak Ektographic IIIB Carousel Projector) set on low power in a darkroom. Projected light intensity was measured by a LI-185B photometer with a LI-190SB quantum sensor (LI-Cor, inc., Lincoln, NE). The sensor was mounted on a stand and placed directly in front of the projection screen, facing the projector. The sensor was positioned within the
Plate I. Color variation in laboratory-reared *Dalbulus maidis* adults.  
a. Adult females, "June-reared" above and "October-reared" below.  
b. Sample pair of adult males with wings removed to expose dorsal  
surface of abdomen where intensity of pigmentation  
was measured; "June-reared" above and "October-reared" below.
projected image of the 3rd-5th segments of the leafhopper abdomen and light intensity reading was measured in \( \mu \text{E/m}^2/\text{s} \).

**Field polyphenism.** Adult *D. maidis* and *D. gelbus* from Mexico were collected with a sweep net and aspirator and preserved in 70% ethanol. Ten males and 10 females of both *D. maidis* and *D. gelbus* were randomly selected from collections made during three trips to Mexico in May 1989, October 1989 and March 1990 (Table 12). Leafhoppers were air dried for 24 hr, and head capsule width, wing cell length, and color intensity were measured as described above. Dry body weight was not measured because preservation in ethanol destroyed body tissues and increased loss of dry weight.

**Lipids Levels.** To measure lipid content, 50 male and 50 female ca. two wk old June-reared and October-reared adults from each species were killed by freezing at -70°C. Leafhoppers were dried at 60°C in a convection oven for 48 hr, weighed in groups of 10 on a Mettler analytical balance, and then defatted by extracting their lipids with petroleum ether (Cockbain 1961). Groups of 10 dried leafhoppers were placed in 5 ml test tubes filled with 3-4 ml of petroleum ether. Ether was changed every 6 hr for 48 hr. Defatted groups of leafhoppers were dried, reweighed, and the resultant weight difference interpreted as the amount of lipids removed.
Table 12. Populations of field-collected *Dalbulus* leafhoppers used for morphological and color comparisons.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Host Plant</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D. maidis</strong></td>
<td>El Grullo, Jalisco, Mexico</td>
<td><em>Zea mays</em></td>
<td>4 May 1989, 25 March 1990</td>
</tr>
<tr>
<td></td>
<td>Laboratorio Natural las Joyas, Jalisco, Mexico</td>
<td><em>Z. mays</em></td>
<td>8 October 1989</td>
</tr>
<tr>
<td></td>
<td>Laboratorio Natural las Joyas, Jalisco, Mexico</td>
<td><em>Z. diploperennis</em></td>
<td>23 March 1990</td>
</tr>
<tr>
<td></td>
<td>Instituto de Botanica, Zapopan, Jalisco, Mexico</td>
<td><em>Z. diploperennis</em></td>
<td>1 May 1989, 10 October 1989, 27 March 1990</td>
</tr>
<tr>
<td></td>
<td>Instituto de Botanica, Zapopan, Jalisco, Mexico</td>
<td><em>T. pilosum</em></td>
<td>1 May 1989, 10 October 1989, 27 March 1990</td>
</tr>
</tbody>
</table>
Results

Morphological differences. A comparison of the five laboratory-reared leafhopper species tested, combining both sex and rearing conditions, showed significant differences exist in the size and weight of the various species (Table 13). For dry body weight, *D. quinquenotatus* was the largest, followed by *D. elimatus*, *D. maidis*, *D. gelbus*, and finally *B. tripsaci* (Fisher’s Protected LSD = 45.7 µg; df = 195; p = 0.05). *D. maidis* and *D. elimatus* did not differ significantly in weight. For head capsule width, *D. quinquenotatus* was the largest, followed by *D. maidis*, *D. gelbus*, *D. elimatus* and *B. tripsaci*. Wing cell length, as a measure of overall wing size, was also significantly different among the five species (F = 18.26; df = 4, 180; P < 0.01). *D. elimatus* had the longest wings, followed by *D. maidis*, *D. gelbus*, *D. quinquenotatus*, and finally *B. tripsaci*.

A comparison of males and females, combining species and rearing regime, showed that females were significantly larger than males for all the morphological traits analyzed (P < 0.05). When separated by species and sex, June-reared adults weighed less and had shorter wings than did October-reared adults. This relationship always was significant for dry body weight and wing length (Table 13). For head capsule width, *D. maidis*, *D. elimatus* and *B. tripsaci*, but
Table 13. Mean ± standard error for dry body weight, head capsule width, and wing cell length of males and females of five leafhopper species reared under June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) conditions similar to those occurring in Jalisco, Mexico.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Rearing Regime</th>
<th>Body Weight (µg)</th>
<th>Head Width (µm)</th>
<th>Wing Length (µm)</th>
<th>Wing Length/Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalbulus maidis</td>
<td>male</td>
<td>June</td>
<td>264.7 ± 2.7</td>
<td>374.5 ± 4.5</td>
<td>1365.7 ± 26.2</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>352.0 ± 2.2</td>
<td>412.0 ± 3.8</td>
<td>1492.9 ± 14.8</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>June</td>
<td>465.0 ± 9.1</td>
<td>416.6 ± 7.9</td>
<td>1482.8 ± 25.5</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>505.3 ± 7.6</td>
<td>456.3 ± 6.1</td>
<td>1575.2 ± 22.8</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>means</td>
<td></td>
<td>396.7 ± 15.4c</td>
<td>414.8 ± 5.4c</td>
<td>1479.2 ± 16.2b</td>
<td>3.93b</td>
</tr>
<tr>
<td>D. elimatus</td>
<td>male</td>
<td>June</td>
<td>253.0 ± 3.8</td>
<td>343.0 ± 5.2</td>
<td>1395.0 ± 18.2</td>
<td>5.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>388.3 ± 3.2</td>
<td>397.4 ± 5.1</td>
<td>1548.7 ± 20.2</td>
<td>3.99</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>June</td>
<td>418.3 ± 7.7</td>
<td>385.5 ± 3.9</td>
<td>1537.7 ± 19.0</td>
<td>3.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>570.5 ± 8.4</td>
<td>422.9 ± 5.8</td>
<td>1678.6 ± 18.8</td>
<td>2.95</td>
</tr>
<tr>
<td></td>
<td>means</td>
<td></td>
<td>407.5 ± 18.3c</td>
<td>387.2 ± 5.2b</td>
<td>1540.0 ± 18.5c</td>
<td>4.04b</td>
</tr>
<tr>
<td>D. gelbus</td>
<td>male</td>
<td>June</td>
<td>254.5 ± 4.5</td>
<td>387.3 ± 3.3</td>
<td>1364.8 ± 14.7</td>
<td>5.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>282.5 ± 3.6</td>
<td>400.1 ± 4.7</td>
<td>1402.3 ± 20.0</td>
<td>4.97</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>June</td>
<td>359.3 ± 5.0</td>
<td>415.2 ± 6.4</td>
<td>1511.2 ± 24.9</td>
<td>4.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>397.3 ± 8.2</td>
<td>428.0 ± 4.8</td>
<td>1591.7 ± 27.4</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>means</td>
<td></td>
<td>323.4 ± 9.6b</td>
<td>407.6 ± 3.4c</td>
<td>1467.5 ± 17.9b</td>
<td>4.64c</td>
</tr>
</tbody>
</table>

(continued)
Table 13. (continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Month</th>
<th>Body Weight</th>
<th>Head Width</th>
<th>Wing Length</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. quinquenotatus</em></td>
<td>male</td>
<td>June</td>
<td>337.2 ± 3.6</td>
<td>441.7 ± 5.5</td>
<td>1317.2 ± 16.9</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>408.0 ± 3.2</td>
<td>444.4 ± 7.1</td>
<td>1409.6 ± 13.9</td>
<td>3.46</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>June</td>
<td>561.0 ± 15.3</td>
<td>505.2 ± 9.1</td>
<td>1468.2 ± 19.2</td>
<td>2.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>628.0 ± 7.7</td>
<td>513.4 ± 5.1</td>
<td>1548.7 ± 20.4</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>means</td>
<td></td>
<td>483.5 ± 19.1d</td>
<td>476.2 ± 6.3d</td>
<td>1435.9 ± 16.0a</td>
<td>3.12a</td>
</tr>
<tr>
<td><em>Baldulus tripsaci</em></td>
<td>male</td>
<td>June</td>
<td>139.0 ± 3.1</td>
<td>299.6 ± 10.9</td>
<td>1273.3 ± 19.3</td>
<td>9.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>196.7 ± 2.4</td>
<td>319.3 ± 8.0</td>
<td>1395.0 ± 14.4</td>
<td>7.10</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>June</td>
<td>258.5 ± 8.3</td>
<td>368.1 ± 9.9</td>
<td>1406.0 ± 24.8</td>
<td>5.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct.</td>
<td>425.0 ± 5.6</td>
<td>414.0 ± 9.8</td>
<td>1656.5 ± 16.8</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td>means</td>
<td></td>
<td>254.8 ± 17.3a</td>
<td>350.3 ± 8.5a</td>
<td>1432.7 ± 24.1a</td>
<td>6.42d</td>
</tr>
</tbody>
</table>

1 Dry body weight.

2 Head capsule width as measured by the distance between the compound eyes.

3 Wing length as measured by the length of the second medial cell of the right elytra.

* Indicates significant difference between June-reared and October-reared adults of the same species and sex (Fisher's Protected LSD; df=180; p=0.05).

Means within a column followed by the same letter are not significantly different (Fisher's Protected LSD; df=195; p=0.05).
not *D. gelbus* and *D. quinquenotatus* had significant size differences between the two rearing regimes.

*B. tripsaci* had the largest wing length to body weight ratio, followed by *D. gelbus*, *D. elimatus*, *D. maidis*, and *D. quinquenotatus* respectively. Within each species, males had a higher wing length to body weight ratio than females, and within each species and sex, June-reared adults had a higher ratio than October-reared adults.

A test of the association between the three measured morphological variables indicated a highly positive correlation between dry body weight and head capsule width (*r*=0.87). Correlations between wing length and body weight (*r*=0.74) and wing length and head capsule width (*r*=0.47) indicated that wing length was a less accurate measure of overall body size.

**Color variation.** Light intensity readings were used to quantify color differences between June-reared and October-reared adult leafhoppers. Light intensity gave a measure of the relative lightness or darkness of exoskeleton pigmentation. A higher intensity indicated a lighter colored morph, while a lower intensity indicated a darker, more pigmented morph. There was no significant difference in the darkness of body pigmentation between males and females for any species or rearing regime (*F*=0.30; *df*=1,180; *p*=0.58). However, there were significant intensity differences in pigmentation color intensity among species
(F=9.54; df=4, 180; p<0.01) and rearing conditions (Plate I; F=44.16; df=1, 180; p<0.01).

The bright yellow *B. tripsaci* and *D. gelbus* had the lightest pigmentation, while *D. elimatus* and *D. maidis* were moderately pigmented as compared to *D. quinquenotatus*, a dark, orange-brown pigmented leafhopper (Fig. 36). Although June-reared adults were lighter than October-reared adults (Plate I), differences were not significant for *D. quinquenotatus* or *B. tripsaci* (Fisher’s Protected LSD=0.876 µE/m²/s; df=180; p=0.05). For the other three species, October-reared adults were darker than June-reared adults (Fig. 36), with *D. maidis* having the most dramatic change in pigmentation intensity. In some instances, the October-reared adults had up to seven spots on the head, but in no case were morphological changes so great that the adults could be misidentified as another species.

**Field polyphenism.** *D. maidis* and *D. gelbus* adults collected during the three trips to Mexico had abdominal swelling and damage, but the head width and wing length were still constant. Head capsule width, wing cell length, and pigmentation of dried, field-collected adults were measured. Head capsule width (F=166.92; df=1, 113; p<0.01) and wing length (F=174.88; df=1, 113; p<0.01), were significantly larger for females than males in *D. maidis* (Fig. 37) and *D. gelbus* (Fig. 38). There was no difference in the color
Figure 36. Intensity (mean ± standard error) of the dorsal surface of the abdomen of June-reared and October-reared adults of five laboratory-reared leafhopper species. A higher value indicates a lighter morph, while a lower value indicates a darker morph.
Figure 37. a. Head capsule width (mean ± S.D.), b. wing cell length (mean ± S.E.), and c. color intensity (mean ± S.E.) of laboratory-reared and field-collected Dalbulus maidis adults. For color intensity, a higher value indicates a lighter morph, while a lower value indicates a darker morph.
Figure 38. a. Head capsule width (mean ± S.D.), b. wing cell length (mean ± S.E.), and c. color intensity (mean ± S.E.) of laboratory-reared and field-collected *Dalbulus gelbus* adults. For color intensity, a higher value indicates a lighter morph, while a lower value indicates a darker morph.
Figure 39. Percent body weight composed of lipids (mean ± standard error) for male and female adults of five leafhopper species reared under June and October conditions.
intensity of males and females within a species collected during the same trip (F=0.001; df=1,113; p>0.05).

Adults collected from the field during the May 1989 trip were similar to June-reared adults in body size and color (Figs. 37, 38). Adults collected during the dry season in October 1989 and March 1990 were darker and larger than those collected during May 1989. Although the pigmentation of dry season field-collected adults was slightly lighter than October-reared adults, possibly due to ethanol bleaching, the differences in pigmentation between wet season and dry season field-collected adults was comparable to the change between June and October laboratory-reared adults.

Lipid levels. Extractable lipids varied significantly among the five species (F=107.22; df=4,80; p<0.01), and between males and females (F=205.03; df=1,80; p<0.01). The October-reared adults always contained significantly higher lipid levels than June-reared adults (F=90.01; df=1,80; p<0.01). Among the various species, D. maidis had the highest lipid reserves, with the lowest lipid concentrations in D. gelbus and B. tripsaci (Fig. 39).

Discussion
Seasonal polyphenism is a common nondiapause adaptation by insects to seasonal climatic changes in the tropics (Tauber et al. 1986). Polyphenism often occurs in response to seasonally predictable, regularly occurring changes in
the environment, of which photoperiod is generally the most reliable cue (Tauber et al. 1984). Seasonal polyphenism is a phenotypic change in the population and can involve changes in both morphology and coloration (Shapiro 1978).

Size of adult leafhoppers has been shown to be influenced during nymphal development by a variety of environmental factors, including temperature, photoperiod, host plant condition, and crowding of conspecifics (Harrison 1980). Differences in environmental conditions in the laboratory under which these five leafhoppers were reared significantly influenced size and weight. For each species, the cooler temperatures, shorter daylength, and development on mature hosts resulted in increased adult size and weight. This increase in size and weight is probably due to a slower rate of development at cooler temperatures, allowing more time for feeding and subsequent growth. This has been demonstrated in work by Simonet & Pienkowski (1980) with the potato leafhopper, *Empoasca fabae* (Harris), and with the blackfaced leafhopper, *Graminella nigrifrons* (Forbes) (Larsen et al. 1990). Photoperiod affects the size of developing adults of the green rice leafhopper, *Nephotettix cincticeps* Uhler (Kisimoto 1959), and the leafhopper *Euscelis incisus* (Kbm.) (Müller 1979). Plant physiological state is another important factor in determining the morphology of developing homopterans. In aphids, host plant condition is important in the regulation of wing development
(Hille Ris Lambers 1966), and in the planthopper Prokelisia marginata (Van Duzee), wing-form is influenced by host plant condition when combined with the effects of crowding by conspecifics (Denno et al. 1985).

It would be expected that leafhopper species within a genus which are active fliers and possibly migrants would have larger wings and a greater wing length to body weight ratio than less active species. A ratio of wing length divided by body weight as an estimate of flight ability suggests that two Tripsacum specialists, B. tripsaci and D. gelbus are the most active fliers of the five species tested (Table 13). This interpretation is contingent upon a relationship between a high ratio and greater flight ability. However, this ratio is misleading in that the maize specialists, D. maidis and D. elimatus, have the largest wings of the leafhoppers tested (Table 13), and are much more active and likely to fly in response to disturbance than are the Tripsacum specialists (Heady & Nault 1985, Nault 1985). D. maidis is endemic to the lower tropical elevations (Triplehorn & Nault 1985), and is thought to disperse into maize at higher elevations as the growing season progresses (Nault 1985), possibly becoming an occasional migrant (Pitre et al. 1967, Triplehorn et al. 1990). If the degree of impermanence of their habitat is an indication of migrant status (Southwood 1962), D. maidis is the best candidate as a migrant among Dalbulus leafhoppers.
One factor which may be responsible for the lower wing length to body weight ratio of *D. maidis* and *D. elimatus* is that both species contain significantly greater amounts of lipids in proportion to body weight than the other three species (Fig. 39). Lipids are important components of cellular membranes, but are probably most important in the form of body fat as an efficient means of storing large amounts of energy. Stored lipids are then used for energy by migratory insects during long flights (Blum 1985). October-reared adults which have a consistently lower wing length to body weight ratio compared with June-reared adults also had higher lipid reserves.

Females were consistently larger than males, and although this may be a function of their slightly slower rate of development than males (Appendix A), egg load is most likely the reason why females are larger and heavier. When *D. maidis* fecundity was measured by the number of developing offspring adults, two week old October-reared females laid almost twice as many eggs as June-reared females (Appendix A). Egg weight could therefore account for the lower wing length to body weight ratio in October-reared females.

For each species, males had significantly higher wing length to body weight ratios than females (Table 13). Although this ratio is apparently inappropriate for comparing flight activity of different species, it may be
evidence within each species that males are more active than females. Active males would be an indication that these species are possibly using the same, localized "call-fly" mate-location behavior reported in the blackfaced leafhopper, *G. nigrifrons* (Hunt & Nault 1991).

*D. quinquenotatus* was consistently larger by weight and head width than the other three tested *Dalbulus* species; however, it had the shortest wing length relative to body weight (Table 13). This leafhopper is a myrmecophile (Larsen et al. 1991) that aggregates within the partially unfolded leaves at the base of its *Tripsacum* hosts (Nault et al. 1983). This leafhopper is less responsive to mechanical stimuli and is much less likely to exhibit escape behavior when disturbed than other *Dalbulus* leafhoppers (Heady & Nault 1985). Because of these behaviors, and the presumed protection of homopteran myrmecophiles from predators by attendant ants (Way 1963, Buckley 1987), there would be little need for long wings, which would be advantageous on an active flier.

There are several associations between overall size and adult survival which should be mentioned. In the large milkweed bug, *Oncopeltus fasciatus* (Dallas), survival is improved with the increased flight ability associated with larger body size (Dingle et al. 1980). Larger adults survive longer than smaller adults, which suggests that larger size may be advantageous during periods of food
deprivation and/or migration (Tauber et al. 1986). Dalbulus nicely fits this pattern in which larger, hardy, overwintering leafhoppers are produced late in the growing season, while smaller, less hardy leafhoppers are produced during the wet season, when food is plentiful.

Body color can have a significant influence on the survival of an insect, especially when the adult is exposed to high and low temperatures. Darkly pigmented objects absorb more radiant solar energy, while lightly colored objects tend to reflect solar energy. At low temperatures, the efficient absorption of available radiant energy is critical for many poikilotherms, such as insects (Stewart 1986). Darker morphs often develop during colder temperatures, whereas high temperatures often result in light-colored morphs (Tauber et al. 1986). These seasonal changes in coloration of a population can represent an adaptation to thermal conditions (Fields & McNeil 1988) and augment the effectiveness of other thermoregulation mechanisms (May 1979).

A dark form of the sycamore aphid, Drepanosiphum platanoides (Schr.), appears during the winter and has been shown to be thermoregulatorily adaptive (Dixon 1972). Darker coloration may similarly aid leafhoppers in cooler habitats by facilitating heat absorption. In combination with increased cold-hardiness (Chapter V), darker colored Dalbulus leafhoppers would be better able to survive the dry
season. Of the leafhoppers we examined for color differences, *D. maidis*, *D. elimatus* and *D. gelbus* all had significantly darker morphs during the dry season (Plate I). This was expected because these species are most likely to be exposed to cold temperatures at higher elevations during the winter (Chapter V).

There is a positive correlation between the development of darker morphs and higher elevations in the leafhopper *Eupteryx uticae* (Fab.), suggesting temperature is an important factor in controlling morph frequency (Stewart 1981). Other environmental factors, such as host plant condition and photoperiod during development also influence the color of adult leafhoppers. Host plant selection by the leafhopper *Oncopsis flavicollis* (L.) determines the color morph of emerging adults (Claridge & Nixon 1981). Photoperiod has also been shown to determine color morphs of adult *N. cincticeps* by Kisimoto (1959) and *S. bicolor* (Whitcomb et al. 1972, Whitcomb & Coan 1989). The lighter pigmented *D. maidis* (from maize and *Z. diploperennis*) and *D. gelbus* (from *Tripsacum* spp.) collected in May 1989, just prior to the wet season (Figs. 37c, 38c), may be an indication of photoperiod control of polymorphism in *Dalbulus* leafhoppers.

Color of insects has functions other than thermoregulation, and can be important in the avoidance of natural enemies (Matthews & Matthews 1978). In Central
America, seasonal color variation in *Rothchildia lebeau* Guerin-Meneville is an adaption to match the moth with backgrounds against which it hides during the dry and wet seasons (Janzen 1984). The advantage of thermoregulation by dark morphs may be offset by the visual selection against dark morphs by entomophagous parasitoids, particularly in warmer, lowland habitats (Stewart 1986). The *D. maidis*, *D. elimatus*, and *D. gelbus* adults collected from maize and *Tripsacum* during the wet season are bright yellow. These species are highly visible, yet are the same size and shape when resting on their hosts as the discarded anthers of the maize, teosinte or *Tripsacum* tassel that litter the upper leaf surfaces late in summer. Resemblance of leafhoppers to discarded anthers may be a defense against visually searching predators.

The dark orange-brown coloration of *D. quinquenotatus* may serve a thermoregulatory function prior to and during local dispersal flights. However, *D. quinquenotatus* is found at lower elevations and is normally shielded from solar radiation by the leaves of its host. Its color, I believe, is more likely cryptic in function. This leafhopper is difficult to see within the shade of partially folded leaves at the base of its *Tripsacum* hosts. Although it is sedentary and would be an easy target for natural enemies, protection from natural enemies by attendant ants, combined with its dark cryptic coloration may serve to
protect this leafhopper from visually searching predators and parasitoids.

Both size and color of laboratory-reared and field-collected adult leafhoppers examined in these experiments were affected by environmental conditions present during development. We consider that the size and color comparison of field-collected \textit{D. maidis} and \textit{D. gelbus} with laboratory-reared adults indicate that June and October conditions in the laboratory closely approximate those conditions that occur in Mexico in the field. However, \textit{D. maidis} and \textit{D. gelbus} adults developing under June conditions in the laboratory and collected in the field during May 1989 at the beginning of the wet season are smaller and lighter in color than adults which would have developed under conditions at the end of the previous wet season and overwintered. These adults were collected from irrigated maize and perennial teosintes, and were likely not representative of overwintering leafhoppers. However, leafhoppers collected on yellow sticky card traps during May and early June at field sites at Las Joyas and Zapopan, Jalisco, Mexico, (Chapter IV) do appear to be smaller and lighter than those trapped in October (Larsen, personal observation).

Among the leafhopper species tested, the maize specialists, \textit{D. elimatus} and \textit{D. maidis}, exhibited the greatest changes in size, color, and lipid levels, indicating that those species are best prepared to deal with
the significant decline of favorable conditions associated with the onset of the dry season. When combined with their ability to tolerate drought (Chapter IV) and cold-hardiness (Chapter V), this seasonal polyphenism adds additional evidence supporting the hypothesis that maize specialist Dalbulus species overwinter as active adults. Tripsacum specialist Dalbulus leafhoppers, however, have a fairly stable but declining resource throughout the dry season. Because of this resource, Tripsacum specializing Dalbulus leafhoppers are less susceptible to the hazardous conditions associated with the dry season and have reduced phenotypic changes as compared with their maize specialist congeners.
EPILOGUE

The chapters in this dissertation represent some of the first studies on effects of the interaction between host plant and temperature on leafhopper egg to adult development, ant-leafhopper mutualistic interactions, and overwintering of deltocephaline leafhoppers. Chapter I provides substantial evidence that temperature plays an important role in determining suitability of plants as developmental hosts of the blackfaced leafhopper.

The fivespotted gamagrass leafhopper, *Dalbulus quinquenotatus* DeLong & Nault, is the only deltocephaline leafhopper known to be attended by ants. Mutualistic interactions between ants and the fivespotted gamagrass leafhopper were the subject of chapters II and III. In chapter II, I reported the first detailed field observations of interactions between ants and *D. quinquenotatus*. Chapter III is unique in that it is the first investigation to compare the interactions of ants with myrmecophiles and their non-attended relatives. I showed that *D. quinquenotatus* behaved differently from its congeners when interacting with ants by excreting more honeydew and allowing extensive physical contact from the ants. To further our understanding of mutualistic interactions
between ants and Homoptera, future studies should address how ants recognize and distinguish between myrmecophiles and non-myrmecophiles.

The ultimate goal of chapters IV-VI was to determine how the corn leafhopper survives the dry season. These chapters compared aspects of the overwintering biology of the corn leafhopper, *D. maidis* (DeLong & Wolcott), with its non-pest congeners. This comparison of the biology of a pest species with its non-pest relatives provides a beginning to understanding factors important in the development of a pest. An understanding of the overwintering biology of *Dalbulus* leafhoppers is essential to being able to comprehensively manage the corn leafhopper.

The population dynamics of *Dalbulus* leafhoppers in the field and their survival in the absence of host plants were studied in chapter IV. These data provide evidence that environmental conditions during development significantly affect the ability of *Dalbulus* leafhoppers to tolerate the absence of host plants during the dry winter. Chapter V showed that the cold-hardiness necessary to survive cooler winter conditions is acquired by *Dalbulus* as a result of developmental conditions. In chapter VI, seasonal variation in differences of the size, weight, and color of *Dalbulus* adults are explained.

It is evident that the overwintering biology of *D. maidis* and its congeners involves a complex interplay of
the ecological strategy of species, their host plants, and environmental conditions. The harsh dry winter conditions and absence of host plants significantly reduce populations of *Dalbulus* leafhoppers present at the beginning of the wet growing season. *Dalbulus* leafhoppers that specialize on *Tripsacum* are not dormant during the dry winter, but continue to develop, feed and reproduce. For maize specialists like *D. maidis*, their intrinsic high fecundity, rapid development, and long-term adult survival with available moisture make it possible to recover rapidly from low populations which survive the winter.

However, to develop a comprehensive management plan to control the corn leafhopper, several questions remain to be answered. The most important task is to find the overwintering site of *D. maidis*. To answer this question, detailed searches of potential alternative hosts around maize fields will be necessary during the middle to late part of the dry season. In addition, a long-term, year-round project monitoring *D. maidis* populations should be in place over a wide part of Latin America. Finally, seasonal dispersal patterns of *D. maidis* in the field, which would include finding their overwintering sites, are necessary to determine if the corn leafhopper is a long distance or local migrant.
APPENDIX A

EGG TO ADULT DEVELOPMENT AND FECUNDITY OF JUNE-REARED AND OCTOBER-REARED DALBULUS AND BALDULUS LEAFHOPPERS

Introduction

The leafhopper genus *Dalbulus* is comprised of 11 neotropical species found in Latin America. The corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott), is the most well known and important member of the genus as a major pest of maize (*Zea mays* L.). Although *D. maidis* is capable of causing feeding damage to maize, (Bushing & Burton 1974), it is most important in Latin America as a vector of three maize disease pathogens (Nault 1990). Other members of the genus utilize maize, the teosintes (*Zea* spp.), or the closely related gamagrasses (*Tripsacum* spp.) as their primary hosts (Nault 1985).

In previous papers, I have examined the effect of seasonally variable developmental conditions on drought tolerance (Chapter IV), cold-hardiness (Chapter V) and polyphenism (Chapter VI) of *Dalbulus* leafhoppers, and discussed the implications of these capabilities on the overwintering of several *Dalbulus* species. Laboratory conditions during these experiments were designed to
simulate environmental conditions occurring in Jalisco, Mexico, at the beginning of the wet season in June and at the beginning of the dry season in October. This paper provides the first information on the combined effects of cooler temperatures, shorter photoperiods, and mature host plants typical of October on the population dynamics of five leafhopper species.

These five species included two maize specialists, the corn leafhopper, *D. maidis* and the Mexican corn leafhopper, *D. elimatus* (Ball), and three *Tripsacum* specialists, *D. gelbus* DeLong, a species that is also found on maize, and two exclusive *Tripsacum* feeding specialists, the fivespotted gamagrass leafhopper, *D. quinquenotatus* DeLong & Nault and a temperate relative, *Baldulus tripsaci* Kramer & Whitcomb. The objectives of this research were to examine the effects of June and October conditions on 1) the egg to adult development and number of resultant adults for these five species, and 2) the fecundity of *D. maidis*.

**Materials and Methods**

Experimental populations were the same as those reported by Triplehorn et al. (1990) and Todd et al. (1991). *Dalbulus* colonies were maintained on sweet corn (cv. Aristogold Bantam Evergreen) in 19.5 x 38.5 x 39 cm rearing cages, while *B. tripsaci* was reared on eastern gamagrass (*T. dactyloides* (L.) L.) in larger 29.5 x 38.5 x 51.5 cm rearing cages (D'Arcy & Nault 1982) in a room maintained at
ca. 26°C, RH of 60-90%, and with a light:dark regime (L:D) of 16h:8h.

To test the effect of environmental conditions during development on survival and development time from egg oviposition to adult eclosion, leafhoppers were reared under two contrasting sets of conditions, designed to simulate those occurring in Jalisco, Mexico, at the beginning of the wet season in June and at the beginning of the dry season in October (Mosino Aleman & Garcia 1974). Laboratory-reared adults were used as a source population. For each species, 50 females and 50 males were placed on host plants in rearing cages kept in an environmental growth chamber at 26°C under 14h:10h L:D. Leafhoppers were given a 48 hr oviposition access period (OAP). Following this OAP, all adults were removed from the cages.

June-reared Dalbulus leafhoppers were reared in 19.5 x 38.5 x 39 cm rearing cages on seedling sweet corn (ca. 3-4 leaf stage) in 10 cm dia pots. June-reared B. tripsaci were reared on young T. dactyloides. Egg hatch and nymphal development was in an environmental chamber set for 14h:10h L:D with a 28°C/20°C temperature cycle, RH of 70-90%, with plants watered daily and replaced with fresh young seedlings when needed.

October-reared leafhoppers were reared in larger 30.5 x 38.5 x 102 cm rearing cages filled with mature sweet corn or Tripsacum growing in 15.5 cm dia pots. Egg hatch and
nymphal development was in an environmental chamber set for 12h:12h L:D with a 23°C/17°C temperature cycle, with plants watered only once per week and replaced by mature plants as needed.

Cages were checked daily for the appearance of newly eclosed adults. Once adults emerged, they were removed daily until all leafhoppers had been taken from the cages. Adults were killed by freezing at -20°C, then sexed and counted.

The experiment was replicated three times. Analysis of variance (ANOVA) of untransformed data was used to determine the effect of species, rearing regime, sex, and replication on the mean development time and the total number of eclosed adults. Experimental design consisted of a factorial with main effects of species, rearing regime, and sex. Sex, however, was treated as a subplot in a split-plot design.

Effects of June and October rearing conditions on the fecundity of the corn leafhopper, D. maidis, was measured by the number of resultant adults emerging under identical developmental conditions. Three groups of 100 June-reared and 100 October-reared two wk old adults (50 females and 50 males each) were placed in 19.5 x 38.5 x 39 cm rearing cages for oviposition. Cages were kept in an environmental growth chamber at 26°C under 14h:10h L:D. Leafhoppers were given a 48 hr OAP on maize seedlings at ca. 3-4 leaf stage. Following the OAP, adults were removed from the cages, and
development of progeny was at 26°C and 14h:10h L:D. Cages were checked three times per week for the appearance of adults. As adults emerged, they were removed and killed in a freezer at -20°C, sexed and counted. Adults were removed until all leafhoppers had been taken from the cages. ANOVA of untransformed data were used to determine the effects of rearing regime on the number of resultant adults and sex ratio.

**Results**

Egg to adult development time ranged from a mean of 24.07 days for June-reared *D. maidis* to 62.03 days for October-reared *D. quinquenotatus* (Table 14). June-reared leafhoppers developed significantly faster than October-reared leafhoppers for all five species (*F*=622.86; *df*=1,20; *p*<0.01). There were significant differences in the mean egg to adult development time among the five leafhopper species (*F*=42.09; *df*=4,20; *p*<0.01). *D. maidis* and *D. elimatus* developed the fastest, followed by *D. aelbus* and *B. tripsaci*, with *D. quinquenotatus* taking the longest to develop. Males developed into adults significantly faster than females by an average of 1.42 days (*F*=101.82; *df*=1,20; *p*<0.01).

There was a consistent relationship in the development rate among *Dalbulus* leafhoppers within each rearing regime (Table 14). Development rate was significantly different among species (*F*=14.76; *df*=4,20; *p*<0.01), and was greatest
Table 14. Population development of five leafhopper species (sexes combined) reared under environmental conditions similar to those in June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) in Jalisco, Mexico, as measured by development time from egg to adult, development rate, and number of developing adults (n=3 replicates).

<table>
<thead>
<tr>
<th>Species</th>
<th>Rearing Regime</th>
<th>Host Plant</th>
<th>Egg to Adult Development Time (days) (mean ± S.E.)</th>
<th>Development Rate (mean %/day)</th>
<th>Number of Developing Adults (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalbulus maidis</td>
<td>June</td>
<td>Z. mays</td>
<td>24.07 ± 1.96</td>
<td>4.15</td>
<td>1008.3 ± 148.8</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>Z. mays</td>
<td>38.96 ± 0.63</td>
<td>2.57</td>
<td>364.2 ± 85.6</td>
</tr>
<tr>
<td>D. elimatus</td>
<td>June</td>
<td>Z. mays</td>
<td>26.06 ± 0.97</td>
<td>3.84</td>
<td>882.7 ± 72.8</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>Z. mays</td>
<td>42.40 ± 1.32</td>
<td>2.36</td>
<td>468.3 ± 102.3</td>
</tr>
<tr>
<td>D. gelbus</td>
<td>June</td>
<td>Z. mays</td>
<td>27.87 ± 1.55</td>
<td>3.59</td>
<td>842.0 ± 106.5</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>T. maizar</td>
<td>50.69 ± 1.97</td>
<td>1.97</td>
<td>240.7 ± 38.7</td>
</tr>
<tr>
<td>D. quinquenotatus</td>
<td>June</td>
<td>Z. mays</td>
<td>32.09 ± 1.15</td>
<td>3.12</td>
<td>161.3 ± 56.1</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>T. maizar</td>
<td>62.03 ± 2.01</td>
<td>1.61</td>
<td>155.4 ± 53.2</td>
</tr>
<tr>
<td>Baldulus tripsaci</td>
<td>June</td>
<td>T. dactyloides</td>
<td>30.87 ± 0.65</td>
<td>3.24</td>
<td>139.4 ± 10.3</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>T. dactyloides</td>
<td>52.58 ± 1.03</td>
<td>1.90</td>
<td>123.1 ± 12.3</td>
</tr>
</tbody>
</table>
for *D. maidis*, followed by *D. elimatus, D. gelbus* and *D. quinquenotatus* for June-reared and October-reared leafhoppers. The overall development rate of 3.61% per day for June-reared leafhoppers was significantly greater than the development rate of 2.09% per day for October-reared leafhoppers (F=256.30; df=1,20; p<0.01).

The mean number of developing adults ranged from 123.1 for October-reared *B. tripsaci* to 1008.3 for June-reared *D. maidis*. There were significant differences in the number of emerging adults by species (F=23.40; df=4,20; p<0.01), by rearing regime (F=43.96; df=1,20; p<0.01) and their interaction (F=7.44; df=4,20; p<0.01). Overall, June-reared adults emerged in significantly greater numbers over a shorter period of time than did October-reared adults for all five species (Figs. 40-44).

Sex of emerging adults was not significantly influenced by species or rearing regime (F=0.05; df=1,20; p>0.05). Sex ratio was approximately 1:1 averaging 218.0 males to 220.5 females over all species and rearing conditions.

Fecundity of two wk old June-reared and October-reared female *D. maidis* adults was measured by the total number of resultant adults completing development following a 48 hr OAP. June-reared *D. maidis* females laid 4.90 ± 0.84 eggs/female/day, significantly fewer than the 7.84 ± 0.28 eggs/female/day laid by October-reared *D. maidis* females (F=10.96; df=1,4; p<0.05). There were no significant
Figure 40. a. Daily mean number of emerging adults and b. cumulative percent of emerging Dalbulus maidis adults over time reared under conditions similar to June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) in Jalisco, Mexico (n=3 replicates).
Figure 41. a. Daily mean number of emerging adults and b. cumulative percent of emerging Dalbulus elimatus adults over time reared under conditions similar to June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) in Jalisco, Mexico (n=3 replicates).
Figure 42. a. Daily mean number of emerging adults and b. cumulative percent of emerging *Dalbulus gelbus* adults over time reared under conditions similar to June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) in Jalisco, Mexico (n=3 replicates).
Figure 43. a. Daily mean number of emerging adults and b. cumulative percent of emerging *Dalbulus quinquenotatus* adults over time reared under conditions similar to June (14L:10D, 28°C/20°C) and October (12L:12D, 23°C/17°C) in Jalisco, Mexico (n=3 replicates).
Figure 44. a. Daily mean number of emerging adults and 
b. cumulative percent of emerging *Baldulus tripsaci* adults over time reared under conditions 
similar to June (14L:10D, 28°C/20°C) and October 
(12L:12D, 23°C/17°C) in Jalisco, Mexico (n=3 
replicates).
differences in the sex ratio of resultant adults ($F=0.004; df=1,8; p>0.05$). There were an average of 3.19 male offspring developing/female/day compared with 3.17 female offspring developing/female/day.

**Discussion**

In an attempt to understand the population dynamics of *Dalbulus* leafhoppers, numerous laboratory studies have looked at the effect of temperature on the egg to adult development of *D. maidis* and other *Dalbulus* species (Barnes 1954, Davis 1966, Pitre 1970, Nault & Madden 1985, Madden et al. 1986, Tsai 1988). Unfortunately, most of these studies utilized primarily seedling maize as a developmental host. This is significant in that most of the non-pest *Dalbulus* leafhoppers utilize the gamagrasses as hosts. In addition, host plant species and physiological age have been shown to influence leafhopper development (Larsen et al. 1990, Todd et al. 1991).

This is the first study to look at egg to adult development of *B. tripsaci*. *B. tripsaci* is a temperate species, found exclusively on eastern gamagrass, *T. dactyloides* (Kramer & Whitcomb 1968). This species overwinters in the egg state (Chapter IV), and is a resident in the stable perennial *Tripsacum* habitat. This species develops at a similar rate to *D. gelbus*, but has fewer offspring produced (Table 14).
For all five species, those leafhoppers reared under
June conditions developed faster and emerged in greater
numbers over fewer days than those reared under cooler
October conditions (Figs. 40-44). Our results confirm
earlier work that the egg to adult developmental period
lengthens at lower temperatures (Barnes 1954, Davis 1966,

We used seedling maize as a developmental host for
June-reared *Dalbulus* gelbus and *D. quinquenotatus*. Previous
studies show these leafhoppers develop faster on maize than
*Tripsacum*, although fecundity of *D. quinquenotatus* is
greater on *Tripsacum* (Nault & Madden 1985). This suggests
host plant is important in determining the rate of
development, and an interaction between host plant and
temperature probably occurs in these species, as occurs in
the blackfaced leafhopper, *Graminella nigritrons* (Forbes)
(Larsen et al. 1990).

Among the four *Dalbulus* species tested, the maize
specialists *D. maidis* and *D. elimatus* had the fastest
development rate, while the *Tripsacum* specialist
*D. quinquenotatus* developed the slowest (Table 14). In
addition, maize specialists produced greater numbers of
resultant adults than did *Tripsacum* specialists. This is
consistent with the r-K continuum proposed for *Dalbulus*
leafhoppers by Nault & Madden (1985), in which maize
specialists develop faster and are more fecund than *Tripsacum* specialists.

The number of resultant adults emerging under June and October conditions can not be considered a measure of parental female fecundity, as females from the same laboratory cultures were used for oviposition in both treatments. However, these numbers can be used to indicate the influence of environmental conditions during development on mortality. For all five species, fewer resultant adults emerged under October-reared than June-reared conditions. It would seem that lower temperatures and more mature hosts in October are less than ideal conditions for development. In the field, longer egg to adult development period would expose the leafhopper nymphs to a higher probability of predation and parasitism, further reducing the number of resultant adults (Price 1984). However, this trend appears to be offset by increased fecundity of October-reared adults. A conservative minimum estimate of fecundity of June-reared and October-reared two wk old females can be made by the numbers of resultant adults developing under identical conditions. For *D. maidis*, two week old October-reared females laid almost twice as many eggs which developed into resultant adults as did June-reared females.

If mating occurs prior to the dry season, only the females would need to overwinter. October-reared *Dalbulus* females are much more tolerant of drought (Chapter IV) and
exhibit better cold-hardiness (Chapter V) than June-reared females. These abilities make October-reared females more fit to survive the harsh environmental conditions of the dry season. If the greater fecundity of October-reared *D. maidis* females is consistent among all *Dalbulus* leafhoppers, it would seem to offset the apparent mortality effects associated with October developmental conditions.
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