

## Influence of developmental conditions on cold-hardiness of adult *Dalbulus* leafhoppers: implications for overwintering

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### Abstract

Little is known about how adults of the corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott), and its congeners survive subfreezing temperatures at high elevations during the dry winter in Mexico. In the laboratory, duration of survival at  $-5^{\circ}\text{C}$  was measured for four Mexican *Dalbulus* species: *D. maidis*, *D. elimatus* (Ball), *D. gelbus* DeLong and *D. quinquenotatus* DeLong & Nault; and a closely related North American species, *Balduus tripsaci* Kramer & Whitcomb. Adult leafhoppers reared under environmental conditions that simulated the beginning of the dry winter season during October in Mexico ('October-reared') were at least twice as tolerant of  $-5^{\circ}\text{C}$  than adults reared under environmental conditions that simulated the beginning of the wet summer season during June ('June-reared'). *Dalbulus* species found primarily at high elevations, such as *D. elimatus*, were seven times more tolerant of  $-5^{\circ}\text{C}$  than *D. quinquenotatus*, a species which overwinters at low to mid elevations on its *Tripsacum* hosts. October-reared *D. maidis* adults survived relatively short periods at  $-5^{\circ}\text{C}$  ( $\text{LT}_{50} = 8.9$  h) compared to October-reared *D. elimatus* adults ( $\text{LT}_{50} = 42.3$  h). This suggests that in Mexico, *D. maidis* either overwinters in protected habitats at higher elevations or it migrates to lower, frost-free regions. October-reared *B. tripsaci* adults, which overwinter in the egg stage, were intolerant of  $-5^{\circ}\text{C}$  ( $\text{LT}_{50} = 2.6$  h). A conditioning period for 1 h at  $+5^{\circ}\text{C}$  before and after exposure to  $-5^{\circ}\text{C}$  significantly improved survival for *D. maidis*. Supercooling points (SCPs) were between  $-23$  and  $-20^{\circ}\text{C}$ , indicating that mortality of these leafhoppers at  $-5^{\circ}\text{C}$  was due to cold shock injury rather than internal ice formation.

### Introduction

*Dalbulus* leafhoppers that specialize on the graminoids (*Tripsacum* spp.) overwinter in southwestern Mexico on their hosts (Larsen *et al.*, 1992). They do so by producing continuous generations on the leaves and stems at the bases of plants that remain green as the dry winter season progresses. In contrast, the maize (*Zea mays* L.) habitat of

the pest species *Dalbulus maidis* (DeLong & Wolcott) and *D. elimatus* (Ball) disappears in late fall at harvest and does not reappear until maize is planted in late spring and early summer. *D. elimatus* survives winter by establishing breeding populations on alternate hosts (Barnes, 1954), whereas *D. maidis* survives as adults in unknown habitats (Larsen *et al.*, 1992).

The dry winter season in Mexico occurs from

October to May (Mosino-Aleman & Garcia, 1974; Moya-Raygoza *et al.*, 1990) and is characterized by low rainfall accompanied by a dieback of green leaves of all annual and most perennial plants. Adult *Dalbulus* leafhoppers that develop at the end of the wet season apparently undergo physiological changes that prepare them to survive winter in the absence of hosts or on host plants of diminished quality (Larsen, 1991; Larsen *et al.*, 1992). Another important feature of Mexican winters is low temperature. During the dry winter season, temperatures during the night can drop to  $-5^{\circ}\text{C}$  or lower, especially at higher elevations. The ability of overwintering adult *Dalbulus* leafhoppers to tolerate short periods of subfreezing temperatures is important to their survival during the coldest months of the dry season.

We hypothesize that leafhoppers that develop into adults late in the growing season will be more tolerant of subfreezing temperatures than those that develop early in the summer. Recently, Larsen *et al.* (1992) reported that adult *D. maidis* and three other *Dalbulus* species experimentally reared under fall conditions (L12:D12 light:dark;  $23^{\circ}\text{C}$  day/ $17^{\circ}\text{C}$  night; on mature, drought-stressed plants) survived longer without food and water or with water only than leafhoppers reared under early-summer conditions (L14:D10;  $28^{\circ}\text{C}$  day/ $20^{\circ}\text{C}$  night; on young seedlings). Thus, as for drought tolerance, we expect that the condi-

tions under which adult leafhoppers develop will influence cold tolerance. Furthermore, we anticipate that *Dalbulus* species found at high elevations in Mexico will be more tolerant of subfreezing temperatures than related species found at low elevations.

In this paper, we report on the survival of several leafhopper species after exposure to  $-5^{\circ}\text{C}$ . These species include: the widely distributed maize pest, *D. maidis*; the high-elevation maize specialist, *D. elimatus*; the high-elevation gamagrass specialist and sometime resident of maize, *D. gelbus* DeLong; and the mid- to low-elevation gamagrass specialist, *D. quinquenotatus* DeLong & Nault. As a control we included *Balduhus tripsaci* Kramer & Whitcomb, a close relative of *Dalbulus* and a widely distributed resident of North America which overwinters in the egg stage (Nault, 1990; Larsen, 1991). In addition, we report on the effect of short conditioning periods at non-lethal, near-freezing temperatures on cold tolerance at  $-5^{\circ}\text{C}$  in *D. maidis* adults and measure supercooling points for each species reared under the two environmental regimes.

## Materials and methods

Laboratory-reared populations of leafhoppers originally were collected in Jalisco, Mexico (be-

Table 1. Location, elevation, host plant, and collection date for populations of leafhopper species used in laboratory experiments

Species	Locality	Host plant	Collection date
<i>Dalbulus maidis</i>	El Grullo, Jalisco, Mexico (elev. 970 m)	<i>Zea mays</i>	4 May 1989
<i>D. elimatus</i>	Piedra ancha, Nevado de Colima, Jalisco, Mexico (elev. 2012 m)	<i>Z. perennis</i>	24 March 1990
<i>D. gelbus</i>	Instituto de Botanica, Universidad de Guadalajara, Zapopan, Jalisco, Mexico (elev. 1675 m)	<i>Tripsacum pilosum</i>	10 October 1989
<i>D. quinquenotatus</i>	Puerto Los Mazos, Jalisco, Mexico (elev. 1200 m)	<i>T. pilosum</i>	7 October 1989
<i>Balduhus tripsaci</i>	Wooster, Ohio, USA (elev. 225 m)	<i>T. dactyloides</i>	10 August 1989

tween 19° 30' and 20° 30' N. Lat.) and Ohio (40° 45' N. Lat.). *Dalbulus* leafhopper colonies (Table 1), including *D. quinquenotatus* which can use 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 × 38.5 × 39 cm rearing cages. *B. tripsaci* was reared on *Tripsacum dactyloides* (L.) L. in 29.5 × 38.5 × 51.5 cm rearing cages (D'Arcy & Nault, 1982). Colonies were maintained at ca. 26 °C, r.h. of 60–90%, with a L16:D8 light:dark regime. Leafhoppers were reared during experiments under two sets of environmental 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').

**June-reared conditions.** June-reared *Dalbulus* leafhoppers were maintained in 19.5 × 38.5 × 39 cm 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 allowed to oviposit on hosts for 48 h. Eggs hatched and nymphs developed in an environmental chamber with a L14:D10 light:dark regime, a 28 °C/20 °C temperature cycle, and a r.h. of 70–90%; plants were watered daily and replaced with fresh young seedlings when needed.

**October-reared conditions.** October-reared leafhoppers were maintained in 30.5 × 38.5 × 102 cm cages filled with mature sweet corn or *Tripsacum* in 15.5 cm dia pots. Adult leafhoppers (n = 150 females, 50 males) were allowed to oviposit for 48 h. Egg hatch and nymphal development occurred in a L12:D12 light:dark regime, a 23 °C/17 °C temperature cycle, and a 20–40% r.h.; plants were watered once per week and replaced with mature plants only as needed.

**Conditioning periods.** To ascertain the importance of non-lethal chilling on cold tolerance of leafhoppers, 42 randomly selected groups of 20–30

two-wk-old *D. maidis* adults (50:50 male to female ratio) from each rearing regime were placed into small 'exposure' cages. Exposure cages were made from 3.3 × 3.3 × 2.5 cm plastic snap pill boxes modified by adding two screened ventilation holes and a corked hole for introduction of leafhoppers. For each rearing regime, six cages were exposed to treatments of –5 °C for 0, 1.5, 3, 6, 12, 24 or 48 h. For each treatment, three cages were placed directly into –5 °C conditions. The other three cages were exposed to +5 °C in an environmental growth chamber under constant light for 1 h before and 1 h after treatment at –5 °C. Following freezing treatments, all leafhoppers were placed on maize seedlings at the 2–3 leaf stage in 15 × 3.8 cm dia plexiglass tubes at ca. 25 °C under constant light. The number of surviving leafhoppers was counted after a 48 to 72 h recovery period on maize seedlings.

**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 the small 'exposure' cages. Because conditioning periods improved survival, all leafhoppers were given a 1 h period at +5 °C before exposure to –5 °C. Groups of three cages were exposed to –5 °C for 0, 1.5, 3, 6, 12, 24 or 36 h for both June-reared and October-reared *D. maidis* and *D. elimatus*, with *D. elimatus* receiving additional treatments of 48, 72, 96 and 120 h. The other three species were exposed to –5 °C for 0, 1, 2, 4, 8 or 24 h, with *D. gelbus* receiving an additional 36 h treatment. Following the –5 °C treatment, leafhoppers were held for 1 h at +5 °C. Following treatment, leafhoppers were placed on maize seedlings in tube cages to recover and surviving adults counted 48 to 72 h later.

**Supercooling points.** The mean supercooling point (SCP) was determined for each of the five species using both June-reared and October-reared adults, using a minimum of 8 individuals (4 females, 4 males) for each species and rearing treatment. The SCP of each individual was measured by positioning a 36-gauge copper-constantan

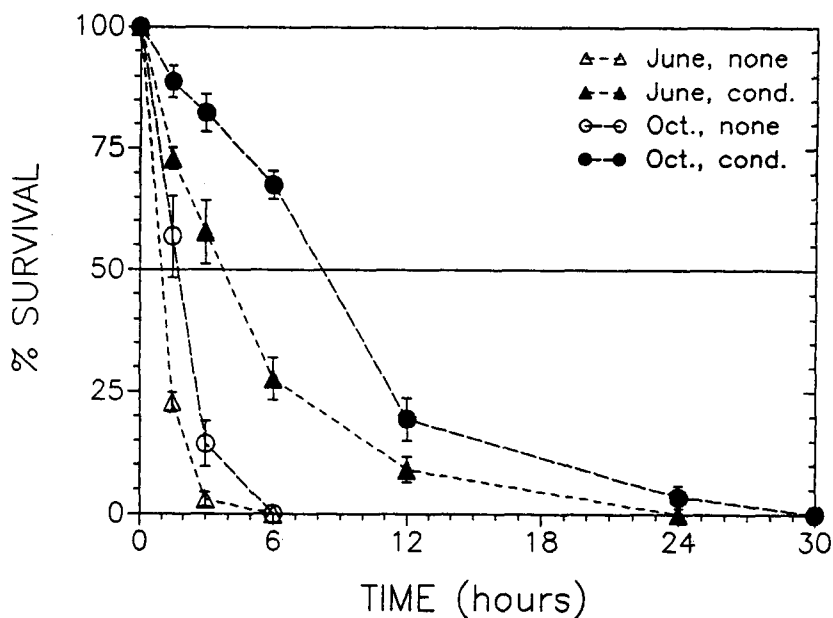


Fig. 1. Survival (mean  $\pm$  s.e.) of June-reared and October-reared *Dalbulus maidis* adults exposed for different periods of time (0–48 h) to  $-5^{\circ}\text{C}$ ; conditioned at  $+5^{\circ}\text{C}$  for 1 h before and 1 h after exposure to  $-5^{\circ}\text{C}$  ('cond.'), or with no conditioning period before or after exposure to subfreezing temperatures ('none').

thermocouple in contact with the insect cuticle. A cooling rate of ca.  $2^{\circ}\text{C}/\text{min}$  was maintained using a foam insulated cooler placed in a  $-80^{\circ}\text{C}$  freezer. Insect temperature was monitored every 5 seconds by a multi-channel data logger (Model OM500, Omega Engineering, Inc., Stamford, CT). SCP was determined as the lowest temperature recorded prior to the release of the latent heat of fusion when body water freezes (Lee, 1989).

**Statistical analysis.** To measure the mean time to 50% mortality of the population ( $LT_{50}$ ), probit analysis was used in the same way as it was to measure cold-hardiness in *Diabrotica* spp. (Eelsey, 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 effects of leafhopper species and rearing conditions on survival time.

## Results

There was a significant increase in the capacity of *D. maidis* adults to survive freezing temperatures if they were conditioned by chilling for short periods before and after exposure to subfreezing temperatures (Fig. 1). This increase was significant for June-reared and October-reared adults ( $F = 217.87$ ;  $df = 1,8$ ;  $P < 0.001$ ). When placed directly into  $-5^{\circ}\text{C}$  conditions, *D. maidis* adult survival as measured by  $LT_{50}$ 's was 1.1 and 1.9 h for June-reared and October-reared adults. However, when conditioned for 1 h at  $+5^{\circ}\text{C}$  prior to and after exposure to  $-5^{\circ}\text{C}$ , survival  $LT_{50}$ 's lengthened to 4.6 and 8.7 h respectively.

Cold-hardiness varied considerably among the five leafhopper species as measured by the percent survival of adult leafhoppers following exposure to  $-5^{\circ}\text{C}$  (Fig. 2). There were significant differences among species in their capacity 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

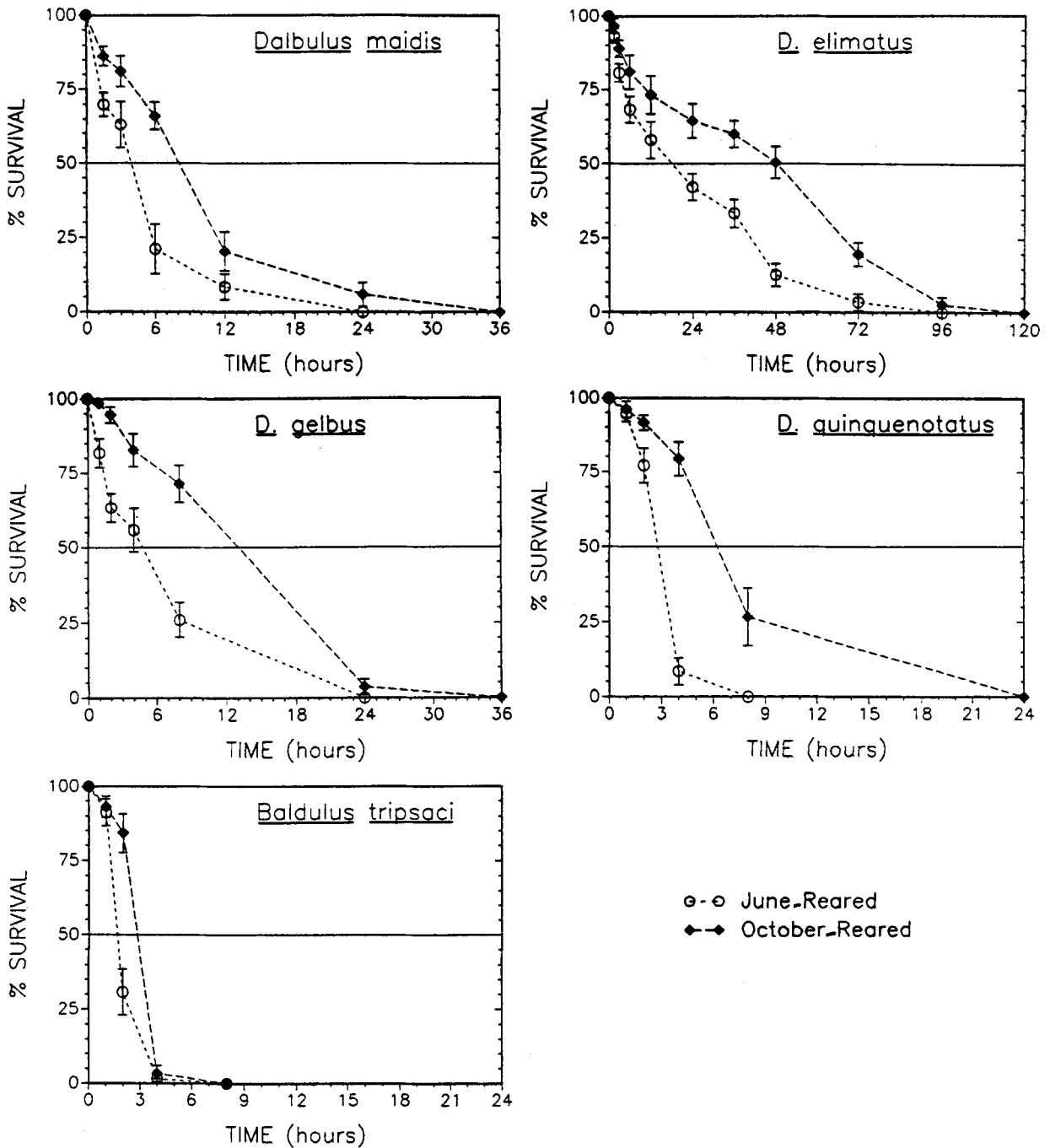


Fig. 2. Survival (mean  $\pm$  s.e.) of June-reared and October-reared adults of four *Dalbulus* species and *Balduius tripsaci* when exposed to  $-5^{\circ}\text{C}$  for various lengths of time (0–120 h) and conditioned at  $+5^{\circ}\text{C}$  for 1 h before and 1 h after subfreezing exposure.

*D. quinquenotatus* and *B. tripsaci* were the least tolerant of freezing temperatures, *D. gelbus* and *D. maidis* were moderately tolerant of  $-5^{\circ}\text{C}$

temperatures, whereas *D. elimatus* was the most tolerant (Table 2).

The  $\text{LT}_{50}$  of October-reared adults was signif-

Table 2. Tolerance to  $-5^{\circ}\text{C}$  as measured by time to 50% mortality ( $\text{LT}_{50}$ ) in hours, and supercooling points (mean  $\pm$  S.E.) for adults of five leafhopper species reared under June (L14:D10,  $28^{\circ}\text{C}/20^{\circ}\text{C}$ , seedlings) and October (L12:D12,  $23^{\circ}\text{C}/17^{\circ}\text{C}$ , mature plants) conditions typical of those in Jalisco, Mexico

Species	Rearing regime	$-5^{\circ}\text{C}$ $\text{LT}_{50}$ * (hr)	Supercooling point ( $^{\circ}\text{C}$ )
<i>Dalbulus maidis</i>	June	4.15 ab	$-20.9 \pm 0.3$
	October	8.96 c	$-20.1 \pm 0.6$
<i>D. elimatus</i>	June	23.51 e	$-20.0 \pm 0.5$
	October	42.34 f	$-21.9 \pm 0.5$
<i>D. gelbus</i>	June	4.84 b	$-21.6 \pm 0.7$
	October	12.11 d	$-20.8 \pm 0.6$
<i>D. quinquenotatus</i>	June	2.76 a	$-20.4 \pm 0.2$
	October	6.20 b	$-22.2 \pm 0.6$
<i>Balbulus tripsaci</i>	June	1.78 a	$-22.7 \pm 0.5$
	October	2.57 a	$-22.4 \pm 0.5$

\*  $\text{LT}_{50}$ 's within a column followed by different letters are significantly different (Fisher's Protected LSD = 2.49;  $\text{df} = 20$ ;  $\alpha = 0.05$ ).

icantly longer ( $F = 174.13$ ;  $\text{df} = 1,20$ ;  $P < 0.01$ ) than that of June-reared adults (Table 2), except for *B. tripsaci* (Fisher's Protected LSD = 2.49 h;  $\text{df} = 20$ ;  $\alpha = 0.05$ ). The  $\text{LT}_{50}$  of October-reared *D. maidis* was 2.16 times greater than that of June-reared adults (Fig. 2). The increase in  $\text{LT}_{50}$  between June-reared and October-reared adults (Fig. 2) was 1.80 times in *D. elimatus* (the most cold tolerant species), 2.50 times in *D. gelbus* (which had the greatest increase in cold tolerance among *Dalbulus* species), and 2.32 times in *D. quinquenotatus* (the least cold tolerant among *Dalbulus* species). The  $\text{LT}_{50}$ 's of 1.7 and 2.6 h indicated *B. tripsaci* (Table 2) was the least tolerant of subzero temperatures among the five species tested. The  $\text{LT}_{50}$  of *B. tripsaci* when reared under October conditions was 1.44 times greater than when reared under June conditions, a smaller change in cold tolerance than exhibited by any of the *Dalbulus* species.

There were no significant differences in the SCPs among the five species tested. For both June-reared and October-reared adults, SCPs were between  $-20.0$  and  $-22.7^{\circ}\text{C}$  (Table 2), more than 15 degrees below  $-5^{\circ}\text{C}$  at which cold tolerance was tested.

## Discussion

During the dry winter season in Mexico, adults of *Dalbulus* leafhoppers are exposed to temperatures that frequently drop for several hours below  $0^{\circ}\text{C}$ , particularly at night at higher elevations (Mosino-Aleman & Garcia, 1974). Under these conditions, cold-hardiness (the capacity of an organism to survive exposure to low temperature) is crucial to overwintering leafhoppers. Cold-hardiness is often influenced by environmental conditions when the insect develops (Salt, 1953; Chen *et al.*, 1987b), and this cold-hardiness is typically enhanced by physiological changes within the insect in preparation for overwintering (Lee, 1991). Environmental conditions characteristic of the later part of the wet summer season in Mexico include shorter photoperiods, lower temperatures, and decreased host plant quality, all of which are important cues in preparing insects for overwintering, particularly cold-hardening and entry into diapause (Danks, 1978, 1987; Tauber *et al.*, 1984). We show that environmental conditions during development have a significant effect on the cold-hardiness of adult *Dalbulus* leafhoppers. October-reared adult leafhoppers that developed under shorter photoperiods, cooler temperatures and on mature host plants were significantly more tolerant of subfreezing temperatures than June-reared adults that developed under longer photoperiods and warmer temperatures on young host plants.

The implications of this seasonal variation in cold-hardiness on overwintering in *Dalbulus* leafhoppers is best understood by an examination of the elevational distribution, host plant utilization patterns, and biology of each species. The Mexican corn leafhopper, *D. elimatus*, is found at higher elevations ( $1792 \pm 59$  m) than other *Dalbulus* species (Nault, 1990), and occurs up to elevations of 2400 m (Barnes, 1954; Triplehorn & Nault, 1985; Nault, 1990). Among the leafhoppers we tested, *D. elimatus* was by far the most tolerant of subfreezing temperatures. This species is found primarily on maize and the teosintes during the summer, but during winter is found regularly on irrigated winter wheat, on *Bromus* spp. and on other herbaceous plants (Barnes, 1954).

Because of its distribution at high elevations, *D. elimatus* is exposed to more subfreezing temperatures during winter than any of its congeners.

The corn leafhopper, *D. maidis*, lives at lower (<1000 m), more tropical elevations (Barnes, 1954; Triplehorn & Nault, 1985), but is widely distributed at higher elevations (up to 2000 m) by the end of the growing season (Larsen *et al.*, 1992; Nault, unpublished). However, *D. maidis* is much less tolerant of freezing temperatures than *D. elimatus*. Our data suggest that *D. maidis* either overwinters in protected microhabitats at higher elevations, or migrates to lower, frost-free elevations.

*D. gelbus* had the greatest seasonal change in its capacity to tolerate subfreezing temperatures. Like *D. elimatus*, this species also is found at high elevations (1645 ± 80 m; Nault, 1990) where potentially it is exposed to frequent subfreezing temperatures. However, *D. gelbus* is much less tolerant of cold temperatures than *D. elimatus*. This might be expected since *D. gelbus* uses *Tripsacum* as a host during the dry season (Triplehorn & Nault, 1985; Nault, 1990). The thickly clumped leaves and stems of *Tripsacum* may provide *D. gelbus* temporary protection from overnight periods of subfreezing temperatures. This type of extensive vegetative cover has been shown to reduce heat loss from plant surface microhabitats (Danks, 1981).

*D. quinquenotatus* was the least cold tolerant of *Dalbulus* leafhoppers, but it too had a significant increase in cold-hardiness when reared under October conditions. This leafhopper is present in mixed-age populations (both nymphs and adults) throughout the year (Larsen *et al.*, 1991) and an increase in cold tolerance would be advantageous during the dry season. *D. quinquenotatus* is found at lower elevations than other *Dalbulus* species used here (125 to 1975 m; Nault, 1990), and it is protected within the leaf sheaths at the base of its hosts (Nault *et al.*, 1983). *D. quinquenotatus* would not be exposed to freezing temperatures as often as its congeners that use exposed leaves in the upper canopy of their hosts or are found at higher elevations.

*B. tripsaci* specializes on *T. dactyloides* in the

eastern U.S. (Kramer & Whitcomb, 1968). Previous studies have shown that *B. tripsaci* overwinters as eggs in leaves of its host, *T. dactyloides*, and that all adults and nymphs are killed during hard frosts (Nault, 1990; Larsen, 1991). This study confirms that June-reared and October-reared *B. tripsaci* adults are intolerant of subfreezing temperatures, as would be expected of a temperate species that overwinters in the egg stage.

In the field, daily temperatures drop gradually rather than suddenly to subfreezing levels at high elevations in Mexico. To partially simulate this gradual drop, we conditioned leafhoppers for 1 h at +5 °C prior to treatment at -5 °C. This rapid, non-lethal exposure to chilling temperatures before exposure to subzero temperatures has been shown to enhance the cold tolerance of several species of insects (Chen *et al.*, 1987a; Lee *et al.*, 1987). Our results with *D. maidis* showed a similar improvement in the freezing tolerance of both June-reared and October-reared adults when leafhoppers were conditioned before subfreezing exposure. We also exposed the leafhoppers for 1 h at +5 °C following the subfreezing temperature treatment, again to simulate the normal gradual rise in field temperatures. It is not known whether such treatment after subfreezing exposure benefits insect survival.

Enhanced survival of subfreezing temperatures after a brief chilling period is likely due, at least in part, to a rapid increase in synthesis of a cryoprotectant (Lee *et al.* 1987). Many insects possess glycerol or an equivalent, such as sorbitol, as a cryoprotectant (Salt, 1961; Lee, 1991). In non-diapausing, freezing-susceptible insects, low temperatures during development often stimulate the gradual accumulation of significant amounts of cryoprotective substances (Baust & Lee, 1981; Somme, 1982; Chen *et al.*, 1987b). Larsen *et al.* (1992) previously showed that *Dalbulus* adults reared in the laboratory under environmental conditions typical of October in the field are more tolerant of drought (i.e. able to survive in the absence of food plants) than adults reared under June environmental conditions. Dehydration or drought, which occurs as plants mature and begin

to senesce, and the resultant deterioration of host plants as a food source has been shown to trigger an accumulation of cryoprotective substances in insects (Salt, 1953; Baust, 1982; Young & Block, 1980; Rojas *et al.*, 1986). Drought can affect the survival of insects, such as leafhoppers, which live entirely on liquid diets (DeLong, 1971), and could be a significant factor influencing the survival of *Dalbulus* leafhoppers at cold temperatures by stimulating the production of cryoprotectants.

The SCP is that subzero temperature at which spontaneous tissue freezing occurs (Salt, 1961). Although SCPs vary seasonally in some insects (Salt, 1961; Knight & Bale, 1986; Lee, 1991), SCPs did not differ significantly among leafhopper species or between the two rearing conditions. Mortality in supercooled, but unfrozen, insects has been reported by a number of workers (Lee & Denlinger, 1985; Knight *et al.*, 1986). Since mortality occurred at  $-5^{\circ}\text{C}$  for all *Dalbulus* species and internal ice formation measured by the SCP occurred at  $-20^{\circ}\text{C}$  or lower, it is apparent that SCP is not an appropriate measure of low temperature tolerance for these leafhoppers.

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### Resumen

*Influencia de las condiciones de desarrollo en la habilidad para sobrevivir el congelamiento de los adultos Dalbulus chicharritas: implicaciones en la sobrevivencia al invierno*

Se conoce muy poco acerca de como la chicharrita adulta, *Dalbulus maidis* (DeLong y Wolcott), y sus congéneres sobreviven las temperaturas de subcongelamiento en las regiones altas de México durante el invierno seco. En el laboratorio, se midió la duración de la supervivencia a  $-5^{\circ}\text{C}$  en cuatro especies mexicanas de *Dalbulus*: *D. maidis*, *D. elimatus* (Ball), *D. gelbus* DeLong, y *D. quinquenotatus* DeLong y Nault, y la especie norteamericana, íntimamente relacionada, *Balbulus trypsaci* Kramer y Whitcomb. Las chicharritas adultas criadas en condiciones experimentales que simulaban el comienzo de la estación seca de invierno en México (criadas en Octubre) fueron como mínimo dos veces más tolerantes a  $-5^{\circ}\text{C}$  que las adultas criadas bajo condiciones medio ambientales que simulaban el comienzo de la estación húmeda de verano (criadas en Junio). Las especies de *Dalbulus* que se encuentran principalmente en regiones muy elevadas tal como *D. elimatus* fueron siete veces mas tolerantes a los  $-5^{\circ}\text{C}$  que *D. quinquenotatus*, una especie que inverna en regiones con elevaciones medias a bajas, en su hospedante *Tripsacum*. Los adultos de *D. maidis* criados en Octubre sobrevivieron relativamente cortos periodos a  $-5^{\circ}\text{C}$  ( $LT_{50} = 8.9$  h) comparados a los adultos de *D. elimatus* criados en Octubre ( $LT_{50} = 42.3$  h). Esto sugiere que *D. maidis* inverna en lugares protegidos en las regiones más elevadas ó migra a regiones más bajas libres de heladas de México. Los adultos de *B. trypsaci* criados en Octubre, que invernan en el estado de huevo, fueron intolerantes a las temperaturas de  $-5^{\circ}\text{C}$  ( $LT_{50} = 2.6$  h). Un período de adaptación de 1 h a  $+5^{\circ}\text{C}$  incrementó significativamente la supervivencia at  $-5^{\circ}\text{C}$  de *D. maidis*. Los puntos de supercongelamiento estuvieron entre  $-23$  y  $-20^{\circ}\text{C}$  indicando que la mortalidad a  $-5^{\circ}\text{C}$  de esas chicharritas se debió al daño por frío más que a la formación interna de hielo.



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