# Pathogenicity of Corn Stunt Spiroplasma and Maize Bushy Stunt Mycoplasma to Their Vector, *Dalbulus longulus*

L. R. Nault, L. V. Madden, W. E. Styer, B. W. Triplehorn,
G. F. Shambaugh, and S. E. Heady

## ABSTRACT

Nault, L. R., Madden, L. V., Styer, W. E., Triplehorn, B. W., Shambaugh, G. F., and Heady, S. E. 1984. Pathogenicity of corn stunt spiroplasma and maize bushy stunt mycoplasma to their vector, *Dalbulus longulus*. Phytopathology 74: 977-979.

*Dalbulus longulus* is the seventh member of the genus reported as a vector of the corn stunt spiroplasma (CSS) and maize bushy stunt mycoplasma (MBSM). *D. longulus* was rediscovered in the eastern Mexican state of Veracruz where it utilizes maize (*Zea mays*), *Tripsacum laxum*, and *T. andersonii* as hosts. CSS and MBSM significantly shortened the longevity of adult *D. longulus* as measured by the quartiles of the survival distribution as well as estimated scale parameter of the Weibull survival model. MBSM caused greater mortality than did CSS. Similar effects of these mollicutes have been reported for other *Dalbulus* spp. We speculate on the relative importance of various Mexican *Dalbulus* spp. as field vectors of CSS and MBSM, based upon their differential sensitivity to these pathogens.

*Additional key words*: survival analyses, vector relations.

The discovery of a new leafhopper species, *Dalbulus tripsacoides* DeLong & Nault, collected from *Tripsacum dactyloides* L. in Mexico, was important evidence to support the hypothesis that leafhoppers in the genus *Dalbulus* evolved with maize (*Zea mays* L.) and its teosinte (*Zea* spp.) and gamagrass (*Tripsacum* spp.) ancestors (9). This hypothesis has been strengthened by the discovery of additional leafhoppers of *Dalbulus* spp., seven in all, including two new species from Mexican teosinte and gamagrass (11).

The corn leafhopper, *D. maidis* (DeLong & Wolcott), is the most widely distributed member of the genus and is considered the principal field vector of the corn stunt spiroplasma (CSS), maize bushy stunt mycoplasma (MBSM), and maize rayado fino virus (MRFV) (5,7,10). The remaining *Dalbulus* spp. are either less widely distributed on maize or utilize teosinte or gamagrass as hosts (11). All *Dalbulus* spp. tested so far are vectors of CSS and MBSM (6). Furthermore, it was discovered that these mollicutes, with a few notable exceptions, are pathogenic to vector *Dalbulus* spp. These exceptions have led Nault (7,8) to speculate that maize stunting mollicutes may have influenced the evolution of their vectors or at least affected the distribution of extant species. These species include the previously mentioned *D. tripsacoides* and recently described *D. quinquenotatus* DeLong & Nault, and *D. guzmani* DeLong & Nault. *Tripsacum* spp. are the only known field hosts for these three leafhoppers. *D. gelbus* DeLong, and *D. guevarai* DeLong, described by DeLong (4) in 1950, have been discovered on maize and several *Tripsacum* spp. Finally, the Mexican corn leafhopper, *D. elimatus* (Ball), which prefers *Zea* spp., can be found with *D. maidis* feeding on maize and teosinte species.

Our previous surveys for *Dalbulus* spp. in Mexico have been confined to the central and western Mexican states of Mexico, Morelos, Guerrero, and Jalisco (9,11). *D. longulus* DeLong was described by DeLong in 1950 (4) from eastern Mexico, but little else has been learned about the species since its discovery and description.

Here we report on its rediscovery and, for the first time, its field hosts. We also report on transmission of CSS and MBSM by *D. longulus* and the pathogenicity of these mollicutes to this vector. Finally, we discuss how the differential pathogenicity of CSS and MBSM to *Dalbulus* spp. may be used to ascertain the relative importance of species as field vectors.

## MATERIALS AND METHODS

In October 1982, maize and *Tripsacum* spp. in the eastern Mexican states of Veracruz and Oaxaca were surveyed for the presence of *Dalbulus* spp. Leafhoppers were collected either by sweeping foliage with sweep nets or directly removing them from leaves with mouth-operated aspirators. Leafhoppers were placed in plastic containers containing leaves of maize or *Tripsacum* and refrigerated until later transported to the vector-virus laboratory at the Ohio State University, Ohio Agricultural Research and Development Center. Leafhoppers were then placed in insect rearing cages (2) containing maize seedlings.

A colony of *D. longulus* was established from 11 females taken from maize at two locations in the state of Veracruz (see Results). These were used in tests for transmission of CSS and MBSM and to determine effects of these mollicutes on adult longevity. Sources of mollicutes are the same as reported previously (6). Third-instar nymphs were exposed to CSS- or MBSM-infected plants. Following exposure, one set of leafhoppers was placed on healthy plants for 2 wk, then individually assayed for ability to transmit CSS or MBSM by caging them on sweet corn (cultivar Aristogold Bantam Evergreen) for an inoculation access period. After 1 wk, leafhoppers were removed and the test plants were placed in a greenhouse to allow symptom development. Plants were held in the greenhouse for 8 wk before final results were recorded.

Following 1 wk of exposure to infected plants, a second set of leafhoppers was divided into three groups of 25 adults each and placed on maize in tube cages (6). Surviving leafhoppers were counted at weekly intervals and transferred to new maize host

plants. The procedure was repeated until all leafhoppers were dead. Unexposed leafhoppers served as controls. In the third week of this experiment, one cage of leafhoppers carrying MBSM was accidently knocked over; therefore, only two replicates of this treatment were included in the analysis.

For each replication of the controls and mollicute exposure, the quartiles of the survival distribution were calculated. These are the times to 75 ($t_{75}$), 50 ($t_{50}$), and 25% survival ($t_{25}$) [6]. Analysis of variance was used to determine the effect of mollicute exposure on the survival quartiles. Duncan's Bayesian least significant difference was used for mean separations [12].

The Weibull model was also fit to the survival distributions by using the procedures explained in Madden and Nault [6]. This model contains two parameters, a scale parameter ($b$) that is inversely related to the rate of population decline, and a scale parameter ($c$) that characterizes the form of the survival distribution.

## RESULTS

*D. longulus* was taken from the following hosts and localities: three males and four females from *Tripsacum laxum* Nash (host deposited in OSU Herbarium, *Doebley 628*, OSU 153633), 2 km E. of Orizaba, VC, 18° 51′N, 97° 03′W at 1,000–1,100 m altitude on 6-X-82; two males and six females from *T. andersonii* Gray (*Doebley 639*, OSU 153644), 19 km ENE of Huatusco near the road to the city of Vera Cruz, VC, 19° 12′30″N, 96° 47′W at 925 m altitude on 7-X-82; four males and five females from a maize field on route 145 between Tierra Blanca and Tinaja, VC, on 8-X-82; and seven males and six females from a maize field on route 150 between Cuitlahuac and Cordoba, VC, on 8-X-82. Both corn fields were at no more than 200 m altitude. *D. maidis* was also collected from these corn fields. Few (<5%) of the maize plants were infected with CSS or MRFV. *D. longulus* was not present in leafhopper samples taken from maize and *Tripsacum* spp. in the state of Oaxaca.

Six of 21 (28.6%) individual *D. longulus* transmitted CSS to test plants, whereas 15 of 17 (84.2%) transmitted MBSM. These two percentages differ significantly, based on a *t*-test ($P = 0.05$). None of 20 unexposed controls transmitted either pathogen. Most exposed leafhoppers were dead following the inoculation access period, a result experienced with several *Dalbulus* species in our previous study [6].

Both CSS and MBSM greatly shortened the lives of *D. longulus* adults as seen in survival profiles (Fig. 1). At all three quartiles (ie, $t_{75}$, $t_{50}$, $t_{25}$) CSS and MBSM significantly shortened adult lifespans. MBSM was more pathogenic to *D. longulus* than was CSS (Table 1).
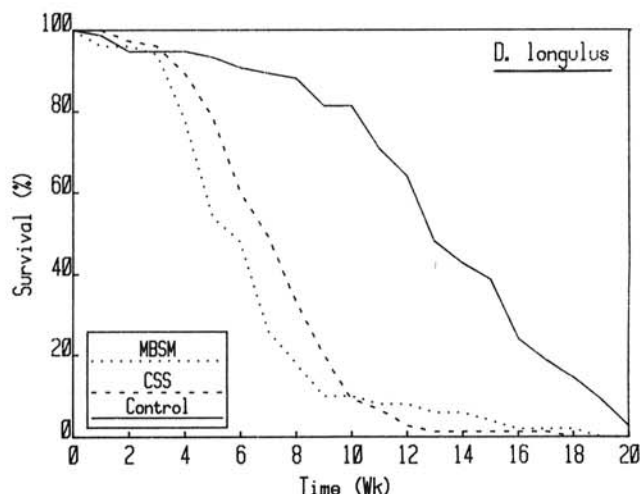


**Fig. 1.** Survival of adults of *Dalbulus longulus* following 1 wk of exposure (as third instar nymphs to eclosion) to maize bushy stunt mycoplasma (MBSM), corn stunt spiroplasma (CSS) infected or healthy maize plants and then transferred at weekly intervals to healthy maize plants.

The Weibull model gave excellent fits to the survival data based on the criteria described in Madden and Nault [6]. Mean estimated scale parameters for leafhoppers exposed to healthy corn, CSS-, and MBSM-infected corn were 14.8, 8.4, and 7.6 wk, respectively. Leafhoppers exposed to healthy corn had a much slower rate of population decline than did leafhoppers exposed to corn infected with either mollicute. The mean estimated shape parameter equalled 3.2, 3.0, and 2.1 for leafhoppers exposed to healthy corn, CSS-, and MBSM-infected corn, respectively. These shape parameters indicate that the hazard rate for this leafhopper species (probability of dying at time *t* given survival up to time *t*) increases over time [6].

## DISCUSSION

The rediscovery of *D. longulus* >30 yr after its description and last report from field collections (1,4) probably reflects a lack of effort to monitor its presence rather than its reappearance. More importantly, we have documented its hosts which, like those of all other *Dalbulus* spp., are members of the Maydeae. This finding further strengthens our claim of an evolutionary link between *Dalbulus* and the ancestors of maize. *D. longulus* belongs to a group of four closely related (sister) species, the other members being *D. guzmani*, *D. elimatus*, and *D. guevarai* [11]. The distributions of these morphologically similar [11] species are generally allopatric. *D. elimatus* occurs in the Nearctic Mesa Central and may utilize species other than *Zea* and *Tripsacum* as food hosts during the dry winter months [1]. *D. guevarai* is a Neotropical species that is found principally in the western Lower Balsas region [11] and has *D. longulus* as its Neotropical eastern counterpart (Veracruzian region). *D. guzmani* has been collected only from *T. pilosum* Scribn. and Merrill, and is endemic to northern Jalisco.

*D. longulus*, with a median survival time ($t_{50}$) of 12.8 wk, had the longest lifespan of the eight *Dalbulus* spp. tested at 26 C on maize seedlings (Table 1; cf. Madden and Nault [6]). Its sister species, *D. guevarai*, had the next longest lifespan with $t_{50} = 8.3$ wk. We do not know why *D. longulus* had such a long survival time compared to other *Dalbulus* spp.

Not surprisingly, *D. longulus* was a vector of both CSS and MBSM. It is the seventh species in the genus demonstrated to transmit both pathogens. Of the seven, we consider *D. maidis* to be the most important field vector of both pathogens, since this species is the only member of the genus known from Central and South America. In Mexico, however, other *Dalbulus* species may play a role in field spread of CSS and MBSM. Not included would be *D. tripsacoides*, *D. quinquenotatus*, and *D. guzmani* because they are found only on *Tripsacum* spp. [11]. This leaves *D. longulus*, *D. guevarai*, *D. gelbus*, and *D. elimatus*, all of which occur on maize, as possible candidates as field vectors of CSS and MBSM.

A careful examination of the pathogenicity of CSS and MBSM to these five leafhopper species provides important clues for speculating on their relative importance as field vectors. We begin with the assumption that these plant pathogenic mollicutes are inherently pathogenic to leafhoppers of *Dalbulus* spp. and the

TABLE 1. Survival of adult *Dalbulus longulus* exposed to corn spiroplasma (CSS), maize bushy stunt mycoplasma (MBSM) or to healthy maize plants[y]

| Treatment | Survival in Weeks[z] | | |
| --- | --- | --- | --- |
| | $t_{25}$ | $t_{50}$ | $t_{75}$ |
| Control | 16.03 a | 12.80 a | 10.47 b |
| CSS | 8.67 b | 6.96 b | 5.13 b |
| MBSM | 7.20 c | 5.80 c | 4.05 c |

[y] Treated leafhoppers were exposed for 1 wk from the time leafhoppers were third instar nymphs to eclosion (adults).

[z] $t_{25}$, $t_{50}$, and $t_{75}$ are times to 25, 50, and 75% survival, respectively. Means followed by the same letter within a column are not significantly different according to Duncan's Bayesian least significant difference ($k = 100$; $P = 0.05$).

closely related *Baldulus* spp. This assumption is supported by the high degree of susceptibility of *D. tripsacoides*, *D. quinquenotatus*, and *B. tripsaci* Kramer and Whitcomb leafhoppers experimentally exposed to either mollicute (6). None of these three species is likely to encounter CSS or MBSM in nature since their natural hosts, the gamagrasses, are immune to these pathogens (7,8). CSS and MBSM are also highly pathogenic to *D. longulus* and *D. guevarai*, suggesting that they only infrequently encounter these mollicutes in nature. For example, the maize fields from which we collected and started our colony of *D. longulus* contained a few CSS-infected plants. Perhaps maize is secondary to *Tripsacum* as a host for *D. longulus* and *D. guevarai*.

*D. longulus* was the only *Dalbulus* spp., except for *D. maidis*, which was more susceptible to MBSM than to CSS. MBSM is more common in the Nearctic regions of Mexico (3) than CSS, and has not been reported from the Neotropical area where we collected *D. longulus*. It should be noted that there are no serological techniques for identifying MBSM, and our knowledge of the extant distribution of this mollicute is therefore inadequate.

*D. maidis* is unaffected by CSS and both vector and pathogen share a Neotropical distribution in Mexico (6). Although it is very likely that *D. maidis* is the principal CSS vector in Mexico, it may not be the primary vector of MBSM in Mexico as it is elsewhere in Latin America. *D. elimatus* and *D. gelbus* are the only two vector species whose adult life spans are not shortened by MBSM. Both leafhoppers occur in the Nearctic regions of Mexico, a distribution that coincides with MBSM. We therefore speculate that either or both of these species may be more important than *D. maidis* as vectors of MBSM in Mexico.

### LITERATURE CITED

1. Barnes, D. 1954. Biologia, ecologia, y distribucion de las chicharritas, *Dalbulus elimatus* (Ball) y *Dalbulus maidis* (Del. & W.). Folleto Tech. 11. Sec. de Agr. y Ganaderia Ofic. de Estud. Especiales, Mexico, D. F. 112 pp.

2. D'Arcy, C. J., and Nault, L. R. 1982. Insect transmission of plant viruses and mycoplasmalike and rickettsialike organisms. Plant Dis. 66:99-104.

3. Davis, R. E. 1974. Occurrence of a spiroplasma in corn stunt-infected plants in Mexico. Plant Dis. Rep. 57:333-337.

4. DeLong, D. M. 1950. The genera *Baldulus* and *Dalbulus* in North America including Mexico. (Homoptera:Cicadellidae). Bull. Brooklyn Entomol. Soc. 45:105-116.

5. Gamez, R. 1980. Rayado fino virus disease of maize in the American tropics. Trop. Pest Management 26:26-33.

6. Madden, L. V., and Nault, L. R. 1983. Differential pathogenicity of corn stunting mollicutes to leafhopper vectors in *Dalbulus* and *Baldulus* species. Phytopathology 73:1608-1614.

7. Nault, L. R. 1983. Origins in Mesoamerica of maize viruses and mycoplasmas and their leafhopper vectors. Pages 259-266 in: Plant Virus Epidemiology: The Spread and Control of Insect-Borne Viruses. R. T. Plumb and J. M. Thresh, eds. Blackwell Scientific Publ., Ltd., Oxford, England. 368 pp.

8. Nault, L. R. 1983. Origins of leafhopper vectors of maize pathogens in Mesoamerica. Pages 75-82 in: Proceedings International Maize Virus Disease Colloquium and Workshop, 2-6 August 1982. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. The Ohio State University, Ohio Agricultural Research and Development Center, Wooster. 266 pp.

9. Nault, L. R., and DeLong, D. M. 1980. Evidence for co-evolution of leafhoppers in the genus *Dalbulus* (Cicadellidae: Homoptera) with maize and its ancestors. Ann. Entomol. Soc. Am. 73:349-353.

10. Nault, L. R., and Knoke, J. K. 1981. Maize vectors. Pages 77-84 in: Virus and viruslike diseases of maize in the United States. Southern Cooperative Ser. Bull. 247. June 1981. D. T. Gordon, J. K. Knoke, and G. E. Scott, eds. Ohio Agricultural Research and Development Center, Wooster. 218 pp.

11. Nault, L. R., DeLong, D. M., Triplehorn, B. W., Styer, W. E., and Doebley, J. F. 1983. More on the association of *Dalbulus* (Homoptera:Cicadellidae) with Mexican *Tripsacum* (Poaceae), including the description of two new species of leafhoppers. Ann. Entomol. Soc. Am. 76:305-309.

12. Waller, R. A., and Duncan, D. B. 1969. A Bayes rule for the symmetric multiple comparisons problem. J. Am. Stat. Assoc. 64:1484-1503.