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Lack of specificity in the interaction between two maize stunting pathogens and field collected *Dalbulus* leafhoppers

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Abstract

We tested hypotheses concerning the specificity of interactions between insect vectors and mollicute plant pathogens in a 22-month study of leafhoppers collected at three agricultural field sites in Mexico. The common species collected, *Dalbulus maidis, D. elimatus, D. gelbus,* and *D. guevari* were equally likely to test positive for corn stunt spiroplasma (CSS) in ELISA, and to transmit maize bushy stunt phytoplasma (MBSP) to test maize seedlings. We documented intraspecific variation in the ability of *D. maidis* to transmit confirmed CSS infections. *Dalbulus guevari* and *D. gelbus* were less successful in transmitting CSS than *D. maidis* from the same population. Our results suggest this vector-plant pathogen interaction is not specific to a single *Dalbulus*-mollicute combination, and that both the range of potential vectors in agricultural fields, and intraspecific variation across populations of these vectors, should be the focus of future work.

Introduction

Dalbulus maidis (DeLong and Wolcott) (Cicadellidae, Macrostelini), the corn leafhopper, is a vector of several maize pathogens, making it the most serious pest of maize in Mexico, Central and South America (Nault, 1990). These pathogens include the corn stunt spiroplasma (*Spiroplasma kunkelii*, CSS) and maize bushy stunt phytoplasma (MBSP), both members of the bacterial class Mollicutes.

Previous laboratory and field work suggests the vector-CSS interaction is specific to *D. maidis*, and MBSP has only two other *Dalbulus* vectors in the field, *viz*., *D. elimatus* (Ball), the Mexican corn leafhopper, and *D. gelbus* DeLong. Of the 13 *Dalbulus* species, most feed on plants (*Tripsacum* species, Gramineae) not susceptible to experimental infection with mollicutes (Pitre, 1970; Nault, 1980; Nault & Styer, 1994). *Dalbulus maidis*, *D. elimatus*, *D. gelbus*, *D. guevari* DeLong, and *D. longulus* DeLong feed on maize in the field, but only *D. maidis* and *D. elimatus* are considered maize specialists (Nault et al., 1983, 1984; Gordon et al., 1985; Nault, 1990). All five can acquire and transmit CSS and MBSP, but because most do not survive the bacterial latent period they are considered poor field vectors (Nault, 1980; Madden & Nault, 1983; Madden et al., 1984; Nault et al., 1984). Effects of CSS and MBSP on the two maize specialists and *D. gelbus* depends on the pathogen: survival is reduced in *D. gelbus* and *D. elimatus* following exposure to CSS, and in *D. maidis* following exposure to MBSP. Adult survival also varies with environment: survival improves in *D. maidis* maintained under conditions similar to the dry season in central Mexico following exposure to CSS and MBSP (Ebbert & Nault, 1994, 2001).

These laboratory studies are consistent with the hypothesis that, among *Dalbulus* collected from field-

grown maize, only *D. maidis*, *D. elimatus*, and *D. gelbus* are likely to tolerate mollicute infection long enough to survive the latent period and spread CSS or MBSP infection among healthy plants. We made the following three predictions related to this hypothesis and tested them by assaying *Dalbulus* collected from three agricultural research stations in central Mexico.

Prediction 1: Dalbulus maidis *will be more successful in transmitting CSS than its congeners.* Based on laboratory survival studies, we expected *D. maidis* would be better able to survive the latent period and thus transmit infection to healthy maize when compared to other *Dalbulus*. A field collection made at one of our sites suggests otherwise: at El Batan, in October, 1982, CSS prevalence was similar in *D. elimatus* and *D. maidis* (Gordon et al., 1985). We tested leafhoppers collected over two years and at three field sites to better document whether CSS prevalence (as estimated by ELISA) or ability to infect healthy seedlings (as estimated in a bioassay) varies among *Dalbulus*.

Prediction 2: MBSP prevalence will be higher in D. elimatus *and* D. gelbus *than in their congeners.* Because the laboratory studies showed reduced survival in most *Dalbulus* following exposure to MBSP infected plants, we predicted that *D. elimatus* and *D. gelbus* would be more likely to survive the MBSP latent period and therefore transmit MBSP than other *Dalbulus*.

Prediction 3: CSS prevalence in D. maidis *will increase over the dry season.* Exposure to CSS or MBSP improves survival in *D. maidis* when the leafhoppers are maintained on moist sand and under either cool (L12:D12; 20 °C:10 °C; average temperature 15 °C) or warm (L14:D10; 35 °C:20 °C, average 31 °C) conditions (Ebbert & Nault, 2001). We choose these conditions for our laboratory study to reflect what *D. maidis* experiences at our El Batan site during the dry season: loss of host plant, low rainfall, and both the highest and lowest temperatures of the year (Mosino-Aleman & Garcia, 1974; see also summary of weather data from field stations in Ebbert & Nault, 2001). We predicted that, as a result of this improved survival, our field collections would show a higher prevalence of CSS in leafhoppers collected in June (at the end of the dry season) when compared to those collected the previous October (at the end of the rainy season).

Materials and methods

We tested our predictions using *Dalbulus* collected at three agricultural research stations of the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. The three sites differ in elevation: Poza Rica (PR) is 60 m above sea level, Tlaltizapan (TL) is 940 m asl, and El Batan (EB) is 2249 m asl. EB and TL are in central Mexico (latitude 19°5′ N and longitude 98°9′ W and 18°7′ N, 104°1′ W, respectively); PR is about 40 km from the Gulf Coast (19°5′ N, 97°5′ W). Various maize cultivars are planted at each site, and a *Tripsacum* garden is maintained at TL. As earlier described in detail (Ebbert & Nault, 2001), the elevational differences between these sites are reflected in cropping practice, temperature, and rainfall: EB is coolest and driest site, PR conditions are the wettest and warmest. Based on previous reports, we expected to collect *D. maidis* and at least one congener at each site: *D. elimatus*, *D. gelbus,* and *D. guevari* at EB (Gordon et al., 1985), *D. gelbus* and *D. guevari* at TL (Nault et al., 1983) and *D. longulus* at PR (Nault et al., 1984).

The observation that two species differ in their success at infecting healthy test seedlings has several potential explanations, and so we took steps to assess confounding factors in our analysis. We made four visits to Mexico, during which we collected at each site on three consecutive days, to account for variation over time and between sites in the prevalence of infected plants. We then assumed that leafhoppers collected at the same time and place were equally likely to encounter and thus acquire infection.

Leafhoppers and pathogens could also vary in their response to our bioassay or ELISA protocol. We controlled for this as much as possible by using the same maize cultivar for all bioassays, matching seedlings for growth stage, conducting all the bioassays for each collection at the same time and under the same greenhouse and walk-in conditions, exposing each leafhopper to three test plants, and using antibody prepared against CSS from one of our sites (PR).

Once they acquire the bacteria, leafhoppers may differ in ability to propagate or survive the infection and thus differ in transmission rates. This variation was the target of our investigation. For MBSP, we assessed infections solely by bioassay. For CSS, we also conducted ELISAs on leafhoppers after the bioassay. This allowed us to compare transmission success among individuals with confirmed infections. We also standardized our collection and testing protocol to

minimize variation in time from collection to the assays. The bioassay began an average of 11 days after collection in the field. The latent period in *D. maidis* under our bioassay conditions is 19 ± 2 days (CSS) and 24 ± 2 days for MBSP (Nault, 1980). By the end of the 9-day bioassay, when insects were frozen for ELISA, an average of 20 days had elapsed since their last opportunity to feed on an infected plant. We expect, then, that our bioassay protocol missed few CSS infections, but may have missed more recent MBSP infections. Using ELISA, we tested individual leafhoppers for infection as described in Ebbert & Nault (2001). We assumed that negative ELISAs reflect a lack of infection, while negative bioassay results in an insect with a confirmed infection indicate a long latent period.

Our sweep net collections provided insects for estimates of relative abundance and infection assays. Each sweep net collection was conducted for 2 h by two people working the same maize plots. We collected leafhoppers at the beginning (October 1992 and 1993) and end (June 1993 and July 1994) of the dry season. Insects were either kept alive for transport to Ohio or killed at the time of collection by air drying them in vented plastic boxes at room temperature. We also used yellow, 12.5 × 20.5 cm sticky traps (Olson Products Inc., Medina, OH 44258) to monitor species composition (presence/absence) between sweep net collections. We suspended traps 3 m above the ground from four metal poles installed in maize plots at each station. The four traps were replaced weekly. Wax paper was placed over exposed cards and the traps were kept under refrigeration until shipment to Ohio for examination. Weeks in which more than two sticky cards were lost in the field, damaged in transit or obscured by mold were removed from the analysis.

In Ohio, we identified leafhoppers to species and tested their ability to inoculate healthy maize (3-leaf seedling stage, cultivar Early Sunglow). All leafhoppers surviving transit from Mexico for each collection were assayed at once. We caged one insect per seedling in screened, 15 cm by 5 cm diameter, butyrate tubes. Carts holding all the test seedlings were then placed in a temperature-controlled, lighted walk-in growth chamber (26 °C, L14:D10 light regime) and watered daily. Leafhoppers and tube cages were moved to new seedlings after three days; after the 3-day exposure to the third seedling leafhoppers were frozen for ELISA. Most leafhoppers survived this 9-day bioassay, leafhoppers that died during this process were also frozen and assayed by ELISA. We then sprayed the seedlings with a synthetic pyrethroid insecticide (resmithrin, Whitmire Micro-Gen, St. Louis, MO 63122) and isolated the plants in dedicated greenhouse rooms maintained between 25 and 30 °C, with ambient and incandescent light supplemented by 400 watt metal halide lamps. Plants were sprayed with resmithrin and examined weekly for CSS or MBSP symptoms; plants without symptoms after eight weeks were scored as healthy (Nault, 1980). A positive result in the bioassay required at least one of the three plants exposed to an individual leafhopper to show typical CSS or MBSP symptoms. Our identification of MBSP infections based on plant symptoms was confirmed using DNA amplification techniques (Harrison et al., 1996).

We categorized each leafhopper collected by species (one of five), site (EB, TL or PR) and collection (one of four). We then compared response variables (positive or negative results from ELISA and bioassays) across these categories. We intended to test these response variables in a complete 3 (site) × 4 (collection) × 2 (species per site) design. Insufficient collections at PR made this analysis impossible: the few *D. longulus* we collected at PR, and all the *D. maidis* collected in 1993, died before they were assayed for infection. Following methods described in Collett (1991) and Feinberg (1980), we therefore tested subsets of the data for heterogeneity in our response variables with logistic regression (lack of fit tests) and likelihood ratio (G) chi-square tests.

We tested for variation among sites and collections using data organized in two tables: one which included all four collections, but excluded PR (a 2-site × 4-collection table) and one which included all the sites, but excluded the 1993 collections (a 3-site × 2-collection table). In each table we tested for interactions between site and collection with a lack of fit test, in which a significant result indicates a model with the interaction term (response = site + collection + site × collection) is a better fit to the data than one without the interaction. Where interactions were not significant, we then tested the effect across sites (regardless of collection time) and across collections (regardless of site). Where these tests produced no evidence that prevalence or transmission ability varied with site or collection, we then pooled all the data and tested for differences among the four species we collected, and among the three populations of *D. maidis*. Where we did find evidence of interactions between site and collection, we assessed the effect of species separately for each collection and site.

Table 1. Dalbulus collected from sticky traps (pooled over three months) at three agricultural field stations in Mexico

Year	Month	El Batan		Tlaltizapan	Poza Rica
		D. maidis	*D. elimatus*	*D. maidis*	*D. maidis*
1992	Oct.–Dec.	16	25	34	154
1993	Jan.–Mar.	5	10	6	27
	Apr.–Jun.	1	5	27	60
	July–Sept.	9	1	16	37
	Oct.–Dec.	211	126	34	
1994	Jan.–Mar.	12	44		
	Apr.–June	5	4		
	July-Sept.	56	42		

We judged differences in response between categories as significant if the probability of obtaining a given chi-square was less than 0.05. In 2 by 2 tables where the average sample size per cell was less than 5, we used Fisher's exact (two-tailed) test and the same criteria for significance (Sokal & Rohlf, 1981). We used JMP for the Macintosh (ver. 3.2.6, SAS Institute, Inc. 1999) for our statistical analysis. Binomial errors greater than 1.5% are reported for proportions (Sokal & Rohlf, 1981).

Results

Abundance and distribution of Dalbulus. We collected and identified to species 2888 *Dalbulus* representing five species: *D. maidis* (present at all three sites), *D. elimatus* (EB), *D. guevari* (EB, TL), *D. gelbus* (EB, PR), and *D. longulus* (PR). Relative abundance varied across sites and sweep net collections (Figure 1). At PR, *D. maidis* was the dominant species, comprising 89% (626 of 701 leafhoppers) of the total catch at that site. *Dalbulus maidis* was only 48 ± 2% (256 of 537) of the TL catch (44 ± 2%, or 236, were *D. guevari*) and 26 ± 2% (167 of 638) of the EB catch, where *D. elimatus* was the most common *Dalbulus* species (67 ± 2%, or 429).

Many of the sticky card traps examined contained few or no *Dalbulus*. We therefore pooled counts within sites into four, three-month periods (Table 1). We identified to species 1012 *Dalbulus* from these traps: 572 from EB, 131 from TL and 309 from PR. These counts showed *D. maidis* was present at all sites during both of the coldest (January to March) and warmest (April to June) periods of the dry season in 1993 and 1994. We recovered only 22 *D. gelbus* (from TL and PR), 3 *D. guevari* (TL) and 20 *D. longulus* (PR) from these traps.

Ability to transmit MBSP. Our bioassays yielded 17 MBSP infections (2.2%, Table 2). We tested for heterogeneity in MBSP transmission among sites and across collections as described in the methods: in neither the 2-site × 4-collection table (lack of fit test, d.f = 3, G = 1.5, P = 0.67) nor the 3-site × 2-collection table (d.f = 2, G = 3.4, P = 0.18) did we find significant interactions between site and collection. The number of MBSP transmissions did not vary with site (pooled across collection, d.f. = 2, G = 1.6, P = 0.44) or collection (pooled across sites, d.f. = 3, G = 3.7, P = 0.30). We therefore pooled across site and collection and found no differences in ability to transmit MBSP among species (d.f. = 3, G = 1.1, P = 0.77). Contrary to our prediction, *D. elimatus* and *D. gelbus* were no more likely (5 of 268 tested, or 1.9%) to survive the MBSP latent period and transmit the pathogen to healthy seedlings than their congeners (12 of 515 tested, or 2.3%, d.f. = 1, G = 0.2, P = 0.67).

Ability to transmit CSS. We detected 13 CSS infections in our bioassay (1.7% of leafhoppers tested, Table 2). There were no significant interactions between site and collection in either the 2-site × 4-collection table (lack of fit test, d.f = 3, G = 5.6, P = 0.13) or the 3-site × 2-collection table (d.f = 2, G < 0.1, P = 0.99). CSS transmissions did not vary with site (d.f. = 2, G = 3.0, P = 0.22) or collection (d.f. = 3, G = 4.4, P = 0.22). We therefore pooled across site and collection and found no differences in ability to transmit CSS among species (d.f. = 3, G = 2.5, P = 0.48). *Dalbulus maidis* transmitted CSS to nine (2.3%) of 396 healthy seedlings tested, a proportion not significantly different from that of its congeners (4 of 387 tested, or 1.0%, d.f. = 1, G = 1.9, P = 0.17).

Comparison of ELISA and bioassay. We tested 783 insects in the bioassay and 694 using ELISA (Table 2); 658 were assayed with both methods. Most (551, or 83.7%) of the ELISA results were consistent with those from the bioassay: 542 were negative and 9 were positive by both methods. Almost all (103) of the remaining 107 insects were positive in the ELISA but not in the bioassay. We considered these as true positives, that is, insects that acquired CSS infection in the field but were unable to transmit it to healthy maize seedlings under our bioassay conditions. We considered the remaining four, in which the ELISA failed to

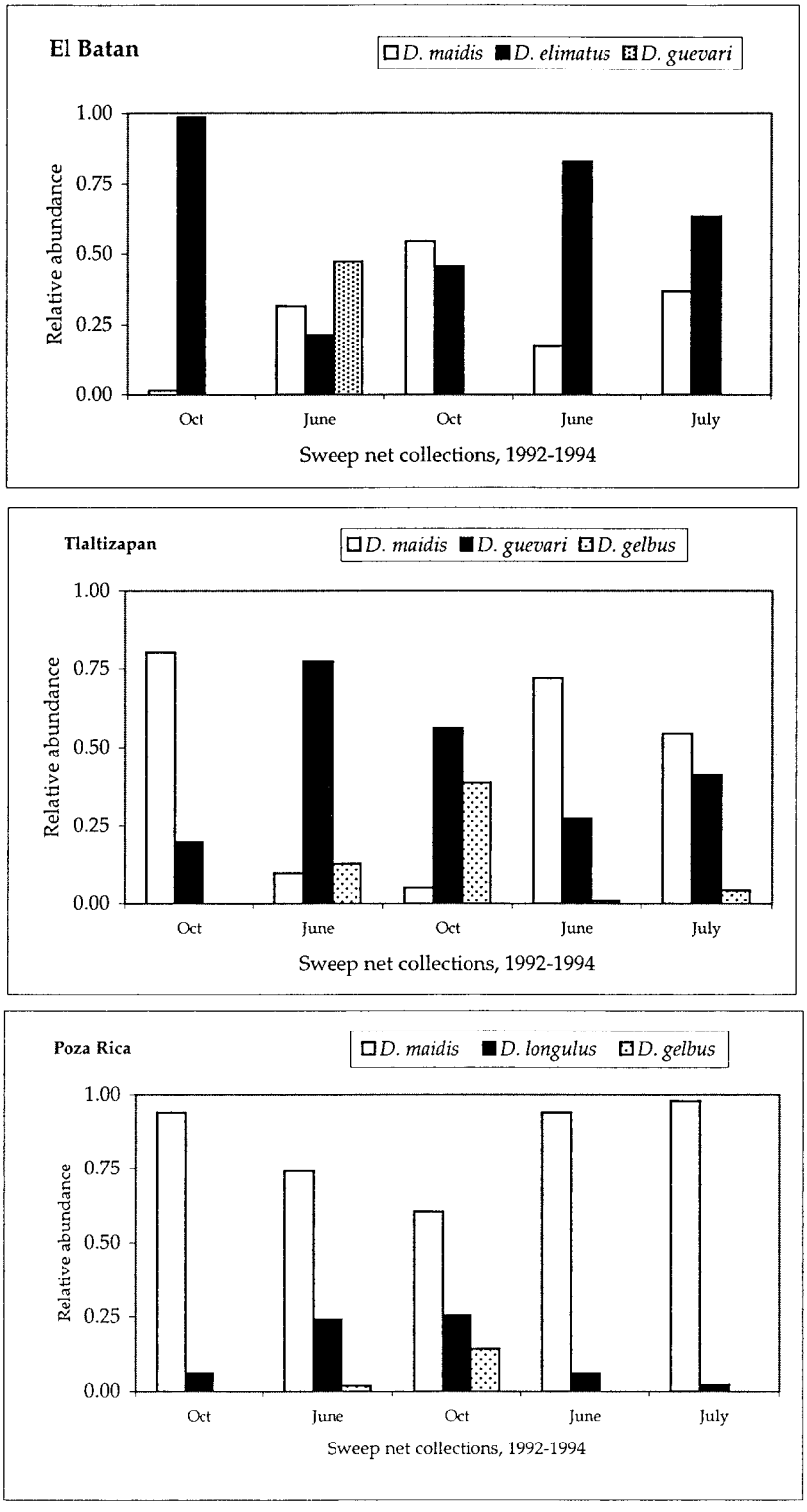

Figure 1. Abundance of *Dalbulus* leafhoppers (relative to the total catch per collection) at three agricultural field stations in Mexico.

Table 2. CSS prevalence (based on ELISA) and ability to transmit CSS and MBSP (bioassay) in *Dalbulus* from sweep net collections at field stations (El Batan, EB; Tlaltizapan, TL and Poza Rica, PR) in Mexico

Species	Site	ELISA		Bioassay		
		Tested	Positive	Tested	CSS	MBSP
D. elimatus	EB	221	54 (24 ± 3%)	245	3 (1%)	5 (2%)
D. gelbus	TL	21	3 (14 ± 8%)	23	0	0
D. guevari	EB, TL	93	15 (16 ± 4%)	119	1 (1%)	3 (3%)
D. maidis	EB	76	27 (35 ± 6%)	91	0	4 (4 ± 2%)
	TL	113	9 (8 ± 3%)	118	5 (4 ± 2%)	3 (3%)
	PR	170	10 (6 ± 2%)	187	4 (2%)	2 (1%)
	All	359	46 (13 ± 2%)	396	9 (2%)	9 (2%)
Total		694	118 (17%)	783	13 (2%)	17 (2%)

signal an infection detected by the bioassay, as false negatives.

As estimated by ELISA, CSS prevalence varied with the interaction of site and collection in both the 2-site × 4-collection table (lack of fit test, d.f. = 3, G = 10.5, P = 0.0151) and the 3-site × 2-collection table (d.f = 2, G = 7.3, P = 0.0262). We therefore tested for heterogeneity among species within sites in both CSS prevalence and ability to transmit CSS: if CSS prevalence varied across sites and collections, but CSS transmission rates did not, this implies that not all infected leafhoppers were equally successful at transmitting an infection detected by ELISA.

At PR, 5.3 ± 3% (3 of 57 tested) of *D. maidis* collected in October of 1992 gave positive ELISA readings, as did 6.2 ± 2% (7 of 113) of those collected in July, 1994. These estimates are not significantly different (d.f. = 1, G = 0.1, P = 0.80). Transmission rates did not differ between collections for either CSS (1 of 68 in 1992, 1.5 ± 2%; 3 of 119 in 1994, 2.5%) or MBSP (1 of 68 in 1992, 1.5 ± 2%; 1 of 119 in 1994, 0.8%. Comparison between years, d.f. = 1: CSS, G = 0.2, P = 0.62; MBSP G = 0.2, P = 0.69). Of the ten *D. maidis* that were positive in the ELISA, three (30.0 ± 14%) were successful in transmitting CSS to healthy seedlings.

Dalbulus maidis from TL showed similar results in the ELISA, regardless of when they were collected (Table 3, d.f. = 2, G = 3.5, P = 0.17). Transmission rates were also consistent across time for both CSS (d.f. = 2, G = 2.3, P = 0.32) and MBSP (d.f. = 2, G = 4.6, P = 0.10). Of the nine TL *D. maidis* testing positive in the ELISA, four (44.4 ± 17%) also were positive in the bioassay. Pooling our ELISA data from June, 1993 and July, 1994, during which we tested all three of the TL *Dalbulus* species, we found no differences (d.f. = 2, G < 0.1, P = 0.98) among the three species in CSS prevalence: 7.4 ± 3% of *D. maidis* (5 of 68), 8.3 ± 8% of *D. gelbus* (1 of 12), and 7.0 ± 3% of *D. guevari* (5 of 71) were positive in the ELISA. However, among those leafhoppers for which we had both ELISA and bioassay data, *D. guevari* and *D. gelbus* were less successful in infecting maize than *D. maidis*: 15 *D. guevari* were positive in the ELISA, but only one of the 14 also tested by bioassay was positive for CSS, and all three of the *D. gelbus* testing positive in the ELISA were negative in the bioassay (comparison of both species, pooled, to *D. maidis*: d.f. = 1, G = 5.5, P = 0.0192).

In July 1994, we collected very few *Dalbulus* at EB, and none gave evidence of CSS infection in either the ELISA or bioassay (Table 4). We therefore excluded this collection from further analysis. Neither *D. elimatus* (d.f. = 2, G = 0.9, P = 0.64) nor *D. maidis* (d.f. = 1, G = 2.6, P = 0.10) differed in ELISA results across the remaining collections. *Dalbulus maidis*, with 27 of 69 insects positive for infection (39.1 ± 6%), was more likely to be infected than *D. elimatus* (54 of 210, or 25.7 ± 3%. d.f. = 1, G = 4.4, P = 0.0365). The two species had similar (d.f. = 1, G = 1.0, P = 0.32) transmission rates. Among leafhoppers with confirmed CSS infections, none of the 27 *D. maidis* and only one of the 50 *D. elimatus* were able to infect healthy seedlings. Ability to transmit MBSP was also similar between the two species (d.f. = 1, G = 1.3, P = 0.26). We have no ready explanation for the contradiction between the ELISA (in which *D. maidis* had a slightly higher rate of infection than

Table 3. Number collected (abundance relative to total catch, RA), CSS prevalence (ELISA) and ability to transmit CSS and MBSP (bioassay) in *Dalbulus* from sweep net collections at Tlaltizapan, Mexico

Oct. 1992	*D. maidis*	89 (80 ± 4%)	45	4 (9 ± 4%)	55	1 (2 ± 2%)	0
Jun. 1993	*D. gelbus*	17 (13 ± 3%)	7	1 (14 ± 13%)	7	0	0
	D. guevari	102 (77 ± 4%)	26	2 (8 ± 5%)	34	0	1 (3 ± 3%)
	D. maidis	13 (10 ± 3%)	7	2 (29 ± 17%)	7	1 (14 ± 13%)	0
Oct. 1993	*D. gelbus*	22 (39 ± 6%)	9	2 (22 ± 14%)	11	0	0
	D. guevari	32 (56 ± 7%)	14	10 (71 ± 12%)	18	0	0
July 1994	*D. gelbus*	5 (4 ± 2%)	5	0	5	0	0
	D. guevari	46 (41 ± 5%)	45	3 (7 ± 4%)	44	1 (2 ± 2%)	2 (5 ± 3%)
	D. maidis	61 (54 ± 5%)	61	3 (5 ± 3%)	56	3 (5 ± 3%)	3 (5 ± 3%)

Table 4. Number collected (abundance relative to total catch, RA), CSS prevalence (ELISA) and ability to transmit CSS and MBSP (bioassay) in *Dalbulus* from sweep net collections at El Batan, Mexico

Date	Species	Number (RA)	ELISA		Bioassay		
			Tested	Positive	Tested	CSS	MBSP
Oct. 1992	*D. elimatus*	267 (98%)	161	42 (26 ± 4%)	172	0	2 (1%)
June 1993	*D. elimatus*	19 (21 ± 4%)	8	1 (13 ± 12%)	9	0	0
	D. guevari	42 (47 ± 5%)	8	0	23	0	0
	D. maidis	28 (32 ± 5%)	11	2 (18 ± 12%)	13	0	1 (8 ± 7%)
Oct. 1993	*D. elimatus*	102 (46 ± 3%)	41	11 (27 ± 7%)	53	3 (6 ± 3%)	1 (2 ± 2%)
	D. maidis	122 (54 ± 3%)	58	25 (43 ± 6%)	71	0	3 (4 ± 2%)
July 1994	*D. elimatus*	12 (63 ± 11%)	11	0	11	0	2 (18 ± 12%)
	D. maidis	7 (37 ± 11%)	7	0	7	0	0

D. elimatus) and the bioassay (in which *D. maidis* with confirmed infections had the same rate of transmission). We speculate this discrepancy is due to a larger sample size, and thus a more accurate result, in the ELISA. Although *D. guevari* was the most common leafhopper we collected at EB in June, 1993, many died in transit. We found no evidence of CSS infection in the EB *D. guevari* in the eight leafhoppers tested in both the bioassay and by ELISA, nor in the 15 tested by bioassay alone.

Comparisons among D. maidis *populations.* *Dalbulus maidis* collected at the three sites did not differ in ability to transmit MBSP to healthy maize (d.f. = 2, G = 3.0, P = 0.22). CSS prevalence showed significant heterogeneity (d.f. = 2, G = 37.1, P < 0.0001); removing EB from the comparison indicated prevalence was similar at PR and TL (d.f. = 1, G = 0.5, P = 0.50). Our data were inconclusive regarding variation between *D. maidis* populations in ability to transmit CSS (d.f. = 2, G = 5.8, P = 0.0539). However, in *D. maidis* with confirmed CSS infections, those from PR and TL were more likely than those from EB to transmit infections to healthy maize (comparison among three populations, d.f. = 2, G = 14.7, G = 0.0007; comparison between PR and TL, Fisher's exact test, P = 0.65).

CSS prevalence in overwintering Dalbulus. In six cases we collected sufficient data to test our hypothesis that CSS infection may benefit vectors by improving their survival over the dry season (Table 5). We compared CSS prevalence at the same site, in the same species and across a single dry season and found either consistent or declining prevalence in overwintering leafhoppers.

Discussion

Our survey of the *Dalbulus* community over two years and three field sites is the most extensive report of mollicute prevalence and relative abundance in agricultural populations of these leafhoppers. The occur-

Table 5. CSS prevalence (based on ELISA data in Tables 3 & 4) in *Dalbulus* collected before and after the dry season in Mexico

Species	Site	Before	After	G[a]	P
D. elimatus	EB	Oct. 1992	June 1993	0.9	0.36
		Oct. 1993	July 1994	6.0	0.0145
D. gelbus	TL	Oct. 1993	July 1994		0.51[2]
D. guevari	TL	Oct. 1993	July 1994	23.4	<0.001
D. maidis	EB	Oct. 1993	July 1994	7.3	0.0068
D. maidis	TL	Oct. 1992	June 1993	1.8	0.18

[a]One degree of freedom for all χ^2 tests.
[b]Fisher's exact test, two-tailed probability.

rence of *D. maidis* throughout the dry season at our sites is of particular importance as it suggests the corn leafhopper can persist in and around maize fields, even at high elevation sites. Such persistence, if coupled with infrequent migration from other distinct populations, allows for genetic differences to accumulate between leafhopper populations. We show here that *D. maidis* collected from EB are much less likely to infect healthy maize with CSS than conspecifics collected from other populations. Whether this difference is due to the vector, the pathogen or the combination of the two is not known. In our earlier study, CSS collected from TL and PR did not differ in their effects on leafhopper survival, but leafhopper colonies established from the three sites varied in their response to common rearing conditions and exposure to mollicutes (Ebbert & Nault, 2001). For example, fewer PR *D. maidis,* when compared to corn leafhoppers from EB or TL, were positive in the ELISA after exposure to CSS from TL.

Based on previous laboratory studies of leafhopper survival following exposure to mollicutes, we made three predictions regarding how vector prevalence should vary between species and over time. We expected CSS prevalence to rise over the dry season, instead we found that it declined or remained constant. We found no evidence to support our prediction that *D. gelbus* and *D. elimatus* would be better able to infect maize with MBSP than would *D. guevari* and *D. maidis*. There are many possible explanations for these differences between our laboratory and field results, perhaps, for example, our sample sizes were inadequate to detect subtle field effects. Because we did not confirm infection with MBSP in the leafhoppers tested in the bioassay, we cannot rule out that similar transmission rates across species mask differences in relative transmission success: EB *D. maidis*, for example, might be more likely than *D. elimatus* to be infected with MBSP, but less likely to transmit the bacteria to healthy plants. With the data at hand, however, the most likely explanation is that our laboratory analyses were unable to accurately predict vector status in the field.

Our results were mixed with regard to our prediction that CSS prevalence and transmission ability would be highest in the corn leafhopper, relative to its congeners. *Dalbulus maidis* and *D. elimatus* were equally likely to transmit confirmed infections, although *D. maidis* had a slightly higher CSS prevalence in the ELISA. *Dalbulus guevari* and *D. gelbus* from TL were just as likely to be infected with CSS as *D. maidis*, but less likely to transmit confirmed infections. This is consistent with our prediction that these two species proved to be less efficient vectors, although not because they are less likely to survive CSS infections.

Our field work suggests that, overall, the four species we tested are equally tolerant of CSS infection, but may differ in success at transmission. Whether they are also equally likely to transmit CSS infection to maize under field conditions will require further study. Clearly, there is much work to be done in untangling the effects of population, species and pathogen on vector efficiency in the interaction between *Dalbulus* and plant pathogenic mollicutes. As of now, we can conclude that the interaction is not as specific as once thought. Strategies to control the substantial crop loss caused by the maize stunting mollicutes should therefore consider the entire vector community and potential variation between vector populations.

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