

## Presence of *Dalbulus maidis* (Hemiptera: Cicadellidae) and of *Spiroplasma kunkelii* in the Temperate Region of Argentina

E. CARLONI,<sup>1,2</sup> P. CARPANE,<sup>1,3</sup> S. PARADELL,<sup>4</sup> I. LAGUNA,<sup>1</sup> AND M. P. GIMÉNEZ PECCI<sup>1</sup>

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**ABSTRACT** “Corn stunt” is one of the main corn (*Zea mays* L.) diseases in the Americas and *Dalbulus maidis* (DeLong & Wolcott) is the key vector of the pathogen *Spiroplasma kunkelii* Whitcomb. In Argentina, the corn-producing area is in the temperate region, where vector and pathogen prevalence levels are unknown. In this study, the prevalence and distribution of *D. maidis* and *S. kunkelii* in the temperate region of Argentina and *D. maidis* overwintering ability in this region were determined. Surveys were conducted in 2005–2006 and 2006–2007 seasons to determine *D. maidis* and *S. kunkelii* presence, and in winter 2006 to determine the vector overwintering ability. The highest *S. kunkelii* prevalence and incidence levels were found in the transition area from the temperate to the subtropical region, related to the highest *D. maidis* prevalence and insects sampled per location. *D. maidis* adults were found in volunteer corn plants and spontaneous vegetation in autumn and winter months, which were inoculative for the pathogen *S. kunkelii*. This overwintering ability was related to detection of *D. maidis* insects in corn crops at early growth stages in the following growing season. This work emphasizes that corn stunt disease is present in the temperate region of Argentina, and this highlights the need to develop proper agronomic practices like monitoring insect vector populations and controlling voluntary plants. This study also indicates that further research is needed to understand the potential yield reduction caused by this pathogen on symptomless plants and population dynamics of the insect vector.

**KEY WORDS** corn leafhopper, pest, overwintering, mollicutes, incidence

Corn stunt is a potentially limiting disease for corn (*Zea mays* L.) in the neotropical Americas, which can cause significant production losses (Bradfute et al. 1981, Hruska et al. 1996, Virla et al. 2004). Causal pathogens of this disease are the mollicutes *Spiroplasma kunkelii* (Whitcomb et al. 1986) and Maize bushy stunt phytoplasma (Nault 1980), and *Maize rayado fino virus* (Gómez 1973). These pathogens may occur alone or in combination and are transmitted in a persistent-propagative way by several leafhopper species, mainly those of the *Dalbulus* (Hemiptera: Cicadellidae) genus or others taxonomically related genera (Nault 1980, 1990; Madden and Nault 1983; Nault and Madden 1985). Plant species affected by *S. kunkelii* in natural conditions belong to the genus *Zea*, including corn and the teosintes *Zea diploperennis*, *Zea perennis*, *Zea mays* x *Tripsacum floridanum*, and *Zea luxurians* (Nault 1980).

The predominant “corn stunt” pathosystem in Argentina is less complex than that described previously.

*Dalbulus maidis* (DeLong & Wolcott) is the only known vector species present in the country (Paradell 1995, Paradell et al. 2001), although *Exitianus obscurinervis* (Hemiptera: Cicadellidae) was recently found to be a vector in experimental conditions (Carlóni et al. 2011). *D. maidis* is endemic to the subtropical region, being the most prevalent leafhopper species associated with corn (Paradell et al. 2001, Luft Albaracin et al. 2008). Corn is the only host plant of importance for *D. maidis*, because this species may complete its life cycle only in corn and teosintes (*Zea* spp.) (Pitre 1967, Nault 1980, Nault and Madden 1985), and there are no wild or cultivated teosintes in Argentina. *S. kunkelii* Whitcomb is by far the most prevalent of the pathogens causing corn stunt, with incidences of up to 100% in the subtropical region (Giménez Pecci et al. 2002a,b), although usually ranging from 12 to 62% (Virla et al. 2004).

The largest area planted with corn in Argentina is located in the temperate region (Ministerio de Agricultura Ganadería y Pesca [MAGYP] 2011), where some adult individuals of *D. maidis* (Paradell et al. 2001, 2005) and few *S. kunkelii*-positive plants (Giménez Pecci et al. 2002a, 2005) are occasionally found in late summer. However, a systematic survey to quantify the presence of corn stunt disease and its associated vectors has not been carried out in Argentina. The adoption of several agronomic practices in this region

<sup>1</sup> Instituto de Patología Vegetal (IPAVE - INTA), Av. 11 de setiembre 4755, (X5020ICA) Córdoba, Argentina.

<sup>2</sup> Corresponding author, edgardocarloni@gmail.com.

<sup>3</sup> Present address: Monsanto Argentina SAIC, Ruta 188 Km 77 (2700), Pergamino, Buenos Aires, Argentina.

<sup>4</sup> División de Entomología, Facultad de Ciencias Naturales y Museo, UNLP, Paseo del Bosque S/N (B1900FWA), La Plata, Buenos Aires, Argentina.

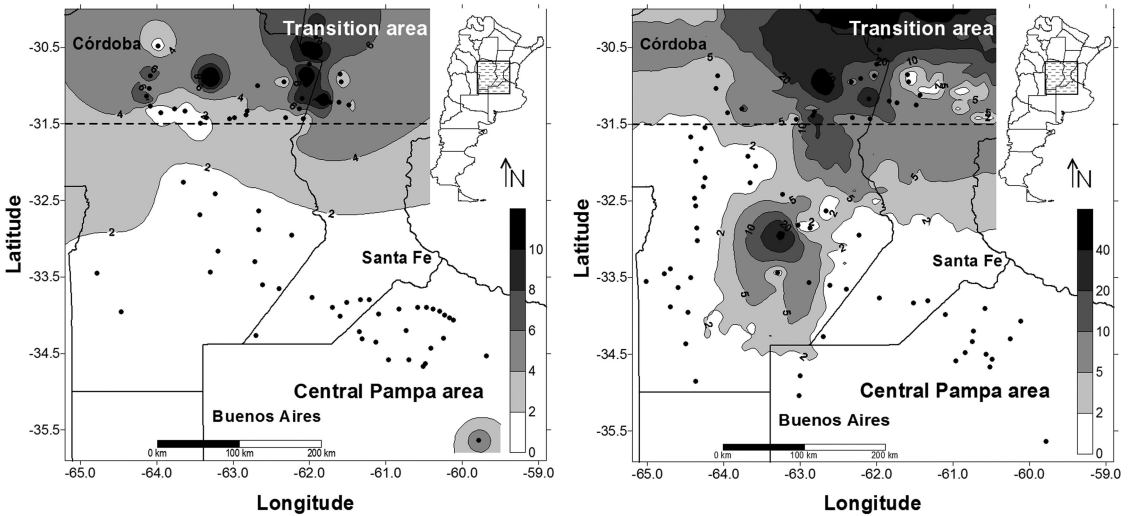


Fig. 1. (left) *S. kunkelii* incidence (percentage of positive plants) and (right) *D. maidis* abundance (number of insects per 30 net sweeps) per location in the temperate region of Argentina in 2005–2006 and 2006–2007 seasons. Points in the map show sampling locations. Dashed lines at  $-31.5^{\circ}$ S indicate the change from the central Pampa area (South) to the transition area to the tropical region (North).

might lead to a higher risk of corn stunt disease, like adoption of no-till systems that increase water use efficiency (Abril et al. 2005, Álvarez et al. 2009) and availability to insect biocontrol technologies that lower insecticide use (Lewi and Rubinstein 2010). These factors increase the area planted with corn (Mercado de Granos 2011) and allow the farming of late-planted or second-crop corn (Portugal 2007), extending the presence of corn with green tissues from early September to the first frost (from late May to early June). This would possibly facilitate *D. maidis* survival in mild winters, resulting in increased prevalence of corn stunt in the temperate area of Argentina. For this reason, it is essential to determine the current geographical distribution and abundance of *S. kunkelii* and of *D. maidis* in the temperate region of Argentina, as a way to infer whether corn stunt may become a limiting disease for corn in this area.

Lack of data regarding the population dynamics of the vector *D. maidis*, mainly its ability to overwinter in Argentina, makes these predictions difficult. Although it is possible that *D. maidis* might migrate from tropical regions (Nault 1990, Moya-Raygoza 2007b), it may also overwinter in temperate zones. Available information suggests that *D. maidis* overwinters as an adult in a wide range of host plants, by using them as a source of shelter, water, and food (Pitre 1967; Larsen et al. 1992; Ebbert and Nault 1994, 2001; Virla et al. 2003), and that it can withstand low temperatures between 0 and  $-4^{\circ}\text{C}$  for short periods (Larsen et al. 1993, Summers et al. 2004, Moya-Raygoza et al. 2007a). Hence, presence of known host plants and mild winters suggest that *D. maidis* might overwinter in the temperate area of Argentina.

The objectives of this study were to determine *D. maidis* and *S. kunkelii* presence in the temperate region of Argentina, and the ability of the vector *D.*

*maidis* as an overwintering host for *S. kunkelii* in this region.

## Materials and Methods

**Geographic Region.** The study was conducted in the temperate region of Argentina (Lorenzini et al. 1995), which includes Córdoba, Santa Fe, and Buenos Aires provinces (Fig. 1). This region has a single corn growing season, in which there may be two planting dates: Early, planted from September to late October and harvested in March–April; and Late, planted from December to early January, harvested in May–June.

The temperate region was separated in two areas for this study, based on different features of their winters: the central Pampa area and the transition area to the subtropical region (Fig. 1). Central Pampa area includes the main corn-producing area (Blanco 2004) and is located between  $-31.50$  and  $-35.50$  S (South latitude). Average minimum winter temperature varies between  $4/6^{\circ}\text{C}$  and the absolute minimum temperature between  $-1/-10^{\circ}\text{C}$ , with 2–27 d with frost (Table 1). Average annual precipitation is  $\approx 800$  mm (Servicio Meteorológico Nacional [SMN] 2011). The transition area was considered for this study between  $-30.00$  and  $-31.50$  S, in the ecotone of the temperate to subtropical climate (Lorenzini et al. 1995). In this region, the winters are milder, with an average minimum temperature between  $4/8^{\circ}\text{C}$ , an absolute minimum between  $-1/-6^{\circ}\text{C}$ , 2–21 d with frost, and average annual precipitation is  $\approx 700$  mm (SMN 2011).

**Selection of Locations for Sampling.** In 2005–2006 and 2006–2007 growing seasons, the vector *D. maidis* was surveyed between November (spring) and April (autumn) of each year, and the pathogen *S. kunkelii* between March and April. *D. maidis* and *S. kunkelii* were sampled in 115 and 140 locations, respectively,

**Table 1.** Average winter, avg min., avg absolute min. temp (°C), and number of days with frost in 2005 and 2006 winters and historical values (avg for 1995–2007) of the temperate region of Argentina, at three locations representative of the central Pampa area and three of the transition area to subtropical region

Area	Location (province)	Year	Avg winter (°C)	Avg min. (°C)	Absolute min. (°C)	No. days with frost
Central Pampa	Junín (Buenos Aires)	2005	10.68	4.63	-6.00	17
		2006	11.47	4.78	-7.00	15
		Historical	10.78	4.57	-8.00	17
	Río Cuarto (Córdoba)	2005	10.99	4.67	-3.50	13
		2006	12.34	5.92	-2.30	5
		Historical	11.34	5.41	-10.60	8
	Venado Tuerto (Santa Fe)	2005	10.74	3.76	-4.30	23
		2006	12.11	5.19	-3.80	9
		Historical	10.82	4.58	-7.00	15
Transition	Ceres (Santa Fe)	2005	13.32	7.05	-1.00	2
		2006	14.16	7.02	-3.00	5
		Historical	12.94	6.87	-6.60	9
	Córdoba (Córdoba)	2005	12.46	5.95	-1.80	5
		2006	13.37	5.83	-1.80	5
		Historical	12.55	5.68	-7.80	9
	Rafaela (Santa Fe)	2005	12.86	6.76	-3.20	9
		2006	13.69	7.11	-4.50	9
		Historical	13.00	6.71	-5.80	8

Data obtained from the National Weather Service (SMN 2005, 2006) and kindly provided by Rafael Rodriguez, Instituto de Clima y Agua (CNIA - INTA) in Castelar, Buenos Aires, Argentina.

throughout the 2-yr sampling period. There was not a full match between vector and pathogen sampling location, both of them being sampled together in only 48 locations. Typically, sampling locations were chosen randomly, with no resampling taking place at each location throughout this study. Locations were georeferenced by using Global Positioning System (Fig. 1).

**Prevalence and Abundance of *D. maidis*.** Insects were sampled in corn and in spontaneous associated vegetation by using insect nets. Sampling size was of 30 net sweeps per location (Virla et al. 2004), in both corn and spontaneous vegetation. Spontaneous vegetation included mainly monocots of *Avena*, *Bromus*, *Cynodon*, *Cyperus*, *Digitaria*, *Echinochloa*, *Eleusine*, *Eragrostis*, *Hordeum*, *Sorghum*, and *Triticum* genera, and dicots of *Amaranthus*, *Bidens*, *Quenopodium*, and *Wedelia* genera. Corn was sampled in transects starting 50 m inside the field and heading to the opposite side of each field. Spontaneous vegetation was sampled in transects parallel to one of the sides of corn fields, separated 20 m from corn border. Global positioning system coordinates were not taken of each individual net sweep in each location, but to the site where corn sampling started. Insects were then killed by placing them in plastic bags containing cotton soaked in ether acetate, and taken to laboratory for identification and counting. *D. maidis* prevalence was estimated as the percentage of locations where at least one individual of this species was found in either corn or spontaneous vegetation (non-corn). Abundance was estimated as the number of individuals per location, and mapped in Surfer Vers.10 (Surfer10 2011).

**Prevalence and Incidence of *S. kunkelii*.** In both seasons, corn fields from milk (R3) to dent stage (R5) (Ritchie and Hanway 1982) were sampled. Fifty plants were sampled in each location (Virla et al. 2004), spaced at 10 m intervals along a diagonal line in the field. The second leaf below the tassel was collected from each plant for *S. kunkelii* detection. Leaves were

stored at 4°C for conditioning and symptom determination (Nault 1980) in laboratory, and then kept at -20°C until tested for the presence of *S. kunkelii* by double antibody sandwich enzyme-linked immunosorbent assay (AGDIA Inc., Elkhart, IN). *S. kunkelii* prevalence was estimated as the percentage of locations where at least one plant yielded a positive reaction. Incidence was expressed as the percentage of positive plants at each location, and mapped in Surfer Vers.10.

***D. maidis* Overwintering in the Transition Area.** Insect samples were taken in winter 2006 to determine whether *D. maidis* overwinters in the transition area. Locations assessed were Colonia Caroya (-30.98 S, -64.10 W) and Guiñazú (-31.31 S, -64.15 W), both in Córdoba Province. Insects were sampled weekly or biweekly, in corn when present or in stubble or volunteer corn when crops were not present. Spontaneous vegetation composed primarily of *Bromus* sp., *Cynodon* sp., *Cyperus* sp., *Digitaria* sp., and *Sorghum* sp. was also sampled. Samplings took place, as previously described, except when corn was not growing, with corn stubble being considered as if it was an actual corn crop. Once collected, insects were conditioned, as previously described, and taken to the laboratory for identification and counting. The relationships between *D. maidis* and weather conditions were assessed by using temperature data obtained from the National Weather Service (SMN 2005, 2006) and the weather station at the Ambrosio Taravella International Airport (Córdoba, Argentina) (-31.31 S, -64.21 W).

In May 2006 (autumn), *D. maidis* individuals were collected from volunteer corn plants and taken alive to laboratory to determine if they inoculated the pathogen *S. kunkelii*. To do so, 395 insects had access to 79 V2-stage corn plants (Ritchie and Hanway 1982), in a ratio of five insects per plant during a 7-d inoculation access period (IAP) (Nault 1980). Sixty days after completion of the IAP, plants were examined for

**Table 2.** Percentage of locations with *S. kunkelii* or *D. maidis* presence in the temperate region of Argentina during 2005–2006 and 2006–2007 seasons

Organism surveyed	Host	Season	Area		
			Transition	Central Pampa	
<i>S. kunkelii</i>	Corn	2005–2006	42 (14/33)	3 (1/39)	$P < 0.0001$
		2006–2007	89 (31/35) $P = 0.0001$	9 (3/33) $P = 0.0557$	$P < 0.0001$
<i>D. maidis</i>	Total	2005–2006	79 (15/19)	38 (16/42)	$P = 0.0002$
		2006–2007	100 (17/17) $P = 0.1165$	24 (9/37) $P = 0.0754$	$P < 0.0001$
	Corn	2005–2006	88 (15/17)	36 (13/36)	$P < 0.0001$
		2006–2007	100 (17/17) $P = 0.3815$	24 (9/37) $P = 0.1213$	$P < 0.0001$
	Non-corn	2005–2006	11 (2/19)	12 (5/40)	$P = 0.8348$
		2006–2007	47 (7/15) $P < 0.0001$	0 (0/30) $P = 0.0661$	$P < 0.0001$

Values between brackets: number of locations with *S. kunkelii* or *D. maidis* presence/number of locations surveyed.  $P$  values in each row indicate  $\chi^2$  (except for *D. maidis* on non-corn, when a Fisher's exact test was used) comparison between areas in each season, whereas those in the last row of each host represent comparisons between seasons. The Total row under *D. maidis* prevalence indicates locations considered as positive for *D. maidis* prevalence when insects were found in either plant host (corn or non-corn).

*S. kunkelii* presence by symptom evaluation (Nault 1980) and DAS-ELISA. To estimate the probability of transmission ( $P$ ) of single individuals, the equation of Swallow (1985) was used:

$$P = 1 - (1 - D)^{1/K}$$

Where  $D$  is the proportion of diseased plants and  $K$  is the number of insects caged per plant. Mortality throughout the IAP was supposed to have little impact on inoculation efficiency because *D. maidis* survival during this IAP was 80% (Carpane 2007). For this reason, mortality was not measured throughout the IAP, and insects eventually dying were not replaced. Insect survival was measured in every cage at the end of the IAP, averaging 78%. As *S. kunkelii* inoculation efficiency was not related to insect survival (results not shown,  $P = 0.9267$  in a  $\chi^2$  test), results were pooled and not described in further detail.

**Statistical Analysis.** *D. maidis* and *S. kunkelii* presence across areas and growing seasons was analyzed by contingency tables, whose marginals were used to estimate the independence of both variables by the  $\chi^2$  test by using InfoStat statistical software (Di Rienzo et al. 2012). The relationship between *D. maidis* and *S. kunkelii* prevalence across locations was analyzed in the same way. In cases where zero values were detected in the contingency tables, the Fisher exact test was implemented by using SAS statistical software (SAS Institute 2004). *D. maidis* abundance was analyzed by using a generalized linear model under a Poisson distribution and log function as a link, followed by a Di Rienzo, Guzmán, and Casanoves means comparison test using InfoStat statistical software (Di Rienzo et al. 2012). *S. kunkelii* incidence was analyzed similarly, but by using a binomial distribution and log function as a link.

### Results

***S. kunkelii* and *D. maidis* Prevalence.** In the two seasons studied, 140 locations were surveyed for *S.*

*kunkelii* presence and 115 for *D. maidis*. The pathogen was found in 49 locations and the vector in 57 (Table 2). A larger proportion of locations with *S. kunkelii* in the transition area than in the central Pampa area for both 2005–2006 ( $P < 0.0001$ ) and 2006–2007 ( $P < 0.0001$ ) seasons were tested by  $\chi^2$  analysis. *S. kunkelii* prevalence increased significantly in 2006–2007 season in the transition area ( $P = 0.0001$ ), but not in the central Pampa area ( $P = 0.0557$ ). *D. maidis* prevalence pattern (including both host types) was similar to that of *S. kunkelii* (Table 2), with higher prevalence in the transition area in 2005–2006 ( $P = 0.0002$ ) and 2006–2007 ( $P < 0.0001$ ) seasons, and no increase in 2006–2007 season ( $P = 0.0754$ ) in the central Pampa area, although prevalence did not increase in the transition area ( $P = 0.1165$ ) either.

When *D. maidis* prevalence was analyzed in corn host, pattern resembled that of total (corn + non-corn) *D. maidis* presence, as in only three locations, insects were not found in corn but in other plant hosts. Prevalence was higher in the transition area than in the central Pampa area ( $P < 0.0001$  in both seasons), and no changes took place between seasons in each area ( $P = 0.3815$  in the transition area,  $P = 0.1213$  in the central Pampa area). *D. maidis* was found in non-corn hosts in 14 locations. Fisher exact test results indicate that prevalence in 2006–2007 season increased in the transition area ( $P < 0.0001$ ), but not in the central Pampa area ( $P = 0.0661$ ). *D. maidis* was also more prevalent in the transition area than in the central Pampa area in 2006–2007 season ( $P < 0.0001$ ), although there were no differences between areas in 2005–2006 season ( $P = 0.8348$ ).

The relationship between *S. kunkelii* and *D. maidis* presence was analyzed in 48 locations where they were both surveyed (Table 3). In 2005–2006, *S. kunkelii* and *D. maidis* presence were significantly related ( $\chi^2$ :  $P = 0.0446$ ), as in 14 of 23 locations, they were both present or absent. *D. maidis* individuals were found, but not *S. kunkelii*-positive plants in nine locations, three in the transition area and six of the central

**Table 3.** Locations with *S. kunkelii* and *D. maidis* presence and absence in the temperate region of Argentina during 2005–2006 and 2006–2007 seasons

Season	<i>S. kunkelii</i>	<i>D. maidis</i>	
		Absent	Present
2005–2006	Absent	5	9
	Present	0	9
2006–2007	Absent	8	1
	Present	2	14

Includes only 48 locations where presence of both *S. kunkelii* and *D. maidis* was surveyed.

Pampa (Table 3). In 2006–2007, the association between vector and pathogen presence was also significant ( $\chi^2$ :  $P = 0.0002$ ), as in most of the locations (22 of 25), they were both present or absent. *D. maidis* individuals were present but not *S. kunkelii* in one location (from the transition area), and in two other locations (both of the central Pampa), *S. kunkelii* was detected but *D. maidis* individuals were not.

Higher *S. kunkelii* incidence and more *D. maidis* individuals per location were found in the transition area than in the central Pampa area (Table 4; Fig. 1) and increased in the 2006–2007 season, except for *D. maidis* in the central Pampa area. Generalized linear model results of *S. kunkelii* incidence showed no significant effect of season  $\times$  area interaction ( $P = 0.6169$ ). However, season ( $P < 0.0001$ ) and area ( $P < 0.0001$ ) effects were highly significant, because of the higher incidence in the transition area than in the central Pampa area and increase in prevalence in both areas in 2006–2007 season. For *D. maidis* number, the season  $\times$  area interaction was highly significant ( $P < 0.0001$ ), because in 2006–2007 season, incidence increased in the transition area like *S. kunkelii* incidence, but decreased in the central Pampa area. *S. kunkelii* incidence was usually higher than the percentage of plants with symptoms (data not shown). This was more noticeable in the central Pampa area, where none of the plants collected had visible symptoms, whereas in most locations of the transition area, a few plants had typical symptoms.

***D. maidis* Overwintering in the Transition Area.** To understand the overwintering ability of *D. maidis* in the transition area to the tropical region, the presence of individuals of this species was studied throughout

**Table 4.** *S. kunkelii* incidence (expressed as percentage of positive plants) and *D. maidis* abundance (number of insects per 30 net sweeps) per location (mean  $\pm$  SE) in the temperate region of Argentina during 2005/2006 and 2006/2007 seasons

Season	Area	<i>S. kunkelii</i>	<i>D. maidis</i>
2005–2006	Transition	1.21 $\pm$ 0.27b	14.65 $\pm$ 0.93b
	Central Pampa	0.05 $\pm$ 0.05c	4.56 $\pm$ 0.36c
2006–2007	Transition	9.36 $\pm$ 0.74a	39.47 $\pm$ 1.52a
	Central Pampa	0.27 $\pm$ 0.13c	0.59 $\pm$ 0.13d

Different letters mean significant differences ( $P < 0.05$ ) in the DGC means comparison test after adjusting a generalized linear model with Poisson distribution with log link function for *D. maidis* and with binomial distribution with log link function for *S. kunkelii*.

the winter in a sweet corn field with sequential planting dates (Guiñazú location) and in a corn field followed by volunteer corn plants (Colonia Caroya location) (Table 5). In Guiñazú, sweet corn crops were managed with frequent insecticide sprays to control corn earworm (*Helicoverpa zea* [Boddie]) larvae, so *D. maidis* adults were found in low numbers, and mostly in associated spontaneous vegetation (*Bromus* sp., *Cynodon* sp., *Cyperus* sp., *Digitaria* sp., and *Sorghum* sp.) or in volunteer corn plants. At the end of June, fields with volunteer corn plants were plowed, so insects were found only in spontaneous vegetation (non-corn host). Temperatures were mild during this period, with an average minimum of 7.5°C and an absolute minimum of 1.2°C. After a frost on 28 July 2006 (−0.9°C), the number of insects collected declined sharply, with only two adults found. At the beginning of the next season, some adults were found in October in corn crops at early vegetative stages. Then, no adults were found until January, when adults were then found throughout summer months.

In Colonia Caroya, during senescence of corn crops (April–July), volunteer corn plants appeared, on which a large number of *D. maidis* adults were found. Temperatures were relatively warm during this period, with an average minimum of 7.0°C and an absolute minimum of 1.5°C. These weather conditions allowed survival of both adults and nymphs throughout July (14 nymphs sampled, data not shown). The frost on 28 July (−0.9°C) greatly decreased the number of insects collected later (only one adult was found in August), but adults were found again early in the next season in corn crops. Insects collected in Colonia Caroya during May 2006 were tested to determine if they inoculated the pathogen *S. kunkelii*. Of 79 plants exposed to field-collected *D. maidis* individuals, 12 had a positive reaction for *S. kunkelii* in symptom determination and DAS-ELISA, resulting in a proportion of 3.2% inoculative insects.

## Discussion

The presence of the insect vector *D. maidis* and of the pathogen *S. kunkelii* in the temperate region of Argentina was confirmed, which is the country's main corn-producing area (MAGYP 2011). In previous studies (Paradell 1995; Paradell et al. 2001, 2005; Giménez Pecci et al. 2002a), the vector *D. maidis* was not found in this region of Argentina, except for a few insects found in the transition area at Helvecia (Santa Fe Province) (Paradell et al. 2001) and Villa María (Córdoba Province) locations (Paradell et al. 2005). This work demonstrates that the insect vector *D. maidis* is present in a wide range of locations of the temperate region of Argentina, where it is also able to overwinter when weather conditions are mild. Together with *D. maidis* presence, the pathogen *S. kunkelii* was found across locations of the temperate region. Presence of both vector and pathogen was higher in the transition area to the subtropical region than in the central Pampa area, both in prevalence and number of insects per location, which in turn was associ-

**Table 5.** Number of *D. maidis* collected between May (mid autumn) 2006 and March (late summer) 2007 at two locations of the transition area

Month-year	Guiñaz			Colonia Caroya		
	Corn crop	Volunteer corn	Non-corn	Corn crop	Volunteer corn	Non-corn
May-2006	0	34	0	ND	395	ND
June-2006	0	24	10	ND	ND	ND
July-2006	ND	ND	51	ND	229	1
Aug-2006	ND	ND	2	ND	1	0
Sept-2006	ND	ND	0	ND	ND	0
Oct-2006	2	ND	0	0	0	0
Nov-2006	0	0	0	0	0	0
Dec-2006	0	ND	0	0	0	0
Jan-2007	0	1	0	1	ND	0
Feb-2007	1	ND	0	4	ND	0
Mar-2007	2	ND	0	6	ND	0

Each location was sampled every 7 or 15 d; the values shown are total number of insects collected in each month. ND, not determined.

ated with a greater corn stunt prevalence and incidence in the former region.

Presence of *D. maidis* in the transition area to the tropical region may be related to its ability to overwinter in this area, at least during mild winters, which is supposed by a consistent *D. maidis* prevalence across locations, a large number of insects per location, and a high *S. kunkelii* incidence, showing symptoms related likely to early inoculations (Scott et al. 1977, Hruska and Gomez Peralta 1997). Winters are often mild in the transition area, with few frosty days and absolute minimum temperatures near  $-2^{\circ}\text{C}$  (SMN 2005, 2006), which is consistent with environmental conditions to which *D. maidis* is known to withstand (Larsen et al. 1993, Summers et al. 2004, Moya-Raygoza et al. 2007a).

Presence of *D. maidis* in the central Pampa area is probably because of the migration of insects from warmer regions, either from the transition area or the subtropical region. Lack of overwintering of *D. maidis* is supported by a low prevalence of this insect across locations, a small number of insects sampled per location, and a low *S. kunkelii* incidence. Lack of symptoms suggests that diseased plants were inoculated at late growth stages (Scott et al. 1977), which is consistent with inoculation by migrant individuals during the growing season. The migratory ability of *D. maidis* is well-known (Nault 1990, Moya-Raygoza et al. 2007b), and winters in the central Pampa area have long periods of minimum temperatures  $<0^{\circ}\text{C}$ , and average minimum temperatures down to  $-7^{\circ}\text{C}$  (Table 1), conditions under which *D. maidis* survival is less likely (Larsen et al. 1993, Ebbert and Nault 1994).

Presence of the pathogen *S. kunkelii* was closely assessed with the occurrence of the vector *D. maidis*. However, individuals of *D. maidis* were indeed detected in some locations, but no *S. kunkelii*-positive plants were found. Few insects were collected in these locations, and so it is likely that a small population size of *D. maidis* either had a low frequency of inoculative insects or inoculated a low proportion of plants. Alternatively, insects found may have gained access to plants at late growth stages, so the exposure to *S. kunkelii* was too brief to be present in the samples

collected (Gussie et al. 1995). In very few locations, plants with positive diagnosis for *S. kunkelii* were found without detecting *D. maidis* individuals like in previous observations (Giménez Pecci et al. 2002a). Incidence of positive plants across these locations was  $<5\%$ . Such a low percentage of positive plants could be inoculated by few insects at each location, which were not detected with the sampling method and intensity used in this work. It is also possible that other vectors for *S. kunkelii* may be present at these sites, including *E. obscurinervis* (Stål, 1859), which is frequently found in corn crops in the temperate region of Argentina (Paradell et al. 2001), and was recently shown to be vector of *S. kunkelii* by using artificial infestation methods (Carlóni et al. 2011).

The results of this work emphasize the role of volunteer corn plants as host for *D. maidis*, extending the period of corn green tissues available in late fall and early winter, which may allow *D. maidis* to survive mild winters (Summers et al. 2004). For instance, in the locations tested during 2006 winter, the availability of maize with green tissues was interrupted for only 50 d (28 July to 16 September), a period that *D. maidis* adults can survive if they have a water and shelter source (Larsen et al. 1992; Ebbert and Nault 1994, 2001). Winter crops such as barley or wheat, or spontaneous vegetation composed of grasses such as *Bromus* sp., *Cynodon* sp., or *Cyperus* sp., may serve these needs (Pitre 1967, Virla et al. 2003, Summers et al. 2004, Moya-Raygoza et al. 2007a). If *D. maidis* survives in mild winters, there could be more insects in the next season, increasing the probability to inoculate *S. kunkelii* at early growth stages, causing greater damage to corn. This same effect was found for the planthopper *Delphacodes kuscheli* Fennah (Lenardon et al. 2007), vector of *Mal de Río Cuarto virus*, whose high population size in the mild 2006 winter correlated to a wide "mal de Río Cuarto" epidemic in the 2006–2007 season.

Corn stunt disease is present in the temperate region of Argentina, but with variable prevalence and incidence across geographic areas and seasons. These results are a warning of a possible increase of corn stunt disease in this region, especially because the resistance level of commercial hybrids used in this

area is uncertain, and agronomic practices such as direct planting and split planting dates could lead to increasing populations of the insect vector *D. maidis*. This increase would take place most likely in growing seasons after mild winters, where the above-zero temperatures would allow not only survival of *D. maidis* individuals but also voluntary corn plants to be used as food and shelter by this vector. These findings create the need to monitor vector populations in growing seasons after mild winters and to understand the effect of corn stunt disease on symptomless plants, both with regard to yield and their potential role as a reservoir for *S. kunkelii*. In addition, it emphasizes the need to control voluntary corn plants to reduce host availability for *D. maidis* insects, as well as to monitor population dynamics of this insect.

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