

Incidence of begomoviruses and climatic characterisation of *Bemisia tabaci*-geminivirus complex in soybean and bean in Argentina

Alemandri, V.; P. Rodríguez Pardina, J. Izaurralde, S. García Medina, E. Argüello Caro, M.F. Mattio, A. Dumón, S.M. Rodríguez and G. Truol

SUMMARY

Since the 1990s geminiviruses have emerged as devastating pathogens in tropical and subtropical regions as well as in temperate regions. Recent studies accomplished molecular hybridization-based detection of three begomoviruses infecting soybean and bean crops in Argentina, *Bean golden mosaic virus* (BGMV), *Soybean blistering mosaic virus* (SbBMV) and *Tomato yellow spot virus* (ToYSV). The aims of the present study were to determine incidence and prevalence of the three known geminiviruses in soybean and bean crops in Argentina following the same procedure and to characterize the sites where *Bemisia tabaci*-geminivirus complex was found based on climate data. The highest incidence values were observed in Salta province. BGMV exhibited the highest prevalence and incidence values in the bean crop, followed by SbBMV. In the soybean crop, SbBMV showed the highest prevalence and incidence values, followed by ToYSV. One hundred and three soybean and bean plots distributed in Argentina, where the *Bemisia tabaci*-geminivirus complex was detected, were characterized employing a Geographic Information System (GIS). The complex was found in warm and low rainfall areas; these climatic characteristics are consistent with those identified in a previously described model. A map with the probability of occurrence of *B. tabaci*-geminivirus, based on the climatic characteristics, was obtained.

Key words: begomoviruses, *Bean golden mosaic virus*, Geographic Information System (GIS), *Tomato yellow spot virus*, *Soybean blistering mosaic virus*.

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RESUMEN

Desde la década de 1990, los geminivirus han emergido como patógenos devastadores en regiones tropicales, subtropicales y templadas. En estudios

recientes, se ha desarrollado un método de detección de tres begomovirus infectando cultivos de soja y poroto en la Argentina, *Bean golden mosaic virus* (BGMV), *Soybean blistering mosaic virus* (SbBMV) y *Tomato yellow spot virus* (ToYSV). El objetivo del presente estudio fue determinar la incidencia y prevalencia de estos tres geminivirus y caracterizar los sitios donde se encontró el complejo *Bemisia tabaci*-geminivirus basándose en datos climáticos. El valor de incidencia más alto se observó en la provincia de Salta. BGMV presentó los mayores valores de prevalencia e incidencia en poroto, seguido por SbBMV. En el cultivo de soja, SbBMV presentó los mayores valores de prevalencia e incidencia, seguido por ToYSV. Un total de 103 sitios distribuidos en la Argentina, donde se detectó la presencia del complejo *Bemisia tabaci*-geminivirus, fueron caracterizados con un sistema de información geográfica (SIG). El complejo se encontró en zonas cálidas y de pocas lluvias; estas características climáticas son consistentes con las identificadas en un modelo descrito anteriormente. Se ha obtenido un mapa con la probabilidad de ocurrencia de *B. tabaci*-geminivirus, sobre la base de características climáticas.

Palabras clave: begomovirus, *Bean golden mosaic virus*, sistemas de información geográfica (SIG), *Tomato yellow spot virus*, *Soybean blistering mosaic virus*.

V. Alemandri, P. Rodríguez Pardina, E. Argüello Caro, A. Dumón, M.F. Mattio, S.M. Rodríguez and G. Truol. Instituto de Patología Vegetal - Instituto Nacional de Tecnología Agropecuaria (IPAVE-INTA). Camino 60 Cuadras Km 5 ½, X5020ICA. Córdoba, Argentina. J. Izaurralde. Instituto de Altos Estudios Espaciales Mario Gulich. Comisión Nacional de Actividades Espaciales (CONAE). Falda del Carmen, Argentina. S. García Medina EEA-INTA. Ruta Nacional 68, km 172-CC 228-4400. Cerrillos, Salta, Argentina. Correspondence: valemandri@ciap.inta.gov.ar

INTRODUCTION

Geminiviruses are devastating pathogens found in tropical and subtropical regions as well as in some temperate regions (Brown, 2001). Begomoviruses (Genus: *Begomovirus*; Family: *Geminiviridae*) are transmitted by a single whitefly species, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (van Regenmortel *et al.*, 2000).

The emergence of the *B. tabaci*-geminivirus complex around the world depends on various factors, such as evolution of variants of the viruses, changes in the biology of vectors, movement of infected planting materials, introduction of new crops and host susceptibility genes through the exchange of germplasm, changes in cropping systems, and climatic factors (Varma & Malathi, 2003). *B. tabaci* populations are drastically affected under climatic conditions characterized by extreme temperatures, low relative humidity (below 60%) and heavy and persistent rainfalls (Singh & Butter, 1984). *B. tabaci* does not adapt well to elevations exceeding 1000 m above sea

level (m asl) in different regions of Latin America (Morales & Jones, 2004).

The first epidemic caused by geminiviruses in Argentina was observed in common bean (*Phaseolus vulgaris* L.) crops in north-western Argentina (NWA) in the 1980s (Morales & Anderson, 2001). The earliest symptoms of geminivirus in soybeans [*Glycine max* (L.) Merr.] were observed during the 1986/87 period in the province of Salta (Ploper *et al.*, 1989) and later confirmed by Sakai *et al.* (1997). In 2006, three geminiviruses were identified in Argentina (Rodríguez Pardina *et al.*, 2006): *Tomato yellow spot virus* (ToYSV) in bean and soybean samples, *Bean golden mosaic virus* (BGMV) in bean, and another virus that exhibited less than 89% homology with other known begomoviruses in bean, soybean and tomato samples. Recently, the latter virus was confirmed as a new species and the name *Soybean blistering mosaic virus* (SbBMV) was proposed (Rodríguez Pardina *et al.*, 2011). Molecular hybridization-based detection of these begomoviruses, BGMV, SbBMV and ToYSV, was accomplished using a general

probe obtained by mixing full-length DNA-A clones of the three begomoviruses, and specific probes comprising part of the common region of each viral genome (Rodríguez Pardina *et al.*, 2011). In that study, only samples with obvious virus-like symptoms were collected and analyzed.

Although these three geminiviruses have been identified and molecularly characterized in two economically important crops in Argentina, incidence in this country is still unknown. Incidence data (with random sampling) are necessary to generate information on the epidemiological aspects of the disease and to design strategies aimed at reducing the economic damage caused by these geminiviruses.

On the other hand, plant disease management practices can be improved by putting epidemiological information in the same format as other farm information using a Geographic Information System (GIS) (Meritt *et al.*, 1999). GIS has been used most extensively for mapping distributions of insects (Everitt *et al.*, 1997) and diseases (Gent *et al.*, 2004).

Aspects of the ecology and epidemiology of whitefly-transmitted viruses in Latin America were studied by Morales and Jones (2004) using GIS. The authors analyzed 304 geo-referenced locations in Mexico, Central America, the Caribbean and South America, where *B. tabaci* and geminiviruses cause significant damage, and constructed a simple model with two climatic variables. The model showed that the suitable climate conditions for the spread of the *B. tabaci-geminivirus* complex are a dry season of at least 4 months with less than 80 mm of rainfall each, and a mean temperature of the hottest month exceeding 21 °C. That work included only a few sites from Argentina where the complex had been detected until then. Subsequent investigations showed the presence of whitefly-geminivirus in other parts of this country, including locations at higher latitudes (Truol *et al.*, 2005; Rodríguez Pardina *et al.*, 2011). Climatic characterization of these new sites with the presence of *B. tabaci-geminivirus* complex would contribute to the knowledge of the epidemiology of these viruses, as well as of their insect vectors. This characterization would allow us to identify areas of disease risk by implementing a GIS.

In this research paper we report the incidence and prevalence of ToYSV, SbBMV and BGMV geminiviruses and the climatic characteristics of locations where the *B. tabaci-geminivirus* complex was detected in soybean and bean crops in Argentina using a GIS.

MATERIALS AND METHODS

Sample collection

The surveys were conducted in five provinces of Argentina: Salta, Jujuy, Tucumán, Santiago del Estero, and Córdoba, in February and March 2007. The area studied was located between latitude 22° 42' 24.12'' and 31° 28' 5.52'' S and longitude 63° 36' 7.92'' and 65° 36' 56.16'' W. Fifty-four fields (36 soybean and 18 bean fields) distributed with a distance greater than 18 km between them in 27 localities were examined. In each field, 30 plants were assessed in a "V" pattern (a plant was randomly collected every 10 steps). Samples were placed in plastic bags, stored on ice during transport to the laboratory and kept at -20 °C until DNA extraction.

DNA extraction and detection of Geminivirus by molecular hybridization

Total DNA from leaves was extracted following Dellaporta *et al.* (1983). Dot blot on nylon membrane with non-radioactive probes was performed using the analysis conditions proposed elsewhere (Rodríguez Pardina *et al.*, 2011). Two types of molecular hybridization probes were used: one general and three specific probes. The general probe detects all known viral species of geminiviruses, whereas the specific probes detect the three different begomoviruses previously described in bean and soybean crops of Argentina: ToYSV, SbBMV and BGMV (Rodríguez Pardina *et al.*, 2011).

The general probe was first used under low stringency condition (5X SSC and 2X SSC during the washing steps). Samples testing positive in this first trial were subsequently analyzed with specific probes under high stringency conditions (2.5X SSC and 1X SSC during the washing steps). An annealing temperature of 65 °C was used in all tests. Total DNA from healthy soybean and bean plants were used as negative controls. Different virus clones were used as positive controls. Samples that were positive with the general probe and negative with the specific probes are here referred to as "Other unidentified Geminiviruses".

Incidence and prevalence

Incidence was established as the proportion of diseased plants in a field. Prevalence was defined as the proportion of fields with presence of disease (Nutter *et al.*, 1991) according to the following

formulas: Incidence (%) = (number of diseased plants per field/total number of plants analyzed per field) x 100; Prevalence (%) = (number of fields with presence of disease /total number of fields analyzed) x 100.

Climatic characterization of sites

Forty-nine sites where the *B. tabaci*-geminivirus complex was detected in previous studies in soybean and bean in the 2004/2006 period in Argentina (Truol *et al.*, 2005; Rodríguez Pardina, 2011) and the 54 sites evaluated in the present study in 2007 were characterized based on climate data (Fig.1). The study area was located between the latitudes 22° 36' 54.36" and 31° 50' 33.36" S and the longitudes 63° 36' 7.92" and 65° 37' 0.12" W. Each of the 103 sites was sampled only once. These points were geo-referenced with a Global Positioning System (GPS), Garmin eTrex Vista.

Climate data

The studied sites were characterized based on climate features using the GIS FloraMap 1.02 (Jones & Gladkov, 1999). This GIS uses climatic data from a 10-minute grid (corresponding to 18 km at the equator). The grids are derived by interpolation from thousands of meteorological stations. A simple algorithm based on the inverse square of the distance between the five nearest stations and the interpolated point is used. Thirty-six climatic variables are used: 12 monthly averages for temperature, rainfall, and diurnal temperature range. Temperature is standardised with elevation using the NOAA TGP-006 (NOAA, 1984) digital elevation model (DEM) and a lapse rate model (Jones, 1991). To adjust for geographic differences in the timing of major seasons, a 12-point Fourier transformation is applied (Jones & Gladkov, 1999; Jones, 1991; Jones *et al.*, 2002).

Climate diagrams

Climate diagrams were recorded for each site studied using the GIS. The following variables were analyzed: Height (m asl), Maximum Average Monthly Temperature (MaxAMT) (°C), Minimum Average Monthly Temperature (MinAMT) (°C), Maximum Average Monthly Rainfall (MaxAMR) (mm), Minimum Average Monthly Rainfall (MinAMR) (mm), and the number of months with less than 80 mm of rainfall (dry period) were studied.

Probability analysis

The climate at the locations where populations of a given taxon were recorded is assumed by the GIS to be representative of the environmental range of the organism. The 36 climate variables described above were first extracted from the pixel at which each site in the input file was located, and the resulting data were subjected to a principal components analysis (PCA). The PCA was performed on the variance-covariance matrix since the Fourier analysis had already transformed the variables to comparable scales. A multivariate normal distribution was fitted to the principal component scores of the sites. The parameters of the multivariate normal distribution were used to calculate the probability of the climate at each pixel belonging to the probability distribution (Jones & Gladkov, 1999; Jones, 1991; Jones *et al.*, 2002).

RESULTS

A total of 1620 plants (30 plants per field x 54 fields) were analyzed. Incidence values of geminiviruses as well as of ToYSV, SbBMV and BGMV are shown in Table 1. Prevalence values are shown in Table 2.

BGMV was detected in the provinces of Salta

Table 1. Incidence of geminiviruses, *Tomato yellow spot virus* (ToYSV), *Soybean blistering mosaic virus* (SbBMV), *Bean golden mosaic virus* (BGMV) in soybean and bean producing areas in Argentina

Crop	Province	General Geminivirus Incidence (%)		Specific Geminivirus Incidence (%)							
		Min.	Max.	ToYSV		SbBMV		BGMV		Other	
				Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Soybean	Córdoba	0	16.7	0	3.3	0	3.3	0	0	0	16.7
	Salta	0	30	0	10	0	6.7	0	0	0	16.7
	Tucumán	0	10	0	6.7	0	0	0	0	0	10
	Santiago del Estero	3.3	23.3	0	3.3	0	10	0	0	3.3	10
Bean	Salta	0	50	0	0	0	16.7	0	36.7	0	13.3
	Jujuy	0	33.7	0	0	0	0	0	16.7	0	17

Table 2. Prevalence of Geminiviruses, *Tomato yellow spot virus* (ToYSV), *Soybean blistering mosaic virus* (SbBMV), *Bean golden mosaic virus* (BGMV) in soybean and bean crops in Argentina

Crop	Number of fields surveyed	Positive fields (%)			
		ToYSV	SbBMV	BGMV	Other Geminiviruses
Soybean	36	16.7	16.7	0	58.3
Bean	18	0	16.7	77.8	66.7

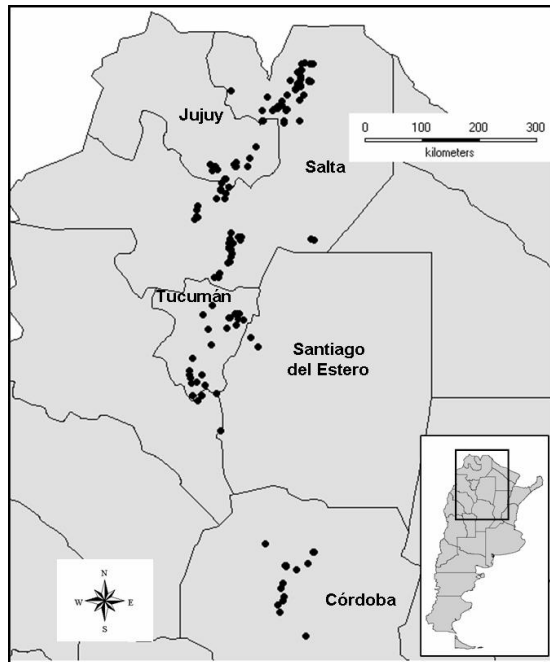


Fig. 1. Sites where *B. tabaci/geminivirus* complex was detected between 2004/2007 in soybean and bean crops in Argentina.

and Jujuy. SbBMV was recorded in Salta, Santiago del Estero and Córdoba, and ToYSV in Salta, Tucumán, Santiago del Estero, and Córdoba. The highest incidence values of BGMV were recorded in the departments of San Martín, Metán and General Güemes (Salta), whereas the highest incidence of SbBMV and ToYSV was detected in the department of San Martín (Salta). The maximum incidence values of geminiviruses of each crop were recorded in the province of Salta; indeed, in this province the highest incidence was 50% in bean and 30% in soybean (Table 1).

BGMV presented the highest prevalence and incidence values (77.8% and 36.7%, respectively), of all viruses detected in the study area, both values corresponding to bean fields. SbBMV exhibited the same prevalence value for both crops (16.7%), whereas incidence was higher in bean (16.7%) than in soybean (10%). ToYSV had prevalence and incidence values in soybean similar to those of SbBMV, and was not detected in bean fields.

Of the 54 fields analyzed in this study, 19 (7 soybean fields and 12 bean fields) were infected with more than one geminivirus. BGMV was present in the 12 bean fields, whereas SbBMV was present in 3 bean fields. Only 19.5% of the soybean fields sampled showed mixed infection with different geminiviruses; of these fields, 4 were infected with SbBMV and ToYSV.

Figures 2 and 3 show AMR and AMT data, respectively, for a field in Embarcación (Salta) where geminiviruses were detected in 2005. Table 3 shows the climate data recorded for the 103 sites studied in the 2004/2007 period where *B. tabaci* and/or geminiviruses were detected. AMT values were found within a range of 6 to 28.7 °C. AMR

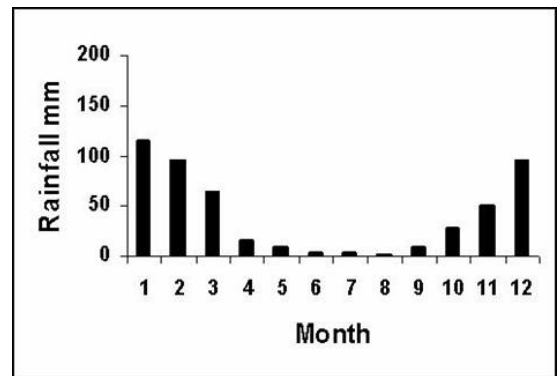


Fig. 3. Average monthly temperature for a plot in Embarcación (Salta), where geminiviruses were detected in 2005.

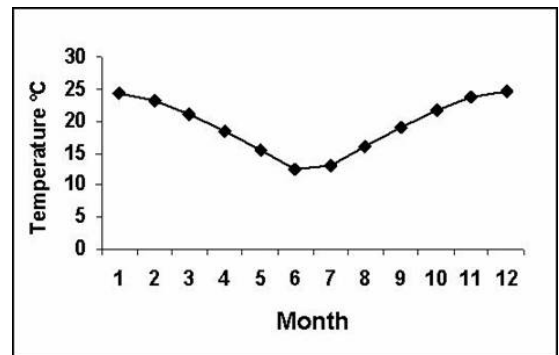


Fig. 2. Average monthly rainfall for a plot in Embarcación (Salta), where geminiviruses were detected in 2005.

Table 3. Climatic data for the sites where the *B. tabaci*-geminivirus complex was detected in in soybean and bean crops in 2004/05/06/07 in Argentina.

Climatic data	Min	Max
Height (m asl)	213	1523
MaxAMT (°C)	19,6	28,7
MinAMT (°C)	6	16,8
MaxAMR (mm)	82	230
MinAMR (mm)	1	12
Dry period (months)	5	10

values were found within a range of 1 to 230 mm.

Because the GIS employed uses the special characteristics of normal distribution to determine probabilities, and because, unlike temperature, precipitation data are remarkably abnormal (Jones & Gladkov, 1999), the latter data were subjected to exponential transformation to achieve an approximately normal distribution. The best exponent was 0.6. The same weights were used

for precipitation and temperature data. The first four components were used and 96.06% of the variability was explained.

Figure 4 shows the map obtained with the probability of occurrence of *B. tabaci*-geminiviruses in Argentina, based on the climatic characteristics of the sites, using data from the 2004/2007 period.

DISCUSSION

Although geminiviruses were detected in the five provinces assessed, Salta presented the highest general geminivirus incidence values (i.e., in samples that were positive with the general probe) in both crops. These results may be attributed to the cultivation conditions of soybean and bean crops in this region. In the traditional bean area in Salta, soybean crops are planted near bean crops (Vizgarra *et al.*, 2006). *B. tabaci* feeds and reproduces easily on this oilseed (Morales

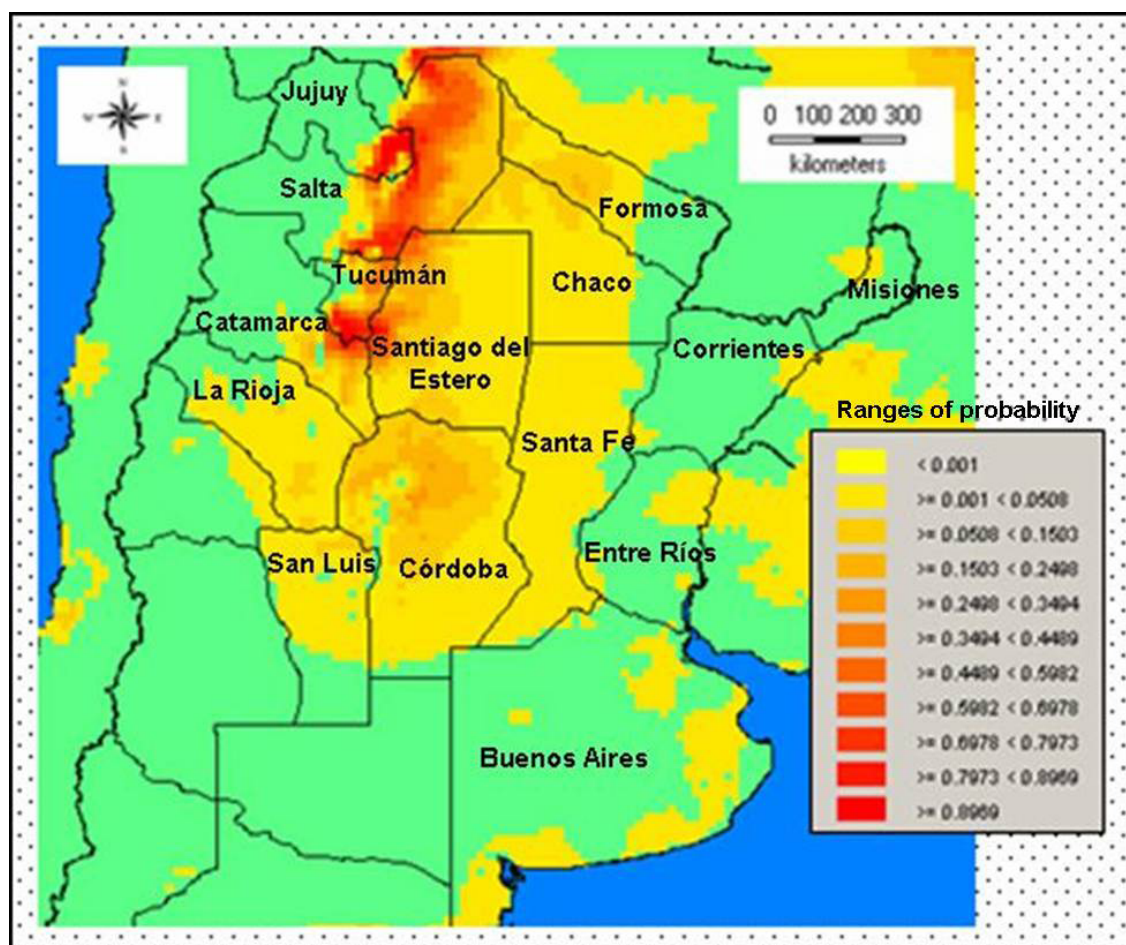


Fig. 4. Map of probability of occurrence of *B. tabaci*-geminivirus in Argentina, generated using the climatic characteristics.

et al., 2006). The physiological maturity stage of soybean usually coincides with the second leaf stage of bean; at this stage, *B. tabaci* migrates to the adjacent bean crops (Morales *et al.*, 2006). It is a critical stage, because the crop is very susceptible to infection by geminiviruses (Schuster *et al.*, 1996). In addition, the province of Salta, of subtropical climate, is well known for the important economic losses caused by these viruses, as it occurs in tropical areas. This situation may explain the incidence and prevalence values detected in the province of Salta.

BGMV was the most widely distributed virus of all the identified viruses that were evaluated. Indeed, most bean fields examined presented the highest BGMV prevalence and incidence values. This is in agreement with records of other South American countries, such as Brazil, and Venezuela, and several countries in Central America, where BGMV is considered the most important bean disease of viral etiology (Morales *et al.*, 1990). On the other hand, BGMV was not detected in the soybean samples analyzed. This is in contrast to previous studies in Brazil, where BGMV was reported in this legume (Fernandes *et al.*, 2009).

SbBMV and ToYSV are newly identified and characterized viruses worldwide (Calegario *et al.*, 2007; Rodriguez Pardina *et al.*, 2011). The present results are one of the first contributions to the knowledge of prevalence and incidence of both viruses. In the year surveyed, SbBMV showed higher incidence in bean than in soybean and prevalence was similar in both crops. ToYSV was not detected in any bean field, although in a previous study it was cited in this crop in Argentina (Rodriguez Pardina *et al.*, 2011). In soybean, ToYSV was detected with the same prevalence and incidence values as SbBMV. Although these two viruses were present at lower values than other geminiviruses, further surveys will be necessary to monitor their presence and evaluate their importance.

"Other unidentified Geminiviruses" were found to be widely distributed, because they were detected in both crops in the five provinces evaluated. The important prevalence and incidence values of "Other unidentified Geminiviruses" found in both crops in the present work show the need for further studies to identify geminiviruses in soybean and bean fields in Argentina (Tables 1 and 2).

Nineteen fields (35%) were found infected with more than one geminivirus. This result is very important because the occurrence of mixed infections would facilitate recombination between different geminiviruses, which may lead to the emergence of new species (Girish & Usha, 2005).

In addition, in mixed infections, some viruses with competitive superiority can displace and eliminate others. In Costa Rica, the *Tomato yellow mottle virus* (ToYMoV) dominated for several years but in recent years it appeared in mixed infections with *Sinaloa tomato leaf curl virus* (STLCV) (Karkashian *et al.*, 1998) and now it has been displaced in several areas of the country (Hilje, 2003). A gradual displacement of *Tomato yellow leaf curl virus* (TYLCV-Sar) by TYLCV from Israel was observed in results obtained in Spain (Navas-Castillo *et al.*, 1999). Another specific case is that of TYLCV, of Mediterranean origin, which was inadvertently introduced to the Americas. TYLCV of Mediterranean origin has the capacity to displace many native viruses presenting, unlike many of them, a wide range of hosts, including wild and ornamental plants (Hilje, 2003). These examples show the need for continuous studies on the identification and distribution of geminiviruses present in Argentina. In this work, we found fields infected with more than one geminivirus. For that reason, any of them is likely to exhibit competitive superiority, displacing and eliminating others, which would require changes in management strategies.

The present results indicate that the climatic characteristics of the soybean and bean fields studied, distributed across five provinces in north-western and central Argentina, are consistent with the pattern obtained from the characterization of 304 sites in Latin America by Morales and Jones (2004), even though the present work includes some locations at higher latitudes. The *B. tabaci-geminivirus* complex was found in warm and low rainfall areas. AMT of the hottest month exceeded 21 °C in 102 of the 103 fields and had a dry period of at least 4 months with less 80 mm of rainfall each.

The *B. tabaci-geminivirus* complex was found at an altitudinal ranging between 213 and 1523 m asl. Although it was suggested that *B. tabaci* does not adapt well to elevations exceeding 1000 m asl in different regions of Latin America (Morales & Jones, 2004), in the present study we found the complex in three sites exceeding this altitude: a bean field in Jujuy at 1523 m asl in 2004 (the site that showed a slightly different AMT, 19.6 °C), a bean field in Salta at 1371 m asl in 2005, and a soybean field in Salta at 1219 m asl in 2007. These results agree partially (because of latitudinal differences) with records of *B. tabaci* at altitudes above 1000 m reported for Guatemala, Costa Rica and Panama (Caballero, 1996).

The map of disease probabilities generated with the climatic data denotes that areas of highest disease probability (depicted in shades of red) were located mainly in the provinces of Salta,

Jujuy, Tucumán and Santiago del Estero. The intermediate probabilities are presented in the province of Córdoba. This map shows that the entire Argentine bean region presents probability of disease risk, as well as a large percentage of the country's soybean region, including two of the main producing provinces of this oilseed, such as Córdoba and Santa Fe.

The map obtained can be used to identify sites of suitable climate conditions for the occurrence of *B. tabaci*-geminivirus complex. Such information will be useful to design strategies for crop protection, as well as to analyze the need for curative interventions, such as the use of insecticides. However, climate conditions are not the only factor involved in disease occurrence. Hirano *et al.* (1995) attempted to identify the factors that influence fluctuations in *B. tabaci* population density. Using data from soybean fields in Indonesia, these authors concluded that climatic variables are not the only factors involved in the fluctuations in population density, but also space-time variations in the number of host plants in the area play a vital role. Taking into account the flight ability and the intrinsic rate of natural increase of *B. tabaci*, Hirano *et al.* (1995) suggested that this vector does not pose a serious problem in environments where host plants are grown discontinuously in time and space. In the north western region of Argentina, in the traditional bean growing area, the cultivation of soybean crop is expanding. This region presents a suitable environment for the virus complex spread. Moreover, there are sites with disease probability that have not been reported yet. However, agricultural, biological or climatic changes, such as the introduction of a particularly suitable reproductive host for *B. tabaci*, the arrival of new biotypes of *B. tabaci*, and extended periods of time without abundant (>80 mm) rain, can lead to *B. tabaci*/geminivirus outbreaks in susceptible crops (Morales & Jones, 2004).

The geo-referenced information generated in this work can be exported into another GIS and can be associated with data of other crops, soils, vegetation, physical barriers, and human intervention to obtain more detailed information for future applications.

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