Acta Tropica 146 (2015) 53–59

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

St. Louis Encephalitis virus mosquito vectors dynamics in three different environments in relation to remotely sensed environmental conditions

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ARTICLE INFO

Article history:
Received 11 October 2014
Received in revised form 5 March 2015
Accepted 7 March 2015
Available online 16 March 2015

Keywords:
SLEV vectors
Culex quinquefasciatus
Culex interfor
Mosquito population dynamic
Remote sensing

ABSTRACT

In Argentina the St. Louis Encephalitis virus (SLEV) is an endemic and widely distributed pathogen transmitted by the cosmopolitan mosquito *Culex quinquefasciatus*. During two outbreaks in Córdoba city, in 2005 and 2010, *Culex interfor* was also found infected, but its role as vector of SLEV is poorly known. This mosquito species is distributed from central Argentina to southern Brazil. The primary aim of this study was to analyze the population dynamic of *Cx. interfor* and *Cx. quinquefasciatus* in three different environments (urban, suburban and non-urban) in relation to remotely sensed environmental data for vegetation (NDVI and NDWI) and temperature (brightness temperature). *Cx. quinquefasciatus* and *Cx. interfor* were found at the three sampled sites, being both the most abundant *Culex* species, with peaks in early and midsummer. Temporal distribution patterns of both mosquito species were highly correlated in a non-urban area of high SLEV risk transmission. *Cx. quinquefasciatus* and *Cx. interfor* were associated with the most urbanized site and the non-urban environment, respectively; high significant correlations were detected between vegetation indices and abundance of both mosquito species confirming these associations. These data provide a foundation for building density maps of these two SLEV mosquito vectors using remotely sensed data to help inform vector control programs.

1. Introduction

Argentina is affected by the emergence and re-emergence of diseases caused by flaviviruses, such as dengue (Estallo et al., 2014), yellow fever (Morales et al., 2011) and human encephalitis by St. Louis Encephalitis virus (SLEV) (Spinsanti et al., 2003) and West Nile virus (Diaz et al., 2011). The SLEV is maintained in nature through biological transmission between birds and *Culex* mosquitoes. Humans are considered dead-end hosts that can suffer from febrile headache to meningoencephalitis and even death (Reisen, 2003). Isolated human cases by SLEV were reported in 1964, 1968, 1971 and 1985 in Argentina (Sabattini et al., 1985). Later, after 17 years of absence of human cases, SLEV reemerged in the central region of the country (i.e., Córdoba province) in 2002 (Spinsanti et al., 2003). In Córdoba, two outbreaks by SLEV were reported in 2005 and 2010 with 47 and 11 individuals infected with this virus, respectively, mostly with neurologic involvement (Spinsanti et al., 2008; Vergara Cid et al., 2011). The isolation of SLEV from *Culex quinquefasciatus* collected in nature at the provinces of Córdoba and Santa Fe (Mitchell et al., 1985; Diaz et al., 2006) and experimental vector competence studies (Diaz, 2009; Flores et al., 2010) indicate that this species is involved as the urban vector in Argentina. *Cx. quinquefasciatus* is widely distributed in America, from United States of America to the central region of Argentina (WRBU, 2014). On the other hand, during the 2005 and 2010 outbreaks, *Culex interfor* was also found infected in Córdoba city with SLEV (Diaz et al., 2006). *Cx. interfor* is present in several provinces

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http://dx.doi.org/10.1016/j.actatropica.2015.03.009

in northern and central Argentina, probably extending into Bolivia and southern Brazil (Dibo et al., 2011; Diez et al., 2011; Mureb Sallum et al., 1996; Rossi et al., 2006; Visintin et al., 2009). However, its role as vector and several aspects of its biology remains largely unknown. Therefore, further investigations regarding vector population dynamics are needed.

Since there is no treatment or vaccine against SLEV, entomological surveillance and mosquito control remain the main public health strategies to prevent human infection. A deeper knowledge about variables affecting vector distribution and abundance leads to the improvement of disease control measures. Environmental alteration by human activities often influences over vector populations dynamics. Deforestation and urbanization increase the proliferation of artificial containers for developing larvae urban mosquito species (Norris, 2004). Certain species are more abundant in urban areas according to landscape characteristics, as *Culex maxi* and *Culex apicinus* that showed opposite patterns depending on the proximity to waterways in Córdoba city (Argentina); *Cx. maxi* abundance was higher near to waterways as the percentages of un-built lots increased (Gleiser and Zalazar, 2009).

The remotely sensed data provides a moderate-cost alternative approach to mapping vector species distribution (Kalluri et al., 2007; Wilschut et al., 2013). Satellite imagery may also improve the efficiency of control programs for mosquitoes, by indicating areas or periods of the year under higher risk of mosquito proliferation (Gleiser and Gorla, 2007). Several authors have observed that the spatial and temporal patterns of mosquito population dynamics are controlled by environmental factors that can be remotely observed (Brown et al., 2008; Chuang et al., 2012; Estallo et al., 2008, 2012). In epidemiology, the use of remote sensing data involves the study of environmental variables that characterize the vector ecosystem such as land use, temperature, vegetation cover and precipitation (Johnson et al., 2008; Tran et al., 2002). The normalized difference vegetation index (NDVI) and the normalized difference water index (NDWI) are two vegetation indices derived from satellite images that can be used as indicative of potential mosquito habitat existence (Brown et al., 2008). Thus, the use of remotely sensed data could aid in detecting foci of virus activity by identifying environmental conditions suitable for SLEV vectors in both urban and nonurban areas. In Argentina, proximity to vegetated areas with NDVI > 0.3 and the presence of low density of urban construction were the main landscape elements that contributed to human infections in the last SLEV outbreak in 2010 in Cordoba city (Vergara Cid et al., 2013). Therefore, this study evaluated the temporal distribution of the vector of SLEV *Cx. quinquefasciatus* and the potential vector *Cx. interfor* in three different environments in relation to remotely sensed environmental data for vegetation and temperature in Córdoba city, Argentina.

2. Materials and methods

2.1. Study area and mosquito collections

Córdoba city (Córdoba Province) has a population of approximately 1,330,000 inhabitants (INDEC, 2010) and is located in the central region of Argentina (64°11′W–31°24′S), at 450 m above sea level. Climate is temperate with mean annual temperature ranging between 16 °C and 17 °C, with a warm and wet season between September and May, and mean annual rainfall of 800 mm (Jarsún et al., 2003).

Adult mosquitoes were collected using CDC CO_2 light-baited traps (three per site) in three areas of Córdoba city: San Vicente (SV), Camino 60 Cuadras (CC) and Bajo Grande (BG). These sampling sites (each separated by >8 km) were selected to represent different environments: SV is urban (SV), CC is suburban and BG is non-urban. The three areas where sampling sites were located were completely different as seen in Fig. 1. At SV, traps were placed at a nursing home located in the south east of the city; vegetation was dominated by *Melia azedarach* (commonly called “paraíso”) and *Pinus* spp.; also ornamental plants and an orchard were present; the nursing home is surrounded by high density of housing and other building constructions. The sampling site is located in an urbanized area characterized by residential, commercial and industrial uses. The suburban site (CC) is located in the south of the city and traps were placed at a family dwelling; vegetation was represented by a mix of autochthonous (*Acacia* spp.) and introduced species (*M. azedarach*, *Pinus* spp., *Ligustrum lucidum*, *Platanus* spp.), surrounding a swimming pool, the house and a fruit tree area. This site is surrounded by some crop areas and is 1 km away from the nearest urbanized settlement. The non-urban site (BG) is located in the eastern of Córdoba, on the outskirts of the city, 2 km away from city urban area and it is characterized by many open areas intended for vegetable and fruit culture. Traps were placed at the sewage treatment plant of the City Hall, which has sewage pool containers and some building constructions, surrounding by ponds with aquatic vegetation, few isolated houses and many open areas. Vegetation is dominated by *M. azedarach*, *Morus alba*, *L. lucidum*, *Pinus* spp., *Ricinus* spp. and grassland.

Traps were placed simultaneously at each sampling site every 2 weeks from 6:00 p.m. to 10:00 a.m. A total of 45 nights of trapping from January 2008 to December 2009 were performed. Captured mosquitoes were frozen in the field and transported to the laboratory. Mosquitoes were then counted and identified to genus and species using the key by Darsie (1985).

2.2. Environmental characterization

Satellite-derived variables were used to characterize and identify suitable environmental conditions for the development of mosquito vectors.

Widely used vegetation indices (NDVI and NDWI) and temperature were obtained from a set of 17 Landsat 5 (L5TM) and 7 (L7 ETM +) path/row 229/82 satellite images (16 days temporal resolution) from January2008 to December 2009. Satellite images were obtained from the Argentine Space Agency (CONAE) catalog by academic cooperation. ENVI (Environment for Visualizing Images, Research Systems) 4.2 software (2004) was used for image processing. The images were geo-referenced using an image from the GLCF (Global Land Cover Facility). Subsequently, a subset of 240 km^2 (800 × 800 pixels) area that included Córdoba city was generated. The images were calibrated to convert Landsat 5 TM or Landsat 7 ETM+ digital numbers to exoatmospheric reflectance (reflectance above the atmosphere) using their respective coefficients (USGS Landsat, 2010).

In order to measure the vegetation coverage and the vegetation water content, we calculated the NDVI and the NDWI, both indices applied for mosquito studies as indicative of potentials mosquito habitat existence (Brown et al., 2008; Estallo et al., 2012, 2008). Values of the earth’s surface temperature were estimated through the brightness temperature (BT) (Landsat images band 6), which gives an approximation of the environmental temperature (Estallo et al., 2008, 2012; Kalluri et al., 2007). A buffer zone of 5 × 5 pixels (150 m) was delimitated around each sampling site in order to calculate the values of NDVI, NDWI and BT of each pixel. From each buffer zone the average values were calculated for each environmental variable.

Since there were only 17 satellite images available for the study (January, February, April, June, July, August, October, November 2008; January, March, April, May, June, July, October, November, December 2009), we used linear interpolation to obtain the missing indices values (March, May, September, December 2008 and

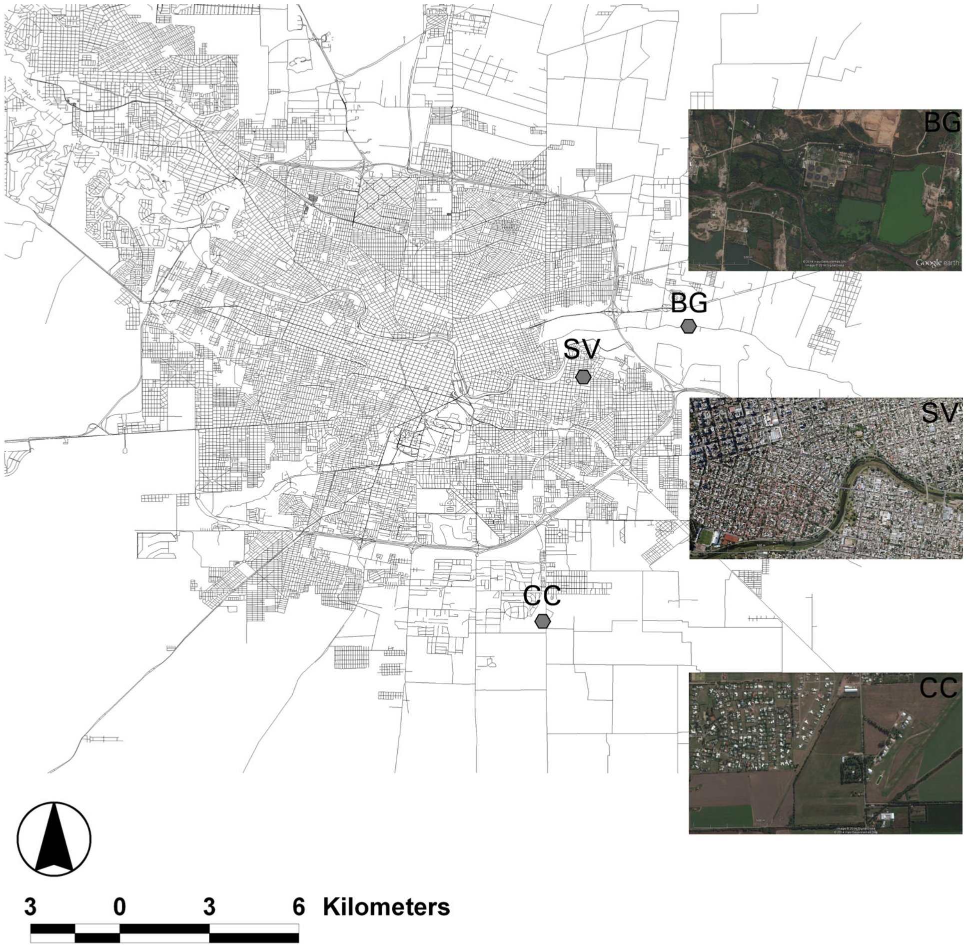

Fig. 1. Trapping sites and sampling area of three sites with different environmental characteristics in Córdoba city, Argentina. BG, Bajo Grande (non-urban); CC, Camino a 60 Cuadras (suburban); SV, San Vicente (urban).

February, August and September 2009) and therefore have 24 environmental values to correlate with the monthly values of mosquito abundances.

2.3. Data analyses

Population dynamics of *Cx. interfor* and *Cx. quinquefasciatus* were characterized through the abundance of both species at each sampling site. Mosquito abundance data were pooled monthly per site and species. A correspondence analysis (CA) was carried out to identify relations between sites according to environmental characteristics (urban, suburban and non-urban environments) and the abundance of mosquito species. An analysis of variance was used to assess significant differences on *Cx. interfor* and *Cx. quinquefasciatus* abundance between sites. Since normality and homoscedasticity were not achieved, the non-parametric Kruskal–Wallis test was performed. Spearman correlations test (R) was performed to evaluate associations between monthly abundance of *Cx. interfor* and *Cx. quinquefasciatus* and remote sensing variables at each site. p-values ≤ 0.05 were considered statistically significant. A Fisher's z-transformation (Z) for correlation coefficients to detect statistical differences was performed. All data analysis was performed with InfoStat software version 2012 (Di Rienzo et al., 2012).

3. Results

A total of 11,974 female mosquitoes belonging to 16 species assigned to *Anopheles*, *Culex*, *Mansonia*, *Ochlerotatus*, *Psorophora*, and *Stegomyia* genera were collected; *Culex* was the most abundant genus represented by nine species (Table 1). The correspondence analysis showed that some species were similarly associated with different sampling sites. The first axis explained 74.63% of the total data variance (Fig. 2). Urban species such as *Cx. quinquefasciatus* and *Stegomyia aegypti* were grouped out together around the most urban site (SV), whereas *Cx. interfor*, *Culex acharistus*, *Culex saltanensis*, *Mansonia titillans* and *Ochlerotatus scapularis* were grouped

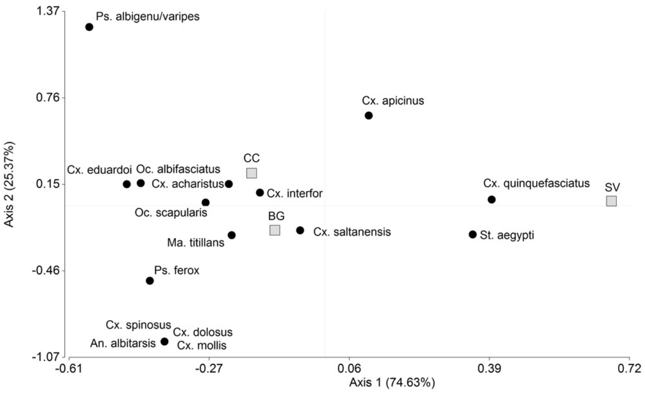

Fig. 2. Correspondence analysis biplot for mosquito species in three sites of Córdoba city with different environmental characteristics from January 2008 to December 2009. For each axis, the amount of variation explained as a part of the total variation in the model is shown. BG, Bajo Grande (non-urban); CC, Camino a 60 Cuadras (suburban); SV, San Vicente (urban).

Table 1
Mosquito species captured in dry ice-baited CDC light traps from January 2008 to December 2009 in three sites with different environmental characteristics in Córdoba city, Argentina. BG, Bajo Grande (non-urban); CC, Camino a 60 Cuadras (suburban); SV, San Vicente (urban).

Mosquito species	Sites			Total
	BG	CC	SV	
Anopheles albitarsis	1	0	0	1
Culex acharistus	9	5	1	15
Culex apicinus	4	35	9	48
Culex dolosus	1	0	0	1
Culex eduardoi	1	1	0	2
Culex interfor	660$^{(a)}$	365$^{(a)}$	44$^{(b)}$	1069
Culex mollis	1	0	0	1
Culex quinquefasciatus[1]	2343	431	962	3736
Culex saltanensis	271	750	28	1049
Culex sp.	351	99	124	574
Culex spinosus	4	0	0	4
Mansonia titillans	1593	4	15	1612
Ochlerotatus albifasciatus	151	411	2	564
Ochlerotatus scapularis	928	2118	12	3058
Psorophora albigenu/varipes	0	1	0	1
Psorophora ferox	9	1	0	10
Stegomyia aegypti	151	19	59	229
Total	6478	4240	1256	11,974

Different letters in parenthesis indicate significant differences ($p < 0.05$) between sites based on a Kruskal–Wallis test on ranked abundance.

[1] No statistical differences were detected between sites ($p = 0.2801$) based on a Kruskal–Wallis test on ranked abundance.

together in association with the non-urban and suburban sites (BG and CC) (Fig. 2).

Cx. quinquefasciatus was the most common *Culex* species (Table 1) and the only species captured during winter in all sites. In SV (76.59%) and BG (36.17%) *Cx. quinquefasciatus* was the most abundant species throughout both sampling years (Table 1). Although we did not find significant differences in abundance between sites ($KW_H = 2.53$; df = 2; $p = 0.2801$), the greatest abundance of *Cx. quinquefasciatus* was observed in BG (62.71%) and SV (25.75%) with peaks in December at both sampling sites (Fig. 3).

Regarding *Cx. interfor*, it was present at all sampling sites representing 8.93% of all collected mosquitoes and it was the second most common *Culex* species (Table 1). The Kruskal–Wallis tests indicated significant difference in *Cx. interfor* abundances between sites ($KW_H = 11.68$; df = 2; $p = 0.0019$). The greatest abundance of *Cx. interfor* was registered at BG (61.74%), followed by CC (34.14%) and SV (4.12%), with peaks occurring in December in BG and CC, and in February at the urban site (SV) (Fig. 3). *Cx. interfor* abundance strongly decreased during cold seasons (May to August), with only a few individuals being captured in BG and CC (Fig. 3). Furthermore, peaks of *Cx. interfor* and *Cx. quinquefasciatus* abundance were coincident in December at BG site. The temporal fluctuation of both species were significantly correlated at all sites, but highest value were detected at BG ($R = 0.82$; $p < 0.01$) and CC ($R = 0.78$; $p < 0.01$) showing a close relation between their abundances and the environmental conditions along the year in these sites.

Cx. interfor and *Cx. quinquefasciatus* abundance was significantly correlated with the environmental variables at each site (Table 2). The NDVI and NDWI average values recorded throughout periods of greatest abundances of both mosquito species were >0.3 and >0.7, respectively, at both non-urban and suburban sites (BG and CC), whereas lower values were registered at SV. Correlations between NDVI, NDWI and *Cx. interfor* abundance at BG and CC were significantly higher according to the Fisher test (NDVI: $Z_{\text{BG-SV}} = 2.422$, $p = 0.0156$; $Z_{\text{CC-SV}} = 3.354$, $p = 0.0008$; NDWI: $Z_{\text{BG-SV}} = 2.510$, $p = 0.012$; $Z_{\text{CC-SV}} = 2.324$, $p = 0.0204$) than those observed in SV. The lowest correlations were detected at the urban site (SV) (Table 2). Concerning *Cx. quinquefasciatus*, correlations between vegetation indices (NDVI and NDWI) and mosquito abundance were significantly higher (NDVI: $Z_{\text{BG-SV}} = -3.865$, $p = 0.0001$; $Z_{\text{CC-SV}} = -3.499$, $p = 0.0005$; NDWI: $Z_{\text{BG-SV}} = -7.770$, $p = 0.00008$; $Z_{\text{CC-SV}} = -10.390$, $p = 0.00008$) at SV (Table 2). Correlations between mean temperature (BT) and the monthly abundance of both species were higher at BG (Table 2). However, only was statistical significant ($Z_{\text{BG-SV}} = 3.803$, $p = 0.0001$) for *Cx. quinquefasciatus.*

4. Discussion

This study addresses the temporal abundance patterns of two mosquito species and the association with environmental factors. *Cx. interfor* and *Cx. quinquefasciatus* abundance were monitored in three sites with different urbanization levels, taking into consideration the relations among their temporal patterns and remote sensing variables. Ecological disturbances and changes in land use, such as deforestation and increased urbanization development, may cause spatial segregation of certain mosquito species through creation or expansion of new breeding habitat (Leisnham et al., 2004; Norris, 2004; Patz et al., 2000). The correspondence analysis performed in this study could differentiate mosquito species groups according to sampling sites. The urban mosquito *Cx. quinquefasciatus* was associated with the urban site (SV), whereas *Cx. interfor* was grouped with the non-urban and suburban sites (BG and CC). Gleiser and Zalazar (2009) demonstrated a positive correlation between the abundance of *Cx. interfor* and the percentage of built surface in Córdoba city, suggesting a preference of this species for densely constructed areas. However, our results reveal that the highest correlations between the abundance of *Cx. interfor* and the vegetation indices were detected in BG and CC, i.e., in vegetated areas. Moreover, the greater abundance was detected in these sites, with peaks in December. Differences between Gleiser and Zalazar (2009) study and the results found in this research, could be due to those authors only considered urban sites as the study area. Our results are consistent with previous studies that showed a higher abundance of *Cx. interfor* in less urbanized sites surrounding Córdoba city in late spring (November to December) and midsummer (February) (Diaz, 2009). According to Almirón and Brewer (1996) ground breeding sites with aquatic vegetation are the most favorable larval habitats for *Cx. interfor* in Córdoba. The area of BG presents suitable conditions for the occurrence of this type of breeding sites. Ponds with aquatic vegetation and open wooded areas with grassland are frequent in this area.

Cx. quinquefasciatus is the SLEV urban vector in central Argentina and it is found both in urban and non-urban environments. This study registered the highest abundance of *Cx. quinquefasciatus* in

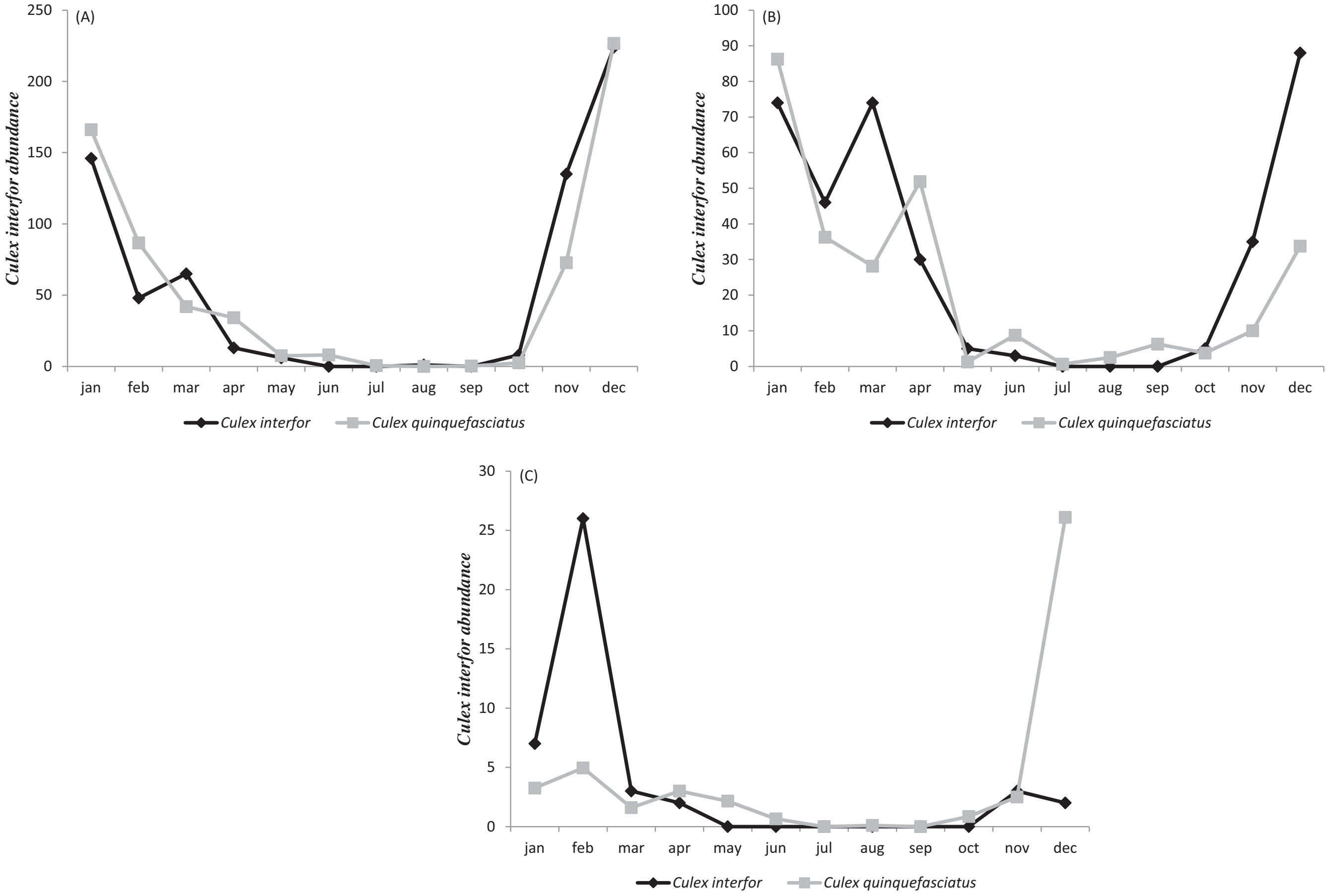

Fig. 3. Annual distribution of adults of *Culex interfor* and *Culex quinquefasciatus* in three sites with different environmental characteristics in Córdoba city from January 2008 to December 2009. (a) BG, Bajo Grande (non-urban); (b) CC, Camino a 60 Cuadras (suburban); (c) SV, San Vicente (urban).

the non-urban site (BG). These results agree with those reported by Diaz (2009), who indicated BG as one locations of high abundance of *Cx. quinquefasciatus*. However, the correspondence analysis revealed an association of *Cx. quinquefasciatus* with the urban site (SV). Moreover, correlations between environmental variables considered in this study and *Cx. quinquefasciatus* abundance were higher in SV. These results indicate that the urban environmental conditions of SV are suitable for the development of this mosquito species. Gleiser and Zalazar (2009) also reported that *Cx. quinquefasciatus* abundance was negatively associated with percentage of unbuilt surfaces. High abundances of *Cx. quinquefasciatus* in BG could be due to the presence of residual waste waters in this sampling area. This species is commonly associated with polluted water with high content of organic components, such as septic tanks, water bodies from storm runoff and sewage (Burke et al., 2010; Ishii and Sohn, 1987; Pires and Gleiser, 2010).

Endemicity of SLEV in urban and suburban areas in Córdoba city has been demonstrated and different viral genotypes are circulating since 2002 (Diaz et al., 2012; Spinsanti et al., 2002). In 2005, during the first outbreak by SLEV, genotype III was detected in *Cx. quinquefasciatus* and *Cx. interfor* (Diaz et al., 2006). In a 3-year study before this outbreak a progressive increase in the abundance of *Cx. interfor* was observed in less urbanized sites surrounding Córdoba city (Diaz, 2009). Two years previous to the outbreak, months of high SLEV activity (November to December) were coincident with peaks of abundance of *Cx. interfor*, being also detected SLEV-positive pools of this mosquito species (Diaz, 2009). In addition, 35% of positive pools in that period were recorded at BG (Diaz et al., 2012). Serological surveys showed the area of BG as a risk area due to high SLEV seroprevalence (14.6%) detected in humans (Spinsanti et al., 2007). Furthermore, another increase in *Cx. interfor* population was registered in peri-urban sites of Cordoba city prior to a high SLEV activity period in 2012 with also three records of SLEV-positive mosquito pools (Batallán, 2013), highlighting the potential role of this species in the SLEV transmission cycles. Before the 2005 outbreak, genotype III had not been detected, suggesting that the

Table 2
Correlations between *Culex interfor* and *Culex quinquefasciatus* abundance and landscape variables from January 2008 to December 2009 in three sites with different environmental characteristics in Córdoba city, Argentina. BG, Bajo Grande (non-urban); CC, Camino a 60 Cuadras (suburban); SV, San Vicente (urban).

Variables	*Culex interfor*			*Culex quinquefasciatus*		
	BG	CC	SV	BG	CC	SV
NDVI avg.	0.76 (<0.01)	0.82 (<0.01)	0.54 (0.01)	0.69 (<0.01)	0.66 (<0.05)	0.76 (<0.01)
NDWI avg.	0.76 (<0.01)	0.75 (<0.01)	0.53 (0.01)	0.68 (<0.01)	0.48 (0.02)	0.81 (<0.01)
BT avg.	0.77 (<0.01)	0.58 (<0.01)	0.70 (<0.01)	0.71 (<0.01)	0.48 (0.02)	0.63 (<0.01)

Values in table are Spearman's *R* correlations. *p*-values are included in parenthesis. Avg, Average. All correlations were statistical different ($p < 0.05$) according to Fisher's z-transformation test with the following exceptions: *Cx. interfor*: $\mathrm{NDWI}_{\mathrm{BG\text{-}CC}}$ and $\mathrm{BT}_{\mathrm{BG\text{-}SV}}$; *Cx. quinquefasciatus*: $\mathrm{NDVI}_{\mathrm{BG\text{-}CC}}$.

introduction of this genotype was an important factor associated to this incident (Diaz et al., 2012). Circulation of different genotypes may occur due to the introduction or reintroduction of new strains from non-urban to urban areas. Different mosquito species could be involved in SLEV transmission according to landscape characteristics. Therefore, SLEV spillover to the urban cycle may occur from enzootic cycles in less urban areas (Weaver, 2005). The less urban sites in this study (BG and CC) showed average values of NDVI > 0.3 throughout periods of greatest abundances of *Cx. interfor* and *Cx. quinquefasciatus*, highlighting areas more likely to experience SLEV infection according to Vergara Cid et al. (2013). Our results also revealed that *Cx. interfor* was more abundant at the less urban sites (BG and CC). Urban outbreaks in Córdoba city may occur by SLEV spillover from enzootic cycles in non-urban areas through biological transmission between birds and *Cx. interfor.* Thus, *Cx. interfor* could act as a link between non-urban and urban environments in Córdoba city, setting a risk to human health. Also, molecular studies revealed that *Cx. interfor* would be an ornithophilic mosquito, feeding on *Zenaida auriculata* (Unpublished results), one of the major SLEV avian host in Argentina, so may contribute to the enzootic virus transmission. Consequently, strategies for mosquito control operations should include non-urban and suburban areas close to the city, like BG and CC, in order to enhance arboviral surveillance. This study also reveals a matching pattern of population dynamics between both mosquito species at the less urban sites. This fact may increase the transmission risk since peaks of abundance of both mosquito species are coincident with months of high viral activity in an area of high SLEV circulation (BG). Viremic birds could be exposed at any given time to more than one SLEV vector. Thus, detecting months of higher *Cx. interfor* and *Cx. quinquefasciatus* abundance, may improve the implementation of appropriate prevention strategies for SLEV transmission. Further studies related to the ecology and the potential role of *Cx. interfor* in the SLEV transmission should be performed.

Remote sensing data provide an excellent tool to detect relations between environmental characteristics and distribution patterns of mosquito species involved in the SLEV transmission cycle. Vegetation indices can be used as proxy variables of local suitable conditions for mosquito population growth. The results reported in this research provides a basis for further in-depth studies that will be useful in the creation of prediction maps for abundance and distribution of vectors that may contribute in the planning of strategies for surveillance, prevention and control of this arbovirosis.

Acknowledgements

We thank CONAE for providing the satellite images as academic cooperation. This study was supported by the Agencia Nacional de Ciencia y Técnica–FONCYT 38060. The authors thank Dr. Andres M. Visintin for helpful suggestions that improved the manuscript. G.P.B. is recipient of a postdoctoral scholarship from CONICET (Resolución No 4498). E.L.E. and W.R.A. are members of CONICET scientific carrier.

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