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SPATIAL AND TEMPORAL DISTRIBUTION OF *CULICOIDES INSIGNIS* AND *CULICOIDES PARAENSIS* IN THE SUBTROPICAL MOUNTAIN FOREST OF TUCUMÁN, NORTHWESTERN ARGENTINA

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Abstract

Culicoides insignis Lutz and *Culicoides paraensis* Goeldi are known bluetongue virus and filariasis (caused by *Mansonella ozzardi* Manson) vectors, respectively. Bluetongue virus appears to be restricted to northeastern Argentina, while filariasis is endemic in the subtropical mountain forest of the Argentine northwest. With the objective of characterizing the abundance and seasonality of both *Culicoides* species, entomological sampling was carried out from Dec 2004 to Nov 2005 in the southern area of the forest of Tucumán province. The specimens were captured using CO2-baited CDC light traps placed in 2 types of environments, wild and anthropized. The abundance of the specimens in relation to environmental variables was analyzed using multiple linear regression. Out of the 2,497 adult specimens collected, 76.9% belonged to *C. paraensis*, 20.4% to *C. insignis* and the 2.5% belonged jointly to *Culicoides debilipalpis* Lutz, *Culicoides lahillei* Lutz and *Culicoides venezuelensis* Mirsa & Ortiz (2.5%), and 0.2% could not be identified. Peaks of abundance of *C. insignis* and *C. paraensis* in decreasing magnitude were observed in the fall, summer and spring, respectively; and the largest number of specimens was found in the anthropized environment. Mean minimum and maximum temperatures and levels of accumulated rainfall were the variables that best explained the abundance of these 2 species. The present work is an important contribution not only to the knowledge of the spatial and temporal distribution and dynamics of these vectors in nature, but also to the elucidation of the implications of anthropization of the forest environment, and the effect of these climatic variables as determinants of the abundance of the species and, hence, as determinants of the possible transmission of filariasis in the subtropical mountain forest of the Argentine northwest.

Key Words: *Culicoides insignis, Culicoides paraensis*, bluetongue virus, filariasis, abundance, seasonality, northwestern Argentina

Resumen

Culicoides insignis Lutz y *Culicoides paraensis* Goeldi son conocidos vectores del virus de la lengua azul (BTV) y de la filariasis (causada por *Mansonella ozzardi* Manson), respectivamente. El virus de la lengua azul aparece reportado para la región del noreste, mientras que la filariasis resulta endémica de la selva subtropical de montaña del noroeste de Argentina. Con el objetivo de caracterizar la abundancia y estacionalidad de ambas especies en el noroeste de la Argentina, se realizó un muestreo entomológico desde diciembre de 2004 a noviembre de 2005 en el área sur de la selva, en la provincia de Tucumán. Los ejemplares fueron capturados mediante trampas de luz CDC cebadas con CO_2 y colocadas en dos tipos de ambientes, silvestre y antropizado. La abundancia de los ejemplares en relación a las variables ambientales fue analizada mediante regresiones lineales múltiples. Se recolectaron 2,497 especimenes adultos, de los cuales el 76.9% pertenecen a *C. paraensis*, el 20.4% a *C. insignis* y el resto a *Culicoides debilipalpis* Lutz, *Culicoides lahillei* Lutz y *Culicoides venezuelensis* Mirsa & Ortiz (2.5%). Se observaron picos de abundancia decrecientes para *C. insignis* y *C. paraensis* en otoño, verano y primavera; resultando el mayor número de ejemplares recolectados para el ambiente antropizado. La temperatura mínima y máxima media y los niveles de precipitación acumulada, fueron las variables que mejor explicaron la abundancia de ambas especies. El presente trabajo resulta una importante contribución no sólo por ampliar el conocimiento de la distribución temporo-espacial y de la dinámica de estos vectores en la naturaleza, sino también por tratar de dilucidar la implicancia de la antropización de los ambientes selváticos y del efecto de las variables climáticas sobre la abundancia de las especies y por lo tanto, de la posible transmisión de la filariasis en la selva subtropical de montaña del noroeste de Argentina.

Translation provided by authors.

Culicoides (Diptera: Ceratopogonidae) are reservoirs and vectors of viruses, protozoa and nematodes that cause diseases of sanitary importance (Spinelli & Wirth 1993). At present, they transmit filarial worms, bluetongue virus (BTV), Oropouche virus (OV), African Horse Sickness virus (AHS), and Epizootic Hemorrhagic Disease virus (EHD), among others, to animals (Linley et al. 1983; Wirth & Dyce 1985; Tanya et al. 1992; Spinelli 1998; Pinheiro et al. 1998; Mellor et al. 2000; Ronderos et al. 2003).

Filariasis caused by the nematode *M. ozzardi*, known also as mansonelosis, is found exclusively in the subtropical mountain forests (Yungas) in Tucumán, Salta and Jujuy provinces, in northwestern Argentina (Biglieri & Aráoz 1915; Mühlens et al. 1925; Romaña & Wygodzinsky 1950; Remondegui et al. 1988; Taranto & Castelli 1988; Zaidenberg 1997; Shelley & Coscaron 2001). *M. ozzardi* is vectored by various *Culicoides* species, i.e., by *Culicoides furens* Poey in Central and South America, St. Vincent Island in the Caribbean (Buckley 1934), Mexico and in Trinidad and Tobago (Biagi et al. 1958; Nathan 1981); and by *Culicoides phlebotomus* Williston in Haiti (Lowrie & Raccurt 1981). In Central America, Mexico and Florida (USA), *C. insignis* has been shown to be a potent vector of bluetongue virus (BTV) (Tanya et al. 1992), but its role in disease transmission in Argentina has not been elucidated. In Argentina, more specifically in the subtropical mountain forest in Tucumán province, *C. paraensis* and *C. debilipalpis* (Romaña & Wigodzinsky 1950) were reported as potential vectors. Later, Shelley & Coscaron (2001) reported *C. lahillei* and *C. paraensis* to be present in the northern area of the forest in Jujuy province. Moreover Shelley & Coscaron (2001) determined that *C. lahillei* served as the primary vector of *M. ozzardi* and *C. paraensis* as the secondary vector.

Despite their great importance as vectors of *M. ozzardi* and bluetongue virus little is known about the bionomics of *Culicoides* vector species, *C. paraensis* and *C. insignis*, in Argentina, as is demonstrated by the few relevant published reports. Since the extensiveness of transmission of these diseases is potentially determined by the level of abundance of these vectors, it is necessary to carry out studies focused on the dynamics and bionomics of these species during different seasons and in different types of environments. This would facilitate the determination of which season and which type of environment poses the most significant risk of filariasis transmission. Thus, the objective of the present work was to elucidate the spatial and temporal dynamics of *C. insignis* and *C. paraensis* in the southern area of the subtropical mountain forest in Tucumán province.

Materials and Methods

Study area

The selection of the study area was based on a bibliographic study of localities in Tucumán province (Fig. 1). In this province cases of filariasis have occurred and its endemicity has been high with a prevalence of 76.25% (Guzmán et al. 1998). Entomological samplings were carried out in 3 localities in the subtropical mountain forest in the northwest of Argentina, Sargento Moya (27.0° 13.0' 31.0"S; 65.0° 39.0 14.0"W; altitude 468 m), La Florida (27.0° 13.0' 10.5"S; 65.0° 37.0' 56.7"W; altitude 452 m) and Capitán Cáceres (27.0° 11.0' 23.2"S; 65.0° 36.0' 18.3"W; altitude 414 m). Data on rainfall, temperature and relative humidity were obtained from the Pueblo Viejo Meteorological Station (27.0° 11.0' 59.0"S; 65.0° 20.0' 59.0"W). The distances between the weather station and the 3 trapping sites are as follows: Sargento Moya (20 km), La Florida (17 km) and Capitán Cáceres (14 km) (Fig. 1). The subtropical mountain forest covers an area of 5.2 million hectares, from the Bolivian border (23 °S) to the north of the Catamarca province (29 °S), passing through Salta, Jujuy and Tucumán provinces (Brown et al. 1993). The climate of this forested region is subtropical with a dry season, average annual temperatures of 18-20 °C and marked seasonality of rainfall. The rainfall has a monsoon regime resulting in rainy summers and dry winters, and with 90% of the rainfall events occurring during 4 mo in the year (Dec, Jan, Feb and Mar) (Sesma et al. 1998). The vegetation is known as "pacará and tipa tree forest" with closed basal arboreal vegetation represented by *Blepharocalyx salicifolius* Berg. (Myrtaceae), *Enterolobium contorsiliquum* (Vell.) Morong (Pacará Earpod Tree, Fabaceae), *Juglans australis* Griseb. (tropical walnut, Juglandaceae) and *Parapiptadenia excelsa* (Griseb.) Burkart (horco-cebil, Mimosaceae). Near the roads and on the stream banks, the vegetation is open with typical species such as *Tipuana tipu* (Benth) Kuntze (tipu tree, Fabaceae), *Jacaranda mimosifolia* D. Don. (jacaranda, Bignoniaceae), *Anadenanthera colubrina* var. *cebil* (Griseb.) (cebil, Fabaceae), *Tabebuia avellanedae* Lor. ex Griseb. (pink trumpet tree, Bignoniaceae), *Heliocarpus popayanensis* Kunth (white moho, Malvaceae), *Zanthoxylum (Fagara) coco* Gillies (cochucho, smelly sauco, Rutaceae), *Tecoma stans* (L.) C. Juss. ex Kunth (yellow trumpet bush, Bignonoiaceae), *Salix humboldtiana* Willd. (Humboldt's willow, Salicaceae) and *Carica quercifolia* (oak-leaved papaya, Caricaceae) (Prado 1995). In the undergrowth can be observed *Piper tucumanum* C. DC (pepper tree, Piperaceae), *Eugenia uniflora* L. (Surinam cherry, Myrtaceae), *Urera baccifera* (L.) Gaudich. (Scratchbush, Urticaceae) and *Solanum riparium* Pers. (Flora del Conosur, Solanaceae), and among the lianas the families Bignonia-

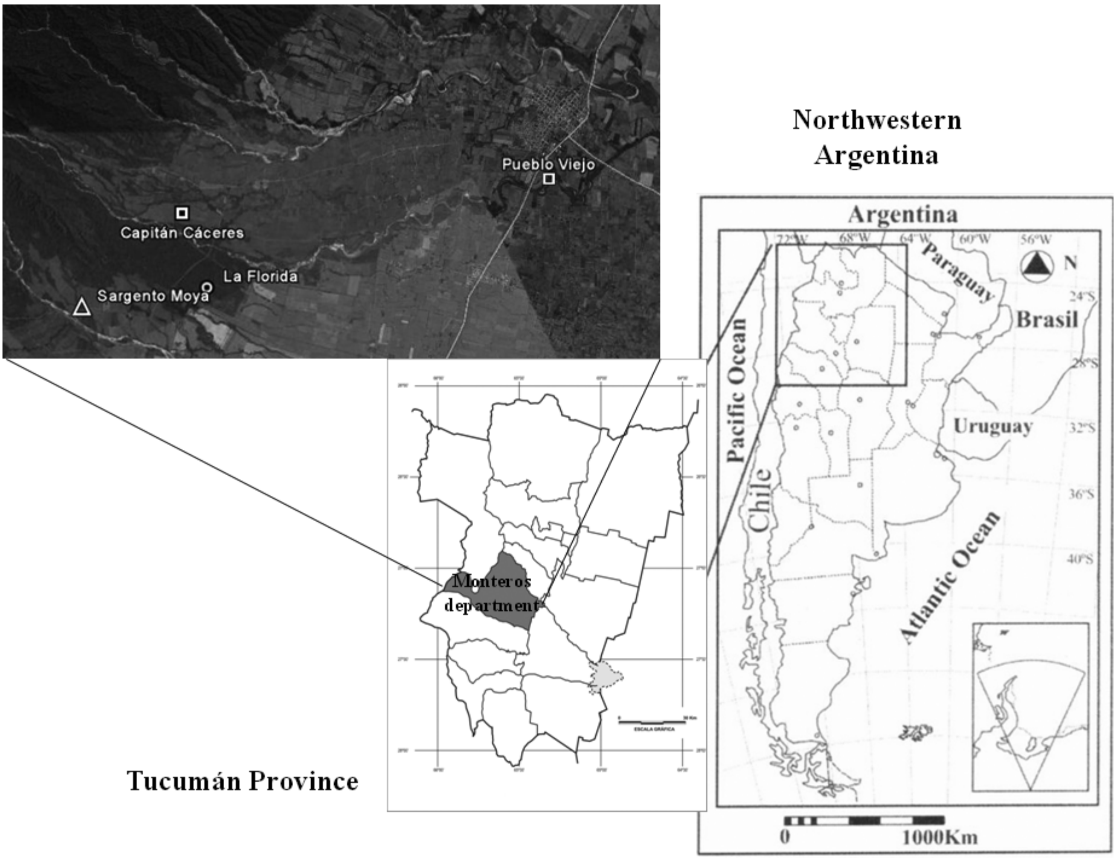

Fig. 1. Maps showing Tucumán province, situated in the Northwest region of Argentina, and the locations of the 3 sampling sites at Sargento Moya, La Florida and Capitán Cáceres, where *Culicoides* populations were sampled, and Pueblo Viejo, the site of the weather station.

ceas, Ulmaceas and Amarantaceas stand out. There are also families of vascular plants with epiphytic habits such as members of Polipodaceae, Asplaniaceae, Piperaceae, Cactaceae and Bromeliaceae (Brown 1995). It is important to emphasize the almost complete anthropic destruction of the area. This was caused mainly by deforestation for permanent and transitory agriculture with sugarcane and soybean culture areas and citrus plantations, and, to a lesser extent, for the rearing of livestock (Prado 1995; Brown & Grau 1995). Thus on vast stretches, the landscape has been totally cleared of its original vegetation; so that the latter survives only along streams where the banks are unsuitable for farming. The degree of degradation varied through the 3 sampling sites. Thus large areas devoted to crop production border on the Sargento Moya and Capitán Cáceres sampling sites. However, at the La Florida site it was possible to find "tipa and pacará" trees as remnants of the original vegetation (Brown & Grau, 1993), because it has been designated as a protected area.

Culicoides Collection and Identification of Species

During the development of a research project based on the bioecological and parasitological aspects of *Anopheles* mosquitoes found in northwestern Argentina carried out from Dec 2004 to Nov 2005, specimens of *Culicoides* were collected by CDC light traps baited with carbon dioxide. The traps were placed every 15 days in Sargento Moya and monthly in La Florida and Capitán Cáceres; and they were operated from early dusk until midnight (16:00-23:00 h). A total of 6 traps were used, 2 per sampling site, in 2 types of environment, i.e., anthropized and wild. The forest edge was considered to be an anthropized environment related to human activity, and characterized by open type vegetation represented by scarce canopy trees and epiphytes. Within this anthropized environment, traps were set approximately 10 m from the edge of a road, 10 m away from the edge of a stream and 15 m away from the edge of sugarcane cultures. The wild natural forested environment was characterized by the presence of native vegetation represented by closed canopy trees (above 25 m), young trees, lianas and epiphytes. The traps were placed in this environment at approximately 100 m from the edge of the road.

Later, the collected specimens were sacrificed with ethyl acetate, separated and placed in properly labeled eppendorf tubes containing 70% alcohol for preservation. In the laboratory, identification of adults was performed following the taxonomic keys of Wirth & Blanton (1959) and Spinelli et al. (2005). The identification of the species was based mainly on the patterns of wing spots, the shapes of the male and female reproductive organs, and the characteristics of certain palpal segments. Some specimens were mounted for microscopic examination on glass slides by the phenol-balsam method of Wirth & Marston (1968). This facilitated the correct identification and/or verification of the species.

Data Analysis

The relative abundance (%) of each *Culicoides* species was calculated as its percentage of all the collected specimens. Further, the abundance of each species was calculated per each sampling site and per each type of environment. The relationship between the number of specimens of each species collected and each of the climatic variables was analyzed by multiple regression analysis. Since only 33 specimens of *C. venezuelensis* (1.3% of total) and only 22 specimens (0.9% of total) of *C. lahillei* were captured (Table 1), these data could not be analyzed rigorously. However, large numbers of *C. parensis* (1,921 specimens) and *C. insignis* (509 specimens) were captured. The species, *C. insignis* and *C. paraensis,* were considered as dependent variables and the monthly climatic variables, mean temperature, maximum and minimum mean temperatures, accumulated rainfall and mean relative humidity, provided by the Pueblo Viejo weather station, were considered as independent variables. For this analysis, the general equation of the models developed was $y = a_0 + a_1X_1 + \ldots + a_nX_n$ where y corresponds to the species of the *Culicoides* genus, a_0 is a constant and $a_1 \ldots a_n$ are the coefficients of the different climatic variables (X_1 to X_n). The climatic variables were designated as follows: X_1—mean monthly temperature, X_2—mean minimum monthly temperature, X_3—mean maximum monthly temperature, X_4—monthly accumulated rainfall, and X_5—monthly mean relative humidity.

Linear regressions were carried out using the Statistica 6.0 (StatSoft 2001) software. The independent variables were included in the multiple linear regression analyses but the variables that contributed least to the explanation of the models were eliminated one by one taking statistical significance ($P < 0.05$) into consideration.

Results

A total of 3,793 adult Ceratopogonidae specimens were captured out of which 2,497 (66%) were *Culicoides* species. Of the *Culicoides* collected, the percent each species contributed to the total in descending order was as follows: *C. paraensis* (76.9%), *C. insignis* (20.4%), *C. venezuelensis* (1.3%), *C. lahillei* (0.9%) and *C. debilipalpis* (0.3%), and undermined *C.* spp. (0.2%) (Table 1). The analysis of the percentage of *Culicoides* specimens collected per sampling site showed that the greatest percentage was found in Sargento Moya (55.5%), followed by La Florida (32.5%) and Capitán Cáceres (12.0%). The various *Culicoides* species showed different patterns of dynamics according to abundance and seasonality. Three main peaks of abundance were observed: 1 large peak occurred during the fall (Apr) and 2 smaller ones during the summer (Feb) and spring (Nov), respectively. At Sargento Moya (Fig. 2 A), *C. insignis* showed peaks of abundance during the fall and spring, while *C. paraensis* was abundant during the fall. At La Florida (Fig. 2 B),

TABLE 1. *CULICOIDES* SPECIES COLLECTED AT 3 DIFFERENT SITES IN THE SUBTROPICAL MOUNTAIN FOREST, TUCUMÁN, BETWEEN DEC 2004 AND NOV 2005. THE SARGENTO MOYA AND CAPITÁN CÁCERES SAMPLING SITES BORDERED ON LARGE AREAS DEVOTED TO CROP PRODUCTION, BUT THE LA FLORIDA SITE HAD "TIPA AND PACARÁ" TREES AS REMNANTS OF THE ORIGINAL YUNGAS VEGETATION.

Species/Localities	Sargento Moya	La Florida	Capitán Cáceres	N (number)	% of Total
C. debilipalpis	1	1	5	7	0.3
C. insignis	301	79	129	509	20.4
C. lahillei	8	4	10	22	0.9
C. paraensis	1,070	627	224	1,921	76.9
C. venezuelensis	9	16	8	33	1.3
C. spp.	0	3	2	5	0.2
Total	1,389	730	378	2,497	100

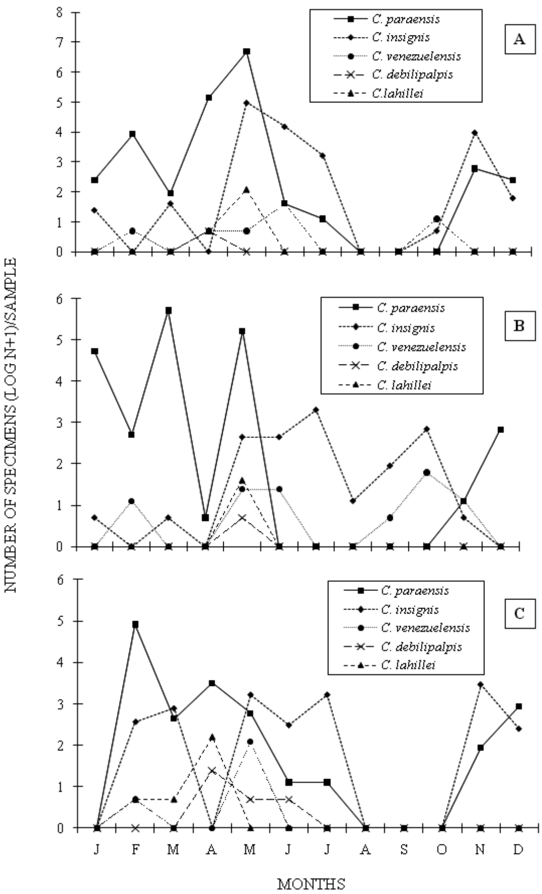

Fig. 2. Population fluctuation of *Culicoides* species at each sampling site from Dec 2004 to Nov 2005. A. Sargento Moya, B. La Florida, C. Capitán Cáceres.

C. insignis was abundant during the fall and winter, while *C. parensis* was abundant during the summer and fall. At Capitán Cáceres (Fig. 2 C), abundance and seasonality of the species were very similar to those found at La Florida, except that *C. insignis* showed peaks of abundance during fall, spring and summer.

When analyzing the abundance of species per type of environment (Table 2), it was noticed that the overall abundance of *Culicoides* species was greater in the anthropized environment (forest edge) than in the wild environment (forest). At Sargento Moya *C. insignis* was more prevalent in the anthropized environment (forest edge) than in the wild environment, while and *C. paraensis* was more abundant in the wild (forest) than in the anthopized environment, while in La Florida and Capitán Cáceres both species were more abundant in the anthropized environment than in the wild environment (Table 2).

Multiple regression analysis allowed obtaining the following descriptive models. For Sargento Moya the regression analysis between *C. paraensis* specimens and the climatic variables showed a significant correlation with mean minimum temperature and accumulated rainfall ($y = 249.95 + 1.30 X_4 - 0.97 X_2$; R^2 ajust. = 0.44; $P < 0.029$) (Fig. 3). In La Florida, mean maximum temperature was the variable more strongly related to *C. insignis* ($y = 110.32 - 2.4 X_3$; R^2 ajust. = 0.60; $P < 0.014$) (Fig. 4). In Capitán Cáceres, none of the climatic variables was significantly correlated with the abundance of *C. insignis* and *C. paraensis* throughout the sampling period.

Discussion

This is the first report concerning *Culicoides* spp. in the subtropical mountain forest of Tucumán since that of Biagi et al. (1958), and it shed lights on the presence of the filariasis vector, *C. paraensis.* Also this study contributed new data concerning the spatial and temporal dynamics of 5 *Culicoides* species in relation to certain climatic variables as well as their interaction with the environment.

With respect to the seasonality of the species, the greatest fluctuations in the abundance of *C. paraensis* occurred during the summer and fall, while such fluctuations in abundance of *C. insignis* occurred during the fall, spring and summer. *C. lahillei* and *C. debilipalpis* were moderately abundant only during the fall, while *C. venezuelensis* was moderately abundant in the fall, spring and summer. However, because of the small number of specimens collected in relation to *C. insignis* and *C. paraensis*, it was not possible to establish a characteristic fluctuation pattern for the sparse species. Clearly these patterns of abundance of the different species must have explanations at least in part in the drastically anthropized environmental conditions of the subtropical forest in Tucumán, which has differentially favored the abundance of the various species during the progression of the seasons. A similar case was reported by De Barros et al. (2007) in Brazil, who found *Culicoides* to be present during almost all the months of the year, with their abundance favored by the hot and humid climate in the study area.

Most of the specimens of *C. insignis* and *C. paraensis* were captured at Sargento Moya. Both species were more abundant in the anthropic environment than in the pristine environment. This pattern may be attributed to what was reported by Prado (1995), i.e., the subtropical forest area in Tucumán and Catamarca provinces has undergone an almost total anthropic destruction compared to the forest in the northern sector (Salta and Jujuy provinces). These changes have inadvertently provided new habitats adequate for the breeding of *Culicoides* complete with sources of blood more readily available than in the wild.

TABLE 2. ABSOLUTE NUMBERS (*N*) AND PERCENTAGES (%) OF *CULICOIDES* SPECIES COLLECTED IN TWO TYPES OF ENVIRONMENTS (ANTHROPIZED-WILD) AT 3 SITES IN THE SUBTROPICAL MOUNTAIN FOREST, TUCUMÁN, ARGENTINA.

	Sargento Moya		La Florida		Capitán Cáceres		
Species	Anthropized	Wild	Anthropized	Wild	Anthropized	Wild	Total
C. debilipalpis	1	0	0	1	5	0	7
C. insignis	209	92	56	23	83	46	509
C. lahillei	6	2	0	4	6	4	22
C. paraensis	487	583	490	137	158	66	1,921
C. venezuelensis	4	5	13	3	8	0	33
C. spp	0	0	2	1	2	0	5
Total	707	682	561	169	262	116	2,497

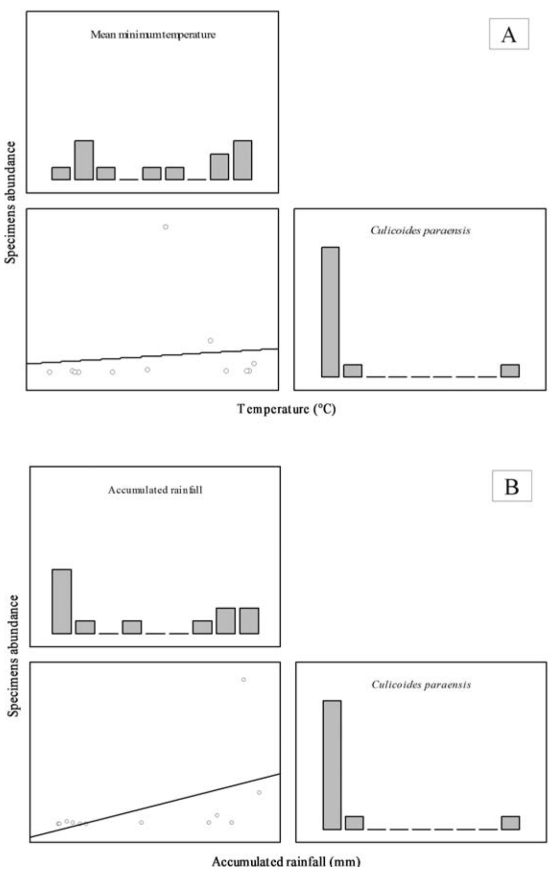

Fig. 3. Regression analysis between A. Mean monthly minimum temperature; B. Monthly accumulated rainfall and number of specimens of *C. paraensis* collected from Dec 2004 to Nov 2005 in Sargento Moya, Tucumán.

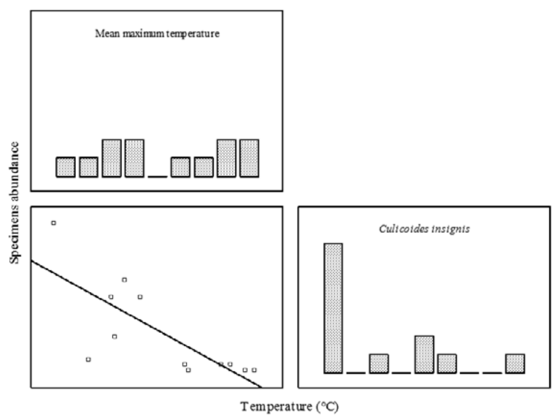

Fig. 4. Regression analyses between mean monthly maximum temperature and number of specimens of *C. insignis* collected from Dec 2004 to Nov 2005 in La Florida, Tucumán.

These results are consistent with those of De Barros et al. (2007), who found that the greatest frequency of *Culicoides* occurred in the urban area. It is important to notice that, although most *C. insignis* and *C. paraensis* specimens were found in the anthropized environment, they were also found in wild environments, although in smaller numbers. It is more likely that these species have remained well adapted for the wild, but that the anthropized environment provides exceptionally favorable conditions for them. The latter observation is an important contribution from the epidemiological viewpoint, since such patterns of distribution in both environments reveal the difficulties that may arise to set up an entomological and epidemiological watch in the area.

With respect to the effects of the climatic variables on the fluctuation of the species, it was observed that mean minimum temperature and accumulated rainfall were the variables that best explained the dynamics of the *C. paraensis* population at Sargento Moya. At La Florida, mean maximum temperature was the variable more strongly related to the prevalence of *C. insignis*. However, at Capitán Cáceres none of the climatic variables was statistically significant with respect to the fluctuation of the species' densities. Maia-Herzog et al. (1988) observed an inversely proportional relationship between the abundance of *Culicoides* and rainfall, and that neither relative humidity nor mean temperature was correlated with the abundance. These findings are similar to the findings in our study. In contrast, Sherlock & Guitton (1964) and De Barros et al. (2007) reported a greater abundance of specimens during the coldest and rainiest months. The study of Santos da Silva et al. (2001), which reported a greater abundance of *Culicoides* during periods of low rainfall preceded by periods of abundant rainfall, agrees with our results since the greatest abundance of specimens was found during the months following strong rainfall, that is, in the fall. The fact that the greatest abundance of specimens occurred in anthropic environments is a matter of concern because it is likely abundance will increase even more as more of the forested land becomes anthropized. Loss of closed canopy, greater drainage, and seasonal changes in vegetation cover are factors that are likely related to changes in temperature and rainfall patterns and vector abundance. These conditions underlie the present situation of these vectors in northwestern Argentina, and they are indicative of vector dynamics in the near future.

Further, given that pathogen transmission is influenced by the abundance of vectors, the population peaks observed during the summer and fall indicate that these periods could be the most important ones with respect to the risk of transmission of *Mansonella ozzardi* by *Culicoides*. It should be noted that the presence of *C. paraensis*, which have been directly implicated in the transmission of this parasite, is likely to cause the emergence or reemergence of the disease. Another point to be considered concerns migratory movements of human populations that might be exposed to these vectors and to the parasite. Human migration is likely to widen the geographical distribution of the parasitosis since the vectors are present in the migration routes.

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