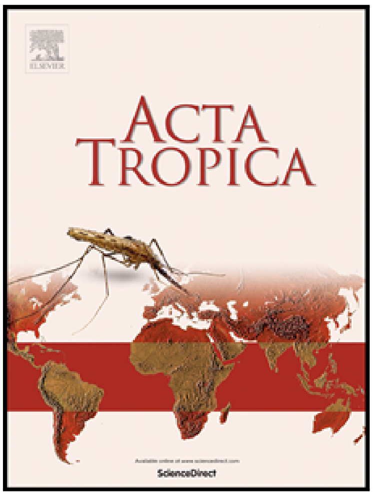

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Impacts of residual insecticide spraying on the abundance and habitat occupancy of *Triatoma sordida* and co-occurrence with *Triatoma infestans*: a three-year follow-up in northeastern Argentina

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ABSTRACT

Triatoma infestans, the main vector in the Gran Chaco region, may competitively displace other sympatric species such as *Triatoma sordida*. We conducted a three-year longitudinal study of site- and house-level infestation and abundance of triatomine bugs before and after an area-wide insecticide spraying campaign followed by sustained vector surveillance in a well-defined rural section of the Argentine Chaco encompassing 368-411 houses. Here, we tested whether insecticide applications targeting and virtually suppressing *T. infestans* reduced the abundance of *T. sordida* and modified its habitat occupancies, and whether their joint spatial distribution was random, aggregated or uniform, and varied over time. Systematic timed-manual searches of 18,031 sites yielded 2,226 *T. sordida* over seven postintervention surveys. *Triatoma sordida* failed to colonize human sleeping quarters after interventions, and its prime and secondary habitats remained virtually unmodified. Residual insecticide spraying and seasonality best described variations in the house-level abundance of *T. sordida* as determined using a generalized estimating equation model. Two-species foci occurred in 3.2% of sites ever positive for any species. The habitat-adjusted relative odds of catching one species was 10.8 times greater when the other species was present, with no evidence of heterogeneity among ORs, suggesting no antagonistic interactions throughout the follow-up. The spatial occurrence of both species was significantly aggregated within 300-500 m before and after interventions, and was random at broader spatial scales. The habitat occupancies of *T. sordida* may be used as a proxy for potential infestation with *T. infestans* and to guide targeted vector control actions.

1. **Introduction**

Triatomine species (Hemiptera: Reduviidae) involved in the transmission of *Trypanosoma cruzi* (Protozoa: Kinetoplastida), the etiological agent of Chagas disease, usually occupy distinct habitat types in association with specific mammalian or avian hosts and genetically defined parasite lineages (Zingales et al., 2012). The reasons underlying the apparent niche partition between sympatric triatomine species remain unclear. Theory and empirical observations support niche partitioning between species is related to differential exploitative capacities of habitats and hosts, antagonistic interactions between species, and fluctuating habitat conditions, among others (Begon et al., 2005). *Triatoma infestans*, the main vector in the southern cone of South America, has been considered capable of competitively displacing other species such as *Panstrongylus megistus* (Pereira et al., 2006), *Triatoma brasiliensis* and *Triatoma pseudomaculata* (Silveira et al., 1984), and *Triatoma sordida* Stål (Noireau et al., 1996, Schofield et al., 1980a). These intrusive species (Noireau and Dujardin, 2010) have extensive sylvatic or peridomestic foci from which they frequently invade domestic premises, attack humans, establish domestic colonies and may transmit *T. cruzi*. The issue of intrusive triatomine species cuts across triatomine species and ecological settings (Guhl, 2009).

Vector control actions (e.g., insecticide spraying) specifically directed against the main target species in systems including other triatomines (which may or may not be affected by control actions) may modify the outcome of interspecific interactions. Residual insecticide spraying campaigns directed against *T. infestans* largely contracted its geographic range and reduced its abundance except in sections of the Gran Chaco region (Schofield et al., 2006). Whether the decline or transient elimination of *T. infestans* may allow the niche expansion and colonization of domestic premises by other triatomines that thrive in sylvatic or peridomestic habitats is a potential threat to successful vector control programs (Guhl et al., 2009; Waleckx et al., 2015). The public-health relevance of intrusive vectors may unexpectedly increase as a collateral effect of insecticide spraying, or it may decrease if they are susceptible to routine control actions. For example, *T. infestans* is sympathric with *Triatoma garciabesi* and *Triatoma guasayana* in peridomestic habitats of the dry Chaco

(Canale et al., 2000; Vazquez-Prokopec et al., 2005). House spraying with residual insecticides targeting *T. infestans* effectively reduced both the relative abundance of *T. garciabesi* and the chances of future site infestation (Rodríguez-Planes et al., 2016), but the relative fraction of occupied sites by habitat type (habitat occupancy) was not modified as *T. infestans* declined.

Triatoma infestans and *T. sordida* live in sympatry in rural houses of the Gran Chaco. *Triatoma sordida* has a much more widespread range up to the Brazilian *cerrado* (Carcavallo et al., 1999), and apparently includes at least two sibling species or subspecies (Calderón-Fernández and Juárez, 2013; Panzera et al., 2015). One is *Triatoma sordida sensu stricto*, which has frequently been collected both in peridomestic habitats and human sleeping quarters in western Paraguay (Rojas de Arias et al., 2012; Sanchez et al., 2016) and Bolivia (Noireau et al., 1995), where it was frequently infected with *T. cruzi* in some rural settings, though not always (Acosta et al., 2017). A similar situation occurred in Brazil (da Rocha e Silva et al., 1977; Falavigna Guilherme et al., 2001; Gurgel-Gonçalves et al., 2010; Maeda et al., 2012), where *T. sordida* has been considered the most relevant of the secondary triatomine species (Schofield and Dias, 1999; Tartarotti et al., 2004).

The second cryptic species is *Triatoma sordida Argentina*, which includes the sylvatic and peridomestic specimens frequently collected in the humid (eastern) Argentine Chaco, apparently unable to colonize human sleeping quarters (Alvarado-Otegui et al., 2012; Gonzalez-Britez et al., 2014). Its prevalence of infection with *T. cruzi* (6.3%) in peridomestic habitats remained unaffected by an area-wide insecticide spraying campaign conducted in Pampa del Indio (Macchiaverna et al., 2015), where it may act as a “bridge” vector between sylvatic and domestic transmission cycles (Maffey et al., 2012). The main (prime) peridomestic habitats of *T. sordida Argentina* are ecotopes used by chickens (Bar et al., 2002; Damborsky et al., 2001; Rodríguez-Planes et al., 2018), but secondary ecotopes (such as various types of corrals) may play a key role in dispersal across the complex network of sylvatic and peridomestic habitats in rural settings (Rodríguez-Planes et al., 2018). Both the heterogeneous ecological and epidemiological patterns described above and taxonomic uncertainties regarding which (sub)species is involved in each case suggest that the exact roles and relevance of *T. sordida* as a secondary vector of *T. cruzi* requires further investigation, more so in the context of rapid land-use changes.

As part of a research program on the eco-epidemiology and control of Chagas disease in Pampa del Indio, we conducted a three-year study of site- and house-level infestation and abundance of triatomine bugs before and after an area-wide insecticide spraying campaign, followed by periodic surveillance and selective re-treatment of every focus of *T. infestans* (Gurevitz et al., 2013). These interventions brought *T. infestans* to the brink of local extinction. We have reported the preintervention habitat (ecotope) occupancy patterns of *T. sordida*, their relevance for spatial colonization dynamics, and the significantly positive association between this species and *T. infestans* over some ecotopes before interventions (Rodríguez-Planes et al., 2018).

Here, using detailed longitudinal data collected at site level over eight surveys, we tested whether i) insecticide applications targeting *T. infestans* reduced the abundance of *T. sordida* and modified its habitat occupancies (i.e., niche expansion); ii) the relative frequency of sites occupied by each of these species differed from those predicted by chance (e.g., because of competitive interference), and iii) the joint spatial distribution of both species was random, aggregated or uniform and varied over time as *T. infestans* tended to become locally extinct. These relevant questions have public health implications beyond this specific study setting and the triatomine species involved.

2. Materials and methods

2.1. Study area

Field studies were carried out in a rural section (denominated Area I for logistic purposes) of the municipality of Pampa del Indio (25° 55'S 56° 58'W), in the Province of Chaco, Argentina, between 2007 and 2010. Rural houses in Pampa del Indio had been last treated with insecticide (targeting *T. infestans*) in 1996-1997. The baseline (preintervention) survey enumerated 368 houses and 24 public buildings in 13 neighboring rural villages in November 2007 (Rodríguez-Planes et al., 2018). In total, 411 houses were enumerated at the last survey conducted in October 2010.

Each house compound (i.e., hereafter denominated house) encompassed a domestic and a peridomestic area. The domestic area nearly always included human sleeping quarters. The peridomestic area included several types of ecotopes defined by its physical structure and use: kitchens, mud ovens, storerooms, granaries, latrines, structures occupied by chickens (trees, nests, corrals), and corrals for livestock (goats and sheep, pigs, cows and horses). Chicken nests consisted in an elevated platform made of wood or bricks where chickens roosted (Gurevitz et al., 2011). Any individual structure that provided eventual shelter and a host-feeding source to triatomines was defined as a site.

2.2. Study design

A prospective dynamic-cohort study of house- and site-level infestation and abundance of triatomines was conducted between late 2007 and October 2010. The preintervention vector survey was carried out in September and November 2007 before a community-wide campaign of house spraying, where all sites from every house were sprayed with suspension concentrate insecticide deltamethrin (K-Othrin, Bayer) at standard dose (25 mg/m^2) by vector control personnel (Gurevitz et al., 2011). Postintervention vector surveys were repeated every 4–6 months postspraying (MPS) over nearly 3 yr, totaling seven surveys, and all houses infested with *T. infestans* were selectively sprayed with insecticide (Gurevitz et al., 2013). In accordance with national vector control guidelines, the catch of *T. sordida* did not prompt an insecticide application unless it was inside human bedrooms.

Timed-manual searches for triatomines were conducted by two skilled field technicians using 0.2% tetramethrin spray (Espacial, Argentina) as a dislodging agent in all sites of each inhabited or uninhabited house and public buildings as described previously (Gurevitz et al., 2011). One person searched each domestic site for 20 min, while another person searched each peridomestic site for 15 min, and both tried to collect as many triatomines as possible. In practice, because most sites were thoroughly inspected before finishing the stipulated time period, the total search effort was roughly similar across sites. Householders were asked to contribute to enhanced vector detection by capturing triatomines and storing them in labeled self-sealing plastic bags until the next survey.

All triatomines collected were identified to species and counted according to stage and sex immediately after capture (Gurevitz et al., 2011; Rodríguez-Planes et al., 2016). Any site with at least one nymph collected was considered to be colonized. The geographic position of each site was registered with a GPS (GeoXM; Trimble Navigation Ltd, Sunnyvale, CA) with an error of 1–3 m. Distances between sites < 3 m were corrected using a sketch map showing the relative position of all sites within each house.

2.3. Data management and analysis

The information collected over the seven postintervention surveys was used to characterize the distribution and relative abundance of *T. sordida* across all ecotopes within each inhabited house over time. We only considered inhabited or occupied houses (i.e., excluded public buildings such as healthcare posts, abandoned huts and other vacant structures) to allow direct comparisons between timed-manual catches and householders' bug collections over time; therefore, the number of house units inspected for infestation differ slightly from those reported before (Gurevitz et al., 2011, 2013). The relation between the future presence of *T. sordida* at site level and insecticide applications directed against *T. infestans* was tested using a three-way contingency table. Data collected at the same sites in consecutive surveys (at t and t+1) were classified according to whether the sites had been sprayed with pyrethroid insecticide between time t and t+1, and for the presence/absence of *T. sordida* at t and t+1. Sites where the presence of *T. sordida* was not registered across the 3-yr period were excluded from the analysis. The effect of insecticide application on the future presence of *T. sordida* conditional on its current presence/absence was tested using a Cochran-Mantel-Haenszel test (see below).

The relation between the total relative abundance of *T. sordida* per house and time-variable factors was analyzed using a generalized linear model with a correlation structure between repeated measures over the same house (generalized estimating equations, GEE) (Ballinger, 2004; Rodríguez-Planes et al., 2016; Zeger et al., 1988). The dependent variable is the total catch over all sites within a house compound, and consists of count data described by a Poisson distribution. All triatomines collected over all sites and ecotopes from each house at a given occasion were pooled for analysis of the temporal pattern of infestation. The explanatory variables were residual insecticidal spraying at house level over the previous six months (a binary factor, with the reference level given by the prior occurrence of an insecticide spray), and seasonality (a binary factor; fall: May and June, taken as the reference level, and spring: October-December, to distinguish between before and after winter bug mortality). By adopting these reference levels, all regression coefficients represent changes in direction of increasing numbers of *T. sordida*. All possible interactions between factors were considered. The database included complete data for 260 identified houses over seven survey occasions. The strength of the correlation over time declined in about 1 to 2 time steps, specifying a first-order autoregressive structure as the working correlation matrix. The goodness-of-fit of the nested full and reduced models was evaluated by the Wald statistic (Quinn and Keough, 2002). A quasi-likelihood information criterion (QIC) showed qualitatively similar results (not shown). All analyses were implemented using the statistical platform R 5.1.0 (R Core Team, 2014), the geepack package and geeglm function (Højsgaard et al., 2006; Yan, 2002; Yan and Fine, 2004).

We analyzed the conditional independence between the relative presence/absence of *T. sordida* in relation to the presence/absence of *T. infestans* adjusted for ecotope relevance to *T. sordida* (classified in three levels: prime, secondary and unsuitable habitats) and stratified by MPS (classified in eight levels, ranging from 0 to 35 MPS) by means of the Cochran-Mantel-Haenszel test implemented in the mhodds command in Stata 15.1 (StataCorp, 2018).

We investigated the bivariate fixed-point pattern of houses infested with triatomines as determined by timed-manual searches. The spatial association (aggregated, random or in repulsion) between houses infested with each and both triatomine species was analyzed with L(r) function (Fortin and Dale, 2005), the linearized version of K-function developed by Ripley (1976). L(r) quantifies coincidence events of both triatomine species inside circles centered on houses and over various radii, which was compared against values obtained by 99 Monte Carlo simulations of a random labeling process (Wiegand and Moloney, 2004). The vector data from postintervention surveys conducted over the same year (2007, 2008, 2009 and 2010) were collapsed into one bivariate annual pattern, with houses infested with both triatomine species being part of each annual point pattern regardless of whether these infestations were concurrent within any of these years or not. The radial distances covered were 300, 500, 1,000, 1,500 and 2,000 m. These analyses were conducted with Programita (2014 version, Wiegand and Moloney, 2004).

3. Results

The area-wide insecticide spraying campaign nearly halved the prevalence of house infestation with *T. sordida* (determined by timed-manual searches) from 20.7% before interventions to 8.3% at 8 MPS, which thereafter fluctuated little from 12.2 to 17.8% between 17 and 35 MPS (Fig. 1A). Householders' bug collections revealed the domestic invasion of adult *T. sordida* at rather similar rates before and after the insecticide spraying campaign. Householders' collections of *T. sordida* totaled 106 adult insects, many of which were caught indoors; occurred in 2.7% of the inhabited houses (range, 0.6-3.8%), and displayed no obvious time trend (Fig. 1B). The total number of *T. sordida* caught by timed-manual catches and householders' bug collections were not significantly related ($r = 0.03$, 6 df, $P > 0.9$). Neither were related the catch of both species by timed-manual searches ($r = 0.02$, 6 df, $P > 0.9$) or householders' collections ($r = -0.45$, 6 df, $P > 0.2$). By contrast, house infestation with *T. infestans* declined from 42.1% to 0.9% and total catch from 2,042 to 19 specimens from 0 to 35 MPS, approximating a stepwise trajectory (Fig. 1A). Householders' collections for *T. infestans* follow an analogous trajectory (Fig. 1B). Detailed analyses on inhabited houses infested with *T. infestans* were published by Gurevitz et al. 2011 (preintervention time) and Gurevitz et al. 2013 (surveillance through 35 MPS).

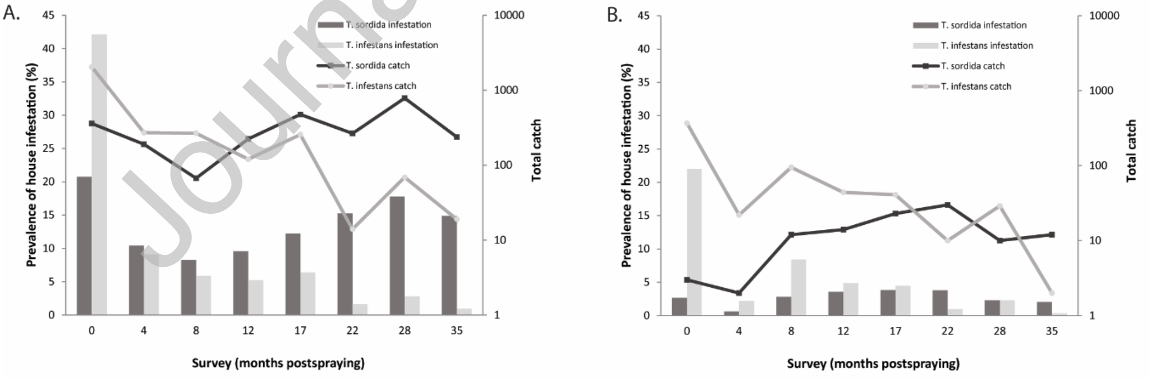

Fig. 1. Prevalence of house infestation with *T. sordida* and *T. infestans* and total catch as determined by timed-manual searches (A) and by householders' bug collections (B) before (late 2007) and after (2008-2010) an area-wide insecticide spraying campaign in Pampa del

Indio, Chaco, Argentina. Full results of survey 0 for *T. infestans* were published in Gurevitz et al. (2011) and for *T. sordida* in Rodríguez-Planes et al. (2018).

Taking together the seven postintervention surveys including 18,031 domestic or peridomestic sites searched for triatomines, the 147 (0.8%) sites positive for *T. infestans* yielded 1,013 insects mostly concentrated over 4-17 MPS (Gurevitz et al. 2013); the 370 (2.1%) sites positive for *T. sordida* yielded 2,226 insects (Table 1). The preintervention survey had revealed 193 sites positive for *T. infestans* (Gurevitz et al., 2011) and 78 or *T. sordida* among 2,177 sites inspected by timed-manual searches (Rodríguez-Planes et al., 2018).

Nearly 75% of *T. sordida* were collected in chicken nests (56.9%) and chicken trees (18.6%), i.e., the prime habitats after interventions (Table 1). Goat or sheep corrals, chicken corrals and pig corrals had similar total bug catches and numbers of infested sites; they are taken as secondary habitats because their indices were lower than those for prime habitats. Despite being rare, granaries had the maximum colonization rate and a rather large mean bug catch. Secondary habitats including granaries accounted for 20.6% of the total bug catch. Taking all surveys together, prime and secondary habitats occurred in 33.7-68.7% of the houses. Nymphs were rare (< 1%) in kitchens, storerooms, latrines, cow or horse corrals and human bedrooms, and no mud oven was ever found to be infested.

Table 1

Ecotope-specific infestation with *T. sordida* over a 3-yr follow-up (2008-2010) after an area-wide insecticide spraying campaign in Pampa del Indio, Chaco, Argentina.

Ecotopes[a]	No. of sites inspected	No. of sites positive[b] (%)	No. of sites colonized[c] (%)	No. of triatomines collected	Mean catch per survey (SD)	Maximum ecotope occurrence (%)[d]
Chicken nest	1041	125 (12.0)	100 (80.0)	1267	181.0 (180.1)	34.3
Chicken tree	2385	99 (4.2)	67 (67.7)	413	59.0 (38.6)	68.7
Goat or sheep corral	1031	38 (3.7)	26 (68.4)	107	15.3 (5.8)	35.0
Chicken corral	1332	31 (2.3)	23 (74.2)	116	16.6 (8.0)	33.7
Pig corral	1412	30 (2.1)	22 (73.3)	146	20.9 (16.9)	41.6
Others[e]	892	14 (1.6)	12 (85.7)	42	8.4 (9.1)	28.4
Granaries	272	10 (3.7)	8 (80.0)	90	22.5 (21.3)	10.0
Human beedrooms	2944	8 (0.3)	2 (25.0)	17	5.7 (7.2)	97.9
Latrines	1957	6 (0.3)	5 (83.3)	19	4.8 (5.6)	69.2

Cow or horse corral	859	4(0.5)	4 (100)	4	1.3 (0.6)	38.3
Storeroom	1016	3 (0.3)	2 (66.7)	3	1	35.2
Kitchen	1506	2 (0.1)	1 (50.0)	2	1	54.5
Mud oven	1384	0	-	0	0	52.5
Total	18031	370 (2.1)	272 (73.5)	2226	318.0 (237.2)	

[a] This table and all subsequent analyses exclude 11 public buildings (e.g., schools, churches, and primary healthcare posts) and uninhabited houses without outbuildings, where no infestation was ever found.

[b] Positive sites in relation to the total of sites.

[c] Colonized sites in relation to positive sites.

[d] Measures the commonness of each type of ecotope across all houses and surveys.

[e] Mainly includes an small chapel, abandoned domiciles or vehicles, and stacked materials where chickens nested.

Prime habitats were consistently the most productive of *T. sordida* on every survey (Fig. 2). The total catch of *T. sordida* declined after the area-wide insecticide spraying (when approximately 2,000 sites were treated) down to the 2008 winter (August); recovered by the 2009 fall, and then peaked at the 2010 fall (when less than 100 sites had been sprayed over the previous 6 months). The composition of the peridomestic environment varied slightly over time, with habitats associated with chickens predominating throughout (Fig. A.1). Chicken trees almost increased twofold over the follow-up, possibly due to improved registration.

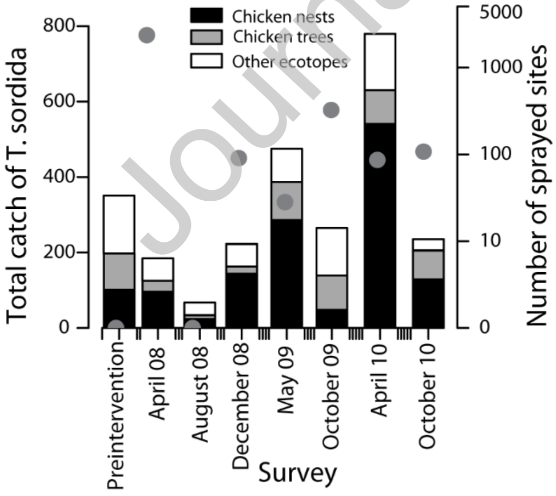

Fig. 2. Total catch of *Triatoma sordida* per unit effort before (late 2007, Rodríguez-Planes et al. 2018) and after (2008-2010) an area-wide insecticide spraying campaign (bars, left axis) in Pampa del Indio, Chaco, Argentina. Black and gray bars identify chicken nests and

chicken trees, respectively. The dots represent the frequency of sites sprayed with insecticide over the six months that preceded each survey (right axis, log scale).

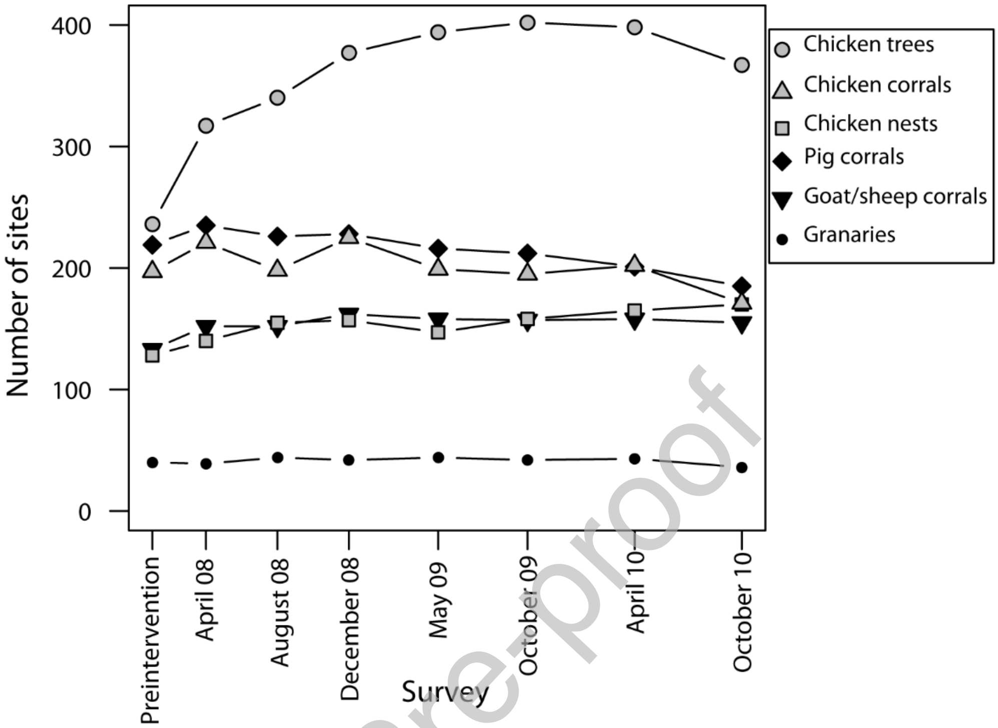

Fig. A.1. Ecotope-specific frequency of peridomestic sites registered in each survey conducted before (late 2007, Rodríguez-Planes et al. 2018) and after (2008-2010) an area-wide insecticide spraying campaign in Pampa del Indio, Chaco, Argentina, regardless of whether the ecotopes were inspected for infestation or not. Habitats associated with chickens appear in gray.

The total catch of *T. sordida* on a given survey occasion was inversely related to the absolute frequency of sites sprayed with insecticide 6, 12 and 18 months before the vector survey, i.e., time lags of 0, -1 and -2, respectively (Fig. A.2). The relative odds of future site infestation with *T. sordida* between two consecutive time steps significantly decreased with the application of insecticide when sites were stratified by ecotope type (Table 2, Cochran-Mantel-Haenszel $\chi^2 = 6.57$, 1 df, $P = 0.01$; OR = 0.63; 95% confidence interval (CI), 0.44-0.90). When *T. sordida*-positive sites were taken separately, a pyrethroid application nearly halved the relative odds of future infestation (OR = 0.49; CI, 0.28–0.88) and reduced the infestation rate from 40.7 to 25.3%, whereas the treatment of sites non-infested with *T.*

sordida did not modify the odds significantly (OR = 0.71; CI, 0.46–1.10) but still reduced infestation from 10.8 to 7.9%.

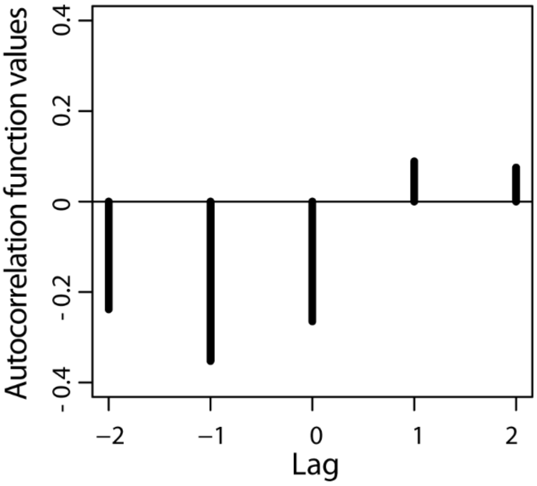

Fig. A.2. Cross-correlation analysis of the total catch of *Triatoma sordida* and the time-lagged number of sites sprayed with pyrethroid insecticide before the vector survey in Pampa del Indio, Chaco, Argentina (2008-2010). Autocorrelation function (ACF) values in the y axis.

Table 2
Relationship between current insecticide application (between time t and t+1) and future site infestation with *T. sordida* (at t+1) according to the current presence/absence of *T. sordida* (at t) over a 3-yr period (2008-2010) in Pampa del Indio, Chaco, Argentina.

T. sordida at time t	Insecticide spray between t and t+1	*T. sordida* at time t+1 Present (%)	Absent	Total
Present	Yes	23 (25.3)	68	91
Present	No	57 (40.7)	83	140
Absent	Yes	27 (7.9)	313	340
Absent	No	129 (10.8)	1068	1197
Total		236 (13.3)	1532	1768

The catch of *T. sordida* at house level was best described by the model that included insecticide application, season, and no interaction term (Table 3). Insecticide spraying reduced the abundance of *T. sordida* by 61% when the estimated coefficients were expressed in terms of total catch per house (i.e., after applying the inverse of the link function *log*). On average, spring vector surveys collected 59% fewer triatomines than fall surveys.

Table 3

GEE analysis of the total catch of *T. sordida* at house level over a 3-yr period (2008-2010) in Pampa del Indio, Chaco, Argentina.

Effects	Estimate	Standard error	Wald statistic	P
Intercept	0.74	0.20	13.47	< 0.001
Insecticide spray	-0.95	0.36	6.88	< 0.01
Season	-0.87	0.25	11.77	< 0.001

The site-level occurrence of *T. sordida* was highly significantly and positively associated with the presence of *T. infestans* adjusted for ecotope relevance over time postspraying (Table 4, Cochran-Mantel-Haenszel χ^2 = 198.13, 1 df, P < 0.001), with no evidence of heterogeneity among surveys (χ^2 = 2.53, 7 df, P > 0.9). On average, the habitat-adjusted relative odds of catching one species was 10.8 times greater when the other species was present over time postintervention (summary OR = 10.80; CI, 7.13-16.37) (Table 5). The adjusted ORs for the presence of *T. sordida* on any survey ranged from 4.75 to 12.78 and were always statistically significant before and after intervention. Thus no evidence of species interference on site occupancy was registered across ecotopes over time. The overall frequency of co-occurrence observed postintervention was low, comprising 16 (3.2%) of 501 site searches of the three ecotope types found positive for any triatomine species (i.e., excluding double-negative site searches).

Table 4

Frequency of sites positive for *Triatoma sordida* and *Triatoma infestans* according to ecotope relevance for *T. sordida* before and after area-wide insecticide spraying (2008-2010) in Pampa del Indio, Chaco, Argentina. Full results of survey 0 were published in Gurevitz et al. (2011) and Rodríguez-Planes et al. (2018).

			Sites positive for							
			Both *T. sordida* and *T. infestans*		Only *T. sordida*		Only *T. infestans*		Negative sites for *T. sordida* and *T. infestans*	
Ecotope relevance for *T. sordida*	Survey (months postspraying)	No. of sites inspected	No.	%	No.	%	No.	%	No.	%
Prime	0	316	12	3.8	19	6.0	14	4.4	271	85.8
	4	666	4	0.	24	3.	9	1.4	629	94.

				6		6				4
	8	678	1	0.1	17	2.5	11	1.6	649	95.7
	12	682	1	0.1	19	2.8	6	0.9	656	96.2
	17	700	1	0.1	37	5.3	5	0.7	657	93.9
	22	711	0	0.0	40	5.6	1	0.1	670	94.2
	28	691	1	0.1	66	9.6	2	0.3	622	90.0
	35	630	0	0.0	44	7.0	1	0.2	585	92.9
Secondary	0	543	10	1.8	24	4.4	9	1.7	500	92.1
	4	386	1	0.3	9	2.3	1	0.3	375	97.2
	8	361	0	0.0	12	3.3	0	0.0	349	96.7
	12	354	1	0.3	11	3.1	0	0.0	342	96.6
	17	363	1	0.3	7	1.9	1	0.3	354	97.5
	22	360	0	0.0	11	3.1	0	0.0	349	96.9
	28	320	0	0.0	11	3.4	0	0.0	309	96.6
	35	299	0	0.0	4	1.3	0	0.0	295	98.7
Unsuitable	0	1311	6	0.5	7	0.5	142	10.8	1156	88.2
	4	1692	1	0.1	5	0.3	34	2.0	1652	97.6
	8	1584	2	0.1	3	0.2	16	1.0	1563	98.7
	12	1435	0	0.0	2	0.1	10	0.7	1423	99.2
	17	1579	0	0.0	9	0.6	21	1.3	1549	98.1
	22	1621	1	0.1	12	0.7	4	0.2	1604	99.0
	28	1483	1	0.1	6	0.4	6	0.4	1470	99.1
	35	1436	0	0.0	5	0.3	3	0.2	1428	99.4
Total postintervention (4 - 35 MPS)		18031	16		354		131		17530	

Table 5

Odds ratios and 95% confidence intervals (CI) for the frequency of sites positive for *Triatoma sordida* and *Triatoma infestans* according to ecotope relevance for *T. sordida* before and after area-wide insecticide spraying (2008-2010) in Pampa del Indio, Chaco, Argentina. Full results of survey 0 were published in Gurevitz et al. (2011) and Rodríguez-Planes et al. (2018).

Survey (months postspraying)	Odds Ratio	95% confidence interval	χ^2	P
0	12.41	6.88 - 22.38	115.8	<0.001
4	12.78	4.64 - 35.19	40.62	<0.001
8	9.57	2.76 - 33.20	19.08	<0.001
12	10.65	2.07 - 54.82	12.5	<0.001
17	4.75	1.03 - 21.78	4.89	0.027
22	11.52	1.49 -89.33	8.8	0.003
28	8.78	1.66 - 46.54	9.53	0.002

Fig. 3 shows the joint spatial distribution of *T. sordida* and *T. infestans* at house level in each of the surveys conducted over 0-17 MPS. Fig. A.3 shows the distribution maps for the period 22-35 MPS. The joint occurrence of both species (collapsed by year) was significantly and positively associated over short distances (300 and 500 m) both before and after area-wide insecticide spraying, remaining virtually invariant over time (Fig. 4). The intensity of the spatial aggregation was greater before interventions and over the first year postintervention (2008). Both triatomine species were always randomly distributed at broader spatial scales ranging from 1,000 to 2,000 m.

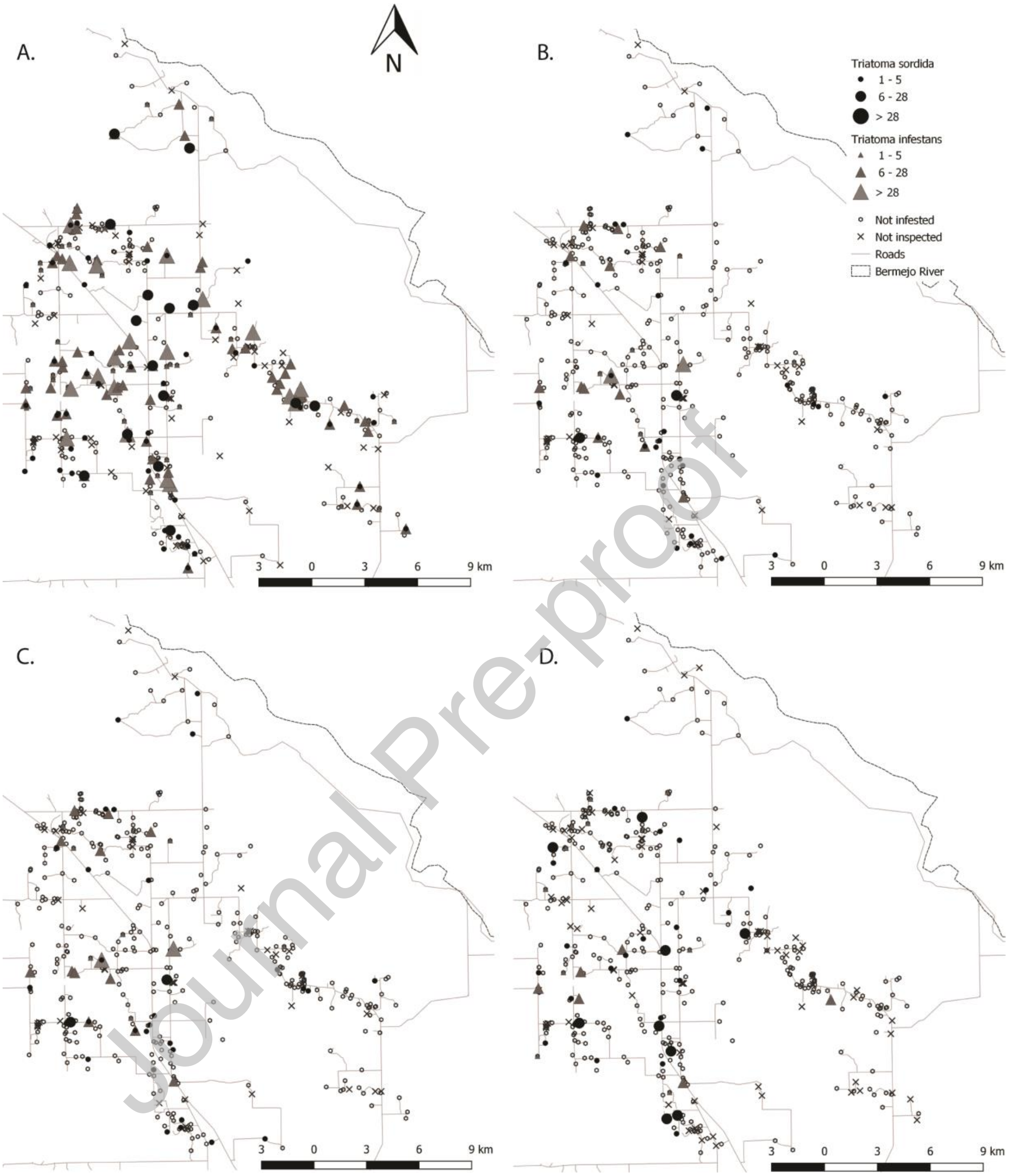

Fig. 3. Joint distribution of the relative abundance of *T. sordida* and *T. infestans* per house as determined by timed-manual collections. Pampa del Indio, Chaco, Argentina, 2007–2008. Full results of survey 0 were published in Gurevitz et al. (2011) and Rodríguez-Planes et al. (2018).

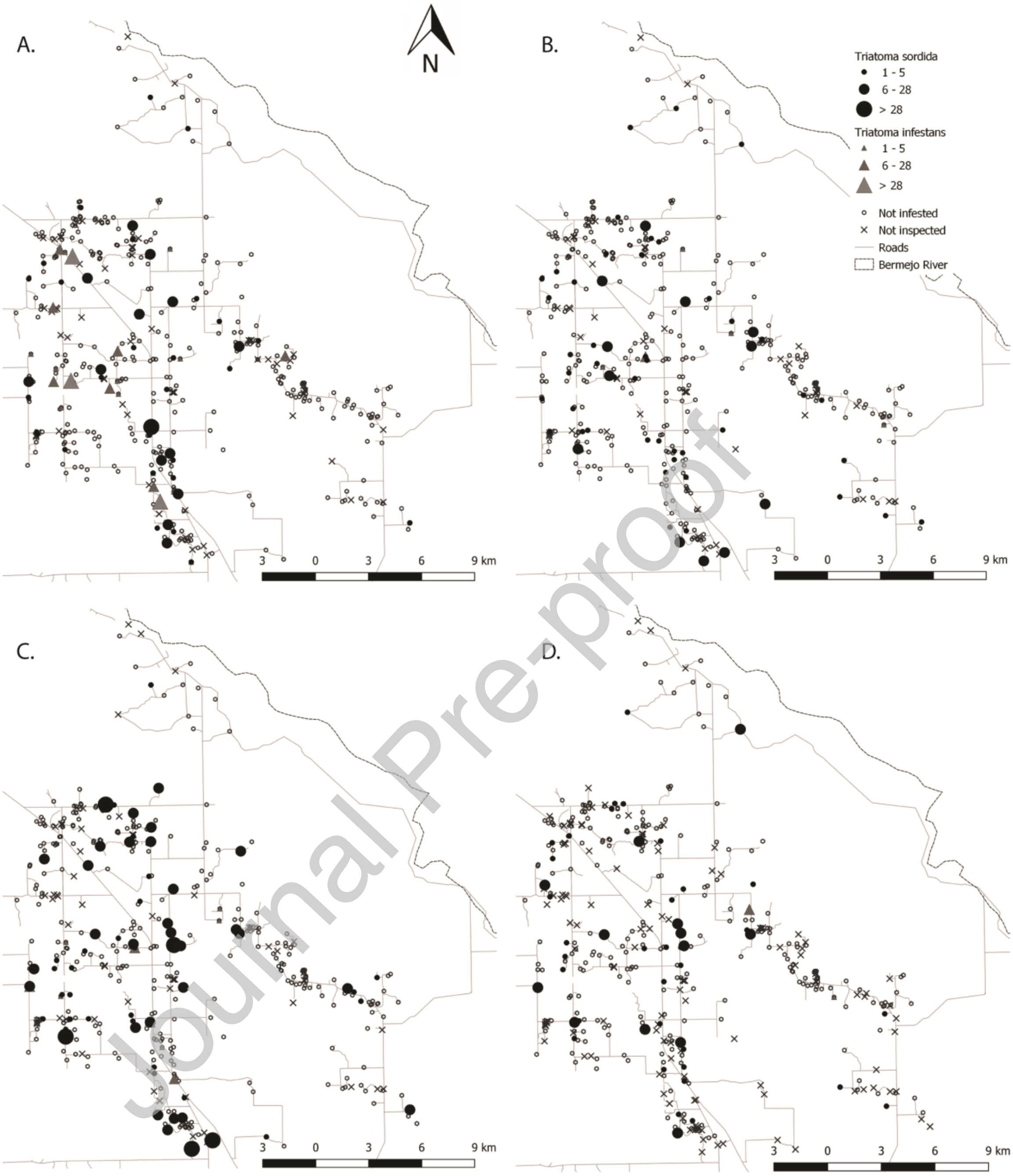

Fig. A.3. Joint distribution of the relative abundance of *T. sordida* and *T. infestans* per house as determined by timed-manual collections. Pampa del Indio, Chaco, Argentina, 2009–2010.

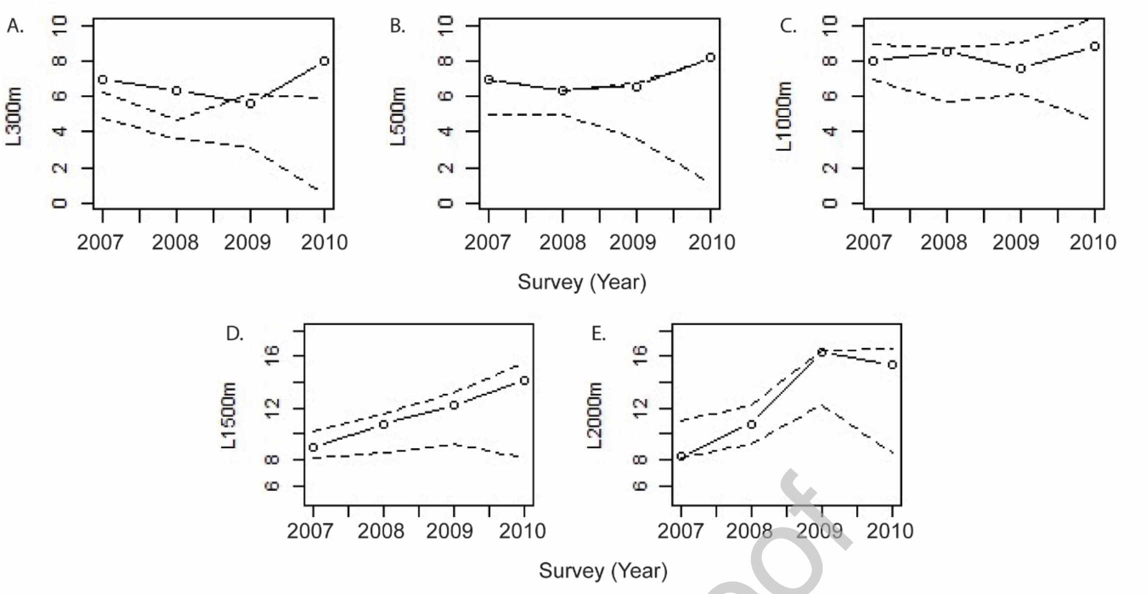

Fig. 4. Bivariate point pattern analysis for house infestation with *Triatoma sordida* and *Triatoma infestans* at house level for different grain sizes (A-E) and years before (2007, Gurevitz et al. 2011 and Rodríguez-Planes et al. 2018) and after (2008-2010) area-wide insecticide spraying in Pampa del Indio, Chaco, Argentina. The observed statistics L1,2 (r) are shown as empty circles; the expected values under the null model as light grey lines, and confidence envelopes as dashed lines.

4. Discussion

Our study documents the invariance of habitat occupancies of *T. sordida* under area-wide and selective chemical control, including the persistent failure to colonize human habitations as *T. infestans* declined (i.e., niche expansion); the short-term reductions in the abundance of *T. sordida* following insecticide treatments targeting *T. infestans*, with subsequent fast recovery and marked seasonal fluctuations, and the strong positive association between both triatomine species at fine spatial scales both before and after interventions.

4.1. Habitat occupancy

The prime and secondary ecotopes of *T. sordida* recorded at baseline remained virtually unmodified after area-wide insecticide spraying combined with selective treatments of *T. infestans*-positive houses. All chicken-related ecotopes were of prime relevance before interventions; goat or sheep corrals and pig corrals were secondary habitats, and the rest unsuitable ones (Rodríguez-Planes et al., 2018). Only chicken corrals changed from being classified as prime to secondary habitat after control interventions. Chicken nests were the most important ecotopes for *T. sordida*, showing maximum levels of infestation prevalence, total catch, and frequency of colonies at every postintervention survey. Chicken trees followed in relevance, with less productive though more frequent colonies occurring in 70% of the houses. Despite the large decline of *T. infestans*, the proportion of *T. sordida*-infested sites in goat or sheep corrals and in pig corrals remained at similar levels after interventions.

Granaries were a special case. Despite being rare, *T. sordida* was especially abundant in granaries, where 80% of infestations included high-density colonies. Granaries usually are filled with corn or other crops, and therefore are seldom inspected for triatomines and sprayed by vector control personnel, thus serving as a persistent source of *T. infestans* (Cohen et al., 2017). Even when they are inspected, the limited sensitivity of timed-manual searches (Abad-Franch et al., 2010; Gürtler et al., 2001) and the intricate nature of stored items increase the chance of “false negative” results unless bug abundance is high. Food storage structures were also considered a risk factor for the presence and abundance of *T. sordida* in Brazil (Rossi et al., 2015).

The broad spectrum of habitats colonized by *T. sordida* may be partially linked to its eclectic host-feeding patterns on a wide array of sylvatic and domestic host species, including birds, humans and other mammals (Diotaiuti et al., 1993; Falavigna et al., 2001; Lorosa et al., 2000; Rabinovich et al., 2011). No host-feeding preference for birds to mammals was demonstrated experimentally (Crocco and Catalá, 1997). In addition, the little variations in habitat ranking before and after area-wide interventions support the notion of secondary habitats as stepping stones and access ways to every peridomestic and domestic site (Rodríguez-Planes et al., 2018), facilitating the arrival of *T. sordida* despite of its rather limited flight ability (Dantas et al., 2018).

Triatoma sordida failed to colonize human sleeping quarters and peridomestic sites built in a similar way (e.g., kitchens, storerooms), except occasionally and ephemerally, during a 3-yr period in which *T. infestans* abundance was sharply reduced and virtually eliminated (Table 4, Fig. A.3C,D), leading to maximum frequencies of uninfested (empty) domestic and peridomestic sites. Both timed-manual searches and householders' collections of *T. sordida* in human sleeping quarters and similar habitats mostly included adult specimens, indicating the species was truly able to invade peridomestic and domestic premises but then failed to colonize them. Nymphs of *T. sordida* were found only on 4 (0.12%) occasions in 3,314 inspections of human sleeping quarters using a dislodgant aerosol: two sites before the attack phase and two at the first postintervention survey, with no subsequent evidence of domestic colonization up to 35 MPS.

This situation differs from the domestic colonization patterns of *T. sordida* in Brazil, where it was apparently close to domiciliation in areas where *T. infestans* had been suppressed or where dramatic land-use changes occurred (Dias, 1988; Diotaiuti et al., 1993; Forattini et al., 1983), and in some areas of Paraguay (Gonzalez-Britez et al., 2014; Sánchez et al., 2016) though not all (Acosta et al., 2017). In contrast, our results qualitatively agree with the occupancy patterns of *T. sordida* in Corrientes, northeastern Argentina (Bar et al., 2002) and in the dry (western) Paraguay (Acosta et al., 2017; Gonzalez-Britez et al., 2014). In our study area, *T. sordida* was predominantly peridomestic or sylvatic (Waleckx et al., 2015), compatible with the new chromosomal taxon *T. sordida Argentina* (Gonzalez-Britez et al., 2014).

4.2. *Insecticide effects and seasonality*

Insecticide applications targeting sites with *T. infestans* significantly reduced the site-level abundance of *T. sordida* and the relative risk of future site infestation but it did not suppress peridomestic colonies nor modified the house infestation prevalence (proportion of infested houses) with *T. sordida* over time. The population displayed fast recovery and marked seasonal fluctuations at site level only. Insecticide effects coincided both at site- and house-level and suggested *T. sordida* was susceptible to pyrethroid insecticides. We note, however, that *T. sordida* populations from Argentina have not been screened for susceptibility to pyrethroids. In Brazil, this species revealed low resistance ratios (2-7) despite it had not been targeted for control (Pessoa et al., 2015). The protective effects of insecticide treatment on future infestation were not detected in *T. sordida*-negative sites, unlike for the sibling species *T. garciabesi* (Rodríguez-Planes et al., 2016). In contrast to *T. sordida* patterns, both the house infestation and relative catch of *T. infestans* by both methods declined steadily over time (Fig. 1).

The relative abundance of *T. sordida* peaked in the fall (before winter mortality) and plummeted by spring, likewise for *T. garciabesi* in the dry Chaco (Rodríguez-Planes et al., 2016). This pattern matches an annual dynamics of slow population recovery towards mid-spring (Forattini et al., 1983; Gorla and Schofield, 1985). Overall infestation numbers before (318 bugs) and after (mean, 355 bugs) area-wide control interventions barely differed despite systematic bug removal. Given the long developmental time of *T. sordida* (6-8 months from egg to adult under near-optimum laboratory conditions: Carcavallo and Martínez, 1972; Guarneri et al., 2000), the fast recovery of population numbers over each four- to six-month period is hard to explain on the sole basis of a pure birth-death process. Although a fraction of all peridomestic triatomines likely survived spraying with pyrethroids (which are less effective outdoors), frequent invasion of adult specimens from peridomestic outhouses or the numerous sylvatic habitats around houses was most likely. *T. sordida* was the only triatomine species found in local sylvatic habitats using Noireau traps (Alvarado-Otegui et al., 2012). Whether land cover around houses and its change over time are positively associated with the occurrence of *T. sordida* foci or house invasion remains untested, and is especially relevant given the long-standing and ongoing deforestation of the Argentine Chaco (Vazquez-Prokopec et al., 2008).

4.3. Species co-occurrence

Our study documents that the site-level occurrence of *T. sordida* or *T. infestans* was approximately 11 times more likely to occur when the other species was present than when it was not, in stark contrast to expectations if antagonistic interactions occurred between both triatomine species.. No evidence of a negative interaction between both species was recorded even after allowing for ecotope and intervention status (before/after). These findings, based on detailed observations at various scales, contrast with the inferences drawn from cross-sectional or long-term occupancies pooled at a village-wide level suggesting that *T. infestans* had the ability to displace *T. sordida* (García-Zapata and Marsden, 1992; Noireau et al., 1996). Moreover, the best experimental evidence available suggesting competitive displacement between these species was obtained in three small brick-made houses kept indoors at room temperature, each harboring one chicken and high-density (two pure and one mixed) triatomine populations (Oscherov et al. 2004). Such abundant populations of *T. sordida* have very rarely been recorded (but see Rodríguez-Planes et al., 2018), and indoor conditions largely differ from those that prevail in peridomestic chicken nests where both species co-occur. Before control interventions, when *T. infestans* was abundant, both species were positively associated at site level in prime and secondary habitats, and occurred independently in other unsuitable ecotopes such as storerooms, kitchens, and human sleeping quarters (Rodríguez-Planes et al., 2018). After the decline of *T. infestans* following area-wide interventions, we also found a strong positive association between species at site level. Both species also co-occurred in chicken nests in the Paraguayan Chaco (Acosta et al., 2017). The spatial autocorrelation between both species was also evident at small distances (300-500 m) both before and long after area-wide interventions, an association probably derived from landscape effects that are beyond the scope of the present study. These patterns do not support an antagonistic interaction and displacement of *T. sordida* in the presence of *T. infestans* at the recorded vector density levels.

The aggregate pattern between both species needs to be examined in the light of several processes. First, although aggregation is expected to increase competitive interactions possibly leading to density-dependent negative effects on mortality and fertility, high-density populations of *T. infestans* under natural climatic conditions (Gorla and Schofield, 1985, 1989) and experimental (Rodriguez and Rabinovich, 1980) or field (Rabinovich, 1985) populations of *Rhodnius prolixus* provided no evidence of density-dependent mortality.

Second, competitive displacement is unlikely to appear in the absence of shared resource constraints (Cecere et al., 2003; Rodríguez-Planes et al., 2018), such as hiding places and host-feeding sources. Limited shelters are the basis on which the use of artificial shelter units as triatomine detection devices is justified (Monroy et al. 1998, Gürtler et al. 2001). Nevertheless, there is *a priori* no reason to expect differential responses for both triatomine species. Limited access to host-feeding sources may cause partial blood meals, subsequent reductions in fertility, and increased developmental times (Schofield, 1980b, 1982). The number and quality of available hosts provide the scenario where competition for blood-feeding occurs. However, the relation between triatomine abundance and host availability is modified by several factors (Gürtler et al., 2014; Levy et al., 2014). For eclectic species such as *T. sordida* and *T. infestans* (Galvão and Justi, 2015), virtually every domestic host represents a potential blood-feeding source. Therefore, the typical host availability in an average rural study house likely exceeds the requirements of all triatomine populations. Competition for blood-feeding sources seems unlikely in scenarios of low-density triatomine populations.

Third, triatomines use chemical signals to mark suitable refuges in a rather unspecific manner (Cruz-Lopez et al., 1993; Mota et al., 2014). Assembling factors in the feces of *T. sordida* and *T. infestans* nymphs induced aggregation in either of them, thus acting as a pheromone and kairomone (Lorenzo Figueiras and Lazzari, 1998). Our current results are consistent with this experimental evidence, and strongly suggest the occurrence of *T. sordida* (or its frass) may signal suitable sites for either species and hence induce a positive association.

The question on whether both triatomine species were under a competitive interaction may also be addressed in the frame of Taylor's Law (TL), which relates the sample variance to the sample mean of population density or abundance through a linear relationship on log-log coordinates. For various triatomine species occurring in discrete habitat types before and after area-wide insecticide spraying in the Argentine Chaco, the data strongly supported the spatial form of TL and showed that insecticide campaigns did not modify the slope coefficients for *T. infestans* and *T. sordida* (Cohen et al., 2017). Although Kilpatrick and Ives (2003) have interpreted such type of outcome as evidence of absence of competition, exploitation competition did not modify the slope of TL in bacteria (Ramsayer et al., 2012). Thus we cannot exclude the occurrence of competitive displacement at much higher population densities than those recorded here or through a different mechanism.

The interpretation of our findings is limited by imperfect detectability, a common issue in presence/absence data, which affects the estimation of abundance, occupancy and occurrence (Rodríguez-Planes et al., 2018). The relevant questions are whether there is a differential detectability between triatomine species, and whether detectability varies seasonally (in response to temperature variations affecting bug activity) and among habitats at postintervention bug densities. Unfortunately, there is no evidence showing whether active manual searches of triatomines are biased toward any species, or whether the response to the dislodgant spray differs substantially between triatomine species (Diotaiuti et al., 2000). Habitat effects on detectability are more likely to occur between prime and secondary habitats (which differ in physical structure) than within each of them; thus temporal comparisons within habitat types would be less affected than between-habitat contrasts.

5. Conclusions

Our results suggest that *T. sordida* currently does not pose any apparent risk of domestic colonization in the humid Argentine Chaco. Its typical association with chickens in peridomestic areas and rather low infection rates, combined with apparent susceptibility to professional residual spraying with pyrethroid insecticides, suggest minimal risks for human health. However, *T. sordida* may contribute to peridomestic transmission cycles in association with several synanthropic mammals, including rodents and opossums (Macchiaverna et al., 2015). The habitat occupancies of *T. sordida* may be used as a proxy of their potential infestation with *T. infestans*, especially in areas where *T. infestans* populations have become rare and vector detection is difficult. Although vector control programs in Argentina routinely collect this information, it is not used for any definite purpose. The postintervention habitat occupancies of *T. sordida* may guide targeted vector control actions, including surveillance and environmental management measures. More research efforts on the precise taxonomic identity and relevance of *T. sordida* as a secondary vector across its large geographic range are required.

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Declaration of competing interests

The authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version

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Fig. 1. Prevalence of house infestation with *T. sordida* and *T. infestans* and total catch as determined by timed-manual searches (A) and by householders' bug collections (B) before (late 2007) and after (2008-2010) an area-wide insecticide spraying campaign in Pampa del Indio, Chaco, Argentina.

Fig. 2. Total catch of *Triatoma sordida* per unit effort before (late 2007) and after (2008-2010) an area-wide insecticide spraying campaign (bars, left axis) in Pampa del Indio, Chaco, Argentina. Black and gray bars identify chicken nests and chicken trees, respectively. The dots represent the frequency of sites sprayed with insecticide over the six months that preceded each survey (right axis, log scale).

Fig. 3. Joint distribution of the relative abundance of *T. sordida* and *T. infestans* per house as determined by timed-manual collections. Pampa del Indio, Chaco, Argentina, 2007–2008.

Fig. 4. Bivariate point pattern analysis for house infestation with *Triatoma sordida* and *Triatoma infestans* at house level for different grain sizes (A-F) and years before (2007) and after (2008-2009) area-wide insecticide spraying in Pampa del Indio, Chaco, Argentina. The observed statistics L1,2 (r) are shown as black dots; the expected values under the null model as light grey lines, and confidence envelopes as dashed lines.

Fig. A.1. Ecotope-specific frequency of peridomestic sites registered in each survey conducted before (late 2007) and after (2008-2010) an area-wide insecticide spraying campaign in Pampa del Indio, Chaco, Argentina, regardless of whether the ecotopes were inspected for infestation or not. Habitats associated with chickens appear in gray.

Fig. A.2. Cross-correlation analysis of the total catch of *Triatoma sordida* and the time-lagged number of sites sprayed with pyrethroid insecticide before the vector survey in Pampa del Indio, Chaco, Argentina (2008-2010). Autocorrelation function (ACF) values in the y axis.

Fig. A.3. Joint distribution of the relative abundance of *T. sordida* and *T. infestans* per house as determined by timed-manual collections. Pampa del Indio, Chaco, Argentina, 2009–2010.

Graphical abstract