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Effect of high-volume insecticide spraying on sand fly vectors in household gardens in Spain

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Abstract

This study investigated the efficacy of high-volume spraying with the adulticide α-cypermethrin alone and in combination with the larvicide diflubenzuron on the density of sand flies in gardens of three detached households in periurban areas in southeast Spain. Treatments were applied four times between June and August 2016, and four nearby sites, two households and two non-urbanized sites, were untreated controls. The number of sand flies collected between May and October 2016 using sticky interception and light attraction traps, was 4446 specimens. Species identified morphologically included *Sergentomyia minuta* ($n=2101$; 48%), *Phlebotomus perniciosus* ($n=1922$; 44%), *Phlebotomus papatasi* ($n=173$; 4%), *Phlebotomus sergenti* ($n=161$; 4%) and *Phlebotomus ariasi* ($n=36$; 1%). Sand flies were detected in both treated and untreated sites. The proportion of positive sticky traps and the median (range) density of sand flies in positive traps were 61% traps and 7 (2–172) sand flies/m^2/day in untreated sites, and 43% traps and 4 (1–56) sand flies/m^2/day in treated sites ($p<0.05$). Similarly, for light traps, it was 96% traps and 30 (3–168) flies/trap/day, and 83% traps

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and 3 (1–12) sand flies/trap/day, respectively ($p < 0.05$). However, sand fly density followed a comparable seasonal pattern in untreated and treated sites and did not consistently decrease following insecticide applications. These results were confirmed with mixed negative binomial modelling of sand fly density adjusted for time since application, month, environmental setting and site. The limited efficacy of the treatments, added to their cost, the impact of insecticides on non-target organisms and human health, and the risk of development of insecticide resistance, should dissuade similar outdoor applications to control sand fly vector populations in residential areas.

KEYWORDS
diflubenzuron, insecticides, *Phlebotomus*, residential estates, sand fly control, α-cypermethrin

1 | INTRODUCTION

Phlebotomine sand flies are hematophagous insects (Diptera, Psychodidae) from tropical and subtropical climates, where they are responsible for the transmission of protozoan parasites *Leishmania* spp. and viruses. The latter include at least 11 *Phlebovirus* species affecting humans and are associated with a generally mild febrile syndrome, except for Toscana virus which causes sporadic cases of meningitis and meningoencephalitis (Lambert & Hughes, 2021). Leishmaniasis is a major disease of humans and animals caused by more than 20 *Leishmania* species. The estimated human leishmaniasis (HumL) annual morbidity rates are 30,000 visceral leishmaniasis cases, and one million cases of cutaneous and mucocutaneous leishmaniasis (WHO, 2023). The incidence of HumL is disproportionally high in developing countries, but it is an emerging disease in Europe associated mostly with acquired immunosuppression syndromes (ECDC, 2022). *Leishmania* spp. infections have been described in many domestic and wildlife mammalian species, although the majority of clinical cases are reported in dogs, caused by zoonotic *Leishmania infantum* infections (Miró et al., 2017; Ribeiro et al., 2018). This is the only endemic *Leishmania* species in Europe, where dogs are the domestic reservoir of infection, and canine leishmaniasis (CanL) is deemed the most important infectious disease of dogs in many areas of southern Europe (ECDC, 2022). One such ecosystem where the impact of CanL is high is wealthy suburban residential estates, where a high density of guard and companion dogs meets ideal conditions for sand flies to breed in protected and organic matter-rich gardens (Athanasiou et al., 2019; Campino & Maia, 2010; Goyena et al., 2016; Iliopoulou et al., 2018; Tarallo et al., 2010).

In the absence of highly effective vaccines, control of leishmaniasis caused by *L. infantum* relies largely on preventive insecticide treatments with high repellent activity on dogs. The most common chemicals used are synthetic pyrethroids such as permethrin and deltamethrin, in the form of collars, spot-on pipettes and sprays (Miró et al., 2017; Ribeiro et al., 2018). Their efficacy is high when the manufacturer's indications are strictly followed, but this may be very difficult to achieve in non-experimental situations (Goyena et al., 2016). Moreover, to attain community-wide control of *L. infantum*, preventive insecticide treatments must reach a large proportion of dogs (Maroli et al., 2001; Reithinger et al., 2004).

Impacts

- Outdoor high-volume spraying with the insecticides α-cypermethrin and diflubenzuron in periurban households had a low efficacy against sand fly vectors.
- Low efficacy was probably due to incomplete insecticide coverage in thick vegetation areas, lack of insecticide residual activity and sand fly repopulation from neighbouring gardens.
- Insecticide impact on non-target organisms and human health and risk of resistance development should dissuade similar outdoor applications to control sand fly vector populations in residential areas.

The alternating application of insecticides in the environment targeting resting adult and larval sand fly stages is an important control option, particularly for *Leishmania* species whose primary reservoirs of infection are humans and wildlife, and in areas with a high incidence of HumL. Insecticide interventions include indoor residual spraying, insecticide-treated bed nets, bed linen, clothes and durable wall lining, and space spray applications (Balaska et al., 2021). Outdoor space spraying around human households is uncommon because sand fly breeding and resting sites are not well characterized and licensed insecticides have short residual effects (Alexander & Maroli, 2003). Depending on whether the active ingredient is diluted or not and the amount delivered per unit area, insecticide application methods may be classified as high, low and ultra-low volume (HV, LV and ULV, respectively). The latter mode of application is the most efficient and the standard method for pest control in agriculture, and it was used against sand fly vectors in open fields in Kenia using organophosphates and pyrethroids (Britch et al., 2011) and pyrethroids in Greece (Chaskopoulou et al., 2018). They achieved 18% and 66% reduction in adult sand fly captures 24 h after treatment with a low and high insecticide dose, respectively, and in the Greek study, a four to six time-fold reduction in the mean sand fly number after six applications over a 4-year period (Chaskopoulou et al., 2018).

There are no similar studies evaluating the impact of space spraying application of insecticides on the local sand fly population in Mediterranean periurban residential areas with a high incidence of CanL. In the present study, we investigated the effectiveness of HV spraying of the chemical adulticide and larvicide pyrethroid, α-cypermethrin, alone and in combination with the insect growth regulator (IGR) diflubenzuron, against sand flies in the exterior plot of three detached homes in periurban residential areas of Murcia City in southeast Spain in 2016. With this method, a high volume of spray is applied and droplets combine producing a uniform liquid film on the treated surfaces (Mathews et al., 2014). Target sand flies were, therefore, primarily resting adults and soil-developing larval stages. A previous study in these sites detected *L. infantum* vectors *Phlebotomus perniciosus* and *Phlebotomus ariasi*, as well as *Phlebotomus papatasi*, *Phlebotomus sergenti* and *Sergentomyia minuta* (Muñoz et al., 2021). Four treatments between May and August were performed by a professional pest control operator using off-the-shelf products licensed for insect pest control. If effective, this was considered a practical and affordable sand fly control strategy for most community residents in the area.

2 | MATERIALS AND METHODS

2.1 | Study design, insecticide treatments and weather conditions

Murcia City, with a population of approximately 460,000 people, is 50 km inland from the southeast Mediterranean coast in Spain, and its geographical coordinates are 37°59′10″ N 1°07′49″ W (Figure 1). The present study was conducted in seven periurban sites on the outskirts of Murcia city where the presence of sand flies had been previously reported (Muñoz et al., 2021). They included the exterior plot (gardens) of five detached family houses (sites 2, 3, 4, 18 and 28 in Muñoz et al., 2021) and two nearby non-urbanized, country sites (sites 1 and 6 in Muñoz et al., 2021) (Figure 1, Table 1) (site pictures presented as supplementary material in Figures S1–S7). More detailed information on the characterization of the sites included in this study can be found in Muñoz et al. (2021).

Sand fly traps were placed continuously between 20/05/2016 and 24/10/2016 except from July 25th to August 26th, when it was not possible to access the house plots. High volume insecticide treatments were carried out in sites 2, 3 and 4, on four occasions in each site on 04/06/2016, 19/06/2016, 09/07/2016 and 27/08/2016, and sites 1, 6, 18 and 28 remained untreated (Figure 2; Table 1). Compounds were used following manufacturers dilution and delivery volume/area indications. These insecticides were α-cypermethrin 6% (Acaritron, Massó, Spain) diluted 1:100 in tap water, on the first and fourth treatments (Figure 2), and a combination of α-cypermethrin 6% at the same dilution with IGR diflubenzuron 48% (Larvigen, Bioplagen, Spain) similarly diluted 1:100, on the second and third treatments (Figure 2). According to label instructions, products act by contact or ingestion, and dilution in water allows them to penetrate through soil, organic matter and porous surfaces. Acaritron has rapid shock and long-term insecticidal effects, acting against adults and larval stages by blocking neural transmission, and some adult insect repellent action. Its environmental persistence was not described, but applications may be repeated every 15–30 days, depending on environmental conditions and pest insect pressure. Larvigen has

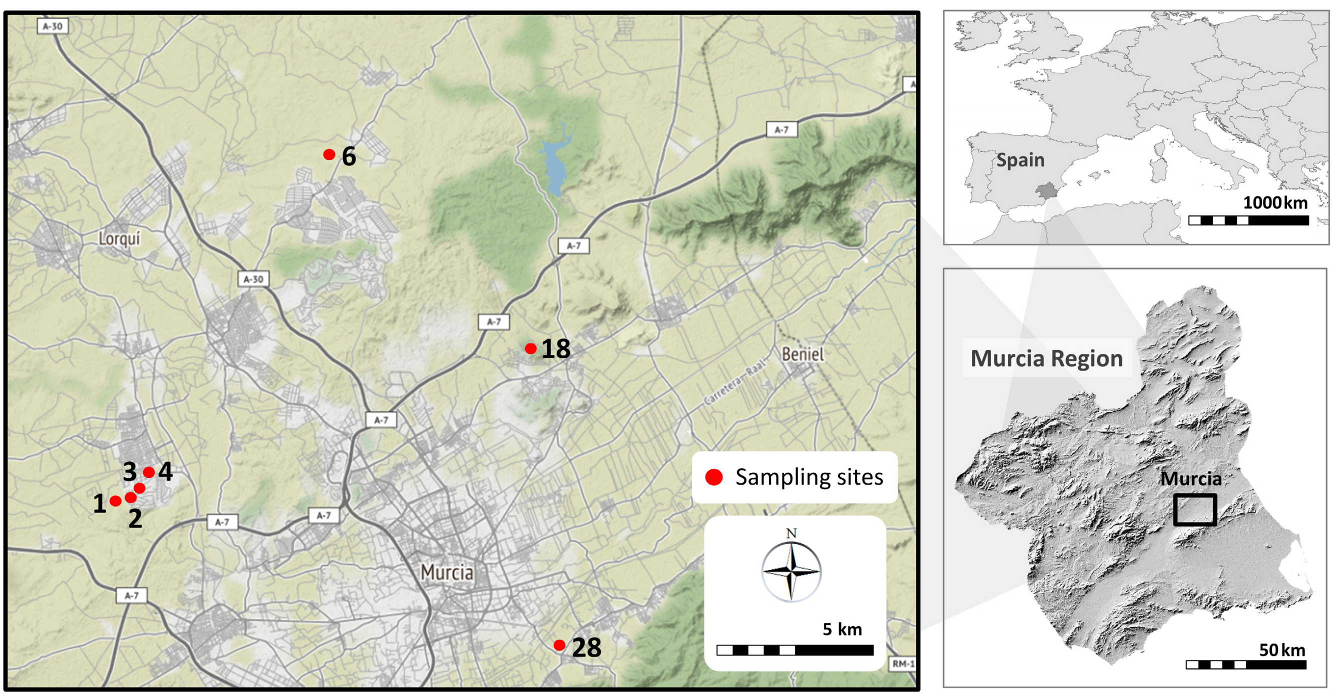

FIGURE 1 Location of sampling sites in periurban households in Murcia, Spain.

TABLE 1 Number of sampling times and locations within house plots, number and percentage of positive traps, and number and density of sand flies in positive traps, by sampling site.

Site no.	Trap type	Environment	Insecticides	No. sampling times	No. sampling locations	Traps		Sand flies	
						No.	% positive	No.	Median (range) density
2	Sticky	House plot	Yes	15	14	209	41	179	5 (2–34)[a]
3	Sticky	House plot	Yes	14	13	166	53	310	5 (1–56)
4	Sticky	House plot	Yes	15	12	175	37	118	3 (1–21)
18	Sticky	House plot	No	15	7	102	57	455	14 (2–92)
28	Sticky	House plot	No	15	12	176	56	316	3 (2–85)
1	Sticky	Non-urban	No	15	6	89	96	986	14 (2–172)
6	Sticky	Non-urban	No	15	12	175	50	496	5 (2–110)
All	Sticky	Both		15	76	1092	52	2860	5 (1–172)
2	CDC	House plot	Yes	8	1	8	75	18	3 (1–4)[b]
3	CDC	House plot	Yes	8	1	8	88	15	2 (1–3)
4	CDC	House plot	Yes	8	1	8	88	40	4 (2–12)
18	CDC	House plot	No	7	1	7	86	71	11 (3–26)
28	CDC	House plot	No	6	1	6	100	108	15 (3–47)
1	CDC	Non-urban	No	8	1	8	100	584	70 (12–131)
6	CDC	Non-urban	No	7	1	7	86	750	139 (19–168)
All	CDC	Both		6–8	7	52	90	1586	12 (1–168)

[a]Density in sticky traps: No. sand flies/m^2/day.
[b]Density in CDC traps: No. sand flies/trap/day.

a fast lethal effect on larvae and pupal stages by inhibiting chitin synthesis, it prevents eggs from hatching, and it is apt for use in organic-rich sites such as septic tanks and dung heaps. The recommended dose of diluted Larvigen and Acaritron is 0.1–0.5 L/m^2. In this study, treatments were dispensed at approximately 1 L/m^2 on surfaces covered in vegetation, aiming at both over- and underleaf coverage, and 0.25 L/m^2 on barren, dry soil surfaces. The volume of diluted product used on each occasion and the approximate areas covered were 100 L in site 2 for 100 m^2 of vegetation, 300 L in site 3 for 250 m^2 of vegetation and 200 m^2 of barren soil surfaces, and 200 L in site 4 for 200 m^2 of vegetation. Barren soil surfaces were not present in sites 2 and 4.

Treatments were carried out by a professional pest control operator using a motorized, high-pressure (20 bar; 300 psi) wheelbarrow sprayer with a hose and single nozzle, in the afternoon between 18:00 and 21:00. The mean (range) temperature, relative humidity and wind speed in this time period in spraying days were 27 (21–33) °C, 47 (29–68) % and 2.3 (0.8–4.0) m/s, respectively. The amount of rain during the whole study period was 72 mm and there was no rain for at least 6 days before and 31 days after spraying.

This work was part of a larger investigation of the epidemiology of human and animal leishmaniasis in Murcia Region, Spain, which was approved by the bioethics and animal experimentation committees of the University of Murcia (Código CEEA: 115/2015; 05/03/2015).

2.2 | Sand fly trapping and identification

Two types of sand fly trapping devices were used: sticky interception traps and light attraction traps. Sticky traps consisted of half an A4 size sheet of tracing paper measuring 210 mm × 148.5 mm, impregnated with castor oil. They were always exposed on both sides, positioned in the same selected spots for an average of 7 days and individually identified. Light traps were battery-operated, miniature Centers for Disease Control and Prevention traps (J. W. Hock Company, Gainesville, FL, USA) (hereinafter CDC traps). They were used once every 2 weeks and left for 24 h. The number of traps placed ranged between 6 and 14 sticky traps per site and only one CDC trap per site (Table 1).

Following collection, individual sticky traps were transported and stored individually between two sheets of A4 paper at 4°C until the sand flies were removed for storage with a small brush dipped in a 70% ethanol solution. Collection cups of the CDC traps were transported to the lab and placed at −20°C for a minimum of 2 h to kill the insects present. Specimens taken from both types of traps were preserved in 70% ethanol at −20°C until morphological identification.

Morphological speciation was based on the external genitalia in males and on the pharynx, cibarium and spermatheca in females, examined under a microscope at 400× magnification (Gállego-Berenguer et al., 1992; Martínez-Ortega & Conesa-Gallego, 1987). Prior to this, sand flies were dissected individually to separate the

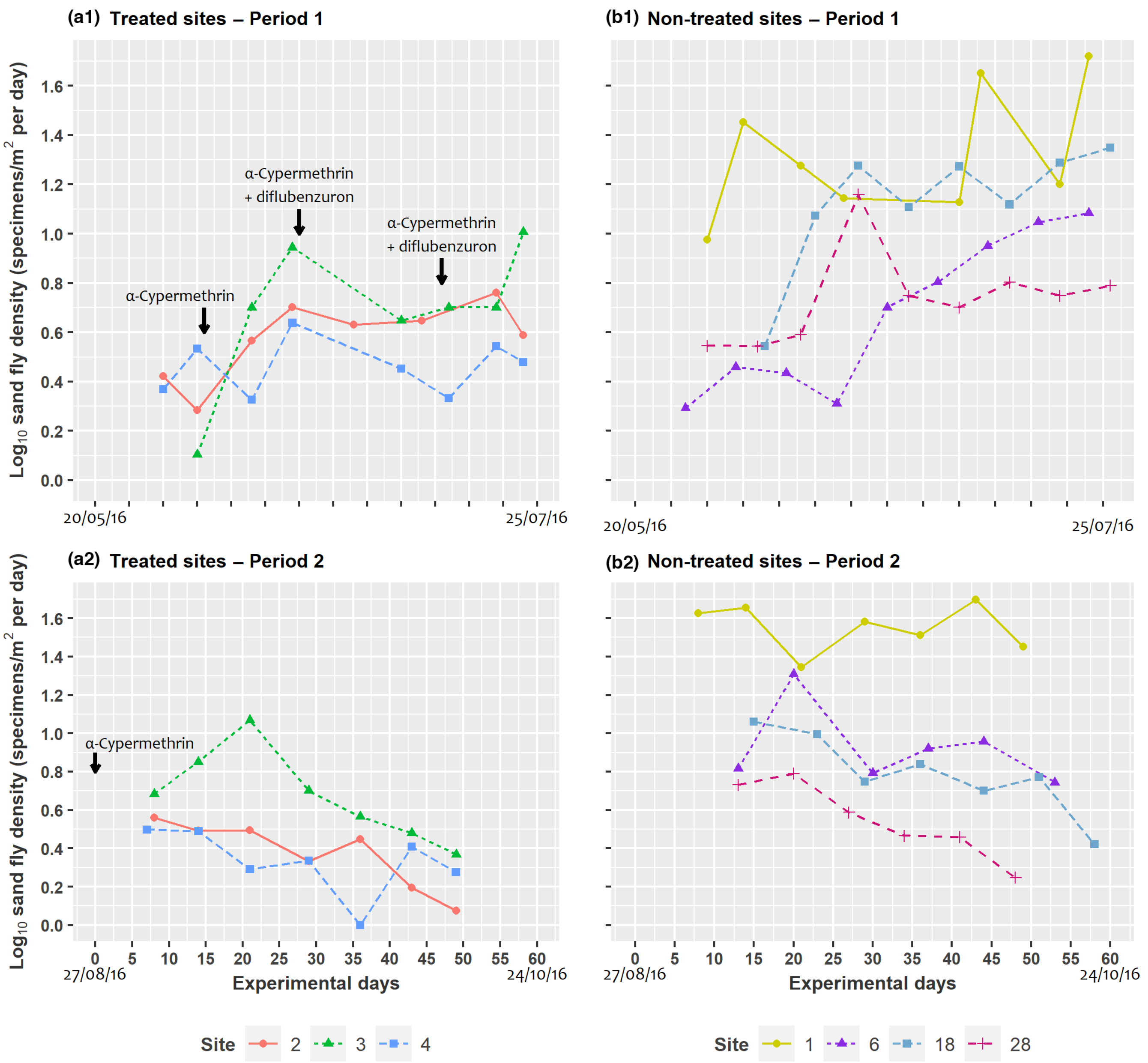

FIGURE 2 Daily, $\log_{10}$-transformed density of sand flies in sticky traps in treated (left panels: a1 and a2) and non-treated (right panels: b1 and b2) sites and the time when insecticides were applied in period 1 (top panels: a1 and b1), from May 20th to July 25th 2016, and period 2 (bottom panels: a2 and b2), from August 27th to October 24th 2016.

head and the last two segments of the abdomen, clarified in Marc André solution (40 g chloral hydrate, 30 mL glacial acetic acid, 30 mL distilled water) and mounted on a glass slide using Hoyer medium (30 g gum arabic, 200 g chloral hydrate, 20 mL glycerol, 50 mL distilled water) (Muñoz et al., 2021).

2.3 | Statistical analysis

Sand fly "abundance" corresponded to the total number of sand flies, and sand fly "species richness" and "species diversity" referred to the number and relative proportion of different species, respectively. "Positive traps" were those with at least one sand fly, and "sand fly density" was calculated by dividing the number of specimens by the sampling effort. The sampling effort was the number of days that the trap was placed in the case of CDC traps, and the number of days multiplied by the surface of the sticky trap (m^2). The corresponding sand fly density units were specimens/day for CDC traps and specimens/m^2/day for sticky traps.

The analysis included investigating the distribution of sand flies and other explanatory variables such as insecticide use, sampling time, site, group (treated or untreated) and environment (urbanized or not). Following, the proportion of positive traps and median sand fly densities in different locations, times and according to insecticide use were compared using Yates-corrected chi-squared test, or when necessary Fisher exact test, and the Kruskal–Wallis test, respectively (Kirkwood & Sterne, 2003).

A mixed-effect multivariate negative binomial model was then developed to investigate the relationship between sand fly density ($\log_{10}$-transformed +1) in sticky and CDC traps, and the time and type of insecticide used or not, adjusted for sampling month, site and location (Demidenko, 2013; Hilbe, 2011). Insecticide type (a 2-level categorical variable: α-cypermethrin 6% and α-cypermethrin 6% + diflubenzuron 48%) and time since application (4 levels: 0–8 days before insecticide application, and 1–14 days, 15–21 days and 23–58 days after insecticide application) were modelled as a single combination variable with ten possible levels. Sand fly density 1–14 days after the application of α-cypermethrin 6% in treated sites was used as the baseline for comparing densities in other periods in treated and non-treated sites. Explanatory variables were included as fixed effects except the site which was treated as a random effect. Parameters were estimated by the maximum likelihood method using the glmer.nb library in the R program, which was the software used for all other statistical tests (R Core Team, 2022). Significance was considered for $p < 0.05$ for a double-sided test.

3 | RESULTS

3.1 | Frequency of positive traps, and frequency and density of sand fly species and sex

Traps collected in the study included 1092 sticky and 52 CDC traps (Table 1), and the corresponding sampling efforts were 460 m^2·days and 52 trap·days, respectively. Sand flies were found in 52% of the sticky traps and in 90% of the CDC traps, and the overall number of sand flies captured was 4446 specimens, including 2860 specimens in sticky traps and 1586 in CDC traps (Table 1). However, the number and median density of sand flies in positive traps differed significantly between and within sites and trap types (Table 1). In sticky traps, the median (range) density (sand flies/m^2/day) varied from 3 (1–21) in site 4 to 14 (2–172) in site 1. Similarly, in CDC traps, the density (sand flies/trap/day) ranged from 2 (1–3) in site 3 to 139 (19–168) in site 6 (Table 1). The proportion of positive sticky traps in urban sites (47%) was lower than in non-urbanized sites (65%) ($p < 0.05$). Similarly, the median (range) density of sand flies in positive traps in urban and non-urbanized sites was 5 (1–92) and 11 (2–172), respectively, in sticky traps ($p < 0.05$), and 4 (1–47) and 92 (12–168), respectively, in CDC traps ($p < 0.05$).

Sand flies identified included 4393 specimens, and the remaining 53 (1%) specimens were damaged and could not be identified. Sand fly richness was comprised of five species, and species diversity included *P. ariasi* ($n = 36$; 1%), *P. papatasi* ($n = 173$; 4%), *P. perniciosus* ($n = 1922$; 44%), *P. sergenti* ($n = 161$; 4%) and *S. minuta* ($n = 2101$; 48%). The relative frequency of sand fly species in sticky/CDC traps was similar and included, respectively, 47%/49% *S. minuta*, 44%/43% *P. perniciosus*, 4%/4% *P. papatasi* and *P. sergenti* and 1%/1% *P. ariasi* (Table 2). However, relative frequencies differed significantly between sites. For example, in sticky traps, the relative frequency of *P. perniciosus* ranged between 17% in site 28 and 69% in site 18, *P. papatasi* represented 9% of sand flies in site 3 and none in site 4, and *P. sergenti* was 13% of sand flies in site 18 and none in sites 4 and 6 (Table 2). The percentage of females was greater in CDC traps (43%) compared to sticky traps (29%), although there were significant differences between species in different sites, particularly for sticky traps and *P. perniciosus* which ranged between 4% in site 1 and 29% in site 2 (Table 2).

3.2 | Bivariate relationship between sand fly density and insecticide use

In sites using insecticides (sites 2, 3 and 4), the proportion of positive sticky traps and the median (range) density of sand flies in positive traps were 43% positive traps and 4 (1–56) sand flies/m^2/day. In sites not employing insecticides (1, 6, 18 and 28), these figures were 61% positive traps and 7 (2–172) sand flies/m^2/day, and differences in proportions and medians between treated and untreated sites were statistically significant ($p < 0.05$). The same comparisons for CDC traps showed 83% positive traps and 3 (1–12) sand flies/trap/day in sites with insecticides and 96% positive traps and 30 (3–168) sand flies/trap/day in sites without insecticides, and the median density of sand flies was significantly lower in sites where insecticides were used ($p < 0.05$).

Figure 2 shows the daily, $\log_{10}$-transformed density of sand flies in sticky traps in treated (left panels: a1 and a2) and non-treated (right panels: b1 and b2) sites, and the time when insecticides were applied in two separate periods: period 1 (top panels: a1 and b1) from May 20th to July 25th and period 2 (bottom panels: a2 and b2) from August 27th to October 24th. In period 1, the application of α-cypermethrin on day 16 was followed by a moderate decrease in site 4 and increases in sites 2 and 3 in the log sand fly density in the next 2 weeks (until day 30) (a1, Figure 2). Instead, treatment with α-cypermethrin and diflubenzuron on day 30 was associated with a decrease in the log-density in all three sites (moderately in site 2) in the following 2 to 3 weeks. The application of this same treatment on day 51 was accompanied by increases in the log density of sand flies in all treated sites (a1, Figure 2). The temporal trend in the log density of sand flies in period 1 in non-treated sites (b1, Figure 2) differed substantially between sites with peaks at different weeks, although it followed an increasing pattern in all four sites.

In period 2, the $\log_{10}$-transformed density of sand flies in sticky traps in treated sites (a2, Figure 2) followed a gradually decreasing trend except in site 3, where it increased and peaked on day 21 and declined thereafter. Temporal patterns of sand fly density in non-treated sites (b2, Figure 2) were comparable to those in treated sites, particularly between sites 18 and 28 (b2, Figure 2) and sites 2 and 4 (a2, Figure 2), and between site 6 (b2, Figure 2) and site 3

TABLE 2 Frequency of sand fly species by sex and sampling site.

		Phlebotomus ariasi			*Phlebotomus papatasi*			*Phlebotomus perniciosus*			*Phlebotomus sergenti*			*Sergentomyia minuta*			All		
Site no.	Trap type	No.	% fem.[a]	% sp.[b]	No.	% fem.	% sp.	No.	% fem.	% sp.	No.	% fem.	% sp.	No.	% fem.	% sp.	No.	% fem.	% sp.
2	Sticky	3	33	1.7	5	40	2.8	59	29	33.0	6	50	3.4	106	60	59.2	179	49	100
3	Sticky	3	33	1.0	28	21	9.2	165	18	53.9	9	22	2.9	101	55	33.0	306	31	100
4	Sticky	3	33	2.6	0	0	0.0	72	22	62.1	0	0	0.0	41	54	35.3	116	34	100
18	Sticky	11	9	2.4	22	9	4.9	311	9	69.1	58	14	12.9	48	48	10.7	450	12	100
28	Sticky	0	0	0.0	3	100	1.0	52	6	16.6	9	100	2.9	249	37	79.6	313	30	100
1	Sticky	5	20	0.5	48	4	4.9	357	4	36.5	20	10	2.0	549	50	56.1	979	30	100
6	Sticky	0	0	0.0	4	50	0.8	239	13	49.5	0	0	0.0	240	54	49.7	483	33	100
All	Sticky	25	20	1	110	12	4	1255	11	44	102	11	4	1334	50	47	2826	29	100
2	CDC	0	0	0.0	2	100	11.1	11	64	61.1	1	0	5.6	4	25	22.2	18	56	100
3	CDC	0	0	0.0	4	100	26.7	11	36	73.3	0	0	0.0	0	0	0.0	15	53	100
4	CDC	1	100	2.5	0	0	0.0	27	37	67.5	0	0	0.0	12	67	30.0	40	48	100
18	CDC	5	40	7.4	3	67	4.4	54	69	79.4	0	0	0.0	6	67	8.8	68	66	100
28	CDC	0	0	0.0	12	67	11.1	59	59	54.6	1	0	0.9	36	47	33.3	108	56	100
1	CDC	5	20	0.9	42	36	7.3	386	17	67.4	56	32	9.8	84	60	14.7	573	26	100
6	CDC	0	0	0.0	0	0	0.0	119	29	16.0	1	100	0.1	625	56	83.9	745	52	100
All	CDC	11	36	1	63	48	4	667	29	43	59	32	4	767	56	49	1567	43	100

[a]Females.
[b]Species.

(a2, Figure 2). The density pattern in non-treated site 1 (b2, Figure 2) was unique in that it remained high and relatively stable throughout period 2.

3.3 | Multivariate relationship between sand fly density and insecticide use

In the negative binomial model, the $\log_{10}$ sand fly density in sticky traps 1–14 days after the application of α-cypermethrin (baseline) was not significantly different compared to non-treated sites at any time, or to treated sites 15–21 days after applying α-cypermethrin and 1–21 days after applying the combination of this product and diflubenzuron (Table 3). In contrast, it was significantly higher than 1–8 days before and 23–58 days after the application of α-cypermethrin (Table 3). The model also indicated substantial unexplained variation in sand fly density in sticky traps between sites (standard deviation = 0.3024), and no remaining significant variation according to month or the environment, urbanized or not. Finally, the $\log_{10}$ sand fly density in CDC traps was significantly lower in urbanized compared to non-urbanized sites and was not associated with any of the other variables considered (Table 3).

4 | DISCUSSION

The present study investigated the impact of HV application of an α-cypermethrin-based adulticide product and its combination with a diflubenzuron-based larvicide product on the local sand fly populations of gardens of private homes in periurban residential estates in a Mediterranean area, during one summer sand fly season. Sand flies were detected in all treated and untreated sites, and although overall density was significantly lower in treated sites, it followed a comparable seasonal pattern in both groups, with no consistent decrease 1 week after insecticide application. There is no evidence of sand fly resistance to the insecticides used in Spain, and failure to significantly reduce sand fly populations suggests the insecticides did not reach most adult resting and larval developing sites. Alternatively, treatments were effective but plots were quickly repopulated by sand flies from neighbouring gardens. In either case, it seems that outdoor HV spraying of insecticides in gardens of individual homes as performed in the present study does not achieve desired levels against sand flies and presumably the risk of sand fly-borne pathogen transmission.

Insecticide spraying is a complex task and its efficiency relies on a good understanding of the ecology of the target insect and depends on a number of factors such as the spraying technique, the time and weather conditions when it is carried out, the spraying technique and the environmental characteristics of the site being sprayed (Mathews et al., 2014). Adult sand fly vectors are frail insects measuring ~1.5–3.5 mm, they feed on plant sugars and females require additional blood meals for oogenesis, which they can take from a wide variety of hot and cold-blooded animals (Muñoz et al., 2019). They are not strong fliers, typically travelling with a short hopping flight over solid surfaces (Killick-Kendrick, 1999), and they have relatively small dispersal areas, ranging from <100 m for engorged females to 1000 m or more for host-seeking females (Pérez-Cutillas et al., 2020). The activity of *L. infantum* vectors in Mediterranean countries began between 19:00 and 22:00, peaked between 23:00 and 2:00 of the following day and ended in the 4:00 to 7:00 time range (Alten et al., 2016). Ultra-low volume applications aiming at host-seeking adults should, therefore, be performed when their activity is highest, and insecticide droplet size must be small enough to impinge on the insect's wings and antennae, as larger ones are more likely to be filtered out by vegetation and other objects in their pathway, and be lost by drip (Bonds, 2012; Knoche, 1994; Mount, 1970; Mount et al., 1996). Spraying private home gardens at nighttime was not an option in this study, and accordingly, treatments targeted mostly resting adults and immature stages. Adult sand flies rest in concealed sites protected from desiccation, and larvae breed in moist, organic matter-rich environments. However, domestic and peridomestic sand fly ecotopes are not well characterized and attempts at recovering larvae from presumed suitable breeding sites have been generally, unrewarding (Alexander & Maroli, 2003; Feliciangeli, 2004). Resting and breeding habitats in Mediterranean include soil in and around human dwellings, cracks in mud and stone floors and walls, basements and cellars of houses, abandoned buildings and animal burrows, among others. Such sites may be widespread and this represents a major limitation for making use of control measures against preimaginal stages (Feliciangeli, 2004). We used a high-volume insecticide treatment system, in fine weather conditions, to attain wide and focused coverage, and made a conscientious effort to spray the insecticides thoroughly on the upper side and undersurface of vegetation. Treatment coverage was monitored visually as spraying was being carried out, by observing the wet sprayed areas, but this was not always possible, particularly in areas concealed by dense vegetation such as thick ivy bushes and other climber plants present in the three treated sites. It is probable that not all adult resting sites and larval developing grounds were reached. Environmental differences between house plots affecting spraying efficacy would explain some of the variations in the sand fly density and temporal patterns. Mount (1998) concluded that the frequency of insecticide application to control mosquitoes in residential areas with moderate to dense vegetation should be 2 to 3 times greater compared to open field applications. Moreover, long-lasting residual activity of insecticides is critical to attain long-term sand fly control (Alexander & Maroli, 2003). The α-cypermethrin and diflubenzuron products used are residual insecticides intended for outside spraying but no claims on the extent of the products residual activity were made by manufacturers.

The difficulty in controlling outdoor sand fly populations by insecticide spraying was exemplified in the trials conducted by Coleman et al. (2006) on a U.S. Military base in Iraq in 2003, who reported a minimal impact on sand fly abundance following 62 days

TABLE 3 Estimates from a random effects negative binomial regression analysing the relationship between sand fly density (log_{10}-transformed) and time since treatments, adjusted for month, environment and site.

Variables	Levels	Estimate	Standard error	*p* value
a) Sticky traps				
Fixed effects				
Intercept		−0.56	0.38	0.1483
Insecticide type; days since application	α-cypermethrin; 1–14 days	0.00		
	α-cypermethrin; −8 to 0 days	−0.68	0.30	0.0232*
	α-cypermethrin; 15–21 days	−0.03	0.27	0.9009
	α-cypermethrin; 23–58 days	−0.59	0.25	0.0183*
	α-cypermethrin + diflubenzuron; 1–14 days	−0.08	0.21	0.7070
	α-cypermethrin + diflubenzuron; 15–21 days	−0.04	0.29	0.8912
	None; −8 to 0 days	−0.13	0.38	0.7198
	None; 1–14 days	0.27	0.29	0.3632
	None; 15–21 days	0.35	0.31	0.2577
	None; 23–58 days	0.18	0.32	0.5633
Month	May	0.00		
	June	0.02	0.25	0.9297
	July	0.09	0.26	0.7252
	September	0.12	0.26	0.6490
	October	−0.09	0.30	0.7689
Environment	Non-urbanized	0.00		
	Urbanized	−0.42	0.28	0.1335
Random effect	Standard deviation			
Site	0.3024			
b) CDC traps				
Fixed effects				
Intercept		0.14	0.49	0.7849
Insecticide type; days since application	α-cypermethrin; 1–14 days	0.00		
	α-cypermethrin; 15–21 days	−0.15	0.93	0.8714
	α-cypermethrin; 23–58 days	0.03	1.08	0.9753
	α-cypermethrin + diflubenzuron; 1–14 days	−0.34	0.79	0.6710
	α-cypermethrin + diflubenzuron; 15–21 days	0.19	0.85	0.8243
	None; 1–14 days	0.56	0.50	0.2634
	None; 15–21 days	0.62	0.55	0.2615
	None; 23–58 days	0.63	0.76	0.4084
Month	June	0.00		
	July	0.04	0.40	0.9233
	September	−0.14	0.45	0.7537
	October	−0.55	0.83	0.5062
Environment	Non-urbanized	0.00		
	Urbanized	−0.62	0.31	0.0463*
Random effect	Standard deviation			
Site	<0.001			

*p-value <0.05.

and 94 days of area and residual spraying with organophosphates and synthetic pyrethroids, between April and September. The authors attributed this to harsh climatic conditions and to adult populations being constantly replenished by maturing larvae (Coleman et al., 2006). Similarly, in a large community outbreak of leishmaniasis in Madrid, Spain (Arce et al., 2013), intensive spraying of potential sand fly breeding and resting grounds with pyrethroids had a short-lived effect, with sand fly populations quickly recovering (Iriso et al., 2017). Successfully treated areas may indeed be rapidly colonized by sand flies from neighbouring areas. Therefore, sand fly control initiatives in residential estates need community-wide involvement and failure to do so is likely to limit success in individual homes, as experienced for example, in mosquito fogging campaigns against dengue in Colombia (Usuga et al., 2019).

Close examination of the seasonal pattern of sand flies offered a more comprehensive understanding of the effect of insecticide application. Abundance in treated sites 2, 3 and 4 peaked at the end of June and July, and in site 3 also in the middle of September. A similar bimodal pattern with peaks in June–July and September has been reported in other sand fly surveys in Murcia (Martínez-Ortega, 1986; Muñoz et al., 2018) and other Mediterranean countries (Alten et al., 2016). However, in a previous survey in sites 2, 3 and 4 in 2014 and 2015, *P. perniciosus* density in sticky traps was highest in May and October and was most abundant in site 4 (Muñoz et al., 2021). In contrast, sand fly density was highest in site 3 in the present study. This site, additionally, had substantially more vegetation than sites 2 and 4. Moreover, the absence of a late peak in September–October may have been caused by the repeated use of insecticides in the previous months. Indeed, September–October corresponds to the 23–58 days period after the application of insecticides when the multivariate model predicted a significantly lower density of sand flies in treated but not in non-treated sites. However, except in site 1 where sand fly density was very high throughout the study, density in non-treated sites in September–October followed a decreasing pattern that was similar to treated sites. Site 1 included a cave, a sheltered environment with a high density of sand flies, which would be less affected by climatic and other environmental changes compared to those in more open sites.

The species richness and the predominance of *P. perniciosus* and *S. minuta* in this study were the same as that found by Muñoz et al. (2021) when investigating sites 1, 2, 3, 4, 6, 18 and 28 the previous 2 years. In contrast, the overall species diversity in sticky and light CDC traps differed in both studies. Here we report a similar density of these two sand fly species in both trap types, whilst Muñoz et al. (2021) described a significantly greater density of *S. minuta* in sticky traps and *P. perniciosus* in CDC light traps. Sticky traps are interception devices that catch passing-by flying insects and are considered to provide unbiased estimates of species diversity in the area where they are placed. Instead, light traps attract phototropic insects and the greater density of *P. perniciosus* in CDC traps was considered a sign of strong species phototropism (Muñoz et al., 2021). The results of the present study suggest that *S. minuta* may have similar phototropism and further studies are needed to clarify this issue for a better understanding of this species' biology. This species is a vector of *Sauroleishmania* of reptiles and its possible implication in the transmission of mammal *Leishmania* spp. has been considered (Daoudi et al., 2020).

5 | CONCLUSIONS

High-volume spraying of α-cypermethrin and diflubenzuron in the outside plot of detached houses as performed in this study does not reduce sand fly density significantly and probably the risk of sand fly-borne infections. This, together with other limitations such as the impact of insecticides on non-target organisms and human health, development of insecticides resistance and cost, should dissuade similar outdoor application of insecticides to control sand fly populations in individual house plots.

AUTHOR CONTRIBUTIONS

Trapping and collection of sand flies were performed by LJB, PFSL, FC, RRY, CMC and EB. Sand fly identification was performed by MO, CMH, JR, ZJ, AF and SV. Environmental data collection, statistical analysis and preparation of tables and figures were performed by MO, CMH, PPC and EB. The first written version of the manuscript was prepared by MO, CMH and EB, and all authors contributed to the submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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