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SPECIAL ISSUE ARTICLE

Spatiotemporal changes in prevalence of *Sodalis glossinidius*, *Spiroplasma* spp. and trypanosome species in wild *Glossina tachinoides* from Sora-Mboum animal African trypanosomiasis focus in northern Cameroon

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Abstract Sterile Insect Technique (SIT) has proven effective to reduce tsetse population density in large infected areas where animal African trypanosomosis (AAT) and human African trypanosomiasis (HAT) elimination was difficult to achieve. However, the decrease in mass production of insectary-reared tsetse and the limited but incomplete knowledge on symbiont–trypanosome interaction over time, impede large-scale use of SIT. We investigated the spatiotemporal changes in symbiont prevalence and symbiont–trypanosome interactions in wild tsetse of Sora-Mboum AAT focus in northern Cameroon, collected in 2019 and 2020, to provide insights into the mass production of refractory tsetse. *Spiroplasma* spp., *Sodalis glossinidius* and trypanosomes were screened with PCR. *G. tachinoides* was the most abundant *Glossina* species found in Sora-Mboum focus. Symbiont prevalences in *G. tachinoides* were higher in 2019 compared to 2020, from 67.6% to 53.5% for *Spiroplasma* spp. and from 28.8% to 8.1% for *S. glossinidius*. These symbionts were also found at higher prevalence in flies from Mouhoun HAT focus in Burkina Faso. Four trypanosome taxa (*Trypanosoma congolense* forest type, *T. congolense* savannah type, *T. brucei* s.l., and *T. vivax*) were found in Sora-Mboum focus and Mouhoun focus, though at lower prevalence in Mouhoun. The presence of *Spiroplasma* spp. in adult tsetse was negatively associated with that of trypanosomes. Our study highlights the potential of *Spiroplasma* spp. as a good paratransgenesis candidate to enhance SIT application. This symbiont is naturally found in high proportions of tsetse and could prevent factory flies from acquiring and transmitting trypanosomes during their lifespan when released for population density control.

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Introduction

Tsetse of the genus *Glossina* are the only known cyclical vectors of trypanosomes, protozoan parasites infecting humans and other vertebrates, causing human African trypanosomiasis (HAT) and animal African trypanosomosis (AAT), respectively (Holmes, 2014; Diall *et al.*, 2017). About 31 *Glossina* species and subspecies are distributed in 37 Sub-Sahara African countries and cover almost 10 million km^2, including *Glossina tachinoides*, the major vector of animal trypanosomes (Alsan, 2015). Great progress has been made toward achieving the WHO goal of interrupting HAT transmission by 2030, with less than 1000 new cases reported every year since 2020 and many countries validating the elimination of the disease as public health problem (Franco *et al.*, 2024). However, as limited prophylactic drugs and no vaccine are available against AAT (Giordani *et al.*, 2016), coupled with the increasing evidence of emergence of resistance to trypanocidal drugs (Ungogo & Koning, 2024), vector control remains a cornerstone strategy for the elimination of this disease (Meyer *et al.*, 2018).

The current tsetse control tools rely on insecticides and traps which face several limitations such as environmental impact by affecting non-targeted organisms (Özkara *et al.*, 2016), and incomplete coverage with large hard-to-reach areas suitable for tsetse development (Laveissière *et al.*, 2011). Therefore, control tools using mass-reared alive flies present an advantage at least regarding the coverage of area under control. The Sterile Insect Technique (SIT) for example, implemented as part of an area-wide integrated pest management (AW-IPM) approach has helped achieving complete eradication of some tsetse populations in Nigeria and in the Unguja Island, Zanzibar (Takken *et al.*, 1986; Oladunmade *et al.*, 1990; Vreysen *et al.*, 2000, 2014). This method relies on the mass-production of tsetse, sterilization of males by ionizing radiation and their release in tsetse areas for mating with wild females resulting in no offspring production. This will gradually reduce the density of the targeted wild tsetse. The SIT requires a sufficient mass production of the targeted wild tsetse, but their abundance use to decrease along generations in insectaries (IAEA, 1990).

During the initial release phase of the SIT program, the large number of sterile males released in the targeted areas could potentially increase the risk of trypanosomes transmission as the irradiation does not affect tsetse susceptibility to develop mature trypanosome infections and transmit the parasites. To mitigate this risk of transmission, sterile male flies are fed with two to three blood meals containing a trypanocide (isometamidium chloride) before being released (Van Den Bossche *et al.*, 2006; Argilés-Herrero and Leak, 2016). This technique can increase the contact between the parasite and the drug at a sub-lethal dose within the vector and could therefore increase trypanosome resistance to trypanocidal drugs. This is why enhancing the refractoriness of irradiated tsetse flies to trypanosome infections by genetically modifying their endosymbionts using paratransgenesis was proposed to be combined with SIT (De Vooght *et al.*, 2014, 2018; Demirbas-Uzel *et al.*, 2018; Kariithi *et al.*, 2018; Ngambia Freitas *et al.*, 2025).

Using modified tsetse symbionts to enhance SIT requires a good knowledge on their biology and their relationships with their host (Abd-Alla *et al.*, 2013; Kariithi *et al.*, 2018). Several bacteria symbionts have been described carrying many biological functions in tsetse, including (i) the intracellular primary symbiont *Wigglesworthia glossinidia* acting on fly's fertility and immune response (Aksoy, 1995, 2014), (ii) *Wolbachia* sp. inducing cytoplasmic incompatibility among tsetse (Alam *et al.*, 2011; Schneider *et al.*, 2013; Brelsfoard *et al.*, 2014), and (iii) the secondary symbiont *Sodalis glossinidius* that helps tsetse digestion process and was found associated to tsetse infections with trypanosomes (Dale & Welburn, 2001; Geiger *et al.*, 2007; Farikou *et al.*, 2010). Besides these major symbionts, *Serratia* and *Burkolderia* were recently identified with high relative abundances of 18% and 13%, respectively, in *G. palpalis palpalis* and *G. tachinoides* from Cameroon (Bouaka Tsakeng *et al.*, 2022). *Spiroplasma* was identified and shown more abundant in larvae than adult *Glossina fuscipes fuscipes* and *G. tachinoides*, suggesting that this bacterium could be a symbiont playing a role in development or nutrition (Doudoumis *et al.*, 2017). The biological functions of these new probable symbionts remain unclear, as a great variation has been observed in their prevalence among adult tsetse of different species and origins (Doudoumis *et al.*, 2017; Schneider *et al.*, 2019; Mulenga *et al.*, 2022; Dera *et al.*, 2023).

Fig. 1 Map showing sites where entomological surveys were conducted in Cameroon (Sora-Mboum) and Mouhoun (Burkina Faso).

This study aimed to (i) investigate the spatial and temporal changes in the prevalence of two symbionts, *S. glossinidius* and *Spiroplasma*, and (ii) explore the symbiont–trypanosome interaction in wild *Glossina* species from Sora-Mboum AAT focus in northern Cameroon and their potential interest as effectors to enhance tsetse mass production and refractoriness to trypanosomes.

Materials and methods

Study areas

This study was conducted on tsetse flies captured in the Sora-Mboum (7°47'N, 15°00'E), a village located in the Mayo Rey Division, North Region of Cameroon (Fig. 1). This Division covers an area of 36 529 km^2 with a total population estimated at about 784 927 in 2024. The climate is of Sudano-Sahelian type with two seasons: a rainy season from April to October and a dry season from November to March. The vegetation is of Savannah type, watered by a dense hydrographic network with fast-flowing streams. Livestock breeding is the main socio-economic activity of rural inhabitants (Bronsvoort *et al.*, 2003). The invasion of tsetse flies impedes the well exploitation of pastures resources, with breeders always complaining from the decimation of their livestock by bovine trypanosomiasis.

Trapping, dissection and preservation of tsetse flies

Two entomological surveys were carried out in March 2019 and March 2020 in the Sora-Mboum AAT focus. Adult tsetse flies captured in March 2020 from the Mouhoun HAT focus in Burkina Faso were also assessed and considered as a comparative group (Fig. 1). Pyramidal traps (Gouteux & Lancien, 1986) were deployed in suitable tsetse biotopes in Rao and Campement sites in Sora-Mboum AAT focus. The geographical coordinates of each trap were recorded with a global positioning sys-

Table 1 Primers used for PCR amplification of trypanosomes' and symbionts' DNA.

Specificity	Primer sequence (5′–3′)	Fragment length (bp)	References
Sodalis glossinidius	TGAAGTTGGGAATGTCG AGTTGTAGCACAGCGTGTA	120	Darby *et al.* (2005)
Spiroplasma spp.	63F: GCCTAATACATGCAAGTCGAAC TKSSsg: TAGCCGTGGCTTTCTGGTAA	455	Mateos *et al.* (2006); Fukatsu and Nikoh (2000)
T. congolense (savannah type)	TCGAGCGAGAACGGGCACTTTGCGA ATTAGGGACAAACAAATCCCGCACA	341	Moser *et al.* (1989)
T. congolense (forest type)	GGACACGCCAGAAGGTACTT GTTCTCGCACCAAATCCAAC	350	Masiga *et al.* (1992)
T. brucei sl	CGAATGAATATTAAACAATGCGCAG AGAACCATTTATTAGCTTTGTTGC	164	Masiga *et al.* (1992)
T. vivax	CTGAGTGCTCCATGTGCCAC CCACCAGAACACCAACCTGA	150	Masiga *et al.* (1992)
Diag-ITS1	TGGACTTCGGATTAAGTACAACA TCATTATGCGCTATTAAGGTAAGC	150–400	Dyer *et al.* (2008)

tem (GPS) and the traps were visited twice a day (from 9:00 am to 11:00 am and 3:00 pm to 4:00 pm) for 5 to 6 consecutive days. The species, sex and teneral status (i.e., whether the fly had already taken its first bloodmeal [non-teneral fly] or not [teneral fly]) of each collected tsetse were assessed using morphological criteria. After species identification, each fly was dissected by separating its legs from the rest of its body (abdomen and thorax). Each sample (legs and bodies separately) was preserved in 95% Ethanol and stored at field temperature (24–30 °C), and at −20 °C in the laboratory until use.

DNA extraction

DNA of the legs and rest of the body of each fly were extracted according to the protocol described by Maniatis *et al.* (1982) and Navajas *et al.* (1998). Briefly, each sample was thawed and air-dried for 90 min, crushed with a pestle in 600 μL of cetyl trimethyl ammonium bromide (CTAB) buffer (CTAB 5%; 1 mol/L Tris, pH 8; 0.5 mol/L EDTA pH 8; 5 mol/L NaCl, 20 μL of β-mercaptoethanol and sterile water), then incubated at 60 °C for 30 min. A mixture of chloroform/isoamylic alcohol (24/1; V/V) was added and the mixture was centrifuged at 8000 r/min for 10 min. The aqueous phase containing nucleic acids was transferred to new labeled tubes and DNA was precipitated by adding isopropanol (V/V) and centrifuged at 10 000 r/min for 10 min. Thereafter, the DNA pellet was rinsed with 70% ethanol, air-dried, and re-suspended in sterile distilled water (30 μL). DNA extracts were stored at −20 °C until PCR amplification.

PCR-based identification of tsetse fly species

The internal transcribed spacer 1 (ITS1) fragments of each tsetse fly were amplified as described by Feudjio Soffack *et al.* (2024) for confirmation of the tsetse species previously identified using morphological criteria. The PCR amplification was carried out in a final volume of 30 μL containing 12.9 μL of double-distilled water, 3 μL of 10× PCR buffer (Bioline), 1 μL of 10 mmol/L dNTP, 2 μL of each of the primer pair Diag-ITS1 (10 μmol/L) (Table 1), 3 μL of 25 mmol/L $MgCl_2$ (final concentration 2.5 mmol/L), 0.5 U (0.1 μL) of BIOTaq DNA polymerase and 6 μL of template (DNA extract from fly legs). PCR amplification was performed with the following cycling parameters: a denaturation step at 94 °C for 5 min followed by 30 cycles, each cycle including a denaturation step at 94 °C for 30 s, an annealing step at 58 °C for 30 s and an extension step at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. The PCR products were resolved on 10% polyacrylamide gel stained with ethidium bromide and visualized under UV illumination. The hyperladders 25 and 100 bp (Bioline) were used to estimate the size of different fragments obtained.

Molecular identification of different trypanosome species

Trypanosoma brucei s.l., *Trypanosoma vivax*, *Trypanosoma congolense* "forest" type and *Trypanosoma congolense* "savannah" type were identified by PCR using specific primers (Table 1), according to the protocol

previously described by Herder *et al.* (2002). PCR amplification of each trypanosome species was carried out in a final volume 15 μL containing 8.64 μL of double-distilled water, 1.5 μL of 10× TBE buffer, 0.3 μL of dNTPs (10 mmol/L), 0.06 μL of Taq DNA polymerase (New England Biolab, 5 U/μL), 0.6 μL of each primer (10 μmol/L), 0.3 μL of $MgCl_2$ (25 mmol/L), and 3 μL of template (DNA extract from fly bodies). PCR amplification was performed following the cycling parameters: a denaturation step at 94 °C for 5 min followed by 40 amplification cycles, each cycle including a denaturation step at 94 °C for 30 s, an annealing step at 60 °C for 30 s, and an extension step at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. PCR products were resolved on 2% agarose gel stained with ethidium bromide and visualized under UV illumination.

Molecular detection of Sodalis glossinidius and Spiroplasma spp.

Sodalis glossinidius and *Spiroplasma* spp. were detected by PCR amplification of a fragment of extrachromosomal DNA as described by Darby *et al.* (2005) for *Sodalis glossinidius*, and a fragment of the 16s rDNA, with a combination of primers designed by Fukatsu & Nikoh (2000) and Mateos *et al.* (2006) and amplification conditions described by Doudoumis *et al.* (2017) for *Spiroplasma* spp. PCR amplification of *S. glossinidius* DNA was performed in a final volume of 15 μL, containing 1.5 μL of 10× PCR reaction buffer, 0.22 μL of each primer (10 μmol/L) (Table 1), 0.27 μL of dNTPs (25 mmol/L), 0.06 μL of Taq DNA polymerase (New England Biolab, 5 U/μL) and 1 μL of DNA extract. PCR amplification of *Spiroplasma* spp. DNA was performed in a total volume of 15 μL, containing 1.5 μL of 10× PCR reaction buffer, 0.51 μL of each primer (10 μmol/L) (Table 1), 0.3 μL of dNTPs (10 mmol/L), 0.3 μL of $MgCl_2$ (25 mmol/L), 0.06 μL of Taq DNA polymerase (New England Biolab, 5 U/μL) and 3 μL of DNA extract. PCR amplification of *S. glossinidius* was performed using the following cycling parameters: a denaturation step at 94 °C for 5 min followed by 40 amplification cycles made up of a denaturation step at 94 °C for 30 s, an annealing step at 52 °C for 30 s and an extension step at 72 °C for 1 min. These amplification cycles were followed by a final extension step at 72 °C for 10 min. The PCR amplification parameters for *Spiroplasma* spp. were like those of *S. glossinidius*, except the number of cycles (35 rather than 40), and the annealing temperature (58 °C rather than 52 °C).

The amplified products were separated by electrophoresis at 100 volts for 30 min on 2% agarose gel stained with ethidium bromide and the amplification products were visualized under UV light. PCR fragments of *Spiroplasma* spp. were cleaned up with Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (NEB, Ipswich, MA, USA) according to the manufacturer's recommendations and sequenced in both directions using Sanger Sequencing (Inquaba Biotech company, South Africa). After sequencing, the forward and reverse sequences for each sample were corrected using the software Chromas Version 2.6.6 (https://technelysium.com.au/wp/chromas/) and aligned to obtain consensus sequences using the software BioEdit V7.1.9 (Hall, 1999). Clean sequences were subjected to nucleotide blast search (Blastn) in the GenBank nucleotide collection (nr) database (https://blast.ncbi.nlm.nih.gov/) for comparison with sequences pre-existing in this database and species confirmation.

Data management and statistical analyses

All data collected as part of this study were recorded into a purpose-built Microsoft Excel database and subsequently exported into the Statistical Package for the Social Sciences (SPSS) Statistics version 26 (SPSS Inc., Chicago, IL, USA) for statistical analyses. Statistical analyses were also performed online using VassarStats computational website.

The apparent density per trap per day (ADT) was used to assess the relative abundance of tsetse during each entomological survey, and was estimated according to the following formula: $ADT = \frac{C}{TD}$, where C is the number of tsetse flies caught, T the number of traps deployed, and D the number of days of trapping (Gouteux & Buckland, 1984). ADTs were compared across the years of trapping (2019 vs. 2020), the trapping sites (Rao vs. Campement) and tsetse sexes (males vs. females) using the Student's *t*-test.

Trypanosome and symbiont prevalence or infection rates in tsetse were expressed as percentages representing the ratio of the number of tsetse harboring trypanosomes or a symbiont species, over the total number of tsetse analyzed in each site/focus; the chi-square test was used to compare trypanosome and symbiont prevalences between the years of trapping, the trapping sites and the tsetse sexes.

A univariate binary logistic regression model (Darby *et al.*, 2005) was used to assess the association between the presence of symbionts (*S. glossinidius* or *Spiroplasma* spp.) and trypanosome infections to determine the potential impact of these symbionts in the different trapping

Table 2 Apparent density of tsetse flies captured in Sora-Mboum, Cameroon.

Variables		No. traps	No. days of capture	No. captured flies	No. teneral flies	ADT
Study sites	Campement	29	5	83	0 (0.0%)	0.57
	Rao	60	6	1355	45 (3.3%)	3.76
Sexes	Males	29 and 60	5 and 6	763	40 (5.2%)	1.51
	Females	29 and 60	5 and 6	675	5 (0.7%)	1.34
Years	2019	50	5	387	12 (3.1%)	1.55
	2020	39	6	1051	33 (3.1%)	4.49

ADT: apparent density per trap per day.

sites. The threshold for significance was set at 5% for all tests.

Results

Apparent densities and species identification of adult tsetse captured during the entomological surveys

A total of 1438 adult tsetse was captured in Sora-Mboum AAT focus, of which 387 (26.9%) were captured during the first entomological survey, and 1051 (73.1%) during the second. The apparent density (ADT) was significantly higher ($P < 0.05$) during the second entomological survey conducted in March 2020 (4.49 flies/trap/day) compared to the first one that took place in March 2019 (1.55 flies/trap/day). Similarly, the number of flies captured, and the ADT were significantly higher ($P < 0.05$) in Rao collection site compared to Campement (Table 2). Although the tsetse ADT was significantly higher in Rao than in Campement or in 2020 than in 2019, it didn't differ between males and females (Table 2). From the 1438 tsetse flies trapped in the framework of this study, 70 (4.9%) were identified as *Glossina morsitans submorsitans* and 1368 (95.1%) as *G. tachinoides* using morphologic characteristics. PCR analysis confirmed the morphological identification of a random subset of 47 *G. morsitans submorsitans* and 238 *G. tachinoides*.

Prevalences of targeted symbionts in Glossina tachinoides

Prevalence of *Spiroplasma* spp. in tsetse Among the 139 and 99 tsetse captured in Sora-Mboum in 2019 and 2020, 94 (67.6%) and 53 (53.5%) were found harboring *Spiroplasma* spp., respectively. Concerning the occurrence of this symbiont in the different sampling sites, 17 flies among 25 (68.0%) and 77 among 114 (67.5%) were harboring the symbiont in Campement and Rao in 2019 respectively, and in 2020, 56.3% (9/16) and 53.0% (44/83) of tsetse flies captured in Campement and Rao, respectively, were found carrying the symbiont (Table 3). Among the 36 samples originating from Mouhoun in Burkina Faso, 24 (66.7%) were also infected with this symbiont. Although no difference was observed between the prevalence of *Spiroplasma* in tsetse from different trapping sites, the prevalence was significantly lower in 2020 compared to 2019 in Sora-Mboum (P-value = 0.027).

Prevalence of *Sodalis glossinidius* in tsetse The prevalence of *S. glossinidius* significantly dropped in tsetse analyzed from Sora-Mboum, from 28.8% (40/139) in 2019 to 8.1% (8/99) in 2020 (P-value < 0.0001). Looking at the different trapping sites in Sora-Mboum, similar prevalences were observed in the two sites in 2019, that is, 24.0% (6/25) in Campement and 29.8% (34/114) in Rao (P-value = 0.72) (Table 3). Although no significant change was observed in the prevalence of *S. glossinidius* in Campement after one year (18.8%, 3 positive flies/16, P-value = 0.69), there was a significant drop in the prevalence of this symbiont in Rao in 2020, (6.0% 5/83, P-value < 0.0001). Concerning the tsetse examined from Mouhoun in Burkina Faso (2020), 16/36 (44.4%) were carrying *S. glossinidius* and this prevalence was significantly higher compared to that observed in Sora-Mboum ($\chi^2 = 24.88$; P-value $< 10^{-4}$).

Global prevalences of *Spiroplasma* spp. and *Sodalis glossinidius* in tsetse The global prevalence of *Spiroplasma* spp. (61.8%; 95% CI = 55.5−67.7) was significantly higher compared to that of *S. glossinidius* (20.2%; 95% CI = 15.6−25.7) ($\chi^2 = 85.14$; df = 1; $P < 0.0001$). This trend was the same looking at the prevalences of these symbionts in the different sites individually. Indeed, in Sora-Mboum, 94 (67.6%) and 40 (28.8%) flies were

Table 3 Prevalence of *Spiroplasma* spp. and *Sodalis glossinidius* in *Glossina tachinoides* from Sora-Mboum, Cameroon.

Variables		No. flies analyzed	No. flies with *Spiroplasma* spp.	No. flies with *S. glossinidius*
Sexes	Males	108	62 (57.4%)	26 (24.1%)
	Females	130	85 (65.4%)	22 (16.9%)
Survey sites/years in Sora Mboum	Campement 2019	25	17 (68.0%)	6 (24.0%)
	Rao 2019	114	77 (67.5%)	34 (29.8%)
	Total 2019	**139**	**94 (67.6%)**[a]	**40 (28.8%)**[b]
	Campement 2020	16	9 (56.3%)	3 (18.8%)
	Rao 2020	83	44 (53.0%)	5 (6.0%)
	Total 2020	**99**	**53 (53.5%)**[a]	**8 (8.1%)**[b]
	Mouhoun 2020	36	24 (66.7%)	16 (44.4%)[c]

[a], [b] and [c] indicate the levels of significance with the chi-squared test; [a]: $\chi^2 = 4.86$, $df = 1$, P-value $= 0.027$; [b]: $\chi^2 = 15.38$, $df = 1$, P-value $= 8.78 \times 10^{-5}$; [c]: $\chi^2 = 23.88$, $df = 1$, P-value $= 1.02 \times 10^{-6}$.

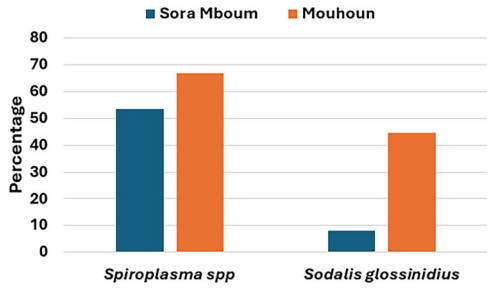

Fig. 2 Prevalence of *Spiroplasma* spp. and *Sodalis glossinidius* in *Glossina tachinoides* captured in Sora-Mboum (Cameroon) and Mouhoun (Burkina Faso) in 2020.

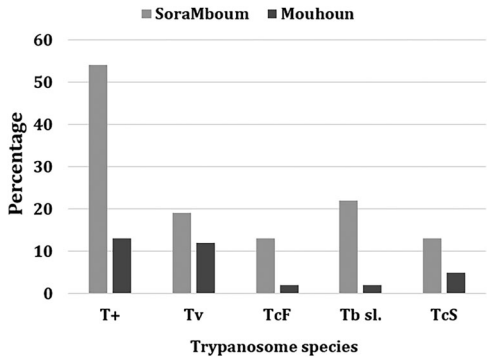

Fig. 3 Prevalence of *Glossina tachinoides* from Sora-Mboum (Cameroon) and Mouhoun (Burkina Faso) infected respectively with: at least one trypanosome species (T+), *T. vivax* (Tv), *T. congolense* Forest type (TcF), *T. brucei sensu lato* (Tb sl.), or *T. congolense* Savannah type (TcS).

found harboring the two symbionts respectively (P-value < 0.0001) and in Mouhoun, 24 (66.7%) and 16 (44.4%) flies were found harboring the two symbionts, respectively (P-value $= 0.057$) (Fig. 2).

Infection rates of different trypanosome species in Glossina tachinoides

Glossina tachinoides infection rates to trypanosomes, as detected using PCR-based assays, were globally higher in 2020 compared to 2019 (54.6% and 35.3% respectively), the difference being significant only for *T. brucei* s.l. (22.2% and 9.4% respectively, $\chi^2 = 7.63$; df $= 1$; $P = 0.0057$) (Table 4). Also, infection rates were globally higher in Rao compared to Campement, the difference being significant only for *T. vivax* ($\chi^2 = 4.24$; df $= 1$; $P = 0.0395$). No significant difference in infection rates to different trypanosome species was observed according to the tsetse sexes (Table 4). Infection rates of *G. tachinoides* by the different trypanosomes' species were significantly higher in Sora-Mboum focus in Cameroon compared to Mouhoun focus in Burkina Faso ($P < 0.001$) (Fig. 3).

Relationship between trypanosome infections and the presence of targeted symbionts

Table S1 displays the relationship between the presence of targeted symbionts (*Spiroplasma* spp. and *S. glossinidius*) and the presence of different trypanosome species in *G. tachinoides* trapped in Sora-Mboum focus in Cameroon or Mouhoun focus in Burkina Faso (Table S1). The significance of these different combinations, measured by Odds ratios with 95% confidence intervals, are presented as supplementary material (Table S2). No significant association was observed between the presence of *Spiroplasma* spp. and/or *S. glossinidius*

Table 4 Prevalence of trypanosomes species in *Glossina tachinoides* from Sora-Mboum, Cameroon.

Variables		No. flies analyzed	No. infected with trypanosomes	No. infected with *T. vivax*	No. infected with *T. congolense forest type*	No. infected with *T. congolense savana type*	No. infected with *T. brucei* s.l.
Years	2019	139	49 (35.3%)	33 (23.7%)	11 (7.9%)	9 (6.5%)	13 (9.4%)
	2020	99	54 (54.6%)	19 (19.2%)	13 (13.1%)	13 (13.1%)	22 (22.2%)
Sexes	Males	108	42 (38.9%)	23 (21.3%)	10 (9.3%)	8 (7.4%)	16 (14.8%)
	Females	130	51 (39.2%)	29 (22.3%)	14 (10.8%)	14 (10.8%)	19 (14.6%)
Survey sites	Campement	41	14 (34.1%)	4 (9.8%)	3 (7.3%)	6 (14.6%)	6 (14.6%)
	Rao	197	89 (45.2%)	48 (24.4%)	21 (10.7%)	16 (8.1%)	29 (14.7%)

s.l.: sensus lato.

and the presence of different trypanosome species, except for a negative association between *Spiroplasma* spp. and trypanosome infected status of flies, regardless the trypanosome species, in tsetse from Sora-Mboum (OR = 0.573; 95% CI = 0.337−0.982; $P = 0.043$). No significant association was observed between the presence of any of the two symbionts and tsetse infection with trypanosomes in Mouhoun (Burkina Faso) (Table S2).

Discussion

To provide more insights for enhancing tsetse's refractoriness to trypanosome infections, we screened *Spiroplasma* spp., *S. glossinidius* and different trypanosome species in wild tsetse caught in 2019 and 2020 in the Sora-Mboum AAT focus in northern Cameroon to better understand the spatial and temporal changes in the occurrence of these symbionts, as well as the symbiont–trypanosome interaction.

Entomological surveys conducted in the Sora-Mboum AAT focus in northern Cameroon revealed the predominance of *G. tachinoides*, indicating their high adaptation to environmental conditions compared to *G. morsitans submorsitans* (Mamoudou *et al.*, 2016; Kame-Ngasse *et al.*, 2018). Indeed, the vegetation of Sora-Mboum is dominated by forest galleries present along streams, offering a suitable and most favorable environment for the development of *G. tachinoides* compared to *G. morsitans submorsitans* which breeds preferentially in savannah environment. This was confirmed in a previous survey conducted in the savannah environment around Lake Iro in southeast Chad where *G. morsitans submorsitans* was the major tsetse species (Signaboubo *et al.*, 2021).

The apparent density per trap per day (ADT) of tsetse caught in the Sora-Mboum AAT focus was higher in Rao compared to Campement, suggesting that some microenvironment components of the trapping sites such as climate, precipitation, humidity, shadow and even host feeding availability could impact the abundance of tsetse. Importantly, the ADT was significantly higher, in the same trapping environments/sites, during the second entomological survey conducted in 2020 compared to the first one carried out in 2019. This suggests that even in the same sites, conditions could drastically change over time, thus modifying the suitability of the environment and habitat of tsetse. In this study, we noticed during the second survey conducted in 2024, the destruction of forest galleries and establishment of agricultural activities that increased host availability for tsetse and therefore the density of the latter, as previously suggested by Ngonyoka *et al.* (2017).

In this study, *Spiroplasma* spp. and *S. glossinidius* were identified in tsetse from all trapping sites, highlighting their role as core tsetse symbionts, as already pointed out in previous studies (Geiger *et al.*, 2011; Doudoumis *et al.*, 2017; Mfopit *et al.*, 2024a; 2024b). Although no difference was found in *Spiroplasma* spp. and *S. glossinidius* prevalence according to the different trapping sites of the Sora-Mboum AAT focus (Rao vs. Campement), a significant difference was found in the abundance of these symbionts between flies collected in Sora-Mboum compared to those captured in Mouhoun HAT focus in Burkina Faso, highlighting the dynamic relationship between tsetse flies and the targeted symbionts as already observed in previous studies (Schneider *et al.*, 2019; Dieng *et al.*, 2022). *Spiroplasma* spp. was more prevalent in tsetse, compared to *S. glossinidius*. This could be due to the suggested nutritive role potentially played by *Spiroplasma* in tsetse flies (Doudoumis *et al.*, 2017), that makes this symbiont more abundant in the flies. Despite the long known evolutionary history between *Sodalis* and tsetse, its prevalence in wild flies has been highly variable depending on tsetse species, and it was not detected at all in a many *G. tachinoides* from East and West Africa (Dieng *et al.*, 2022).

Regarding tsetse infection with trypanosomes, four trypanosome taxa (*T. congolense* forest type, *T. congolense* savannah type, *T. vivax* and *T. brucei* s.l.) were found in Sora-Mboum AAT focus in northern Cameroon as was already reported in Faro-et-Deo, another AAT focus in northern Cameroon (Paguem *et al.*, 2019). *Trypanosoma vivax* was the most prevalent trypanosome species as previously reported in clinically healthy cattle in northern Cameroon (Mamoudou *et al.*, 2016), likely because of their relative short life cycle in the mouthparts of tsetse flies where mature trypanosomes ready to infect potential hosts are found (Solano *et al*., 1996; Paguem *et al.*, 2019). *Trypanosoma brucei* s.l. and *T. vivax* were also found to be more prevalent in 2020 than in 2019, and in Rao compared to Campement, respectively, likely because of differences in host availability, cattle management practices and environmental conditions. Nevertheless, it is worth mentioning that species-specific primers were used for the detection of trypanosome species and would have led to an under-detection of all trypanosome species present in trapped tsetse flies, such as *T. grayi* already reported in northern Cameroon (Ngomtcho *et al.*, 2017).

Regarding the association between trypanosome infections and symbionts, no significant association was observed between *Spiroplasma* spp. or *S. glossinidius* and different trypanosome species infections in tsetse. This result is in line with previous studies carried out in Cameroon and the neighboring Nigeria to assess the relationship between *S. glossinidius* and trypanosomes (Kame-Ngasse *et al.*, 2018; Kanté Tagueu *et al*., 2018; Odeniran *et al.*, 2019), or *Spiroplasma* spp. and trypanosomes (Ngambia Freitas *et al.*, 2021). However, a negative association was found between the presence of *Spiroplasma* spp. in tsetse flies collected in the Sora-Mboum focus, and the trypanosome-infected status of these flies, regardless the trypanosome species, corroborating other studies by Schneider *et al.* (2019) or Dera *et al.* (2023), who found the same negative association. The lack of association found considering each trypanosome species separately could just be due to the low prevalence of each species that can affect the power of association tests as reported by Peduzzi *et al.* (1996). Therefore, *Spiroplasma* infections might induce resistance of tsetse flies to infections with trypanosomes (Schneider *et al.*, 2019; Son *et al.*, 2021). The mechanism by which *Spiroplasma* spp. induces refractoriness to trypanosomes in flies remains unclear but could be related to the competition for the same proliferation niches (midgut or hemolymph), competition for the same specific nutriments essential for their development, induction of an immune response or specific gene regulation in tsetse as previously suggested (Snyder *et al.*, 2010; Snyder & Rio, 2013, 2015). Regarding the association between *S. glossinidius* and trypanosomes in flies, our results are in contrast with previous studies, which highlighted a positive association between *S. glossinidius* and different trypanosome species, the presence of *S. glossinidius* favoring trypanosome infections (Farikou *et al.*, 2010; Wamwiri *et al.*, 2013). Despite the fact that more investigations seem highly needed to better understand the tripartite interaction between tsetse flies, their symbionts and trypanosomes, at the current stage, *Spiroplasma* spp. seems helpful in SIT application, as it can help impairing the ability of factory flies released during control operations to acquire and transmit trypanosomes and therefore appear as an added value for the SIT application.

Conclusion

This study revealed a high spatial and temporal variations in prevalence of *Spiroplasma* spp., *S. glossinidius* and various trypanosome species among tsetse flies collected in the Sora-Mboum AAT focus in northern Cameroon. Although no association was found between *S. glossinidius* and trypanosomes, *Spiroplasma* spp. was negatively associated with the presence of trypanosomes, indicating that this symbiont could be a suitable candidate for paratransgenesis to improving mass production

of trypanosome-refractory tsetse in insectaries for usage in SIT programs.

Author contributions

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Disclosure

The authors declare no conflict of interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Contingency table showing the relationship between the presence of targeted symbionts (*Spiroplasma* spp. and *Sodalis glossinidius*) and trypanosome infections in *Glossina tachinoides*.

Table S2 Odd ratios measuring the relationship between the presence of targeted symbionts (*Spiroplasma* spp. and *Sodalis glossinidius*) and trypanosome infections in *Glossina tachinoides*.