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Daily and seasonal variation of the nuisance caused by *Simulium damnosum* s.l. in three epidemiological facies of onchocerciasis (Soubré, Bouaflé and Touba) in Côte d'Ivoire

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ABSTRACT

The stings of the blackflies create a nuisance that hinders the agricultural and fishing activities of rural populations. In order to respond to the concern of the latter, this study was carried out in three epidemiological facies of onchocerciasis in Côte d'Ivoire. The aim of the study was to determine the daily and seasonal variation of blackfly nuisance. To do this, blackflies were captured on humans, identified and dissected. The entomological data revealed a total of 4244 blackflies captured. The comparison between the daily periods and the blackfly nuisance reveals averages of 42.4; 2.03; 22.3 bites/man/ hour and 5.04; 10.06; 27.1 bites/man/hour recorded in the morning, afternoon and evening respectively in Soubré and Bouaflé. In Touba the respective averages of 12.06 and 83.06 bites/man/hour were recorded in the morning and evening. A significant difference was observed between the bite density received during the rainy and dry seasons ($\chi^2 = 4.81$; $p = 0.022$). The bite density recorded in the rainy season was about twice as high as in the dry season (OR=1.87). A significant nuisance due to blackflies appeared in the divisions of Soubré, Bouaflé and Touba and varied seasonally between localities. In order to eradicate this blackflies nuisance, the onchocerciasis control system should eliminate blackflies by various means such as the destruction of breeding sites.

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Keywords: Blackfly, seasonal variation, onchocerciasis, blackfly nuisance, Côte d'Ivoire.

INTRODUCTION

Blackflies are the vectors of onchocerciasis or river blindness in Africa, Central and South America (WHO, 2013). In Africa, the most important species are *Simulium neavei* and members of the *Simulium damnosum* complex. These blackflies create a

considerable nuisance because of their painful bites and their sometimes huge mass attacks. These bites cause local swelling and inflammation with intense skin irritation lasting from a few days to several weeks (WHO, 2013). Within the framework of the Onchocerciasis Control Programme in West

Africa (OCP), the basic strategy has been to interrupt the transmission of the blinding strain of *Onchocerca volvulus* by destroying *S. damnosum* s.l at its larval stage (Hougard et al., 2000). Thus, in some areas, the significant reduction in blackfly bites from the start of the programme has been a great relief to the local population (WHO, 2009). Due to the success of this strategy, vector control operations were stopped in 2002 in the basins of the initial area. Since 2003, the eleven participating countries of the OCP have assumed responsibility for residual disease surveillance and control activities. Unfortunately, in Côte d'Ivoire, the cessation of OCP activities and the start of the APOC coincided with the military-political crisis of 2002 followed by the post-electoral crisis of 2011. These armed conflicts led to an interruption of entomological monitoring for more than 10 years (Yapi et al., 2014). This has led to a re-invasion of *Simulium* along rivers in the cotton zone on the Léraba (Ouangolodougou), Bou and Foubou (Dikodougou) rivers (WHO, 2013). Furthermore, an entomological assessment carried out in 2014 indicates the return of blackfly populations in the division of Bouaflé (Yapi et al., 2014). These authors insist on the aggressiveness of the blackflies and mention several cases of bites. Although blackflies are less infectious (Simaro et al., 2019), their reappearance is perceived by the population as a real despair as they are once again confronted with difficult living and/or working conditions. Indeed, blackflies bites cause significant nuisance with sometimes considerable economic consequences. In order to respond to the concern of the populations, the OCP has encouraged individual actions against the blackflies, which will make it possible to permanently protect the populations of the regions subject to this re-invasion. This is the reason for this study in three epidemiological facies in the west of Côte d'Ivoire, namely Soubré (south-west), Bouaflé (centre-west) and Touba (north-west). The general objective of this work was to determine the daily and seasonal variation of blackflies nuisance in order to contribute to the protection of the health of farmers in areas with high blackflies populations.

MATERIALS AND METHODS

Study zone

Division of Soubré

Soubré department is located at 05° 47' 12.6'' and 05° 47' 08'' LN and 06° 36' 0'' and 06° 36' 30'' LW (Figure 1). This area is marked by dense rainforest that is now giving way to remnants of forest and huge plantations of perennial crops such as coffee, cocoa, oil palm and rubber (Sorokoby et al., 2010). The climate of this division is sub-equatorial, with two rainy seasons from April to June and September to November, and two dry seasons, from July to August and December to March (Ohou-Yao et al., 2017). In this area, the average temperature is 24.6°C and rainfall is high with an annual average of 1800 mm (Sorokoby et al., 2010). The Soubré division has a hydrographic network made up of permanent watercourses. The current of this river is marked by numerous rapids that make it impassable to river navigation. These rapids are conducive to the creation of innumerable small, functional pre-imaginal deposits.

Division of Bouaflé

The Bouaflé division is located at 06° 58' 50.2'' and 06° 50' 30.2'' LN and 05° 45' 17.9'' and 05° 40' 12.7'' LW (Figure 1). The division is divided between dense forest in the south and west and wooded savannah in the north and east. Extensive and shifting agriculture, together with logging, have profoundly modified the flora, leaving large areas of fallow land and perennial crop plantations (Kouamé, 2008). This anthropic pressure makes Bouaflé an essentially agricultural region with a production of 46,803 tonnes of cocoa in 2014, 912 tonnes of coffee, 1090.7 tonnes of cotton and 10212.79 tonnes of food products. The division of Bouaflé belongs to the pre-forestry sector which has four seasons, including two rainy seasons from March to June and from September to October. The average rainfall is 1421 mm per year, with an average temperature of around 25°C (Kouamé, 2008). The hydrographic network of the Bouaflé division is essentially composed of the Marahoué or red Bandama river, which divides into several tributaries, bounded by outcrops of bedrock.

Division of Touba

Located at 08° 23' 04.4'' and 08° 15' 18.4'' N and 07° 37' 49.2'' and 07° 22' 34.2'' LW, Touba division is covered by a wooded savannah with some forests in places (Figure 1). It is a highly agro-pastoral area. The recurrent food crop is maize. This crop is supplemented by cassava and yams. Cattle breeding and fishing are practiced in this division. The climate is Sudano-Guinean, with harmattan in December and January. The long dry season (November to March) precedes the rainy season marked by two maximum rainfall periods, one in April and the other in October. Annual rainfall varies between 1600 and 2000 mm with an average annual temperature of 30°C (Simaro et al., 2019). The division of Touba is drained by three rivers and some small streams (Kouamé, 2005). These rivers are tributaries of the Sassandra. There are streams and marshes, which together with the Sassandra River and its three tributaries (the Bagbé, the Bafing and the Boa) at the rapids, form pre-imaginal *Simulium* deposits (Bellec et Hébrard, 1977).

Sampling methods

Capture of blackflies

The technique of capturing blackflies from humans using plastic haemolysis tubes has been used (Le Berre, 1966 ; Philippon, 1977 ; Simaro et al., 2019). A two-person team was used to capture the blackflies. Each person took turns working for one hour. The seated catcher collected in haemolysis tubes all the female blackflies that wanted to gorge themselves on him. Due to the low location of the fly bites, only his legs were stripped and exposed. At the end of the day, the number of blackflies captured was quantified. The captures were carried out throughout the day, from 7 a.m. to 6 p.m. without interruption thanks to the rotation of the catchers (WHO, 2009). Three capture points were selected per sampling site. In Côte d'Ivoire, climates were divided into two rainy seasons (May to August and September to November), a low humidity season (February to April) and a dry season (November to January). For the study of the season factor, the catches of the dry season

were made in the month of December 2016. Then, those of the intermediate season were carried out during the months of March and October 2017. As for the rainy season captures, they were carried out during the month of July 2017. In each season, the captures were carried out for three consecutive days in the same place.

Morphological identification of *Simulium damnosum* s.l. females

The identification of captured blackflies was established on the basis of the micro-morphological criteria used by the OCP program to distinguish the different species of the *S. damnosum* s.l. complex (Quillevere et al., 1977). Indeed, it consisted in observing and identifying the different parts of the blackflies, namely the colour of the antenna, the colour of the wing tuft and arculus setae, the colour of the scutellum setae, the colour of the first article of the foreleg or procoxa and the colour of the setae of the 9th abdominal tergite, using a binocular magnifier.

Determination of the maturity of dissected *Simulium damnosum* s.l. females

The blackflies, after being killed with chloroform, were dissected on a slide bearing a 4.5% sodium chloride solution. The dissection technique was that of Lewis (1957). The abdomen was incised in its posterior part with a dissecting needle, and then the different organs were pulled backwards. The appearance of the ovaries was used to differentiate between parous and nulliparous females (Ovazza et al., 1965; Duke, 1968c ; Simaro et al., 2019). Females with clear ovaries, with larger, bulkier, more spherical and swollen follicles, are nulliparous. The uppermost ovary is yellowish, with a mottled appearance. The follicles are slightly smaller and more oval.

Determination of blackflies infection by *Onchocerca volvulus*

The head, thorax and abdomen of the parous females were separated and soaked in a 4.5% sodium chloride solution and then diluted. They were carefully examined for *Onchocerca volvulus* larvae, the numbers and stages of

which will be used to establish infestation rates and parasite loads of *Simulium damnosum* complex females.

Data analysis

The data obtained were analysed using R software (Ri 368 3.4.4 version). A Chi-2 test of independence was used to compare the estimated parameters. This test made it

possible to compare the average number of bites received between the different capture sites and also between the dry and rainy seasons. The results are considered significant when the probability value p (p-value) is less than or equal to 0.05 ($p \leq 0.05$).

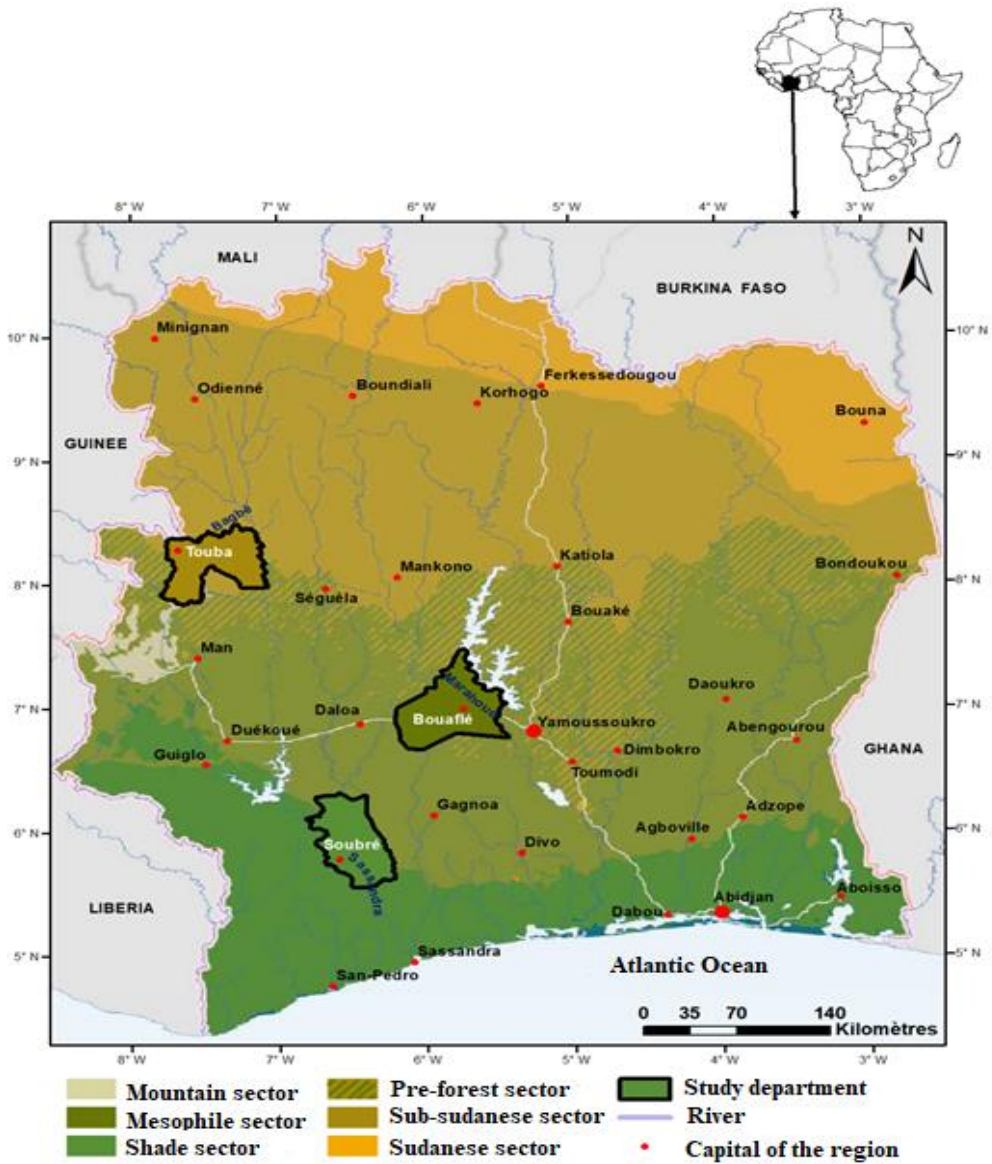


Figure 1: Study area.

RESULTS

Simulidian fauna

A total of 4244 blackflies were captured during this study, with 1333 in Soubré, 881 in Bouaflé and 2030 in Touba (Table 1). Morphological identification of blackflies reveals the existence of two groups of species. These are the species of the savannah group (*Simulium damnosum* s.s and *Simulium sirbanum*) (2900 blackflies) and the species of the forest group (*Simulium squamosum*, *Simulium sanctipauli*, *Simulium soubrense*) (1344 blackflies). The forest group is made up of 1333 blackflies captured in Soubré, 5 in Bouaflé and 6 in Touba (Table 1). As for the savannah group, 876 and 2024 blackflies were captured in Bouaflé and Touba respectively (Table 1). However, no blackflies from the savannah group was captured in Soubré.

Simulidian parturity

Of a total of 4244 blackflies captured, 2150 were dissected, including 672 in Soubré, 448 in Bouaflé and 1030 in Touba (Table 2). The dissection rate was 50.41% in Soubré, 50.85% in Bouaflé and 50.73% in Touba. Of the number dissected, 367, 272 and 682 blackflies were found to be parous in these respective localities. The average parturity rate was 54.61 % in Soubre, 60.71% in Bouaflé and 66.21% in Touba (Table 2).

Natural infection rate

No dissected blackflies was found to be parasitized in Soubré, Bouaflé and Touba. The monthly transmission potential (MTP) was zero during the study period.

Daily period and blackfly nuisance

Soubré area

The evaluation of the average density of blackflies bites received according to daily hourly variations revealed a significant difference between the number of bites received and the different periods of the day at Soubré ($p = 0.0121$) (Figure 2). This significant

difference was observed between the number of bites received in the morning and in the afternoon ($\chi^2 = 3.681$; $p = 0.027$) on the one hand and between the afternoon and evening hours on the other ($\chi^2 = 6.88$; $p = 0.018$). The averages of 42.4, 2.03 and 22.3 bites/man/hour were recorded in the morning, afternoon and evening respectively (Table 3).

Bouaflé area

The evaluation of the average density of blackflies bites received according to daily hourly variations reveals a significant difference between the number of bites received and the periods of the day at Bouaflé ($p = 0.0015$) (Figure 3). The significant difference was observed between the number of bites received in the afternoon and evening ($\chi^2 = 16.81$; $p = 0.006$) on the one hand, and between the evening and morning hours ($\chi^2 = 36.88$; $p = 0.0015$) on the other. The average of 5.04 bites/man/hour was recorded in the morning, 10.06 bites/man/hour in the afternoon and 27.1 bites/man/hour in the evening (Table 3).

Touba area

The evaluation of the average density of blackflies bites received as a function of daily hourly variations reveals a significant difference between the number of bites received and the periods of the day in Touba ($p = 0.029$) (Figure 4). This significant difference was only observed between the number of bites received in the morning and the number of bites received in the evening ($\chi^2 = 8.97$; $p = 0.012$). The respective averages of 12.06 and 83.06 bites/man/hour were recorded in the morning and evening (Table 3).

Comparison of seasonal variation and blackflies nuisance in the localities studied

The comparison between seasonal variation and the average density of blackflies bites received in the study localities indicates that

there is a significant difference between the number of blackflies bites received per individual in the dry and rainy seasons (Table 5). In Soubré, this difference was $p = 2.2 \cdot 10^{-16}$, in Touba, it was $p = 2.2 \cdot 10^{-16}$ and in Bouaflé, it was $p = 1.4 \cdot 10^{-3}$ (Table 5).

Seasonal variation and blackflies nuisance between seasons

No significant difference was obtained between the bite density received during the rainy season (July) and the intermediate season

(October) ($\chi^2 = 37.22$; $p = 0.95$) (Table 5). The same is true between the bite density received during the dry season (December) and the intermediate season ($\chi^2 = 42.60$; $p = 0.83$). However, a significant difference was observed between the bite density received during the rainy season and the dry season ($\chi^2 = 4.81$; $p = 0.022$). The results obtained show that the bite density recorded in the rainy season is about twice as high as in the dry season (OR = 1.87).

Table 1: Results of blackflies capture in the different localities.

Localities				
Population	Soubré	Bouaflé	Touba	Total
Forest blackflies	1333	5	6	1344
Savannah blackflies	0	876	2024	2900
Total	1333	881	2030	4244

Table 2: Parturity rate in the study localities.

Localities				
	Soubré	Bouaflé	Touba	Total
Number of day	12	12	12	36
Number of females dissected	672	448	1030	2150
Dissection rate	50.41	50.85	50.73	50.66
Parous female	367	272	682	1321
Parous female rate	54.61	60.71	66.21	60.21

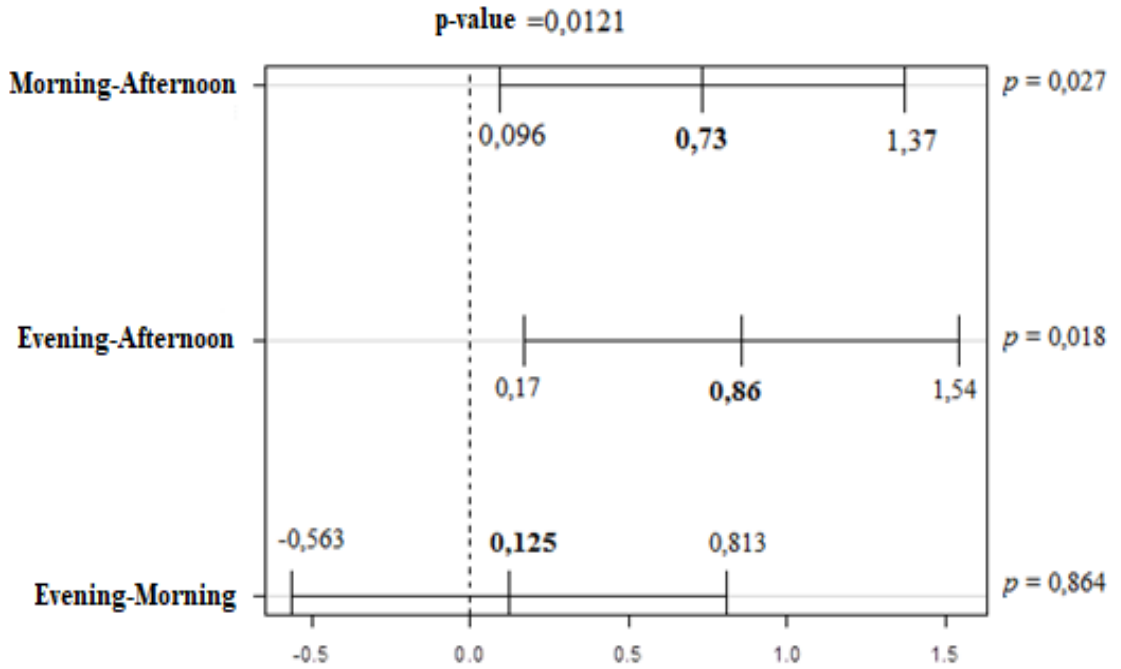


Figure 2: Daily variation in average blackflies bites in Soubré.

Table 3: Assessment of blackflies nuisance according to daily hourly variations in the study localities.

Period	Localities								
	Soubré			Bouaflé			Touba		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening	Morning	Afternoon	Evening
Bites number	210	6	66	25	30	81	60	0	415
Average (\pm sd)	42.4 \pm 0.5	2.03 \pm 0.05	22.3 \pm 0.6	5.04 \pm 0.04	10.06 \pm 0.05	27.1 \pm 0.1	12.06 \pm 0.06	0	83.06 \pm 0.05
CV (%)	1.17	2.46	2.69	0.99	0.47	1.4	0.66	0	0.06

CV : coefficient of variation, sd : standard deviation

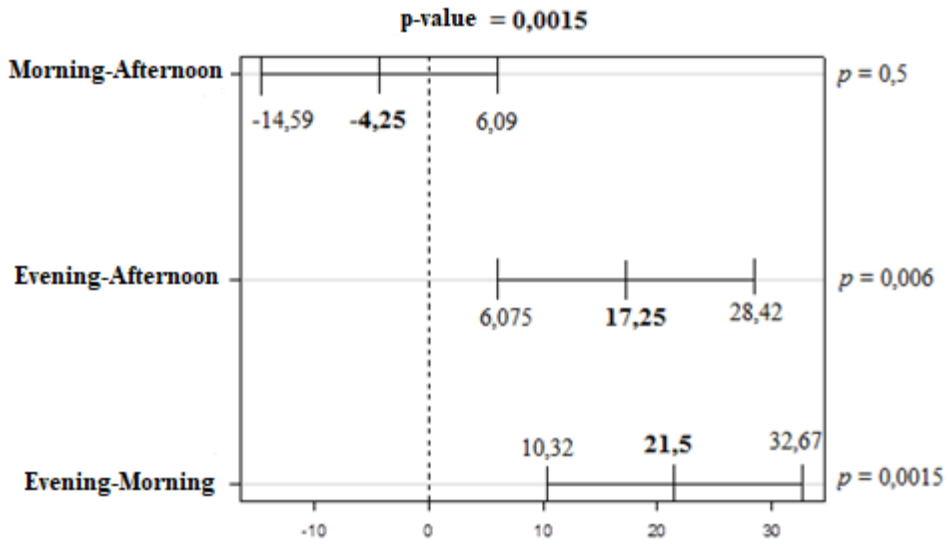


Figure 3: Daily variation in the average blackflies bites in Bouaflé.

Table 4: Comparison between seasonal variation and simuliidae nuisance in the study locations.

	blackflies captured		χ^2	p-value
	dry season	rainy season		
Soubré	196	908	459,19	$2,2 \cdot 10^{-16}$
Bouaflé	268	347	10,14	$1,4 \cdot 10^{-3}$
Touba	442	1107	285,49	$2,2 \cdot 10^{-16}$

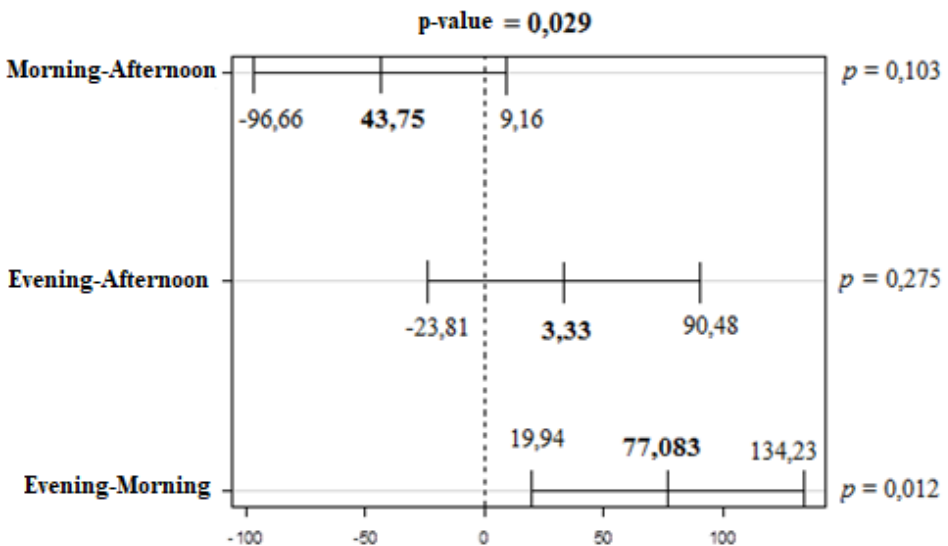


Figure 4: Daily variation in the average blackflies bites in Touba.

Table 5: Seasonal variation of blackflies nuisance in the study area.

Period	Est Coef	OR	CI 95 %	p-value
Rainy season – Dry season	0,47	1,87	1,07 - 2,38	0,022
Rainy season – Shoulder season	0,013	1,013	0,68 - 1,51	0,95
Dry season – Shoulder season	0,044	0,96	0,64 - 1,43	0,83

Est Coef : Estimated Coefficient, OR : Odd ratio, CI 95% : Confidence Interval 95%

DISCUSSION

The blackflies captured during this study belong to the *Simulium damnosum* complex. Specific identification indicates that savannah-type species constitute the bulk of the captures at the Bouaflé and Touba sites. At the Soubré site, forest-type species were the only ones encountered. These results indicate that savannah species of *S. damnosum* s.l. are predominant in these bioclimatic zones. Indeed, this was shown in the study by Adjami et al. (2004) on the identification of vectors and parasites of onchocerciasis, carried out in order to better understand the vector/parasite relationships in a forest and forest/savannah transition zone in Côte d'Ivoire. These authors attested that the forest species of *S. damnosum* s.l., although present in all seasons in the upper Bandama and middle Bandama, are in very low proportions, 2% and 4% respectively in the dry and wet seasons. The predominance of the savannah species of *S. damnosum* s.l. would cause a problem because of the nuisance of blackflies, which would greatly disrupt agricultural and fisheries activities, resulting in a drop in annual productivity (Coulibaly, 2007). Simulidian parturity results indicate that Touba has the highest parturity rate. In contrast, the lowest parturity rates, which are indicative of a young population of blackflies, are recorded in Soubré and Bouaflé. The variation in the parturity of blackflies females according to the zones studied could be explained by the fact that during the dry season in the savannah zone, the variations in climate and vegetation are more marked, which forces the females to confine themselves to a restricted biotope (Mavoungou et al., 2013).

This only allows for limited movement. The reserves of the females would then be depleted less quickly, allowing for a longer life span in the savannah zone. Therefore, the average physiological age of blackflies in the savannah zone would allow them to easily maintain the parasite cycle. The daily variation in the number of blackfly bites is thought to be closely related to the variation in temperature during different times of the day in different localities (Tekle et al., 2012). The temperature in these localities exceeds 30°C at midday and early afternoon. This is because blackflies only bite during the day and their activity ceases when it rains or the temperature is too high (Routledge et al., 2018). According to Basáñez et al. (2006) and Coffeng et al. (2014), temperature is the limiting factor for blackflies activity. Furthermore, the bite rates recorded in this study are above the tolerable threshold of 30 bites/man/day (Coffeng et al., 2014). These numbers of bites received per man/day in both the dry and rainy seasons show that the nuisance is real and would be particularly intolerable in the Touba area where the populations live in almost permanent contact with the blackflies. The results of the seasonal variation of simuliid nuisance indicate a significant difference in the density of blackflies bites between the rainy and dry seasons. Indeed, from the beginning of the rainy season, the floods lead to an increase in submerged vegetation constituting innumerable sites favourable to the development of pre-imaginal stages of blackflies, hence an increase in the blackflies population (Traoré et al., 2006). In contrast, during the dry season, the water level in rivers

is very low, resulting in a scarcity of plant supports used by larvae and nymphs for their development. Under these conditions, the blackflies population does not have the same possibilities of renewal. The results are similar to the observations of Enyong et al., 2006b. Indeed, these authors recorded in the Kumba and Ngambé areas of Cameroon, densities of blackflies biting females varying synchronously with the water level of the Mungo and Sanaga rivers. However, these observations are often contradictory. Yapi et al. (2014), noted the increase in blackflies density in the dry season, while Eyong et al. (2006) and Traoré et al. (2006) reported it in the rainy season. The simuliid density observed in Touba in the rainy season is, on average, almost twice as high as that recorded in Soubré and Bouaflé. In fact, in the Soubré and Bouaflé areas, the rivers are very uneven, with more or less large rocky masses, submerged or emerged according to the periods of flooding or recession. These non-navigable regions are invaded by a particular vegetation of plants. Attached to the rocks by powerful roots, these plants invade the rapids (Adéléké et al., 2010). In periods of high water, they are also the almost exclusive support for the larvae and pupae of *Simulium damnosum* s.l. (Hopkins et al., 2005). Water level variations during flood periods are slow and do not result in the sudden destruction of pre-imaginal larval and pupal sites by drowning (Simaro et al., 2019). According to Adéléké et al. (2010), the existence of these huge pre-imaginal nests results in the presence of considerable numbers of blackflies during the rainy season. In the Touba locality, human activities are almost exclusively agricultural and cultivation practices are essentially seasonal and more intense during the rainy season. During this period, simulium are generally more abundant (Simaro et al., 2019). This abundance results in a disruption of agricultural and fishing activities of populations in contact with simulium (Coulbaly, 2007). However, the low blackflies densities recorded during the rainy season in Bouaflé compared to Soubré and Touba could be explained by the fact that the Marahoué River is a very flat and dense

watershed. The first rains cause significant variations in the water level of these river systems, variations that drown the pre-imaginal breeding sites and do not allow the development of many larval populations (Hopkins et al., 2005). At the end of the rainy season, the water level stabilises and recedes slowly, allowing the larvae to develop on the many floating supports.

Conclusion

During the entomological surveys, it was found that the blackflies population in the study areas is mainly composed of savannah species. The high percentage of savannah vectors indicates a phenomenon of "savannisation" of onchocerciasis in Côte d'Ivoire. This phenomenon constitutes a risk of extension of blinding onchocerciasis and requires surveillance. In addition, the Simuliid nuisance disrupts the agricultural and fishing activities of populations in contact with the blackflies. However, no blackflies infected with *Onchocerca volvulus* were found during the study. This is not synonymous with zero transmission over a longer observation cycle. Finally, seasonal variations totally alter the nuisance of blackflies between sites. To eradicate this simuliid nuisance, the onchocerciasis control system should eliminate blackflies by different means such as destruction of breeding sites.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SS is the principal investigator; SS and AAB designed the experimental protocol; AAB, KKI and KBJ participated in the biological and statistical analyses. KM, DA, TM and SDP participated in writing and proofreading the manuscript.

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