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Parasitoids of fruit flies (Diptera: Tephritidae) and their distribution along mango production zones in Senegal

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ABSTRACT: In this study, the diversity and distribution of fruit fly parasitoids and their reservoir plants along a latitudinal gradient in three zones of mango production in Senegal were reported. Fruits were collected from wild and cultivated plants at 15 different sites (5 sites per production zone) over a period of seven months. In the northern and middle zone (Niayes and Centre). Our results showed a significant contrast in the distribution of parasitoid species between zones and fruit types. In Niayes, *Psytallia cosyrae* (Hymenoptera: Braconidae) was the dominant species, while in Centre it was *P. cosyrae* and *Diachasmimorpha* spp. (Hymenoptera: Braconidae). The native plant species that were hosting the highest densities of fruit flies and parasitoids were *Capparis tomentosa*, *Ziziphus mauritiana*, *Kedrostis hirtella*, and *Momordica balsamina*. In the southern zone (Casamance), we measured parasitism rate by the introduced parasitoid *Fopius arisanus* (Hymenoptera: Braconidae) and found an average of 5% parasitism rate in mango orchards.There, the most abundant parasitoids found were *F. caudatus* and *F. sylvestri*. Additional research is needed to characterize the factors affecting the effectiveness of biological control with hymenopteran parasitoids and determine the role played by native host plants in the control of fruit flies.

Keywords: Hymenopteran parasitoids, parasitism, host fruits, fruit flies, mango, *Bactrocera dorsalis*, Senegal

INTRODUCTION

In sub-Saharan Africa, the horticultural sector is seriously affected by losses caused due to several fruit fly infestation. In addition to native species, the exotic species *Bactrocera dorsalis* (Hendel) has invaded and spread rapidly in this sub-region (Hernández-Ortiz *et al.,* 2006; Khamis *et al.,* 2009; De Meyer *et al.,* 2002). Besides direct losses, quarantine measures have a significant impact on exports for producing countries. In the absence of effective management approaches, these pests can lead to a total loss of production (Ekesi *et al.,* 2016). Management practices for fruit flies rely on pesticides and are now being questioned due to the strict regulation of pesticides residues on fruits and vegetables by export markets as well as their harmful effects on public health and ecosystems.

To tackle these issues, management approaches have evolved towards more sustainable alternatives, such as food baits associated with biopesticide applications, sterile insect technique (SIT), or orchard sanitations. Many studies showed that fruit fly populations are naturally controlled by parasitic hymenopterans (Silvestri 1913; Vargas *et al.,* 2016) and in the African tropical zone, several species of parasitoids exhibited potential as biological control agents (Silvestri, 1914; Vayssières *et al.,* 2011a; Billah *et al.,* 2008; Ndiaye *et al.,* 2015; Rousse and Quilici 2009; Ekesi and Billah, 2006). In addition to native species, exotic species have also been successfully introduced to control of fruit flies (Sivinski *et al.,*1997; Wharton and Yoder, 2018). An important step toward using parasitoid as a tool against fruit flies will be to characterize their diversity and effectiveness for biological control (Manrakhan *et al.,* 2015; Vayssières *et al.,*2011a). Despite some attempts (Manrakhan *et al.,* 2015; Vayssières *et al.,*2011a), in Senegal, little information remain available regarding fruit flies natural enemies and the last survey was carried eight year ago in Casamance (Vargas *et al.,* 2007). The only known species of parasitoids are *Diachasmimorpha fullawayi, Psyttalia dexter* (Silvestri, 1913) and *Coptera silvestrii* (Wharton *et al.,* 2000 ; Rugman-Jones *et al*., 2009). In the present study, we reviewed the diversity and distribution of native hymenopteran parasitoid species, in relation to their host plants in two important areas of mango (*Mangifera indica*) production: Niayes and Centre. In Casamance, we evaluated the parasitism rate of fruit flies by *F. arisanus* as it has now been eight years since its introduction as a biocontrol agent (Sivinski *et al.,* 2000). Our goal is to gather knowledge that will help set more effective and sustainable strategies for fruit fly control by examining the potential of native fruit plants to host parasitoids that can be used for augmentative releases.

MATERIALS AND METHODS

Study sites and zones

GPS coordinates for the sample sites are presented in Table 1. The Niayes zone is a coastal strip that runs from Saint Louis to Dakar and is about 180 km long and its width varies from 5 to 30 km inland (Cissé, 2000). It is characterized by dunes and depressions and a coastal climate favorable to fruit and vegetable production. Considered one of Senegal's most important agro-ecological zones, it accounts for nearly 80% of the national horticultural production. However, intense wind erosions have impacted its agro-sylvo-pastoral potential (Cissé, 2000; Gravaud, 1988; Mau *et al.,* 2007). Furthermore, the Sudan-Sahelian drought that started in the 70's led to a rainfall deficit and a reduction in groundwater levels as well as a rise in the salinity of soils. This imbalance is enhanced by anthropogenic actions such as growing urbanization and deforestation (Fall et *al.,* 2001).

The Centre zone covers about 180,000 hectares and extends from the the Petite-Côte south of Dakar to the natural region of Sine Saloum in northern Gambia. The Delta Saloum National Park is ranked as a World Heritage Site, a Biosphere Reserve, and a wetland of international importance under the Ramsar Convention. The huge delta, formed by the confluence of two rivers, the Sine and the Saloum, brings salty water into the land (Mau *et al.,* 2007). The Centre zone is an emerging area for mango production. . There, the plantations are smaller than in Niayes and are scattered in islets. Trees that are over-grafted with improved varieties are typically common (Fall et *al.,* 2001).

The Casamance zone is in the southernmost part of Senegal and covers 35,680,000 hectares (18% of the national territory). It has a sub-Guinean tropical climate with relatively high access to water, including a rainy season that lasts about five months (May to October). Its diversified water system includes the 300 km of the Casamance River and its tributaries, and a 86 km coastline bordered by 70,000 ha of mangroves (Ba, 2004; Mau *et al.,* 2007). Casamance is one of the main mango production areas in Senegal. Familial farms and traditional practices are the most common style of production. Mangoes and citrus fruits are the predominant fruit species on farms (Fall et *al.,* 2001).

Sampling of infested fruits

Fruit samples were collected weekly from April to October 2018 in and around mango orchards. We used convenience sampling (type of non-probability sampling) to select fruits from cultivated and wild tree species, mature or immatures: fruits were being drawn

Table1. Details of sample sites within mango zones, Senegal along with geographical coordinates

Zone	Location	Latitude	Longitude	Altitude (m)
Niayes	Sindia	14, 35076 N	17, 01329 W	30
	Wayembane	14, 77486 N	17, 21621 W	15
	Keur Moussa	14, 77486 N	17, 12503 W	22
	Sébikotane	14, 74116 N	17, 14115 W	23
	Niaga	14, 79566 N	17, 26257 W	22
Centre	KeurBabou Diouf	13, 57520 N	16, 23093 W	7,4
	Néma Nding	13, 53447 N	16, 22354 W	12,2
	Médina Sangako	13, 48289 N	16, 23028 W	17,4
	Ndramé Macoumba	13, 49033 N	16, 26514 W	20,9
	Tallène	13, 48289 N	16, 23028 W	15,2
Casamance	Djibélor	12, 55556 N	16, 32008 W	6
	Koubanack	13, 12593 N	16, 36594 W	16
	Petit Camp	12, 53051 N	16, 27899 W	11
	Diouloulou	13, 03161 N	16, 35717 W	14
	Bourofaye	12, 53296 N	16, 29838 W	7

from that part of the population that was close to hand. We selected the fruits based on the presence of fruit fly puncture marks on the skin. Size and composition of the sample varied, depending on fruit availability and tree phenology. We collected a maximum of ten fruits per tree species (Vargas *et al.,* 2016). Samples were brought back to the laboratory where fruit fly incubation and pupa collection were carried according to the procedure described by Vayssières *et al.* (2011a), Vayssières *et al.* (2012) and Ekesi and Billah (2006).

Incubating samples of infested fruits

Fruit incubation provides data on species-specific fruit fly infestation rates as well as host plant species. For this, we counted and weighed the fruits and placed them in incubation units. Each incubation unit consisted of a small box containing the fruits (≈ 20 cm diameter) placed within a larger box (≈ 30 cm diameter) that contained a thin layer of fine sand (about two to three cm thick) to allow mature larva to pupate. The unit was then covered with fine cloth to prevent larva escape. For ten days, pupa were extracted from the sand by sieving every two to three days. We counted the pupa and placed them in Petri dishes lined with toilet paper and we kept them in cages until the emergence of flies and/or parasitoids. The emergences were also counted and dated (Ekesi and Billah, 2006). Adult flies and parasitoids were fed with water and honey until their morphological characteristics allowed for identification. The temperature in the laboratory was maintained at 27 ± 1 °C and 60.5 ± 2% relative humidity and a photoperiod of 12:12 (L:D) hours (Vargas *et al.,* 2016).

Parasitoid rearing and releases

In mango orchards, chemical treatment to control fruit flies is not a common practice. Furthermore the agro-ecological conditions are suitable to biodiversity and the development of biological control approaches. For these reasons, the Senegalese government has allowed the introduction of the ovo-pupal parasitoid *F. arisanus*in the territory. *Fopius arisanus* were obtained from the Hawaii Entomology Laboratory (USDA-ARS). (The rearing methods for *F. arisanus* on *B. dorsalis* is outlined by Khamis *et al.* (2009). Approximatively 500,000 wasps were shipped by airplane to Senegal between 2013 to 2018, parasitoids were transferred from the Dakar International Airport to the National Crop Protection Service Entomology Laboratory (Direction de la Protection des Vegetaux).

We placed approximately 2000 wasps per cubical cage (26 x 26 x 26 cm). Wasps were fed with honey, raw sugar, and water and held inside the cages until release.

We released the parasitoids in Casamance in 15 mango orchards (**Table 1**). During the release, the cubical cages were placed under host trees and opened gently, allowing parasitoids to disperse to nearby ripe host fruits (Sivinski *et al.,* 1997).

Summary of the literature available on parasitoids for the Casamance zone

Data used in this study was articles published in scientific journals. We examined the eight known références as primary sources of information of the country. Major sources were recorded in a database, including the identity of host plants, parasitoids and associated fruit flies (Table 5).

Data analysis

The fruit infestation index was computed as the total number of pupa obtained from the infested fruits divided by the total mass of the samples. This index provides information on fruit damage and allows for comparison of infestation levels, although the number and size of the fruits collected can vary considerably throughout the sampling period (Cowley *et al.,* 1992). Parasitism rate (T) was calculated only for solitary parasitoids and calculated as follow:

$$Tx = \frac{Np}{Nm+Np}$$

Np = number of parasitoids emerged from the pupa
Nm = number of fruit flies emerged from pupa

We used a one-factor analysis of variance to compare mangoes'mass for each site and a regression analysis between fruit mass and parasitism rate. We performed a multiple regression analysis between parasitoid species, fruit fly species, and host plant species.The data were analyzed with JMP Pro 14. The significance level was set to α = 0. 05.

Identifications

Identification of the wild trees was done with the Flora Guide of West African Dried Areas (Arbonnier, 2004) and the Flora Guide of Senegal (Berhaut, 1971-1979). Parasitoids species were identified at the Entomology laboratory of the IFAN (Institut Fondamental d'Afrique Noire) in Dakar thanks to voucher specimens.

RESULTS

In the Niayes zone, we found eight species of host plants: five cultivated species and three wild species. Among them, *Mangifera indica* (mango), *Capparis*

Table 2. Native hymenopteran parasites of fruit flies and associated host plants in the Niayes zone, Senegal

Parasitoid	Host plant	Number of sampled fuits	Total mass (kg)	Fruit fly species
Psyttalia cosyrae (Hym. :*Braconidae*)	*Capparis tomentosa* (Capparaceae)	1354	58,663	*Carpomya bipustulata* *Bactrocera dorsalis*
Psyttalia cosyrae (Hym. :*Braconidae*)	*Capsicum annum* (Solanaceae)	742	1,758	*Ceratitis capitata*
Psyttalia cosyrae (Hym. : Braconidae)	*Momordica balsamina* (Cucurbitaceae)	566	2,994	*Dacus ciliatus*
	Citrus limon (Rutaceae)	*631*	*4,925*	
Aganaspis sp (Hym. :Figitidae) *Trichopria* sp (Hym. :Diiapridae)	*Citrus maxima* (Rutaceae)	155	67,824	*Bactrocera dorsalis* *Zeugodacus cucurbitae* *Ceratitis cosyra*
	Citrus sinensis (Rutaceae)	142	46,263	
	Psidium guajava (Myrtaceae)	91	0,677	
	Anona muricata (Annonaceae)	47	25,544	

Table 3. Native hymenopteran parasites of fruit flies and associated host plants in the Center zone, Senegal

Parasitoid	Host plant	Number of sampled fruits	Total mass (kg)	Fruit fly species
Diachasmimorpha spp. (Hym. :*Braconidae*)	*Ziziphus mauritiana* (Ramnaceae)	589	1,354	*Carpomya bipustulata*
	Cordyla pinnata (Fabaceae)	78	18,798	
	Anacardium occidentale (Anacardiaceae)	130	11,661	
Psyttaliac osyrae (Hym. : *Braconidae*)	*Capparis tomentosa* (Capparaceae)	836	5,116	*Carpomya bipustulata ;* *Bactrocera dorsalis ;* *Ceratitis capitata*
	Capsicum annuum (Solanaceae)	*188*	*0,582*	
	Citrus limon (Rutaceae)	217	1,152	
	Citrus maxima (Rutaceae)	89	47,944	
	Citrus sinensis (Rutaceae)	73	16,352	
Psyttalia cosyrae (Hym. : *Braconidae*)	*Sclerocarya birrea*	345	3,208	*Ceratitis cosyra* *Ceratitis silvestri*
	Cucumis melo (Cucurbitaceae)	25	7,567	
	Cucumis metilifer (Cucurbitaceae)	67	15,812	
	Ficus ingens (Moraceae)	*162*	*0,301*	
Dirhinus sp. (Hym. : *Chalcididae*) *Fopius concolor* (Hym. : *Chalcididae*)	*Kedrosti shirtella* (Cucurbitaceae)	51	32,691	*Zeugodacus cucurbitae*
	Mangifera indica (Anacardiaceae)	332	146,416	
	Momordica balsamina (Cucurbitaceae)	175	2,544	

tomentosa (African caper), *Capsicum annum* (chili pepper), *Citrus maxima* (pomelo), and *Momordica balsamina* (basalm pear) were the only species yielding both parasitoids and flies. We also identified 3 parasitoid species, namely *Psytallia cosyrae*, *Aganaspis* sp. and *Trichopria* sp. (Table 2). The African caper (*C. tomentosa*) was infested by both *Carpomya bipustulata* and *B. dorsalis.* 74.63% of these fruit flies were parasitized by *Psytallia cosyrae*. The basalm pear (*M. balsamina*) was infested by *Dacus ciliatus* and 15.91% of these flies were parasitized by *P. cosyrae*. The chili pepper was infested by *Ceratitis capitata* and 4.88% of these flies were parasitized by *P. cosyrae*. The pomelo (*Citrus maxima*) was infested by *B. dorsalis*, *B. cucurbitae,* and *Ceratitis cosyra.* Those flies were parazitised by *Aganaspis* sp. (2.03%) and *Trichopria* sp. (1.94%).

In the Centre zone, 14 host plants were sampled belonging to 5 cultivated species and 9 wild species. Among them, we found 12 species hosting fruit flies and three species hosting both flies and parasitoids: *Ziziphus mauritiana* (Indian jujube), *C. tomentosa* (African caper), and *Kedrostis hirtella.* Four species of parasitoids were identified: *Diachasmimorpha spp*, *P. cosyrae*, *Dirhinus sp,* and *Fopius concolor* (Table 3). The Indian jujube (*Ziziphus mauritiana*) was infested by *C. bipustilata;* 13.65% of these flies were parasitized by *Diachasmimorpha spp*The mockernut hickory (*C. tomentosa*) was infested by *C. bipustilata*, *B. dorsalis,* and *C. capitate*; 67.19% of these flies were parasitized by . *P. cosyrae. Kedrostis hirtella* was infested by *Zeugodacus cucurbitae*; 11.83% of flies were parasitized by *Dirhinus sp.* and 7.33% by *Fopius concolor*.

Host plant species had a substantial effect on parasitism identity and rate, and there were several highly significant interactions between host fruits and flies species (Table 4).

We summarized the number of native and introduced hymenopteran parasitoids of fruit flies and associated host plants that were reported for Casamance (Table 5). The most recent report by Vargas *et al*. (2007) shows that the most abundant parasitoid species are *Fopius caudatus* (63.97% emergences) and *F. silvestrii* (14.10%), with a population peak between July and September. In our own survey for survival of *F. arisanus* under natural conditions in Casamance, we collected 614.33 kg of infested fruits from April to October 2018, which yielded 13,344 pupa. The mean overall parasitism rate was 5.21±1.8%. From April to June, 0.94 ± 7.77% pupa were parasitized by *F. arisanus*. Gradually, the parasitism level increased between July and October to reach 4.18 ± 4.07% (Fig.1).

DISCUSSION

From our results and existing data that we reviewed, we found several trophic relationships between parasitoids and wild plants species that are mainly infested by ceratite flies. Our data showed the occurrence of parasitoids from July to October, coinciding with abundant rainfall and mango maturity (Fig 1). We found a large variation in the distribution of parasitoid species between mango zones and host plants. We found that *Psytallia cosyrae* was the most dominant species in the northern zone of Niayes. In the Centre zone, *Diachasmimorpha spp*., and *P. cosyrae* were the two most abundant species. Finally, from the

Table 4. Relationships between parasitoid species, fruit fly species, and host plant species by Multiple Regression analysis

Zone	Parasitoid species	Host fruits			Host flies		
		df	*F*	*P*	*df*	*F*	*P*
Niayes	*Psyttalia cosyrae*	26	6.3	**0.0001**	26	2.8	0.23
	Aganaspis sp.	26	4.6	**0.0000**	26	6.3	1.19
	Trichopria sp.	26	3.8	**0.0000**	26	9.1	2.01
Centre	*Diachasmimorpha* spp.	29	7.3	**0.0000**	29	3.5	0.25
	Psyttalia cosyrae	29	6.3	**0.0000**	29	7.5	1.11
	Fopius concolor	29	4.6	**0.0000**	29	4.4	0.43
	Dirhinus sp.	29	9.2	**0.0001**	29	5.1	0.61

literature survey, *Fopius caudatus* and *F. sylvestri* were the most important parasitoids reported for the southern part of the country (Casamance). Previous surveys had only highlighted the occurrence of native parasitoids belonging to the genus *Fopius* and *P. cosyrae* in Niayes and the Plateau de Thiès (Vargas *et al.,* 2007; Montoya *et al.,* 2000). Therefore this is the first report of the importance of *Diachasmimorpha* spp. in the Centre zone.

Within all mango zones, there was phenological differences between the fruiting period of mango trees and alternative host plants. It was only when mangoes were not available that other plants (wild or cultivated) served as alternative food sources for some fruit fly species hosting parasitoid wasps. These alternative host plants are thus a potential reservoir for populations of native parasitoids. In the Centre and Niayes zones, the parasitism level ranged from 0 to about 70% depending on the plant species but the exact factors behind this variation are unclear. For example, *P. cosyra* parasitized more flies on fruits of the African caper (*C. tomentosa*) than on chili peppers (*Capsicum annuum*). These observations match those of Vargas *et al.* (2007) but remained to be explained. It appears that fruits of some native species such as the African caper (*Capparis tomentosa*) , the Indian jujube (*Ziziphus mauritiana*) , *Kedrostis hirtella* , and the basalm pear (*Momordica balsamina*) can host high densities of both fruit flies and parasitoids. Therefore, these wild plant species are good candidates for the application of augmentoria, mass production, and augmentative releases of biological control agents. According to Maya (2006) increasing releases near native host plants may improve parasitism levels in the field and significantly reduce damage to fruits during certain times of the year.

The absence of parasitoids in many plant species may be due to the difficulty in localizing immature fruit flies in some fruits (Silvestri, 1912; Vargas *et al.,* 2007; Vayssières *et al.,* 2012). As a result, a parasitoid such as *F. arisanus* may prospere only in habitats that contain small fruit hosts where the flies may be easier to localize. Mangoes are large fruits, thus the poor performances of *F. arisanus* that have been confirmed by our study might explain the low efficacy of previous field releases that

Table 5. Native and introduced parasitoids with host plants and associated fruit flies. Extract of references published in the Casamance area

Parasitoïd	Fruit fly species	Fruit species	Reference
Fopius caudatus *F. silvestrii* *F.desideratus* *Diachasmimorpha fullawayi* *D.carinata* *Psyttalia cosyrae* *P. concolor* *Pteromalidae* *Eulophidae*	*Ceratitis cosyra* *C. silvestrii* *C. punctata*	Wild species	Ndiaye *et al.* (2015) Vayssières *et al.* (2012)
F. desideratus		*Anacardiuum occidentale* *Anona senegalensis* *Icacina senegalensi* *Saba comorensis* *Sarcocephalus latifolius*	Vayssières *et al.* (2012)
F. arisanus	*B.dorsalis* Ceratitflies	*Mangifera indica* *Citrus* spp. *Anacardium occidentale* *Psidium guajava* *Saba senegalensis* *Landolphia heudelotii*	Vargas *et al.* (2016) Ndiaye *et al.* (2015)

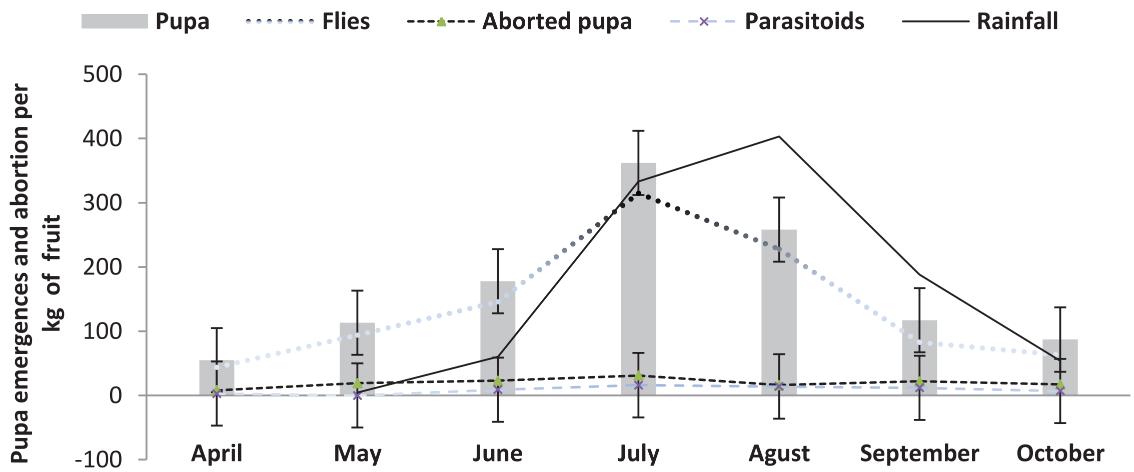

Fig. 1. Average number (± sd) of pupae per kg of mango fruit and average percentage (± sd) of *F. arisanus* emerged from mango fruits sampled in Casamance zone between April and October 2018. Rainfall data are presented in solid blackline

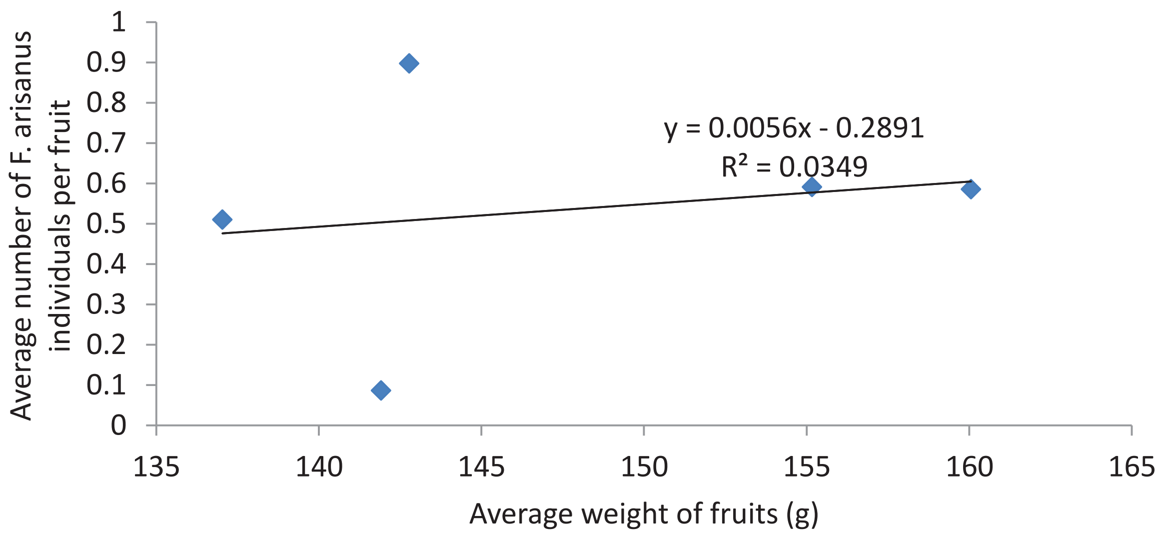

Fig. 2. Linear regression between *F. arisanus* emergence and weight of mango fruit sampled from April to October 2018.

have been performed by the DPV entomology laboratory. These observations are similar to those of Sivinski *et al.* (1997) and Montaya *et al.* (2000) who reported a low level of parasitism within the same area. Likewise, parasitism by *F. arisanus* on *M. indica* fruits and other wild fruits infested by *B. invadens* and other fruit fly species was low Montaya *et al.* (2000). A linear regression analysis has shown a weak link between the fruit weight and parasitism rate (Fig 2). Similarly, Sivinski *et al.* (2000) has documented a negative correlation between fruit size and braconid parasitism, presumably because host larvae in larger fruit are able to feed at greater depths and are more difficult for parasitoids to reach with their ovipositors.

Furthermore, there may be unfavorable conditions to parasitism in mango orchards due to cultural practices like cleaning or application of insecticides (Hernández-Ortiz *et al.,* 2006).

CONCLUSION

This study, provides essential reference data for future conservation actions involving native parasitoids as well as introduced species like *F. arisanus* to regulate the populations of *B. invadens*. All species of parasitoids identified in this study were reported from West Africa. We found that some native plants were hosts for both flies and their parasitoids while other plant species appeared to harbor only uncontrolled populations of fruit flies. It could potentially be interesting to identify those species, as their presence near mango orchards might be unwanted. Therefore, assessing the distribution of host plants in the vicinity of orchards is crucial for the management of polyphagous fruit fly populations. We suggest that native plants could be selected and planted around the orchards to help reduce the populations of fruit flies in the field using augmentative methods.

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