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Survey of potential insect vectors of Rice Yellow Mottle Virus in the Southern and Central rice basin of Benin

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ABSTRACT

Objectives: Insects are considered as the main vectors of Rice Yellow Mottle Virus (RYMV) in Africa. However, in Benin, little is known about the abundance and diversity of potential insect vectors of RYMV in rice fields to prevent and manage disease impact on rice production.

Methodology and Results: The inventory of the potential insect vectors of this disease was carried out in the rice basin of the Southern and Central of Benin where three sites namely AfricaRice station, Koussin and Ouedeme, were prospected. The sweep net technique, visual observation and yellow plates trap were used to conduct the surveys. Eighty insect species belonging to 28 families and 8 orders were recorded at rice tillering, booting, heading and maturation stages. Variation of insect diversity was low among the sites. The short-horned grasshopper *Oxya hyla* (Serville), the ladybird beetle *Chnootriba similis* (Mulsant), the rice white leafhopper *Cofana spectra* (Distant) and the stalk-eyed fly *Diopsis thoracica* (Westwood) were the most important species considering their relative abundance (10.62%, 5.19%, 7.99% and 7.01%, respectively) and frequency of occurrence (90%, 80%, 73.33% and 80%, respectively). These insects were mostly present at tillering and booting stages.

Conclusion and application of results: Seventy-five (75 %) of the recorded species were not identified as RYMV vectors before. Special attention must be paid to the distribution and importance of these insects in the fields. Studies on their ability to transmit RYMV according ecological conditions should be done to know high-risk production areas and to prevent large epidemics of RYMV.

Keywords: Rice-feeding insects, West Africa, virus transmission, relative abundance, occurrence, rice ecology

INTRODUCTION

Rice is one of the most produced and consumed cereals in West Africa. Similarly, to all cereals, rice production is constrained by several diseases. Several studies found that Rice Yellow Mottle Virus (RYMV) is one of the main constraints to rice production (Séré *et al.*, 2008; Onwughalu *et al.*, 2010, 2011). Yield losses due to RYMV were estimated between 5-100% (Banwo *et al.*, 2004; Alegbejo *et al.*, 2006). In Benin, this disease was identified for the first time in 1999 (Konaté *et al.*, 2008). Its presence was confirmed later by the AfricaRice pathology unit (AfricaRice, 2008, unpublished data). It has been proven that insects are the main vectors of RYMV (Bakker, 1971; Nwilene *et al.*, 2009). Rats, birds (Peters *et al.*, 2012), domestic animals like cattle and donkeys (Sarra & Peters, 2003), and manure (Calvert *et al.*, 2003) are RYMV vectors. Rice plant is an ideal host for most insect species. All plant parts are fed by various insect species. In West Africa, some prospections carried out mainly in Côte d'Ivoire, Senegal, Nigeria, Guinea and Guinea-Bissau reported approximately 330 insect species in rice fields (Heinrichs & Barrion, 2004). They are all considered as potential vectors of RYMV due to their presence in these fields (Nwilene, 1999). In Benin, Little research has been done on the diversity of insect pests in rice fields and are mainly related to stem borers (Togola *et al.*, 2010; Togola *et al.*, 2011), root termites (Togola *et al.*, 2012) and storage insects (Togola *et al.*, 2014). However, for a better understanding of the RYMV epidemiology and its distribution, the entomofauna of potential vectors should be known.The present study aims therefore at inventorying the insect pests, potential vectors of RYMV in Southern and Central basin of the rice production in Benin.

MATERIALS AND METHODS

Study region: Surveys were conducted from November 2009 to September 2010. Three sampling sites in the Southern and Central rice basin where RYMV isolates were identified (AfricaRice unpublished data, 2010). The sites were localized in Ouedeme (N 06°42'57", E 01°40'54"), Koussin (N 07°14'34", E 02°17'19") and AfricaRice-Cotonou station (N 6° 25'10", E 2°19'83") (Figure 1).

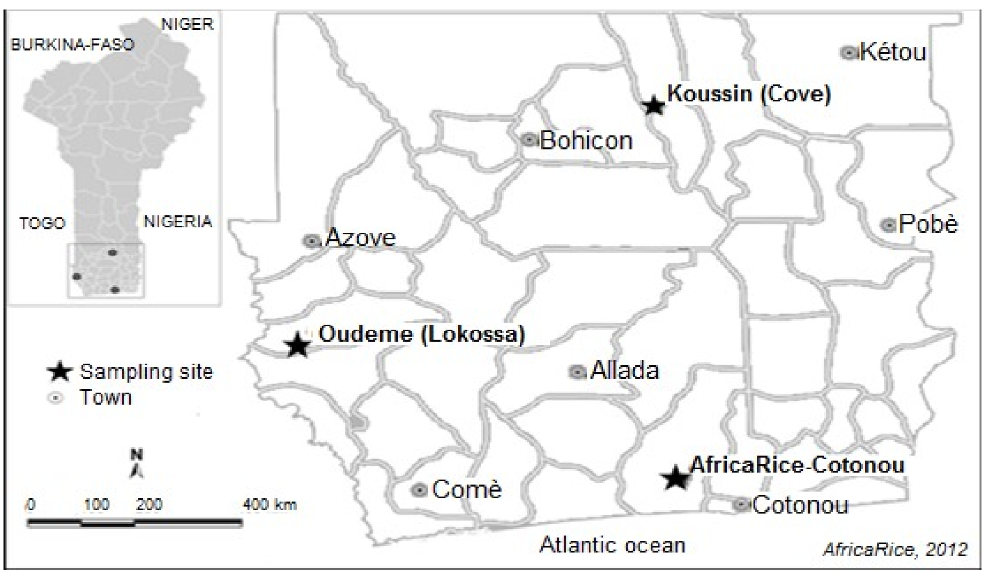

Figure 1. Rice basin in Southern and Central Benin.

Ouedeme and Koussin sites were irrigated lowland ecosystem where rice is essentially cultivated. AfricaRice site is research station with irrigated lowland and upland ecosystems of rice production. Maize, cowpea, cassava and market gardening were also grown besides rice fields in this station. For the three sites, rice is continuously growing throughout the year due to favorable climatic conditions.

Sampling Methods: For each site, surveys were carried out during tillering, booting, heading and maturation stages of rice development in lowland system. A plot of 3600 m^2 was randomly selected for each stage. No insecticide was applied on such plots. Each developmental stage was sampled using the techniques of sweep net (Sadou *et al.*, 2008), visual observation and yellow plates trap. For sweet net, 360 mowing were carried out for each developmental stage. Due to the high mobility of some insect species, visual observation was done. It entails counting all observed insects on one the diagonal of the field. These insects were reported and briefly described. The small and low mobile insects were collected using a small mouth aspirator. The last technique used, was catching the maximum number of insect species with yellow plates (Roth, 1963; Rolot, 2005). Yellow color is known to attract most flying insects (Dupriez *et al*., 2001). Nine plastic plates of 20 cm width each and 10 cm depth (Sylvie, 1995), were filled with soapy water and placed on tutors at each rice stage. To prevent the decomposition and discoloration of trapped insects, 10 g of table salt were added in the soapy water (Limoges, 2003). Each developmental stage was sampled once for each site.

Period and sequence of sampling: For each rice developmental stage, visual observation was firstly done. Thereafter, yellow plate traps were placed in the sampling area for two days before net sweeping was performed. The sweep net was used only during tillering and booting. Trapped insects with the plates were carefully recovered by water filtering through a fine mesh. Trapped insects with sweep net were conserved into fine mesh cages containing the rice plants and brought back to the laboratory where sorting was done to separate and rear insect larvae not yet identifiable. Trapped adult specimens were also preserved in 70% ethanol considering the site, development stages, technique and data of sampling before the identification.

Identification of insect specimens: Identification of insect specimens was done in the laboratory of entomology at AfricaRice-Benin using the identification key of insects (Heinrichs, 1983), the key of insect families (Delvare & Aberleng, 1989) and the manual of the insect pests of rice and their natural enemies in West Africa (Heinrichs & Barrion, 2004). In addition, the reference collections of the IITA-Benin insectarium were used for fine-tuning the identification. Insects were thereafter counted and classified by species.

Statistical analysis: To determine the relative proportion of species and orders of the identified insects, Chi-square test was performed. The relative abundance and frequency of occurrence of the insect species were calculated to assess the importance and distribution. The relative abundance (A) of a species is the ratio of individuals of the species to the total number of specimens of all species. It is computed using the following formula:

$A = \frac{\text{Na}}{\text{Na+Nb+Nc+N...}} * 100$ Where *A* is the Relative abundance and Na, Nb, Nc are the number of specimens respectively for species a, b and c.

The frequency of occurrence (F) is the sample percentage in which a given species was present relative to the total of samples collected (Dajoz, 1985; Alhmedi *et al*., 2007). The formula used is as follow: $F = \frac{Pa}{P} * 100$, where *F* is the frequency of occurrence of each species, *Pa* is the number of samples containing the species and *P* is the total number of samples collected. There are four (4) groups of frequency of occurrence (Dajoz, 1985): Group 1 (constant species with 50% or more of the total sample), Group 2 (accessory species that occur between 25-49% of the total sample), Group 3 (accidental species that occur in 10 to 24% of the total sample) and Group 4 (sporadic species which occur in less than 9% of the total sample). The diversity indices were determined for the rice growing stages and for each site. The Shannon-wiener index (1) and its related Evenness Index (2), Simpson's index (3) and its related Evenness Index (4). The reciprocal form of Simpson index was used as indicated by Magurran (2004):

(1) $H' = -\sum_{i=1}^{S} p_i \ln p_i$;

(2) $J' = H'/H_{\max} = H'/\ln S$;

(3) $D = \sum_i^S \left(\frac{n_i\,(n_i-1)}{N\,(N-1)}\right)$; (4) $E_{1/D} = \frac{(1/D)}{S}$ where $p_i = n_i/N$; n_i is the number of individuals in the *i*th species; N = the total individuals in the sample and S = the total number of species.

RESULTS

Variation of insect diversity in the rice basin: The values of related Evenness of Shannon (near 1) and Simpson (near 0) indices showed low variation on diversity of insect species based on rice developmental stages in (Table 1) and sites (Table 2). All these indices were similar in value and did not show a significant difference on pest richness. In total, 80 insect species belonged to 8 orders, 28 families were found. A significant difference was observed between the different orders in the three sites (P < 0.0001). Generally, Homoptera, Orthoptera, Coleoptera and Diptera orders were the most predominant (P < 0.0001) (Figure 2). Thirty-nine insect species distributed into 20 families were reported in both sites.

Table 1: Insect diversity Indices at different rice developmental stages

Stage	Site	S	H'	J'	1/D	$E_{1/D}$
	AfricaRice	46	2.84	0.74	23.20	0.50
Tillering	Koussin	41	2.88	0.77	12.31	0.30
	Ouedeme	40	2.94	0.79	12.82	0.32
	AfricaRice	54	3.42	0.86	23.52	0.43
Booting	Koussin	45	2.80	0.74	10.50	0.23
	Ouedeme	44	2.99	0.79	13.17	0.30
	AfricaRice	42	2.30	0.62	5.29	0.12
Heading	Koussin	35	2.81	0.79	9.56	0.27
	Ouedeme	35	2.92	0.82	13.26	0.38
	AfricaRice	22	1.91	0.61	4.60	0.21
Maturation	Koussin	20	2.30	0.77	6.99	0.35
	Ouedeme	18	1.98	0.69	3.98	0.22

S: Max diversity, H': Shannon-wiener index, J': related Evenness index of Shannon, 1/D: Simpson's index, $E_{1/D}$: related Evenness of Simpson

Table 2: Insect diversity Indices in the different prospection sites

Site	S	H'	J'	1/D	$E_{1/D}$
AfricaRice	68	3.43	0.81	17.33	0.25
Koussin	58	3.04	0.75	12.85	0.22
Ouedeme	53	3.21	0.81	16.34	0.31

S: Max diversity, H': Shannon-wiener index, J': related Evenness index of Shannon, 1/D: Simpson's index, $E_{1/D}$: related Evenness of Simpson

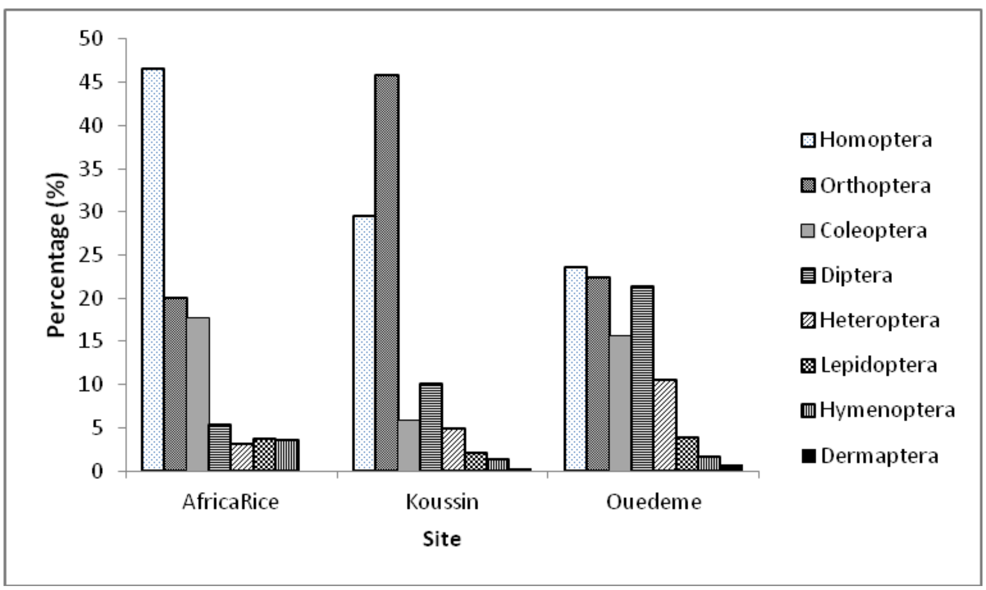

Figure 2. Insect orders distribution in the Southern and Central Benin rice basin.

Diversity and abundance of insect orders by rice growing stage at AfricaRice: At AfricaRice, 7 orders, 26 families and 68 species were identified. Despite the non-significant difference (*P* = 0.12) between them, Orthoptera (20.59%), Coleoptera (20.59%) and Homoptera (17.65%) were the most frequent considering insect species. Diptera (4.41%) were less sampled. Homoptera order was dominant in all developmental stages (Figure 3).

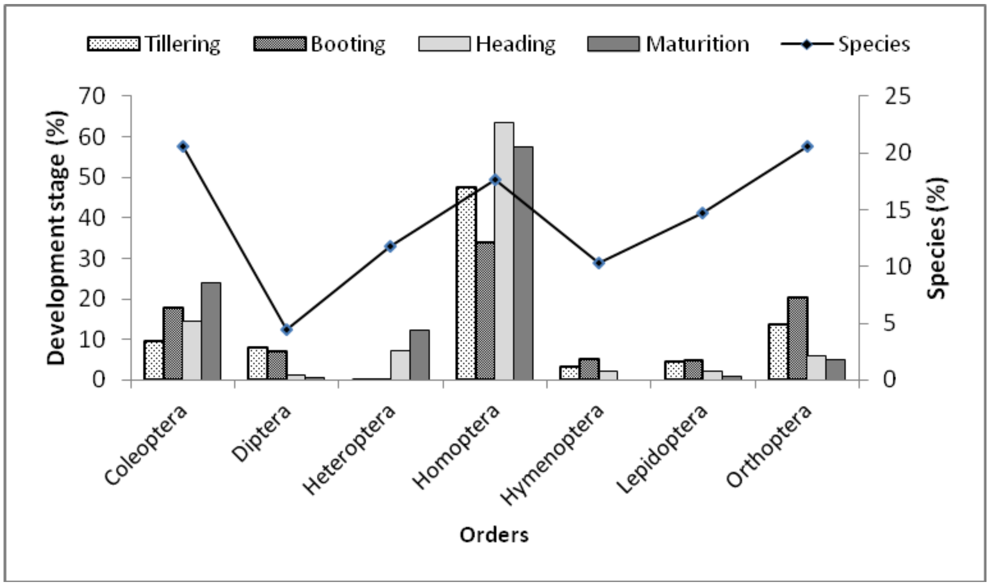

Figure 3: Diversity and abundance of insect orders according rice developmental stages in AfricaRice site.

Diversity and abundance of insect orders by rice growing stage at Koussin: Eight orders, 25 families and 58 species were reported at Koussin. Orthoptera were the most abundant order with 34.45%, 54.76%, 46.88% and 38.89% of species respectively at tillering, booting, heading and maturation. A significant difference (*P* = 0.03) was observed between orders in terms of number of species with predominance of Homoptera (22.41%), followed by Orthoptera (18.97%), Coleoptera (15.52%) and Lepidoptera (15.52%) orders (Figure 4).

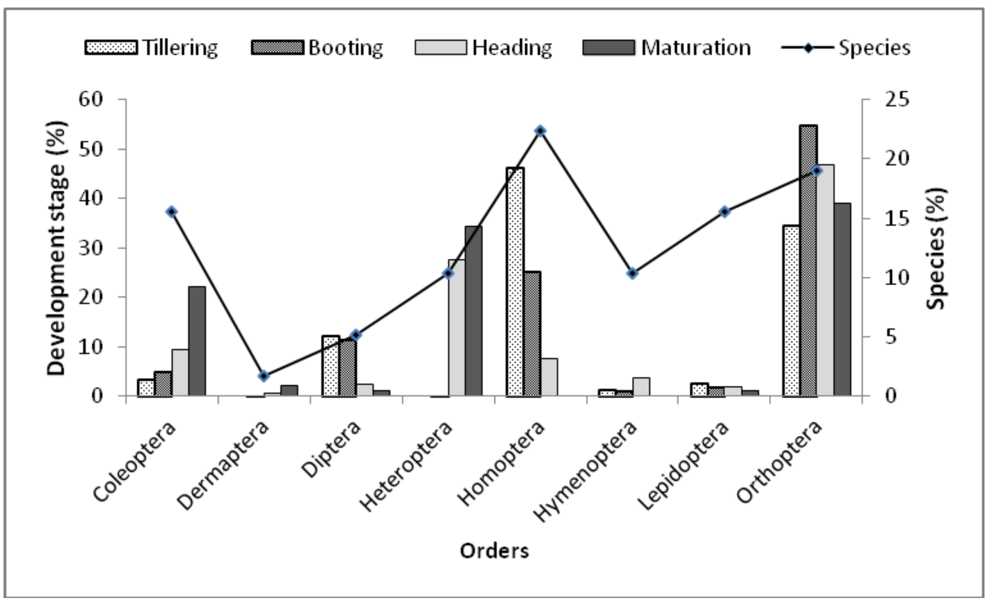

Figure 4: Diversity and abundance of insect orders according rice developmental stages in Koussin site.

Diversity and abundance of insect orders by rice growing stage at Ouedeme: Eight orders, 25 families and 53 species of insects were collected with preponderance at booting compared to other stages. Homopterans were the most abundant at tillering (41.67%) and booting (21.94%). Heteropterans were the order mostly found at heading (28.19%) and maturation (67.09%). As regards the number of species (*P* = 0.03), Homoptera order, was mostly reported amounting to 22.64%. They were followed by Coleoptera (18.87%) and Orthoptera (18.87%) orders. Dermaptera order was represented by only one species (1.89%) (Figure 5).

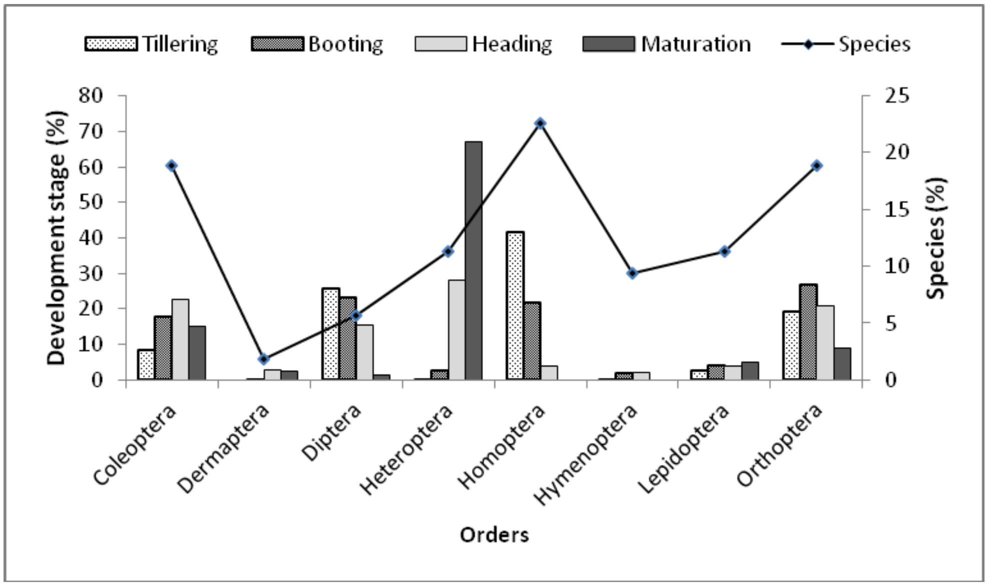

Figure 5: Diversity and abundance of insect orders according rice developmental stages in Ouedeme site.

Frequency of occurrence and relative abundance of collected insect species collected in all sites: These insects were phytophagous (87.75%), parasitoids (8.75%) and predators (7.5%) (Figure 6). The most frequent and abundant insects belonged to the Homoptera, Coleoptera, Orthoptera and Diptera orders with the relative abundance and frequency of occurrence higher than 2% and 50% respectively. Approximately 80 insect species, 23.75% were already identified as RYMV vectors (Table 3). *Cofana spectra* (Distant), *Cofana unimaculata* (Signoret) and *Nephotettix modulatus* (Melichar) were the most important homopterans. Among the coleopterans, *Chnootriba similis* (Mulsant), *Aulacophora foveicollis* (Lucas) and *Xanthadalia effusa* (Erichson) were the most common species found. In the Orthoptera order, the major species were *Oxya hyla* (Serville) and *Conocephalus longipennis* (de Haan). *Oxya hyla* was the most reported species in all the survey area with 90% and 10.62% of occurrence and abundance, respectively. In the Diptera order, *Diopsis thoracica* (Westwood) and *Diopsis apicalis* (Dalman) were the most common species (Table 3).

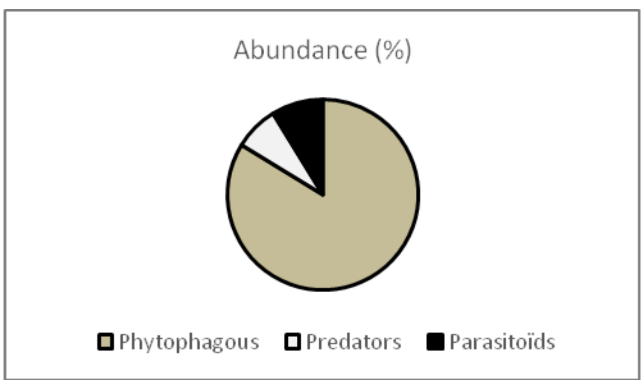

Figure 6. Abundance of insect species according the feeding mode.

Table 3. Frequency of occurrence and relative abundance of insect species in all sites

Orders	Species	A (%)	F (%)
Homoptera	*Poophilus costalis* Walker*	2.29	46.67
	Poophilus sp.	0.91	23.33
	Cofana unimaculata Signoret*	3.94	63.33
	Cofana spectra Distant*	7.99	73.33
	Deltocephalus schmidtgeni Wagner	0.65	30.00
	Nephotettix sp.	0.87	50.00
	Nephotettix modulatus Melichar*	4.45	53.33
	Nephotettix afer Ghauri	1.29	40.00
	Hortensia sp.	0.11	10.00
	Empoasca fascialis Jacobi	0.38	23.33
	Locris rubra Fabricius*	0.31	23.33
	Locris maculata Fabricius*	0.09	10.00
	Nilaparvata maeander Fennah	0.13	10.00
	Sogatodes cubanus Crawford	0.56	33.33
	Rhopalosiphum padi L.	6.08	20.00
	Sitobion graminis Takahashi	5.14	23.33
Coleoptera	*Cryptocephalus* sp.	0.07	6.67
	Aulacophora foveicollis Lucas*	1.34	63.33
	Chaetocnema pusilla Laboissiere	0.20	13.33
	Chaetocnema pulla Chapuis*	0.76	16.67
	Chaetocnema sp.	0.24	20.00
	Lema rubricollis Klug	0.11	10.00
	Lema sp.	0.18	13.33
	Medythia quaterna Fairmaire	0.22	20.00
	Dicladispa viridicyanea Kraatz*	0.29	23.33
	Sitophilus oryzae L.	0.18	23.33
	Sitophilus zeamais Motschulsky	0.11	13.33
	Chnootriba similis Mulsant*	5.19	80.00
	Chilocorus sp.	0.22	20.00
	Xanthadalia effusa Erichson*	3.49	60.00
	Cheilomenes lunata Fabricius*	0.42	36.67
	Tenebrio sp.	0.04	6.67
Orthoptera	*Oxya hyla* Serville*	10.62	90.00
	Oxya sp.	1.40	50.00
	Paracinema tricolor Thunberg*	0.87	30.00
	Acrida bicolor Thunberg*	1.25	40.00
	Acrida turrita L.	0.22	16.67
	Acrida sp.	1.58	53.33
	Stenohippus aequus Uvarov*	1.20	20.00
	Stenohippus sp.	0.78	40.00
	Conocephalus longipennis de Haan*	4.74	90.00
	Phaneroptera sp.	0.96	53.33
	Zonocerus variegatus L.*	0.22	23.33
	Attractomorpha sp.	0.45	33.33
	Metioche sp.	0.33	20.00
	Gryllotalpa africana Beauvois	0.09	13.33
	Paratettix sp.	2.78	23.33
	Euscyrtus sp.	2.18	36.67
Heteroptera	*Aspavia armigera* Fabricius	3.36	43.33

	Aspavia sp.	0.11	10.00
	Agonoscelis versicolor Fabricius	0.02	3.33
	Nezara viridula L.	0.36	43.33
	Rhinocoris albopilosus Signoret	0.24	26.67
	Mirperus jaculus Thunberg	0.40	26.67
	Riptortus dentipes Fabricius	0.65	50.00
	Cletus fuscescens Walker	0.11	6.67
	Anoplocnemis tristator Fabricius	0.20	13.33
	Creontiades sp.	0.04	6.67
Diptera	*Diopsis thoracica* Westwood*	7.01	80.00
	Diopsis apicalis Dalman	2.76	63.33
	Dasyphora sp.	0.04	6.67
	Argyrophylax nigrotibialis Baranov	0.96	40.00
Lepidoptera	*Nymphula depunctalis* Guenée	0.65	43.33
	Marasmia trapezalis Walker	0.07	10.00
	Chilo zacconius Bleszynski	0.60	33.33
	Chilo sp.	0.29	30.00
	Eldana saccharina Walker	0.13	16.67
	Maliarpha separatella Ragonot	0.45	36.67
	Sesamia calamistis Hampson	0.71	46.67
	Sesamia sp.	0.04	6.67
	Spodoptera sp.	0.07	6.67
	Sitotroga cerealella Olivier	0.20	23.33
Hymenoptera	*Bracon* sp.	0.24	20.00
	Stenobracon deesae Cameron	0.13	13.33
	Cotesia sesamiae Cameron	0.49	26.67
	Opius sp.	0.24	33.33
	Pediobus furvus Gahan	0.29	26.67
	Telenomus sp.	0.58	40.00
	Apis mellifera L.	0.33	23.33
Dermaptera	*Diaperasticus* sp.	0.29	30.00
Total	80	100	

In total, 80 insect species were identified in survey area. Insect species known as RYMV vectors in this area were reported with asterisk (*). A: Relative Abundance, F: Frequency of Occurrence

Apart from these major insects, the homopterans *Poophilus costalis* (Walker), *Rhopalosiphum padi* (L.) and *Sitobion graminis* (Takahashi), the orthopterans *Paratettix* sp., *Euscyrtus* sp., *Acrida bicolor* (Thunberg) and *Paracinema tricolor* (Thunberg), the heteropteran *Aspavia armigera* (Fabricius), the lepidopterans *Sesamia calamistis* (Hampson), *Nymphula depunctalis* (Guenée) and *Chilo zacconius* (Blesynski) and the hymenopterans *Telenomus* sp. and *Cotesia sesamiae* (Cameron) were also frequently found in the survey area (Table 3).

DISCUSSION

To know the potential insect vectors of the RYMV in the rice basin of the Southern and Central of Benin, prospections were done in the three rice production sites. Eighty species belonging to 8 orders were identified in the rice basin of Southern and Central Benin. About a quarter of recorded insect species were already identified as vectors (Nwilene, 1999; Koudamiloro *et al*., 2014; Koudamiloro *et al*., 2015). Sixty species representing three quarter of the total species were found at least in two sites. Indeed, rice plant is a preferred host for many insects. Each part of this plant (from root to panicle) can be damaged by various insects. In West Africa, 330 insect species were identified in rice fields (Heinrichs & Barrion, 2004). The rice basin of south and central Benin was characterized by favorable climatic and environmental conditions such as moderate temperature (24–30 °C), rainfall (80.0-120.0 cm year^{-1}), heavy vegetation and high relative humidity (85-90%) (MAEP, 2009). These conditions are appropriate for insect development. Moreover, data of Shannon and Simpson indices pinpointed that pest diversity was closer within development stages and sites. Thus, it can be stated that the distribution of insect species in different sites of the basin is homogeneous. However, the higher number of insect species observed at AfricaRice research station compared to Koussin and Ouedeme might be due to the simultaneous presence of two ecosystems of rice production (upland and irrigated lowland) and other crops (maize, cowpea, cassava, and vegetables) in the site. These crops can serve as alternative or associated host for a wide range of rice insects such as *C. similis, Nephotettix* spp., *Poophilus* spp., *Sitophilus* spp., *S. calamistis*, *Eldana saccharina* (Walker) and *Diopsis* spp. The abundance of insect species in the AfricaRice station may also be due to the varietal diversity of cultivated rice. Indeed, several varieties of New Rice for Africa (NERICA) and rice populations such as International Rice (IR), Tropical *Oryza glaberrima* (TOG) and Near Isogenic Lines (Nil) are grown in many experimental trials. The insects will be able to select the most appropriate rice variety to their development cycle and multiplication as reported by Nwilene *et al.* (2008). For the distribution of insects by orders, homopterans were the most abundant due to the high number of aphids *R. padi* and *S. graminis* in the AfricaRice field. They were followed by orthopterans and coleopterans, which were observed in all rice growing stages especially at tillering and booting. These stages are high yielding biomass steps, which are favorable for both insect feeding and refuge. Dipterans were highly represented by *Diopsis* spp. These stem borers can grow easily and even become sedentary in the event of continuous cropping in the same environment (Banwo, 2002; Togola *et al.*, 2010), as reported in this study. Other important stem borers (lepidopterans) commonly found on rice in Benin such as *S. calamistis*, *Chilo* spp. and *E. saccharina* (Togola *et al.*, 2010), were not significant among the recorded samples perhaps because the dissections of rice tiller were not performed in this study, even though their typical symptoms such as dead hearts and white panicles were observed in the fields. All these orders were among the most important rice pests in West Africa as reported by Brenière (1983) and Heinrichs and Barrion (2004). Regarding the species distribution in the basin, *O. hyla* was the most frequent and the most abundant. The locust was found in all rice ecosystems (Dale, 1994). According to Nwilene *et al.* (2011), it was one of the most important pests of rice in fields. Some studies reported that this insect was present throughout the year in other rice growing areas like Côte d'Ivoire and Guinea where similar studies were conducted (Heinrichs & Barrion, 2004). During their prospections, Paraïso *et al.* (2012) recorded this insect in all ecological zones of Benin with high density in humid areas such as the study basin. For the beetle, *C. similis* was the most present. The ladybird beetle was one of the major rice pests especially in the upland ecosystem as specified by Sadou *et al.* (2014). It was found at the vegetative stages of rice in all sites, probably due to the presence of many host plants such as rice, maize, sorghum, millet (Heinrichs, 1991; Tuey, 1999). For the homopterans, *C. spectra* was the most abundant. It is a cosmopolitan species, from the tropics of Africa to Australia according Wilson and Claridge (1991). In West Africa, this polyphagous insect is present in the lowland ecosystem similarly to most rice leafhoppers (Dale, 1994; Heinrichs & Barrion, 2004). The dipteran *D. thoracica*, was also one of the major insects found in the basin. It is one of 10 main insect pests of rice in West Africa and one of the four major rice stem borers in Africa (Heinrichs & Barrion, 2004; Nwilene *et al.*, 2011). It is mainly found in irrigated lowlands as confirmed in this study. These 4 important insect species were also RYMV vectors according several studies (Koudamiloro *et al.*, 2015). Among the entomofauna in the South and Central rice basin of Benin, natural enemies found were predators and parasitoids. The most important predators belonged to the Orthoptera and Coleoptera. Among them were sampled *Metioche* sp., and *C. longipennis*, which attack bugs (Shepard *et al.*, 1991; Van den Berg *et al.*, 1992), *C. lunata* and *X. effusa* are aphids predators (Brown, 1972; Sadou *et al.*, 2014). The parasitoids belonged to the Hymenoptera and Diptera orders, which contain the most important rice parasitoids in West Africa (Polaszek, 1998; Heinrichs & Barrion, 2004). They were mostly found at the AfricaRice station where stem borers were also more frequent. *Telenomus* sp. was the most abundant probably because being the larval parasitoid of *C. longipennis* (Agyen-Sampong, 1980) which was found at high density in the basin.

CONCLUSION

Diversity and abundance of insect species were studied in the Southern and Central rice basin of Benin and 80 insect species belonging to 8 orders were found. During the vegetative stage i.e. tillering and booting when rice is more susceptible to RYMV, the following four orders Homoptera, Orthoptera, Coleoptera and Diptera were the most frequent and abundant. Among the most frequent species were *O. hyla*, *C. similis*, *C. spectra* and *D.*

thoracica. Future research efforts should focus on the determination of RYMV vector transmission ability of the main insect species found in the rice basin.

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