Journal of

Entomology

ISSN 1812-5670

Academic
Journals Inc.

www.academicjournals.com

Journal of Entomology 6 (4): 198-206, 2009
ISSN 1812-5670

New Records of Insect Vectors of Rice Yellow Mottle Virus (RYMV) in Côte d'Ivoire, West Africa

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Abstract: The study aimed to investigate the vectorial capacity of twelve insect species to transmit Rice Yellow Mottle Virus (RYMV) from diseased seedlings of a susceptible rice variety (Bouaké 189) and a perennial wild rice (*Oryza longistaminata*) to seven alternative host plants. Results indicated that *Trichispa sericea*, *Chaetocnema pulla*, *Chnootriba similis*, *Conocephalus longipennis*, *Oxya hyla*, *Paratettix* sp., *Zonocerus variegatus*, *Euscyrtus* sp., *Cofana spectra*, *Cofana unimaculata*, *Locris rubra* and *Locris maculata* were capable of transmitting RYMV from infected Bouaké 189 and *Oryza longistaminata* to alternative weed hosts *Leersia hexandra*, *Imperata cylindrica*, *Digitaria horizontalis*, *Echinochloa colona*, *Echinocloa crus-pavonis*, *Eleusine indica* and *Brachiaria lata*. Only *Chaetocnema pulla*, *Trichispa sericea*, *Chnootriba similis*, *Oxya hyla*, *Zonocerus variegatus*, *Euscyrtus* sp., *Parattetix* sp., *Cofana spectra*, *Cofana unimaculata* and *Locris rubra* played an important role in transmitting the disease from rice to *O. longistaminata*, *Leersia hexandra* and *Imperata cylindrica*. The present study confirmed the vectorial capacity of these vectors out of which eight were reported for the first time in West Africa.

Key words: RYMV, insect vectors, rice, alternative hosts, epidemiology

INTRODUCTION

Rice Yellow Mottle Virus (RYMV) is one of the most devastating diseases of irrigated and lowland rice, occurring in the sahel, tropical humid and sub-humid ecosystems of West Africa. However, it does occur in mangrove and inland swamps in Guinea and in upland rice in Sierra Leone and Côte d'Ivoire (Awoderu, 1991). The disease is characterized by mottling and yellow symptoms of varying intensities depending on the genotype. The virus is environmentally stable, highly infectious and endemic to Africa and surrounding islands. The host range of RYMV is narrow and restricted to gramineous species mainly in the rice tribes Oryzeae and Eragrostidae. The pathogen belongs to the sobemovirus group and transmitted mechanically through rice plants injuries and by insect vectors (Abo *et al*., 1998). Virus transmission by insects with biting and chewing mouthparts is commonly explained

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by an effective dose of the virus on contaminated mouthparts (Abo *et al.*, 1998). The regular occurrence of insects in rice fields in West Africa has prompted close examination of these species as possible vectors (Abo *et al.*, 1998). Although, there has been considerable effort to identify resistant rice varieties and to develop an integrated management of RYMV for the region's farmers, the poor understanding of virus variability, epidemiology and role of vectors has hindered progress in the control of the disease. Previous studies in East Africa and West Africa to understand the epidemiology of the disease concentrated on insects feeding on diseased rice plants where the insects collect the virus and then pass them on to the next healthy plants that they feed on (Bakker, 1970, 1971; Abo *et al.*, 2000a). Since rice is not cultivated in the fields continuously for 12 months in a year, there is need to know where, the virus spends the off-season. In this case, the alternative host plants may have a role to play. Therefore, the present study was designed to assess the vectorial capacity of insects found within rice fields to transmit the disease from rice to alternative hosts. This is important because in the absence of rice in the field, the vectors fly and take refuge on alternative hosts around the bunds or in nearby fields (Abo *et al.*, 2000a). A better understanding of RYMV epidemiology requires information on the insects that serve as vectors and alternative hosts that act as reservoirs. This could then provide a basis for the development of appropriate methods of control that would improve sustainable intensification of lowland rice-based systems as well as the resource-use efficiency in irrigated rice-based systems.

MATERIALS AND METHODS

Study Site

The study was carried out between year 2000 and 2001. The trial sites were located at Sakassou and Gagnoa in Côte d'Ivoire. Sakassou is at latitude and longitude of 5°06 W and 7°52 N. The areas experienced a bimodal rainfall pattern, mechanized upland cultivation and double cropped lowland rice with dam-irrigation. Gagnoa is located in the forest zone with a latitude and longitude of 6°00 W and 6°06 N. Bimodal rainfall, single and double cropped lowland rice with stream irrigation.

The Alternative Host

Eight gramineous weeds including a wild rice commonly found in rice fields in West Africa were selected for the trials (Table 1). Each of these hosts was selected because (1) they represent annual and perennial grasses found along the toposequence, (2) they are diverse enough to capture the spread of the disease to rice at the beginning of the cropping season and (3) the grasses occur naturally from the free-draining uplands, through hydromorphic areas, to the seasonally flooded lowlands which may maintain the build-up of insect populations.

Table 1: Names, grass type, plant height and ecology of seven alternative host plants used in the screening trials

Grass species	Grass type	Height (cm)	Ecology
Digitaria horizontalis Willd.	Annual	30-75	Upland
Eleusine indica (L.) Gaertner	Annual	60	Upland
Brachiaria lata (Schum.) C.E. Hubb.	Annual	20-60	Upland
Echinochloa colona (L.) Link	Annual	60	Lowland and hydromorphic
Echinochloa crus-pavonis (Kunth) Schultes	Annual	75-200	Lowland
Oryza longistaminata A. Chev. and B. Roehr	Perennial with rhizomes	200	Lowland and hydromorphic
Leersia hexandra Sw.	Perennial with rhizomes	30-100	Lowland and hydromorphic
Imperata cylindrica (L.) Raeuschel	Perennial with rhizomes	120	Upland

Johnson (1997)

Insect Species

Adult insects were collected from rice fields using a new trapping net cage method and maintained on Bouaké 189 seedlings in rearing net cages measuring (1.0×0.8×0.5 m) according to Abo *et al*. (2000b) with some modification. Insects were allowed to undergo a generation in rearing cages before being transferred to a second net cage (0.8 ×0.6×0.4 m) containing diseased seedlings of RYMV susceptible rice variety, Bouaké 189.

Transmission Tests

The viral transmission test was according to Sere *et al*. (2008) with some modification. Two groups of insects (leaf biting and leaf sucking) found in and around rice fields were tested for their ability to transmit RYMV from rice to alternative hosts and from a wild host to other alternative host plants. The insect were allowed to feed on diseased Bouake 189 for 48 h, after a group of five adult insect were removed having acquired the virus and caged to feed on healthy seedlings of each host plant. A control with virus-free Bouaké 189 and *Oryza longistaminata* was also set up. The cage experiment was replicated three times. Three potted plants were used per cage and the seedlings were kept under irrigation. The caged adult insects were allowed to feed on healthy seedlings for 10 days. At the end of 10 days, the insects were removed from the cage and killed by exposure under freeze temperature for 30 min. The ability of the five adult insects to transmit RYMV to each host plant was checked serologically using enzyme-linked immunosorbent assay (ELISA) test (Clark and Adams, 1977). The leaf samples from the three replications were analyzed separately in ELISA to detect the virus. In order to relate the results of artificial inoculation tests to the natural sources of RYMV in wild insects and alternative hosts, a monthly survey was carried out in August, 2000 through July, 2001 at Sakassou and Gagnoa. The insects were collected using the trapping net cage method and the plant samples were also taken within the area of the net cage. Five samples were taken per field at every visit. All insects caught and plant samples were put in small plastic containers and plastic bags, respectively, for further laboratory ELISA test (Clark and Adams, 1977).

RESULTS AND DISCUSSION

In the artificially inoculated screen house cages, 12 insect species (*Trichispa sericea*, *Chaetocnema pulla*, *Chnootriba similis*, *Conocephalus longipennis*, *Oxya hyla*, *Paratettix* sp., *Zonocerus variegatus*, *Cheilomenes lunata*, *Euscyrtus* sp., *Cofana spectra, Cofana unimaculata* and *Locris rubra*) were found capable of transmitting RYMV from diseased Bouaké 189 seedlings to 8 alternative host plants (*Oryza longistaminata*, *Leersia hexandra*, *Imperata cylindrica*, *Digitaria horizontalis*, *Echinochloa colona*, *Echinocloa crus-pavonis*, *Eleusine indica*, and *Brachiaria lata* (Table 2). Insect species capable of transmitting RYMV from wild rice (diseased *Oryza longistaminata*) to other alternative host plants were also identified (Table 3). From wild hosts under natural conditions in Gagnoa, RYMV infection was detected in the following insect vectors: *Chaetocnema pulla*, *Cheilomenes lunata*, *Conocephalus longipennis*, *Oxya hyla*, *Cofana spectra* and *Cofana unimaculata* (Table 3-7). Out of these insect vectors, four are known vectors (*Trichispa sericea*, *Chaetocnema pulla*, *Chnootriba similis* and *Conocephalus longipennis*) (Bakker, 1971; Abo *et al*., 2000b). The remaining eight are new and reported for the first time in West Africa.

Virus transmission by insects is a common way for viruses to travel between different host plants and this is possibly as a result of a protein that plant viruses attach to as they hitch an insect ride between plants (Uzest *et al*., 2007). The leaf feeding beetles

Table 2: Insect species capable of transmitting RYMV from diseased Bouaké 189 to alternative hosts after 48 h acquisition feeding period

		Alternative hosts							
		Annual grass weeds					Perennial grass weeds		
Insect species	A B	C	D	E	F	G	H	I	J
Leaf feeding beetles									
Chaetocnema pulla	**100**	**67**					**33**		**67**
Trichispa sericea	**100**		**33**		**33**			**33**	**33**
Chnootriba similis	**100**	**100**	**67**	**33**	**33**	**33**		**67**	**100**
Cheilomenes sp.	**100**								
Leaf feeding grasshoppers									
Conocephalus longipennis	**100**	**67**							
Oxya hyla	**100**	**67**		**100**				**33**	
Zonocerus variegatus	**100**		**33**						**33**
Euscyrtus sp.	**100**						**33**		
Parattetix sp.	**100**	**67**			**33**	**33**		**100**	
Sucking bugs									
Cofana spectra	**100**		**33**	**67**	**100**	**33**		**33**	**67**
Cofana unimaculata	**100**	**33**	**100**	**67**					**100**
Locris rubra	**100**	**100**	**67**		**33**	**33**		**33**	**100**

The symbols A: Control, B: *Oryza longistaminata*, C: *Digitaria horizontalis*, D: *Eleusine indica*, E: *Brachiaria lata*, F: *Echinochloa colona*, G: *Echinochloa crus-pavonis*, I: *Leersia hexandra* and J: *Imperata cylindrica*. Bold values are percent infected plants in ELISA test

Table 3: Insect species capable of transmitting RYMV from wild rice (diseased *Oryza llongistaminata*) to alternative hosts after 48 h acquisition feeding period

		Alternative hosts							
		Annual grass weeds					Perennial grass weeds		
Insect species	A B	C	D	E	F	G	H	I	J
Leaf feeding beetles									
Chaetocnema pulla									
Trichispa sericea	**100**	**100**		**67**				**67**	**67**
Chnootriba similis	**100**	**67**	**67**	**100**	**67**			**100**	**67**
Cheilomenes sp.									
Leaf feeding grasshoppers									
Conocephalus longipennis									
Oxya hyla									
Zonocerus variegatus									
Euscyrtus sp.	**67**				**67**				**67**
Parattetix sp.	**100**		**67**	**100**		**67**			**100**
Sucking bugs									
Cofana spectra	**67**		**67**	**100**	**100**	**100**		**33**	**67**
Cofana unimaculata	**67**	**33**	**67**	**100**	**67**	**33**		**67**	**67**
Locris rubra	**100**		**100**	**100**	**100**			**67**	**100**

A: Control, B: *Oryza longistaminata*, C: *Digitaria horizontalis*, D: *Eleusine indica*, E: *Brachiaria lata*, F: *Echinochloa colona*, G: *Echinochloa crus-pavonis*, I: *Leersia hexandra* and J: *Imperata cylindrica*. Bold values are percent infected plants in ELISA test

(*Chaetocnema pulla*, *Trichispa sericea* and *Chnootriba similis*), the leaf feeding grasshoppers (*Oxya hyla*, *Zonocerus variegatus*, *Euscyrtus* sp. and *Parattetix* sp.) and the sucking bugs (*Cofana spectra*, *Cofana unimaculata*, *Locris rubra*) play important role in transmitting the disease from rice to perennial wild rice. These vectors carried the virus in the field. Perennial hosts with rhizomes could play an important role, because they act as reservoirs for the spread of the disease. However, the annual grass *Digitaria horizontalis* appears to be favoured over other grasses by the leaf-biting and leaf-sucking vectors. Furthermore, all the vectors transmitted RYMV to Bouaké 189 which was used as a control plant.

Table 4: Natural sources of RYMV found in wild vectors and grass weeds at Gagnoa and Sakassou, Côte d'Ivoire

			Insect vectors					
Month and year	Location	Grass 5 m^{-2}	A	B	C	D	E	F
Aug. 2000	Gagnoa	*Echinochloa obtusifl*	25	10	0	2	0	0
		Cyperus distans	0	39	0	2	6	0
		Oryza longistaminata	10	6	0	0	1	0
		Eleusine indica	12	7	0	1	5	3
		Digitaria horizontalis	21	36	0	2	0	0
		Panicum repens	0	3	0	0	3	0
		Paspalum scrobiculat.	39	38	2	5	15	0
		Panicum laxum	5	9	1	0	0	0
		Cynodon dactylon	26	7	1	1	0	0
		Imperata cylindrical	32	11	0	1	4	0
			170	166	4	14	34	0
	Sakassou	*Setaria pallide-fusca*	0	5	0	1	0	0
		Leersia hexandra	49	24	0	8	1	0
		Digitaria horizontalis	83	0	0	0	2	0
		Imperata cylindrical	79	2	0	3	2	0
			211	31	0	12	5	0
Sep. 2000	Gagnoa	*Echinochloa obtusifl*	71	0	2	2	6	0
		Paspalum vaginatum	74	0	**2**	1	0	0
		Oryza longistaminata	68	12	0	4	0	0
		Eleusine indica	0	1	0	1	0	0
		Digitaria horizontalis	**38**	**8**	**2**	**5**	**4**	**2**
		Echinochloa stagnina	0	3	0	0	1	0
		Paspalum scrobiculat.	1	1	0	0	2	0
			252	25	6	13	13	0
	Sakassou	*Diplachne fusca*	25	16	0	3	9	0
		Imperata cylindrica	13	1	0	1	1	0
			38	17	0	4	10	0

A: *Chaetocnema pulla*, B: *Conocephalus longipennis*, C: *Oxya hyla*, D: *Cofana spectra*, E: *Cofana unimaculatus* and F: *Cheilomenes* sp. Bold values are infected vectors and plants in ELISA test

Table 5: Natural sources of RYMV found in wild vectors and grass weeds at Gagnoa and Sakassou, Côte d'Ivoire

			Insect vectors					
Month and year	Location	Grass 5 m^{-2}	A	B	C	D	E	F
Oct. 2000	Gagnoa	*Eleusine indica*	3	0	0	0	3	0
		Vossia cuspidate	2	3	1	0	3	0
		Pennisetum sp.	7	0	**1**	0	4	0
		Cynodon dactylon	29	4	0	1	5	0
		Digitaria horizontalis	20	2	0	1	1	0
		Cyperus sphacelatus	1	4	1	0	1	0
		Echinochloa crus-pav.	0	1	0	5	6	0
		Echinochloa stagnina	0	**4**	**1**	1	14	0
			62	18	4	8	37	0
	Sakassou	*Imperata cylindrica*	4	0	0	1	2	0
		Panicum laxum	2	0	0	1	1	0
		Leersia hexandra	3	0	0	1	0	0
		Paspalum vaginatum	0	1	0	2	0	0
			9	1	0	5	3	0
Nov. 2000	Gagnoa	*Echinochloa crus-pav*	0	3	0	1	0	0
		Eleusine indica	3	5	0	0	2	0
		Brachiaria lata	3	6	1	0	2	0
		Cynodon dactylon	6	21	0	2	0	0
		Sacciolepis africana	0	14	1	1	0	0
			12	49	2	4	4	0
	Sakassou	*Imperata cylindrica*	2	12	1	9	0	0
		Panicum laxum	0	0	0	11	7	0
		Leersia hexandra	0	0	0	11	35	0
		Paspalum vaginatum	5	0	0	2	4	0
			7	12	1	33	46	0

Table 5: Continued

Month and year	Location	Grass 5 m^{-2}	Insect vectors A	B	C	D	E	F
Dec. 2000	Gagnoa	*Paspalum conjugatum*	7	5	4	0	2	0
		Paspalum vaginatum	2	2	8	0	2	0
		Pennisetum sp.	5	0	**4**	0	0	0
		Cynodon nlemfuensis	15	1	0	2	2	0
		Cynodon dactylon	7	3	0	1	1	0
		Panicum laxum	0	4	6	1	1	0
			36	15	22	4	8	0
	Sakassou	*Panicum laxum*	1	0	0	2	0	0
		Imperata cylindrica	4	0	0	2	0	0
		Digitaria horizontalis	0	1	0	1	0	0
		Pennisetum sp.	2	0	0	0	0	0
			7	1	0	5	0	0

A: *Chaetocnema pulla*, B: *Conocephalus longipennis*, C: *Oxya hyla*, D: *Cofana spectra*, E: *Cofana unimaculatus* and F: *Cheilomenes* sp. Bold values are infected vectors and plants in ELISA test

Table 6: Natural sources of RYMV found in wild vectors and grass weeds at Gagnoa and Sakassou, Côte d'Ivoire

Month and year	Location	Grass 5 m^{-2}	Insect vectors A	B	C	D	E	F
Jan. 2001	Gagnoa	*Paspalum vaginatum*	2	3	13	2	12	0
		Echinochloa crus-pav	0	0	1	0	0	0
		Digitaria horizontalis	29	1	6	1	11	0
		Vossia cuspidate	0	21	14	14	13	0
			31	25	34	17	36	0
	Sakassou	*Eleusine indica*	0	1	0	0	0	0
		Imperata cylindrica	0	2	0	0	0	0
		Ageratum conyzoids	1	16	0	0	0	0
			1	19	0	0	0	0
Feb. 2001	Gagnoa	*Sporobolus pyramide*	0	2	2	2	2	0
		Paspalum vaginatum	0	1	3	0	0	0
		Cynodon dactylon	0	4	5	0	2	0
		Digitaria longiflora	0	1	0	0	0	0
		Digitaria horizontalis	9	38	6	0	3	2
		Pennisetum purpure.	1	2	**12**	3	3	0
		Echinochloa crus-pav	0	5	22	1	3	0
			10	53	50	6	13	0
	Sakassou	*Imperata cylindrica*	3	5	0	0	0	0
			3	5	0	0	0	0
Mar.2001	Gagnoa	*Diplachne fusca*	0	27	18	0	0	0
		Digitaria horizontalis	0	0	**16**	1	3	0
		Panicum laxum	0	5	3	0	0	0
		Pennisetum purpure.	0	0	2	5	2	0
		Eleusine indica	0	0	2	0	0	2
			0	32	41	6	5	0
	Sakassou	*Imperata cylindrica*	0	0	0	1	0	0
		Digitaria horizontalis	0	0	0	4	1	0
		Leptochloa caerules.	0	1	0	0	0	0
			0	1	0	5	1	0
Apr. 2001	Gagnoa	*Cynodon dactylon*	0	22	32	68	64	0
		Digitaria horizontalis	0	12	14	23	56	0
		Leptochloa caerules.	0	5	8	51	128	0
			0	39	54	142	240	0
	Sakassou	*Imperata cylindrica*	0	0	0	1	0	0
		Panicum repens	0	0	0	4	1	0
		Sporobolus pyramide	0	1	0	0	0	0
			0	1	0	5	1	0

A: *Chaetocnema pulla*, B: *Conocephalus longipennis*, C: *Oxya hyla*, D: *Cofana spectra*, E: *Cofana unimaculatus* and F: *Cheilomenes* sp. Bold values are infected vectors and plants in ELISA test

Table 7: Natural sources of RYMV found in wild vectors and grass weeds at Gagnoa and Sakassou, Côte d'Ivoire

			Insect vectors					
Month and year	Location	Grass 5 m^{-2}	A	B	C	D	E	F
May 2001	Gagnoa	*Sporobolus pyramide*	0	5	5	0	1	0
		Panicum repens	0	4	4	1	2	0
		Digitaria horizontalis	0	5	3	5	1	0
		Cyperus exaltatus	0	0	0	0	0	0
		Leptochloa caerules.	0	5	18	0	1	0
			0	19	30	6	5	0
	Sakassou	*Leersia hexandra*	0	5	0	1	1	0
		Imperata cylindrica	0	29	8	13	4	0
			0	34	8	14	5	0
Jun. 2001	Gagnoa	Paspalum conjugatum	0	2	0	0	0	0
		Leptochloa caerules.	0	14	**16**	0	**3**	0
		Paspalum vaginatum	0	1	4	0	0	0
			0	17	20	0	3	0
	Sakassou	*Eleusine indica*	0	0	0	2	1	0
		Brachiaria lata	0	0	0	0	0	0
		Leersia hexandra	0	0	2	7	4	0
			0	0	2	9	5w	0
Jul. 2001	Gagnoa	*Paspalum vaginatum*	4	3	4	1	2	0
		Digitaria horizontalis	0	5	9	1	40	0
		Brachiaria lata	0	0	2	10	17	0
			4	8	15	12	59	0
	Sakassou	*Brachiaria deflexa*	0	0	0	24	12	0
			0	0	0	24	12	0

A: *Chaetocnema pulla*, B: *Conocephalus longipennis*, C: *Oxya hyla*, D: *Cofana spectra*, E: *Cofana unimaculatus* and F: *Cheilomenes* sp. Bold values are infected vectors and plants in ELISA test

Variable virus transmission efficiency by vector species has been demonstrated. For instance, *Frankliniella schultzei* was more efficient in transmitting Tomato spotted wilt virus (TSWV) than Scirtotrips dorsalis (Rosello *et al*., 1996). Burrow *et al*. (2006) indicated that the difference between populations in their ability to transmit virus was demonstrated for the first time with *Ciccadiluna mbila* and the *Maize streak* virus. Gray *et al*. (2002) indicated that clonal populations of *Schizaphis graminum*, a vector of Barley yellow dwarf virus, could differ in their ability to transmit viruses. Although the potential of three RYMV insect vectors, *Oxya hyla*, *Locris rubra* and *Chnootriba similes* has been evaluated (Sere *et al*., 2008), little is known about the role of other insects found in farmers' rice fields in the transmission of the virus.

Natural sources of RYMV were found in grasses belonging to the annual species *Digitaria horizontalis*, *Echinochloa crus-pavonis* and *Leptochloa caerulescens* at Gagnoa and perennial species *Imperata cylindrica* at Sakassou from August 2000 to July 2001. In September 2000, the grass *Digitaria horizontalis* and all the vectors sampled from it were RYMV positive by ELISA test. This provides strong evidence that wild hosts must be the main source of infection for rice crop.

CONCLUSION

Out of 12 insect vectors found to transmit RYMV, *Chaetocnema pulla*, *Oxya hyla* and *Conocephalus longipennis* are the most important because they are short feeding insects. Insects that have short feeding periods before moving to another plant are highly mobile and are considered to be the most efficient vectors of viruses. The implications of leafhoppers (*Cofana spectra* and *Cofana unimaculata*) and spittle bug (*Locris rubra*) in RYMV spread suggest that virus transmission goes beyond the boundaries of biting and chewing insects in West Africa. In earlier study, the stalk eyed flies, leafhoppers and spittlebugs were unable to transmit RYMV to susceptible rice host. This could be as a result of emergence of resistance breaking virus and presence of two serotypes of Rice yellow mottle virus reported in Côte d'Ivoire.

The fact that the virus found in alternative hosts under artificial inoculation corresponds to virus carriers in the wild indicates that sources of virus infection for the spread of the disease to rice crop are mainly from wild hosts. The abundance of grasses found in the forest (Gagnoa) and derived savanna (Sakassou) zones reflects the diversity of the natural flora in these locations. This diversity, coupled with their overlapping maturity periods, may help maintain high population densities of RYMV vectors in these zones where the infection reservoir of the virus gradually increases under conditions of year-round cropping with irrigation.

ACKNOWLEDGMENTS

We express our appreciation to Dr. Sidi Sanyang and Mr. Guy Manners for their useful suggestions and reviews of the manuscript. The technical assistance of Mr. Lekpa Jean Baptiste and Mr. Etienne Dessieh is gratefully acknowledged.

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