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Inventory of the mealybug species associated to the cocoa tree *(Theobroma cacao* L.) in four producing areas infected with the swollen shoot disease in Côte d'Ivoire

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Abstract
The objective of this study was to catalog the mealybug species associated to the cocoa tree in four producing areas (Bouaflé, Soubré, Duekoué and Sinfra) infected with the swollen shoot disease in Côte d'Ivoire. The study consisted in counting mealybug colonies in infected farms, collecting mealybug specimens, and identifying then. The results of the investigation revealed the presence of eight species of mealybugs: *Planococcoïdes njalensis*, *Planococcus citri*, *Ferrisia virgata*, *Pseudococcus longispinus*, *Planococcus kenyae*, *Phenacoccus hargreavesi*, *Dysmicoccus brevipes*, *Maconellicoccus hirsutus*. Significant differences ($P < 0.05$) were revealed between species with regard to the mean number of colonies per tree in the cocoa farms. *P. njalensis* was the most abundant species in the cocoa farms surveyed, with 14.58 colonies per tree. The mealybugs were mainly recorded on leaves, shoots and pods, however, the pods appeared to be the most colonized. Moreover, the mealybugs identified were associated with various species of ants.

Keywords: Mealybugs, cocoa, swollen shoot, *Planococcoides njalensis*

1. Introduction
For many years, the main phytosanitary constraints cocoa farming in Côte d'Ivoire were constituted by the attacks of cocoa mirid (*Sahlbergella singularis and Distantiella theobromae*) and pod rot due to *Phytophthora palmivora*. All research efforts were devoted to these pests and the disease of pod rot. [6, 19, 20, 21, 25, 26, 27,]. However, over the last decade, the emergence of new diseases and pests such as the cocoa swollen shoot [18] and cocoa shoots borers attacks are a serious threat to cocoa farming in Côte d'Ivoire [27, 30].
Swollen shoot is a viral disease characterized by the swelling of the shoots. This disease is well known in some producing countries of West Africa such as Ghana, Togo and Nigeria [1, 6, 23, 21, 32, 33]. Swollen shoot has caused incalculable losses to the cocoa industry in West Africa after the destruction of millions of cocoa trees [37]. In Côte d'Ivoire, after the uprooting of 150,000 cocoa trees from 1945 and 1948, the disease had completely disappeared [24,].
In the other producing countries of West Africa, where the disease has been raging for several years it has been the subject of concern [6, 12, 19, 20, 21, 25, 26, 27,]. These works revealed that the spread of swollen shoot is done by mealybug which transmit the virus from diseased trees to neighboring healthy trees [36]. In Ghana and Togo, it was shown that several species of mealybugs are involved in the transmission of swollen shoot virus [9, 11, 13, 33, 34,].
The resurgence and the development of the cocoa swollen shoot in the ivorian orchard is a major concern for producers. If nothing is done to prevent or minimize its impact, this disease could in the short term affect the volume of domestic production and sustainability of cocoa culture. It was therefore important and urgent to undertake research projects to determine the health of the orchard, study the viral strains involved, identify the virus vectors in the ivorian context, know the host plants other than cocoa and develop appropriate control methods. It is in this context that the present study was conducted to inventory the mealybug species in the cocoa orchards in Côte d'Ivoire in order to identify those that are vectors of ivorian strains of swollen shoot virus.

2. Materials and Methods

2.1- Study area: The study was conducted from November 2009 to October 2010 in four cities of Cote d'Ivoire in which cocoa farms are infected with swollen shoot disease. These are Bouafle, Sinfra both located in the centre of the country, Soubré and Duekoué are in southwest and west parts respectively.

2.2 Choice of trees: In each town, three cocoa farms infected with swollen shoot were chosen. In each of them, 200 trees were spotted around a swollen shoot source. Each has been carefully inspected up to two meters tall in search of mealybugs and ants associated to these mealybugs.

2.3 Mealybugs collection: mealybugs species found in the cocoa farms visited were collected and taken to the laboratory for identification. A brush was used to loosen the mealybug attached to the plant organs. These insects were gathered in a Petri dish and labeled. When the mealybugs were securely attached to the organs, a pointed toe clip was used to take them off. The samples were then stored in small bottles containing alcohol at 70 °C.

2.4 Preparation of mealybugs: The technique used was that of Balachowsky and Gosselin of 1950. It consisted in thinning the mealybugs and their staining by the basic fuchsin. After thinning and staining, the samples were mounted to binocular microscope between slide and cover glass for observation.

2.5 Mealybugs Identification: the keys that were used for identification are Balachowsky [2] Bland et Jaques [3]; Entwistle, [13]; Foua Bi [15]; John et Douglass 1977 [17]: William [38]; William, [39].

2.6 Statistical analyses. All data collected were subjected to analysis of variance using the GLM (General Linear Model) procedure of the software SAS. The comparison of means was performed by the Waller Duncan test at the threshold of 5%.

3. Results

3.1 Species inventoried

Eight mealybugs species belonging to the family Pseudococcidae were inventoried in the cocoa farms of the four cities (Bouaflé, Soubré, Duekoué, Sinfra). Three species were observed in Bouaflé, five in Soubré, six in Duekoué and seven in Sinfra. Among the eight species found, two species (*Planococcoïdes. njalensis*, *Planococcus. citri*) were observed in the four cities. The other species were present in two or three cities (Table 1).

Table 1: List of mealybugs observed in the cocoa farms infected with swollen shoot of the four cities

N°	Family	Species	Cities			
			Bouaflé	Soubré	Duekoué	Sinfra
1	Pseudococcidae	*Planococcoïdes njalensis* Laing	+	+	+	+
2		*Planococcus citri* Risso	+	+	+	+
3		*Ferrisia virgata* Cockerell	-	+	+	-
4		*Pseudococcus longispinus*Targioni Tozzetti	-	-	+	+
5		*Planococcus kenyae* Le Pelley	+	-	+	+
6		*Phenacoccus hargreavesi* Laing	-	-	+	+
7		*Dysmicoccus brevipes* Cockerell	-	+	-	+
8		*Maconellicoccus hirsutus* Green	-	+	-	+

(+) : Presence (-) : Absence

3.2 Relative abundance of mealybugs observed in the four cities

In total 275 colonies of mealybugs were counted. *P.njalensis* were majority with 175 colonies representing 63.63 % of the total colonies counted. The other species (*P. citri, F. virgata, P. longispinus, P. kenyae, P. hargreavesi, D. brevipes, M. hirsutus* represented 36.37 % of the total colonies (Table 2).

3.3 Average abundance of mealybugs observed in the four cities

P. njalensis were the most abundant in cocoa farms with 14.58 colonies per tree. This species were followed by *P. citri* with 4.66 colonies per tree. The other species were relatively rare with less than one colony per tree. The Analysis of variance performed on colonies of species showed significant differences ($P < 0.05$).

Table 2: Relative abundance (%) of mealybug cocoa farms infected with swollen shoot disease in the four cities

			Cities			
N°	Family	Species	Relative abundance (%)			
			Bouaflé	Soubré	Duékoué	Sinfra
1	Pseudococcidae	*Planococcoïdes njalensis* Laing	52.24	44.48	39.44	38.84
2		*Planococcus citri* Risso	10.27	8.13	23.11	8.15
3		*Ferrisia virgata* Cockerell	-	6.47	9.96	-
4		*Pseudococcus longispinus*Targioni Tozzetti	-	-	2.32	2.79
5		*Planococcus kenyae* Le Pelley	5.54	-	3.33	3.45
6		*Phenacoccus hargreavesi* Laing	-	-	2.37	3.21
7		*Dysmicoccus brevipes* Cockerell	-	2.30	-	2.47
8		*Maconellicoccus hirsutus* Green	-	3.65	-	2.48

3.4 Organs of the cocoa tree attacked by mealybugs

Mealybug species observed were recorded on leaves, shoots and pods. Most of the time, mealybug species were found on the pods. The Statistical analysis revealed significant differences ($P < 0.05$) (Table 3).

3.5 Ants species associated with mealybugs

Several species of ants were associated to the mealybug species encountered. These are *Pheidol sp*., *Crematogaster sp*., *Iridomyrmex sp*.. The Comparison of mealybug colonies associated to ants and those without ants revealed significant differences ($P < 0.05$) for only one species (*P. njalensis*,).

Conversely, for all other species, no significant difference (P > 0.05) were recorded between colonies with ants and colonies without ants (Table 4).

Table 3: Number of colonies of mealybugs on the differents organs

				Organs	
N°	**Family**	**Species**	**mean number of colonies per tree**		
			Leaf	Shoot	Pod
1	Pseudococcidae	*P. njalensis*	0.2 ± 1.81 a	0.68 ± 1.2 a	13.7 ± 2.21 b
2		*P. citri*	1.2 ± 0.7 a	1.05 ± 0.14a	2.41 ± 1.0 b
3		*F. virgata*	0 ± 0 a	0.1 ± 1.17 a	0.85 ± 0.4 b
4		*P. longispinus*	0 ± 0 a	0.1 ± 0.12a	0.2 ± 2.3 a
5		*P. kenyae*	0 ± 0 a	0.2 ± 0.26a	0.1 ± 0.14 a
6		*P. hargreavesi*	0.1 ± 0.07 a	0 ± 0 a	0.2 ± 0.12a
7		*D. brevipes*	0 ± 0 a	0 ± 0 a	0.4 ± 0.29 a
8		*M. hirsutus*	0 ± 0 a	0.2 ± 0.63a	0.3 ± 1.21 a

For the same specie the averages followed by the different letters are significantly different. (Waller –Duncan test, P < 0.05)

Table 4: Number of colonies of mealybugs with ants and without ants

N °	**Family**	**Species**	**mean number of colonies with ants per tree**	**mean number of colonies without ants per tree**
1	Pseudococcidae	*P. njalensis*	14.0 ± 7.5 a	0.58 ± 0.29 b
2		*P. citri*	2.90 ± 1.03 a	1.76 ± 1.6 a
3		*F. virgata*	0.4 ± 0.03 a	0.55 ± 1.7 a
4		*P. longispinus*	0 ± 0 a	0.3 ± 0.11 a
5		*P. kenyae*	0.2 ± 0.01 a	0.1 ± 0.32 a
6		*P. hargreavesi*	0.1 ± 0.33 a	0.2 ± 0.15 a
7		*D. brevipes*	0.1 ± 1.28 a	0.3 ± 1.2 a
8		*M. hirsutus*	0.2 ± 0.54 a	0.3 ± 0.53 a

For the same specie the averages followed by the different letters are significantly different. (Waller –Duncan test, P < 0.05)

4 Discussion

The survey carried out on cocoa farms infected with swollen shoot disease enabled the recording of eight species of mealybugs belonging to the family Pseudococcidae in all four cities. Except *M. hirsutus*, all species had already been identified in cocoa farms in Ghana and Togo [10]. Duffour [10] showed by transmission tests that these species were vectors of swollen shoot virus and could transmit the forms of the virus known in Ghana and Togo. Moreover, these species are on the list of the fourteen species vectors of swollen shoot virus reported by several authors, [5, 13, 31, 33, 34]. Among the species recorded, *P. njalensis*, *P.citri* and *F. virgata* are known as the most important vectors [22].These are also the three species that were used as vectors in a study carried out by Duffour [9] on transmission methods for the characterization of Togolese forms of swollen shoot virus. These three species were those generally used for the development of inoculation tests in the screening of plant material for the resistance swollen shoot [8, 11, 14].

In the four cities (Bouafle, Soubré, Duékoué, Sinfra), significant differences were revealed between the species surveyed in terms of the number of colonies per cocoa tree. *P. njalensis* were the most abundant species. Our results corroborate those obtained in Ghana by Strickland [36]. Indeed, this author studying the relative abundance of species of mealybugs in Ghana showed that, *P. njalensis* represented the most abundant species. Our results similar to those of Duffour [10] who, in his study of the variation of relative abundance of species Pseudococcidae in Togo, showed that *P. njalensis* constituted 80% of the mealybugs total number. The abundance of *P. njalensis* could be explained by the fact that all cocoa farms surveyed had favorable conditions for their development. This argument is similar to that of Duffour [10] who reported that *P. njalensis* grows better in cocoa farms with luxuriant vegetation formed either by the forest cover, or by the cocoa farm canopy or both at once. The *P. njalensis* population abundance was also attributed to the cocoa farm age by Nguyen- Ban [31] who reported that older cocoa farms abound *P. njalensis* colonies than the young cocoa farms.

Mealybug species were encountered on three types of organs which are leaves, shoots and pods. Our results corroborate those of Boulard [4] and Nguyen-ban [31]. Indeed, Boulard [4] showed that the mealybug species tend to grow more on pods than other organs. Moreover, Nguyen-ban [31] in his study of variation in populations of vectors of swollen shoot disease in Togo, showed that the pods, generally constitute the privileged gathering points for mealybugs, with 60-80% of the population. The pod is probably the preference organ mealybugs on the cocoa tree. According to Boulard [31], it serves both as settling site and food. The sap flows in all parts of the cocoa tree, however, the pod seems to be one of the parts of the plant rich in sap. This may partly justify the large number of mealybugs on that organ.

The survey has revealed that several species of ants were associated with mealybugs in cocoa farms. *P. njalensis* was almost always encountered with ants (*Pheidole sp*., *Crematogaster sp*., *Iridomyrmex sp*). Other mealybugs were often found in association with ants, but in several cases in the absence of ants. These results confirm those of Strickland [36] that showed that in Ghana, there are two distinct biological groups of Pseudococcidae regarding their association with ants. The first group is represented by *P. njalensis* which lives in close association with some species of ants. The second group for which the association with ants is optional, is composed of the other species of mealybugs. The association of ants with mealybugs would be linked to the production of honeydew by mealybugs. Indeed, the ants are attracted by the honeydew produced by mealybugs on which they feed. In return, the ants provide mealybug cleaning and protecting colonies against physical agents (rain, wind, sun, etc.) and natural enemies [4, 22, 10]. The ants also play an important role in moving mealybugs. In pineapple farms, it was shown that the ants carry the mealybugs from one foot to the other in the same plot and from a plot to another, which would explain in part their dissemination [16].

5. Conclusion

This study enabled to inventory several species of mealybugs in cocoa farms infected by swollen shoot disease. The majority of these species found in the cacao orchard in Côte d'Ivoire are known in Ghana and Togo as the swollen shoot virus vectors. *P. njalensis* have been the most numerous species in cocoa farms compared to the seven other species. Generally, there were+ mealybugs mainly on pods and less on the shoots and leaves. Moreover, mealybugs were observed in association with ant species. Associations of *P. njalensis* with ants seem to be mandatory while those of the other species are rather optional. The knowledge of these mealybugs that may be subject to insecticide treatments as part of a strategy against the swollen shoot virus is certainly an important achievement which can be popularized.

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