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Myrmecofauna of cocoa trees infested by Loranthaceae genus *Phragmanthera* in Sodecao seed fields of Nkoemvone (South of Cameroon)

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The present study was carried out in order to identify the ant fauna of cocoa fields leeched by Loranthaceae, genus *Phragmanthera* in Nkoemvone seed farms in the South region of Cameroon. The collection of ants was conducted between November 2013 and January 2014. A total of 15 ant species divided into two sub families (Formicinae and Myrmicinae) have been identified, the subfamily of Myrmicinae being the most represented with 8 genera and 13 species. Fourteen of the 15 ant species harvested, were found on infested cocoa trees. *Tetramorium aculeatum* has emerged as the most abundant ant species on infested cocoa trees with an index of Berger-Parker dominance equal to 0.32 and 0.34 for cocoa parasitized by *Phragmanthera capitata* and *Phragmanthera nigritana*, respectively.

Key words: Ants, inventory, cocoa plants parasitized, Loranthaceae, Cameroon.

INTRODUCTION

Cocoa is a cash crop for all producing countries, and an important source of income for farmers' families. In Cameroon, cocoa has a prominent place in the economy; this is one of the main exported products. Along with coffee, Cocoa represents approximately 28% of non-oil exports and 40 % of exports from the primary sector. Cocoa is the main source of income for millions of people in rural areas. Today cocoa production faces many

constraints of which the most important are related to parasites that cause annual losses estimated at 40% of world production (Eskes and Lanaud, 1997). Among the many parasites that cause damage to cocoa, Loranthaceae are the most recurrent.

Loranthaceae are epiphytic, chlorophyllous hemiparasites living on trees and shrubs branches, wild or cultivated (Balle, 1982). These plants in the form of

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tufts, are anchored in the wood of the host through a sucker that establishes functional links with the driver of the host device (Sallé et al., 1998). Therefore, the parasite draws water, minerals and the additional organic matter it needs. This trophic embezzlement generally leads to a reduction in the biomass of the host which in turn leads to a reduction in yield. When one part of the host is intensively attacked by Loranthaceae, the reproductive and photosynthetic potential of the part distal to the infestation declines leading to death of the part. Parasitism by Loranthaceae is a highly prevalent environmental problem worldwide (Polhill and Wiens, 1998). These epiphytic chlorophyllous parasitic plants are responsible for changing economic and morphogenetic damages depending on woody species parasitized (Salle et al., 1998). More serious damage occurs in poorlymanaged orchards and/or under drought stress conditions, situations that often lead to the death of the host (Boussim et al., 2004). Their distribution and the damages they cause are variable (Dibong et al., 2009a; Monv et al., 2009).

Loranthaceae are widely distributed in tropical areas: America, Africa, and Australia; they extend to temperate regions with few representatives (Balle, 1982; Barlow, 1983). They are grouped into 950 species, distributed in 77 genera in temperate regions. Polhill and Wiens (1998) counted over 500 species in Africa and Arabia while Balle (1982) identified 7 genera and nearly 26 species in Cameroon. In seed fields of Nkoemvone, 6 species of Loranthaceae and a percentage of parasitism equal to 10.44% have been noticed, *Phragmanthera* genus being the most common with two species: *Phragmanthera* capitata and *Phragmanthera* nigritana representing 94.14 and 5.5%, respectively of all parasites found in the seed fields (Ondoua et al., 2015).

According to their habits, the ant species harmful to human activities may directly or indirectly damage plants. The workers of some ant species exploit the sap or corrode the internal tissues of the plant damaging thereby the aerial and underground portion of the plant. The damages caused by the workers can range from simple injuries to cut into pieces of the soft parts of the plant while causing death of the plant (Kenne et al., 1999). Mony et al. (2009) reported that ants are responsible for Loranthaceae flowers falling, reducing therefore their spread.

Ants also have biological control agents. In USA, there are decapitating flies which are used as biological controls agents against imported fire ants. Some of these parasitic flies attack large workers, whereas others attack small to medium-sized workers. These flies hover over fire ant workers and in an instant swoop down, attacking the worker and depositing an egg in the thorax of the ant. The egg hatches in a few days and the larvae begin chewing their way forward toward the head region. After reaching the head, the larvae completely eat the fire ant glands and muscles and release an enzyme that causes

the head to fall off (Morrison and Gilbert., 1998).

The objective of this study is to contribute to the knowledge of the myrmecofauna associated with the main species of Loranthaceae that parasitize cocoa seed fields of Nkoemvone.

MATERIALS AND METHODS

Study area

The selected studied area was the locality of Nkoemvone. It houses the main cocoa seed production station of the state company in charge of developing cocoa (SODECAO) in Cameroon. The choice of this site was motivated by the high frequency of Loranthaceae in seed fields and increasingly significant yield losses. This station contains 11 biclonal fields on an area of over 50 hectares and produces over 200,000 pods per year.

Nkoemvone is located in the south region of Cameroon between 11.1° and 12.2° longitude West and between 2.4° and 2.8° North latitude (Figure 1), at 630 m of altitude and 15 km from Ebolowa on the Ebolowa - Ambam road. This site is subject to a humid and equatorial climate with four seasons: a long dry season (December - March), a short dry season (June - August), a long rainy season (September - November) and a short rainy season (April - May). Its precipitations reach 1755 mm and the average temperature is 25.5°C (Fines et al., 2001).

Harvesting and identification of myrmecofauna

Observations on the field were made at different times of the day to determine the forage period of various ant species on cacao trees. Quadrats of 5×5 cm were delimited on cocoa trees as well as on the different species of Loranthaceae, to collect and estimate the density of all the ants.

Samples of ants were collected on 45 cocoa trees parasitized by *P. capitata*, 45 parasitized by *P. nigritana* and 45 not infested cocoa trees. A total of 135 cocoa trees whose diameter was between 10 and 20 cm were selected. These samples were taken both during a period of flowering and fruiting of Loranthaceae, and a period of non-flowering as well. The collecting was carried out between November 2013 and January 2014, when cocoa trees were not in bloom in the site.

The presence or absence of ants was noticed. Worker ants were collected in the morning (between 8 am and 12 pm). Ant labourers present in plants were harvested after using machete to lift fixation parts. These ants were collected using a mouth aspirator (for labourers of medium size) or with a set of collecting devices for big labourers and kept in labelled tubes containing 70°C alcohol and kept at the laboratories of animal biology at the Faculty of Science of the University of Douala or brought to the laboratory of zoology at the Faculty of Science of University of Yaounde 1 for identification.

Identification of the myrmecofauna

Identification of ants has been made possible by the use of the identification keys (Hölldobler and Wilson, 1990; Bolton, 1994) based on the morphology. Ants were observed to the classic binocular microscope. These ants were cleaned to remove plant detritus and separated according to the external anatomy. The petiole and number of antenna section were the main criteria used to identify the ants. Ants were cleaned with paper towel, and then introduced into a petri dish before being under scrutiny. They were identified by species or morph species. A few specimens of each

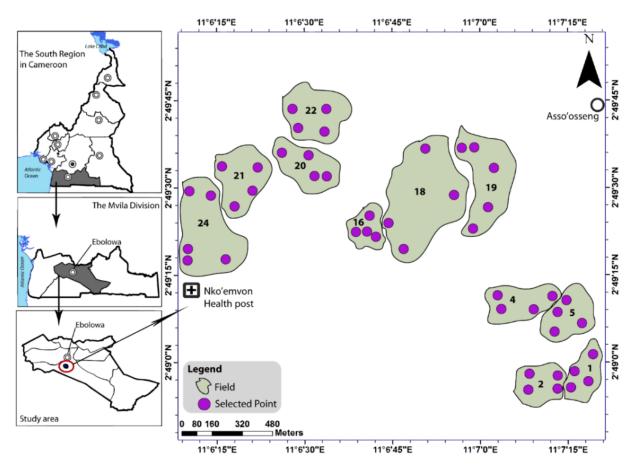


Figure 1. Location map of the study area.

species or morph species were mounted on card sharp and deposited in the different laboratories of the Universities of Douala and Yaounde 1.

Statistical data analysis

Some indexes were used for statistical data analysis:

Absolute Abundance (n_i): For a given species, is the number of times it has been found on plants with ants.

The relative abundance $(p_i) = n_i/N \times 100$ (where $n_i =$ number of species of the rank i, N = total number of each species at the three sites).

Percentage of trees occupancy: The percentage of occupancy is defined by this relationship:

$$P_{OCC} = \frac{Fe_i}{Fe_*} \times 100$$

With Fe_{i} , relative frequency and Fe_{t} , total number of plants with ants.

Measurement of the diversity

Diversity of species: It expresses the total number of species collected at the site (shaft area) marked "S".

Shannon-Weaver diversity index H': Shannon-Weaver diversity index is based on the information theory and is a measure of the sum of the degree of "uncertainty" when we need to predict which species belongs to an individual taken at random from a collection of S species and N individuals. H ' has two properties that reflect its popular use by ecologists to measure species diversity: (1) H' = 0 if and only if the community is formed only by a single species; (2) H' has the maximum value only when all the species are represented by the same number of individuals that is to say a situation of perfect specific regularity of abundances. This index is determined by the relationship:

$$H' = -\sum_{i=1}^{S} (P_i Log_2 P_i)$$

Shannon-Weaver index increases when the number of community species increases and theoretically it may reach high values.

Pielou Index of Equitability (E): Equitability or equal distribution is the balance between the numbers of different species of the site. An environment will have a maximum equitability if all species are of the same numbers.

$$E = \frac{H'}{H'max} = \frac{H'}{\log_2 S}$$

Where H'= predetermined index of Shannon, S= number of species noticed, H' max = $\log_2 S$ and $0 \le E \le 1$.

Equitability index measures the distribution of individuals within species, regardless of species diversity. Its value ranges from 0 (dominance of one species) to 1 (equipartition of individuals in the species).

The degree of similarity between communities was expressed by the Sorensen index, ES:

$$ES = (2c \times 100) / (a+b)$$

where a, number of species in site 1; b, number of species in site 2; c, number of species common to both sites. This index also varies between 0 and 1(Barbault, 1992).

Measurement of biological dominance: Berger-Parker index expresses the importance of the main species. It is the relative abundance of the dominant species in relation to the abundance of all species in the environment. The low value of "d" indicates a high diversity of the medium. It is determined by the relationship:

$$ID_{\text{Berger-Parker}} = \frac{n_{\text{max}}}{n}$$

 $n_{\text{max}},$ number of $\,$ most common species in the middle; n, total abundance.

This index ranges from 0 (corresponds to a high diversity or negligible dominance of a particular species) to 1 (low diversity and dominance of the most common species).

RESULTS

Out of the 135 cocoa trees of *Theobroma cacao* retained, ants were found on 118 (87.4%), while the other 17 showed no presence of ants (12.6%). Cocoa trees carrying no ants were those not infested.

Inventory of the myrmecofauna present on *T. cacao* L.

Individuals ants collected from cocoa trees in seed fields belong to two subfamilies (Formicinae and Myrmicinae) and comprise 9 genera and 15 species (Table 1). The subfamily of Myrmicinae is the most represented with 8 genera (Axinidris, Cataulacus, Crematogaster, Monomorium, Myrmicaraia, Pheidole, Terataner and Tetramorium) and 13 species (Axinidris murielae Shattuck, Cataulacus kholi Mayr, Cataulacus species, Crematogaster (Atopogyne) africana Santschi... Crematogaster (oxygyne) Crematogaster sp. 1, (oxygyne) sp. 2, Crematogaster sp. Crematogaster (sphaerocrema) gabonensis Emery, Myrmicaria opaciventris Emery, Pheidole mayri Forel, Terataner piceus Menozzi and Tetramorium aculeatum Smith) while the subfamily Formicinae is the least represented with the only Camponotus genus and 2 species [Camponotus flavomarginatus Mayr and Camponotus (Myrmotrema) vividus F. Smithl.

The species *C. myrmopelta vividis*, *Crematogaster* sp., *C. (Atopogyne) africana* Santschi., *C. sphaerocrema*

gabonensis, Monomorium noxitum Bolton, M. opaciventris, P. mayri, T. aculaetum and T. piceus Menozzi were found on cocoa in Nkoemvone seed field without any preference of the host; contrary to C. flavomarginatus Mayr, C. kholi, Cataulacus spp., C. oxygyne sp.1 and C. oxygyne sp. 2 which preferred hosts parasitized both by P. capitata, and P. nigritana. Only the species A. murielae Shattuck was found on not infested cocoa (Table 1).

Relative frequencies and occupancy of plant by the collected ant species

T. aculeatum is the most common species as well when *P. capitata* has flowers or not; with an occupancy rate of 26.67% during both periods followed by *Crematogaster* sp. with 15.56% occupancy rate. Other ant species were found on *P. capitata* with high occupancy rate only when it was in flowers; such as *Camponotus Myrmopelta*, *C. flavomarginatus*, *C. kholi* and *M. opaciventris*, *M. noxitum* and *T. piceus* were harvested only when *P. capitata* was blooming (Table 2).

In the case of *P. capitata*, *T. aculeatum* is the most common both when *P. nigritana* is blooming or not; with an occupancy rate of 30.23% during the two periods, followed by *C. oxygyne* sp.1 with 13.95% occupancy rate. Other species of *Crematogaster* and *Cataulacus* have the same occupancy rate both during flowering and nonflowering period of the parasite (Table 2). It is the same with species like *P. mayri*. However, *M. opaciventris* was found on *P. capitata* with a higher occupancy rate when the latter was in bloom. *A. murielae*, *C. flavomarginatus*, *M. noxitum* and *T. piceus* were harvested only when *P. capitata* was blooming.

Diversity and specific dominance of ants collected depending on the host

On the different host trees where the myrmecofauna was collected, diversity is the same with 14 species each.

According to the determination of Shannon-Weaver index, cocoa parasitized by P. capitata are more diversified (H' = 2.62), than those parasitized by P. nigritana (H' = 2.60) and not parasitized ones (H' = 2.52).

The determination of the Berger-Parker index of dominance in Table 3 has shown that *T. aculeatum* is the most abundant ant found on *Phragmanthera* with ID=0.34 for both cocoa parasitized by *P. capitata* and those parasitized by *P. nigritana*. These values showed a lack of dominance of any species as it appeared that this value approaches zero; contrary to uninfected cocoa where *C. phaerocrema gabonensis* is the most common (ID = 0.43).

The distribution of individuals within species was evaluated by the Pielou index equitability which enabled to demonstrate that cocoa parasitized by both *P. capitata*

Table 1. Inventory of the myrmecofauna on parasitized cocoa and not parasitized cocoa trees by *Phragmanthera capitata* and *P. nigritana* in Nkoemvone seed fields.

Sub family Formicinae		Geni	us		- Host species		
	N1 1	% 11.11	Camponotus	N2	%	Camponotus Myrmopelta vividus F. Smith	CPPC, CPPN, NPC
				2	13.33	Camponotus flavomarginatus Mayr.	CPPC, CPPN,
			Axinidris			Axinidris murielae Shattuck.	NPC
					Cataulacus Kholi Mayr.	CPPC, CPPN,	
			Cataulacus			Cataulacus sp.	CPPC, CPPN,
Myrmicinae						Crematogaster(Atopogyne) africana Santschi.	CPPC, CPPN, NPC
		8.89	Crematogaster	13	86.67	Crematogaster oxygyne sp. 1	CPPC, CPPN,
	8					Crematogaster oxygyne sp. 2	CPPC, CPPN,
	0	0.09		13	00.07	Crematogaster sp.	CPPC, CPPN, NPC
						Crematogaster sphaerocrema gabonensis Emery.	CPPC, CPPN, NPC
			Momorium			Monomorium noxitum Bolton	CPPC, CPPN, NPC
			Myrmicaria			Myrmicaria opaciventris Emery.	CPPC, CPPN, NPC
			Pheidole			Pheidole mayri Forel.	CPPC, CPPN, NPC
			Terataner			Terataner piceus Menozzi.	CPPC, CPPN, NPC
			Tetramorium			Tetramoruim aculeatum Smith.	CPPC, CPPN, NPC
Total	9	100	-	15	100	-	-

CPPC: Cocoa parasitized by *Phragmanthera capitata*; CPPN: cocoa parasitized by *P. nigritana*; NPC: Not parasitized cocoa. N1, number of genera present in each sub-family; N2, number of species in each subfamily.

Table 2. Relative frequencies and occupancy rates of ants genera harvested on cocoa parasitized by *P. capitata* and *P. nigritana* in seed fields of Nkoemvone.

Ants collecting zone		P. capitata (pf)		itata (pnf)	P. nigritana (pf)		P. nigritana (pnf)	
Species	Fei	Pocc	Fei	Pocc	Fei	Pocc	Fei	Pocc
Axinidris murielae Shattuck.		0	0	0	0	0	0	0
Camponotus Myrmopelta vividus F. Smith	5	11.11	2	4.44	4	9.30	4	9.30
Camponotus flavomarginatus Mayr.	3	6.67	1	2.22	2	4.65	0	0
Cataulacus Kholi Mayr.	4	8.89	1	2.22	3	6.98	3	6.98
Cataulacus sp.		6.67	3	6.67	5	11.63	5	11.63

Table 2. Contd.

Crematogaster (Atopogyne) africana Santschi.	3	6.66	3	6.66	2	2.65	2	2.65
Crematogaster oxygyne sp.1	5	11.11	5	11.11	6	13.95	6	13.95
Crematogaster oxygyne sp. 2	5	11.11	5	11.11	4	9.30	4	9.30
Crematogaster sp.	7	15.56	7	15.56	5	11.63	5	11.63
Crematogaster sphaerocrema gabonensis Emery.		8.88	4	8.88	2	4.65	2	4.65
Monomorium noxitum Bolton		2.22	0	0	1	2.36	0	0
Myrmicaria opaciventris Emery.		13.33	3	6.67	5	11.63	3	6.98
Pheidole mayri Forel.		6.67	3	6.67	1	2.33	1	2.33
Terataner piceus Menozzi.		2.22	0	0	2	4.65	0	0
Tetramoruim aculeatum Smith.		26.67	12	26.67	13	30.23	13	30.23
Total		45		45		43		43

Fei: Relative frequency; Pocc: occupancy rate, Fp: flowering period, Pfp: parasite flowering period, Nfp: not flowering period.

Table 3. Number of individuals per ant species collected based on various hosts.

Species	CPPC	CPPN	NPC
Axinidris murielae Shattuck.	0	0	3
Camponotus Myrmopelta vividus F. Smith	12	10	3
Camponotus flavomarginatus Mayr.	3	4	5
Cataulacus Kholi Mayr.	5	6	8
Cataulacus sp.	3	2	1
Crematogaster (Atopogyne) Africana Santschi.	17	11	15
Crematogaster oxygyne sp. 1	36	40	6
Crematogaster oxygyne sp. 2	92	85	78
Crematogaster sp.	18	20	6
Crematogaster sphaerocrema gabonensis Emery.	135	127	142
Monomorium noxitum Bolton	1	1	6
Myrmicaria opaciventris Emery.	19	15	18
Pheidole mayri Forel.	6	3	0
Terataner piceus Menozzi.	1	2	1
Tetramoruim aculeatum Smith.	179	168	41
Taille de l'échantillon (N)	527	494	333
Richesse spécifique (S)	14	14	14
Shannon-Weaver (H')	2.62	2.60	2.52
ID _{Berger-Parker}	0.34	0.34	0.43
Pielou Index of equitability (E)	0.69	0.69	0.66

Table 3. Contd.

	CPPC and CPPN: S _s =100
Similarity Index (Ss)	CPPC and CNP: $S_s = 92.85\%$ p
	CPPN and CNP: $S_{s = 92.85\% p}$

CPPC: Cocoa parasitized by *P. capitata*, CPPN: cocoa parasitized by *P. nigritana*; NPC: Not parasitized cocoa.

and P. nigritana were in a situation of communities homogeneity; Indeed, the values of Pielou index of equitability obtained (E = 0.688 and E = 0.683, respectively in the two species of *Phragmanthera*) tended more towards one than towards 0. Not parasitized cocoa come next with a less perfect situation of communities homogeneity, that was E = 0.662.

The similarity of studied communities was assessed using the Sorensen index which established that the trees infected both by *P. capitata* and *P. nigritana* shared 100% of the recorded ant species (the same species existed on parasitized cocoa). On the contrary, 92.85% ant species harvested were present on both the uninfected and cocoa parasitized by one and/or the other species of *Phragmanthera*.

DISCUSSION

Parasitic plants as Loranthaceae are mostly associated with one or a few species of specialized ants and association is mandatory for the survival of the partners. The loyalty of these interactions is fostered by the housing and/or the food offered by plants. In return, the ants protect their host against defoliators and competitors and can even supply it with nutrients. On the plant side, the production of housing for ants (domatia) is a fundamental character of myrmecophilous species. These domatia are hollow structures that

can be localized on the trunk, petiole, stipules or leaf lamina. Moreover, colonization of some myrmecophytes requires a drilling of the domatia entry from ants (Brouat et al., 2001).

Many ants exploit Hemiptera and/or Lepidoptera Lycaenidae larva (Banks et al., 1991). These associations cause considerable damage to the plants vegetative organs (Dejean et al., 2007). Associations as *P. capitata*-ants were observed on fruit trees in the orchard of the Ndogbong chiefdom in Douala (Mony et al., 2009; Dibong et al., 2010a).

The two main species of Loranthaceae subject of the study in seed fields of Nkoemvone, namely, *P. capitata* (Sprengel) S. Balle and *P. nigritana* (Hook.f. ex Benth.) Balle are not specific to the Nkoemvone area that belongs to the semi-deciduous Guinean-Congolese dense forest area, semi-deciduous sector in the strict sense and are also reported in Benin, Cameroon, Gabon, Ghana, Guinea, Liberia, Mali, Niger, and Senegal (Dibong et al., 2009b).

It appears that the species of ants *T. aculeatum* and *Crematogaster* sp. live on multiple hosts, including those infested by Loranthaceae, it is the dominant ants. Mony et al. (2011) found that the ants of the *Crematogaster* genus were most abundant at 90.19% in house orchards and gardens in Logbessou. Noutcheu et al. (2013) reported that *Crematogaster* genus was the most abundant ants associated with trees parasitized by *P. capitata* in the orchard of the Ndogbong

chiefdom in Douala, Cameroon. Dibong et al. (2012) also reported the presence of these two dominant species in the locality of Lokomo in the east region of Cameroun, in orchards infested by Loranthaceae. Ants of *Crematogaster* genus are often dominant in their communities. In New Guinea, a species living in the canopy (*Crematogaster major*), represents 56 to 99% of collected ants on trees (Leponce and Missa, 1998). *Crematogaster irritabilis* can cover, meanwhile, more than 99% of trees in an area of one hectare in New Guinea (Leponce et al., 1999). *C. parabiotica* overwhelmingly dominates the Peruvian Amazon rainforest.

Formicinae and Myrmicinae are the two sub families of our study. These results are reminiscent of earlier work in tropical Africa by Dejean et al. (1994, 1999) and Asia (Savitha et al., 2008; Ramesh et al., 2010). Myrmicinae were more abundant than Formicinae subfamily (Table 2). Their dominance matches their abundance within the world fauna (Hölldobler and Wilson, 1990; Bolton, 1994). Most Myrmicinae species are characterized as typical leaf litter inhabitants. Some renowned predatory ants associate to their diet plant extracts (honeydew of Hemiptera and plants nectar); in the case with *Pheidole megacephala* (Fabricius), *T. aculeatum Mayr, C. flavomarginatus* Mayr and *Crematogaster* sp.

T. aculeatum is a myrmicine that has already reported abundant and prevalent in various agroecosystems including cocoa farms in Cameroon

(Jackson, 1984). It is more abundant on cocoa trees when Loranthaceae are flowering. It feeds flowers, ovarian tissues, seeds and fruits of tree crops (Veeresh, 1990). The majority of plant species produce seeds equipped with elaiosomes. These feeder bodies are attractive to ants which will consume them after transporting in their nest. The workers of this species also harvest the sweet liquids such as extra floral nectaries which are an important part of their diet (Völkl et al., 1999). These nectaries localized in leaves, branches, external parts of flowers and suckers, contain amino acids or lipids. Plants having such structures will attract thousands series of ant species whose presence induces a reduction in the intensity of defoliation by herbivore insects (Ness, 2003; Vesprini et al., 2003). Nutrients of host plant species are crammed into domatia form of food reserve (Hölldobler, 1976). The suckers Phragmanthera are suitable nests to T. aculeatum; formed by an impressive network of galleries dug in Phragmanthera living wood, they eventually dry out and cause their death (Dibong et al., 2010b). According to Peakall (1994) and Koptur et al. (1998), the majority of interactions between epiphytes and ants came from nesting sites offered by the root networks.

The comments related to ants activity in seed fields of Nkoemvone showed ants foraging on flowers, fruits and suckers of both *P. capitata* and *P. nigritaria*. In both, the *Phragmanthera* species identified, galleries dug in the suckers of the host timber finally get rotten. The sucker plays the role of ants forage. The ants will forage burrowing inside to look for sweet substances, causing dryness of the sucker and will leave a gateway into the heart of the host and rotting wood will continue (Dibong et al., 2012).

Conclusion

Interactions between cocoa parasitized by the *Phragmanthera* genus and ants are those with *T. aculeatum* where the suckers of the parasite serve as nests. In seed fields of Nkoemvone, parasitism with *Phragmanthera* is the site of an important forage activity of two main types of ants, *Tetramorium* and *Crematogaster*. This activity results in dessication of suckers and death of parasites (Loranthaceae) and is a hope in the development of biological control, a major asset in biodiversity conservation.

Conflict of Interests

The authors have not declared any conflict of interests.

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