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Ecology of Myrmecofauna (Genus: *Crematogaster*) Associated to *Helixanthera mannii* (Oliv.) Danser (Loranthaceae) to Cocoa Trees in Oboa: Center Region of Cameroon, Africa

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Abstract

In order to find an effective control method against *Helixanthera mannii* (Oliv) Danser, parasite of agroecosystems in the northern part of the city of Yaoundé, a study of the myrmecofauna associated with this plant was carried out in an orchard of Oboa in Soa district. The total parasitism rate by *Helixanthera mannii* Danser was 20.43% for the entire orchard. 168 ants belonging to 12 species including ten genera and four subfamilies were collected from unparasitized and parasitized host individuals of *Theobroma cacao*. 86 ants belonging to two subfamilies were collected from selected branches of *Helixanthera mannii* from parasitized cocoa trees, namely Formicinae (*Camponotus flavomarginatus*) and Myrmicinae (*Crematogaster* (*oxygyne*) sp., *Crematogaster* (*decacrema*) sp. and *Cataulacus* sp.) or 58.14% of the myrmecofauna during the flowering of this Loranthaceae and 41.86% during its fruiting. The ants of the genus *Crematogaster* proved to be the most active and abundant, *i.e.*, 76.83% on non-parasitized hosts and 93.03% on hosts parasitized by *Theobroma cacao* by *Helixanthera mannii*. *Crematogaster* (*oxygyne*) sp. had an occupancy rate of 69.77% followed by *Crematogaster* (*decacrema*) sp. with 23.26% or 93.03% of the myrmecofauna recorded during the flowering and fruiting of *Helixanthera mannii*. *Crematogaster* (*oxygyne*) sp. proved to be the most active ant on these hosts. Note that the dominant ant status recognized for ants of the genus *Crematogaster* and their foraging activity on the flowers and fruits of *Helixanthera mannii* can contribute to the reduction of these parasitisms.

Keywords: ecology, *Crematogaster*, *Helixanthera mannii*, *Theobroma cacao*, Oboa, biological control

1. Introduction

Ants have very varied and complex relationships with plants, ranging from loose associations to specific associations depending on the needs they seek from the latter. The ignorance of the parasites of our ligneous species and of the biology of their natural enemies are, among other things, real.

The latter and natural woody plants are subject to severe attacks limiting the expansion of orchards by Loranthaceae. Thus, through their mode of attachment to host plants, they significantly reduce yields or affect the quality of harvests (Sonké et al., 2000; Engone et al., 2005). These parasites, which have not been sufficiently studied by research and extension structures, constitute today a real constraint for the promotion of home gardens, orchards and spontaneous species in Cameroon (Dibong et al., 2008).

The importance of myrmecofauna in ecosystems is well recognized. Ants are sensitive to variations in the environment and are therefore generally used as bio-indicators in environmental remediation programs (Dejean et al., 2014). They are biological control agents in agrosystems. Ants are predators that regulate the populations of many other insects, scavengers that remove the corpses of insects or small animals, pollinators and essential players in the dispersal of seeds and in the enrichment of the soil (Wilson, 1987; Tobin, 1994; Floren et al., 2002; Wilson & Hölldobler, 2005). In environments with little human activity and in agrosystems, ants limit herbivore communities and increase plant growth and reproduction (Schmitz et al., 2000, Symondson et al., 2002). The arboreal environment is a generally dry environment where water retention is much lower than on the ground (Orivel, 2000). The life and community structure of arboreal ants is most often found within the subfamilies

Dolichoderinae, Formicinae, Myrmicinae and Pseudomyrmicinae which are also considered to be the most evolved (Hölldobler & Wilson, 1990; Orivel & Dejean, 1999, Djiéto-Lordon, 2004). Competition and predation are the predominant factors in the structuring of the myrmecofauna (Andersen & Patel, 1994). Thus we note a vertical stratification of the habitat of the ants. Ground-dwelling species nest on the ground and may forage on trees. Tree ants are present on trees where they also nest. Food resources from plants play a key role in the ecology of arboreal ants. The latter generally consume a greater quantity of liquids than soil-dwelling ants. Some species feed on nectar from extra-floral nectaries or Hemiptera honeydew, which themselves feed on plant sap (McKey et al., 1999). In plant-ant relationships, ants typically receive shelter or food from the plant, and in return protect the plant against herbivores or even against other plants (Beattie & Hughes, 2002; Heil & McKey, 2003; Passera & Aron, 2005; Dejean et al., 2007). Overall, between plants and ants, mutualistic interactions are much more abundant and diversified than antagonistic interactions. Optional or obligatory mutualisms vary depending on whether the partners can survive without each other or not (Bronstein, 2004).

Given the many damage caused by the Loranthaceae which represent a real gangrene in our plantations, gardens and orchards in sub-Saharan Africa; since that this parasite severely harms *Theobroma cacao* hosts, research into the ecology of the myrmecofauna linked with *Helixanthera mannii* is of economic, environmental and ecological significance.

2. Materials and Methods

2.1 Site and Study Period

The study was carried out in Cameroon in the northern part of the city of Yaoundé in a cocoa plantation in the village of Oboa (traditionally called Endongo) in the district of Soa Department of Mefou and Afamba with geographical coordinates 04°05′54.5″ North latitude, 11°37′46.3″ East-West longitude and 593 m altitude (Figure 1) over the period from July to September. The cocoa plantation is more than fifty year with an area of approximately 110 m × 130 m (14300 m^2), the area of the plot studied being 40 m × 60 m or 2400 m^2. The Oboa village has a climate which belongs to the equatorial domain with four seasons: a short rainy season (mid-March to June), a long rainy season (September to mid-November), a long dry season (mid -November to mid-March) and with a short dry season (July to August). The average annual rainfall is 1600 mm and the average annual temperature varies between 19 to 33 °C (Suchel, 1987; Abossolo et al., 2015).

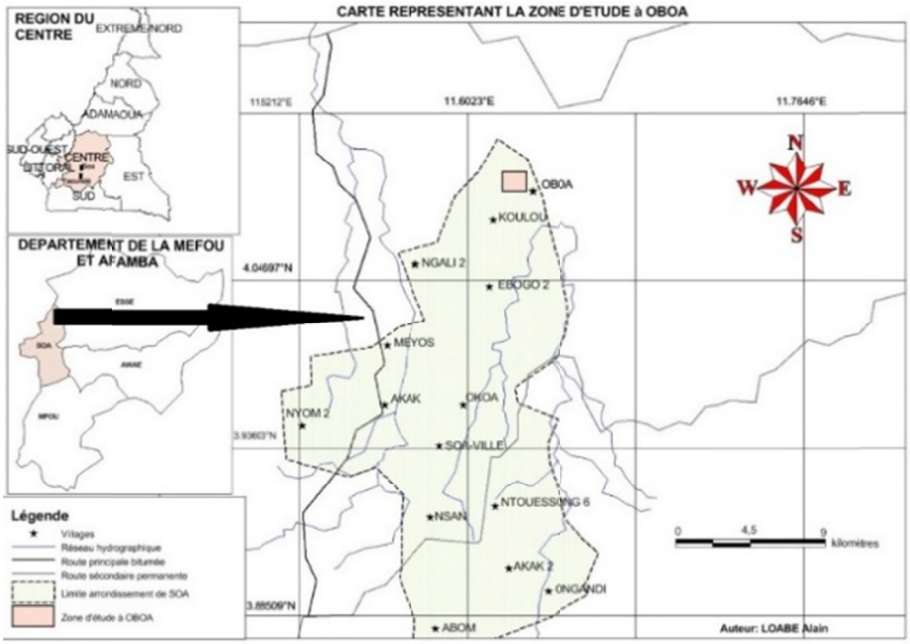

Figure 1. Location of the Oboa orchard in the district of Soa
(map of the National Institute of Cartography modified)

2.2 Methods

For the control trees, samples were taken only at the level of the trunk once each week (in the morning from 8 a.m.); however, for the parasitized trees, samples were taken at various levels: trunk, leaves and clump(s) of *Helixanthera mannii*. As a sampling technique we used manual collection and as a sampling device we used quadrats. The grid of the site consisted in delimiting the sampling surface to make small squares of 5 m × 5 m (25 m^2), *i.e.*, a total of 96 squares for an overall surface of 2400 m^2 (40 m × 60 m). Then we identified the floristic diversity and the associated myrmecofauna. We then proceeded to the selection of three parasitized trees and three control trees (*i.e.*, non-parasitized) of *Theobroma cacao* whose trunk diameter was greater than or equal to 15 cm in order to carry out the general collection of the myrmecofauna in the quadrats.

2.3 Floristic Inventory

The floristic inventory consisted of counting all woody species with a diameter greater than 15 cm parasitized or not by *Helixanthera mannii*. For the parasitized trees, the foraging activity of the myrmecofauna associated with them was evaluated.

2.4 Collect and Identification of Myrmecofauna

On the stem, flowers, leaves, fruits and suckers of *Helixanthera mannii*, foraging activity of the myrmecofauna was observed. Subsequently, ant were collected during foraging periods in order to assess their diversity and their abundance. The ants were collected from the host individuals of *Theobroma cacao* and *Helixanthera mannii* between 8 a.m. and 12 p.m. every Sunday. The capture of the small ant was done using a mouth aspirator and for the large one using soft forceps then kept in labeled boxes containing alcohol at 70°. The identification was made in the laboratory thanks to the identification keys of Hölldobler and Wilson (1990), Taylor (2011), Bolton (2003, 2016) and with the help of the identification keys of the database African ants (www.antbase.org) and their nomenclature approved by qualified entomologists from the Department of Animal Biology and Physiology of the Faculty of Sciences of the University of Yaoundé I.

2.5 Data Analysis

In this work, the relative abundance (Ra) is the ratio of the number of individuals (n_i) of a given species to the total number (N) of individuals of all species combined (Dajoz, 2000) allowed to calculate the frequencies of the myrmecofauna present in the orchard. $Ra = (n_i/N) \times 100$ is the equation for it. Regardless of the number of individuals or the mass represented by each taxon, the number of species (or morphotypes) listed in an ecosystem or community is its total specific richness (S) (Peet, 1974). The rate of parasitism noted as Pp made it possible to assess the level of infestation of *Helixanthera mannii* in the Oboa orchard. It was calculated using the following formula: Pp = (Number of infected trees/Total number of trees) × 100.

- Shannon Weaver's index (H') gives an idea of the diversity of a stand by taking into account not only its specific richness but also the number of individuals in each population (Frontier, 1983). It varies from 0 (when only one species composes or dominates the community) to lnS (when all species have the same abundance); its formula is as follows: $H' = -\sum_{i=1}^{S} (p_i \times Log_2 p_i)$, with $pi = n_i/N$. Pi = proportion of individuals of species i; S = total number of species in the environment.

- The Simpson index (λ) represents the proportion of the abundance of a species i within a stand (Pearson & Rosenberg, 1978). Its formula is: $D = -\sum_{i=1}^{S} [n_i(n_i - 1)]/[N(N - 1)]$, where, n_i = number of individuals of species "i", n = total number of individuals in the sample. It varies from 1 (in case of maximum diversity) to 0 in case of minimum diversity.

- The chi-square test allowed for the determination of the myrmecofauna's importance between the non-parasitized and parasitized plants in the orchard, as well as the myrmecofauna connected with each group.

- The regression curves show the correlation between flowering, fruiting and ants. The coefficient of determination (R^2) varies between 0 and 1. The closer R^2 is to 1, the stronger the linear correlation and vice versa.

3. Results

3.1 Floristic Inventory in Orchard of Oboa

A 230 hosts trees belonging to ten species including ten genera and ten families, parasitized or not by *Helixanthera mannii* were inventoried on the site (Table 1). The most represented families were those of Meliaceae and Sterculiaceae with 215 individuals or 93.48% of the listed flora. The least represented families are those of Euphorbiaceae and Lauraceae with two individuals or 0.86% of the listed flora.

Table 1. Parasitized and non-parasitized woody species with Helixanthera mannii recorded in the Oboa orchard.

Families	Host species	Unparasitized host individuals	Parasitized host individuals	Abundance	Relative abundance (%)
Anacardiaceae	*Mangifera indica* Linné	3	0	3	1.30
Burseraceae	*Dacryodes edulis* (G. Don) H. J. Lam	2	0	2	0.87
Fabaceae	*Pterocarpus soyauxii* Taub	2	0	2	0.87
Lauraceae	*Persea americana* Mill	0	1	1	0.43
Meliaceae	*Entandrophragma cylindricum*	4	0	4	1.74
Moraceae	*Milicia excelsa* (Welw.) C. C. Berg	2	0	2	0.87
Rutaceae	*Citrus sinensis* (Linné) Osbeck	2	0	2	0.87
Sterculiaceae	*Theobroma cacao* Linné	165	46	211	91.74
Euphorbiaceae	*Ricinodendron heudeulotii* (Müll. Arg)	1	0	1	0.43
Sapotaceae	*Baillonnella toxisperma*	2	0	2	0.87
Total		183	47	230	100

The average parasitism rate is 21.80% in the sampling area. The cocoa tree's productivity, and consequently the productivity of the entire orchard, was impacted by the distribution of *Helixanthera mannii* clumps on its two sides.

3.2 Inventory of Myrmecofauna

168 ants belonging to 12 species including ten genera and four subfamilies were identified on the host individuals: *i.e.*, 82 on *Theobroma cacao* and 86 on cocoa trees parasitized by *Helixanthera mannii* (Tables 2 and 3). The Ponerinae with a single species *Hyponera* sp., Dolichoderinea with *Technomyrmex* sp., the Formicinae with four species including *Camponotus flavomarginatus*, *Oecophylla longinoda*, *Lepisiota* sp. and *Cataglyphis* sp. and the Myrmicinae with six species including *Monomorium* sp., *Crematogaster* (*Dececrema*) sp., *Tetamorium gabonense*, *Crematogaster* (*Oxygyne*) sp., *Crematogaster* (*Orthocrema*) sp. and *Cataulacus* sp.. The Formicinae and Myrmicinae made up 164 individuals, or 98.23% of the myrmecofauna gathered, of the parasitized and non-parasitized host of *Theobroma cacao* individuals. With $X^2 = 35.035$, ddl = 11, and a p-value = 0.00024456, the chi-square test demonstrates a very significant difference between the myrmecofauna composition of parasitized and non-parasitic cocoa plants. According to Shannon Weaver's diversity index, the myrmecofauna diversity of unparasitic plants is $H'_2 = 1.513$ and $H'_{2Max} = 3.58$. Hence the myrmecofauna present on non-parasitized host individuals is not diversified. $H'_{2'} = 0.8245$ and $H'_{2'Max} = 2$ for that of parasitized plants which was also not diversified. Simpson's index gives ($1\text{-}D_2 = 0.6178$) for the composition of the myrmecofauna of non-parasitized cocoa trees against ($1 - D_{2'} = 0.4567$) for that of parasitized cocoa trees.

Table 2. Ants harvested from healthy and parasitized cocoa trees during the months of July, August and September in the Oboa orchard

Ant species	Samples taken from non-parasitized hosts	Samples taken from parasitized hosts	Abundance	Relative abundance (%)
Crematogaster (*oxygyne*) sp.	49	60	109	64.88
Crematogaster (*decacrema*) sp.	5	20	25	14.88
Cataulacus sp.	3	3	6	3.57
Tetamorium gabonense	1	0	1	0.60
Crematogaster (*orthocrema*) sp.	9	0	9	5.36
Monomorium sp.	6	0	6	3.57
Camponotus flavomarginatus	1	3	4	2.38
Lepisiota sp.	3	0	3	1.79
Cataglyphis sp.	1	0	1	0.60
Oecophylla longinoda	1	0	1	0.60
Technomyrmex sp.	2	0	2	1.19
Hyponera sp.	1	0	1	0.60
Total	82	86	168	100

Table 3. Ants harvested on selected branches of *Helixanthera mannii* from parasitized cocoa trees during the months of July, August and September in the Oboa orchard

Ant species	Abundance	Relative abundance (%)
Crematogaster (*Oxygyne*) sp.	60	69.77
Crematogater (*Decacrema*) sp.	20	23.26
Cataulacus sp.	3	3.49
Camponotus flavomarginatus	3	3.49
Total	86	100

The myrmecofauna associated with plants is depicted in Figure 2 with actively foraging on ripening *Helixanthera mannii* berries, reflecting their premature drop. In contrast, Figures 3 and 4 depict their activities on and inside the Loranthaceae haustorium, causing the host plant parasite to die back by drying it out.

Figure 2. Picture of *Crematogaster* (*Oxygyne*) sp. in forage at the base of *Helixanthera mannii* berries (according to YEBGA)

Figure 3. Picture of *Crematogaster* in full forage on the haustorium of *Helixanthera mannii* (according to YEBGA)

Figure 4. Picture of the *Helixanthera mannii* haustorium with nest sites of *Crematogaster* having dried up a branch of *Theobroma cacao* (according to YEBGA)

3.3 Correlation Between Flowers, Fruits of Helixanthera mannii and Ants

The coefficient of determination during flowering ($R^2 = 0.969$) was very close to 1 due to the close link between the quantity of fallen flowers of *Helixanthera mannii* and the ants (Figure 5). When harvesting ants on Loranthaceae decreased, more blooms fell to the ground.

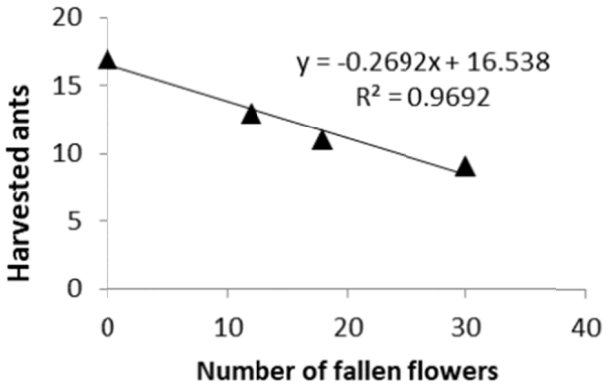

Figure 5. Relation between ants and *Helixanthera mannii* flowers

The association between the ants and the falling fruits of the Loranthaceae, on the other hand, is weak, and as a result, the coefficient of determination during fruiting ($R^2 = 0.341$) is far from 1 (Figure 6).

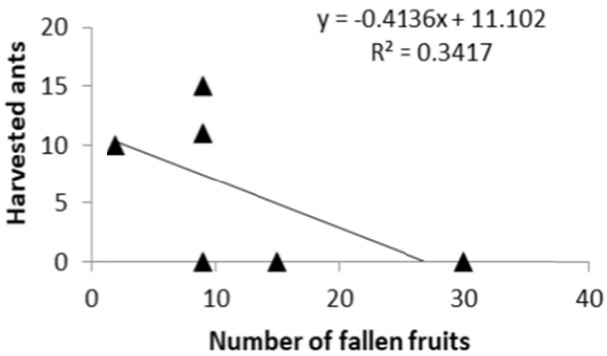

Figure 6. Relation between ants and the fruits of *Helixanthera mannii*

4. Discussion

Ponerinae, Formicinae, Dolichoderinae and Myrmicinae were present on *Theobroma cacao* in the Oboa orchard. These results differ from those obtained by Jueya et al. (2019) who instead obtained five ant subfamilies (Pseudomyrmicinae, Dolychoderinae, Ponerinae, Formicinae and Myrmicinae). Among these four subfamilies, only the Myrmicinae is most diversified, as also reported by the work of Ladoh et al. (2013), Noutcheu et al. (2013) in the chiefdom of Ndogbong in Douala, Ondoua et al. (2016) in the sodecao orchard in Nkoemvone and those of Jueya et al. (2019) in the orchard of Nkolbisson in Yaounde. 86 ants were sampled on *Helixanthera mannii* (flower bud nodes, branches and berries), *i.e.*, 50 ants during flowering and 36 ants during fruiting. *Crematogaster* (*Oxygyne*) sp. proved to be the most active on *Helixanthera mannii* with a rate of 69.77% followed by *Crematogaster* (*Decacrema*) sp. with 23.26%. These results differ from those obtained by Jueya et al. (2019) who instead obtained *Tetramorium acculeatum* as an active ant on Loranthaceae *Tapinanthus preussii* (Engler) Van Tieghem and *Phragmanthera capitata* (Sprengel) S. Ball in the Nkolbisson orchard in Yaounde. Some of these ants, notably the genus *Crematogaster*, have already been found in various Loranthaceae (Noutcheu et al., 2013; Ladoh et al., 2013).

The ants mentioned above have a mutualistic relationship with their host plant. Generally, the plant provides food and shelter. These are therefore obligatory interactions for both partners. Thus plants are highly dependent on their hosts to defend themselves against phytophagous and some do not survive their absence very long. However, a low foraging activity of ants was noticed during flowering when almost all the flowers reach maturity with very few early drops. It is not the same during fruiting when ants are almost absent from the branches bearing the berries. A slight fall of ripe berries was observed during this period and some were rotting. Monitoring the flowering and fruiting of *Helixanthera mannii* shows that ants of the genus *Crematogaster* participate in the fall of its flower buds and its berries. This result corroborates those obtained by Ladoh Yemeda

(2011), Ladoh et al. (2013) and Massako Mayuk (2011) in the orchards of Ndogbong and Logbessou in Douala where the foraging activity of ant workers reduced parasitism by Loranthaceae.

The absence of ants on the twigs that bore terminally developed fruits during the previous three weeks was notable, aside from the ants that were sampled. In the haustorium of *Helixanthera mannii*, *Crematogaster* nests were found (Figure 4). There are nests in the suckers of *Phragmanthera capitata*, according to the studies of Mony et al. (2009) and Dibong et al. (2010). The Myrmicinae was mainly represented by dominant species of the genus *Crematogaster*. These ants have already been reported on Loranthaceae (Murase et al., 2002, Mony et al., 2009, Dibong et al., 2012). The latter are characterized by very populous societies and intra- and inter-specific aggressiveness, which limits the access of other ants to colonized species (Noutcheu et al., 2013). The genus *Crematogaster* tolerates abundant Hemiptera on the fatty leaves of Loranthaceae, often exploited for their honeydew. According to Hossaert-Mckey et al. (2001) and Blüthgen et al. (2004), trophobiosis with Hemiptera is a primordial character of dominant arboreal ants of the genus *Crematogaster*. *Crematogaster* by association with Hemiptera, limit the spread of Loranthaceae on host species, thus contributing to their biological control. All this could explain the low rate of parasitism encountered in this orchard, *i.e.*, 20.43%. This result differs from those obtained by Mony et al. (2009), Dibong et al. (2008, 2009, 2010, 2011, 2012), Noutcheu et al. (2013), Ladoh (2011), Ladoh et al. (2013) and Massako (2011) who respectively obtained a slightly higher rate of parasitism in the orchards located in Douala. The particularity of this orchard is that the parasitism is specific to *Helixanthera mannii*. However, this is the first known case of occupation of this species on cocoa trees in Cameroon. The work of Mony et al. (2009), Dibong et al. (2011), Lodoh et al. (2013), Ondoua et al. (2016), and Jueya et al. (2019) rather reported the presence of *Phragmanthera batangue*, *Tapinanthus preussii*, *Tapinanthus bangwensis* as well as *Phragmanthera capitata* as Loranthaceae recorded respectively in the camp of the chiefdom of Ndogbong in Douala, of Sodecao in Nkoemvone and of Yaounde in Nkolbisson.

5. Conclusion

The work carried out on the Oboa site aimed to determine the relationship between ants of the genus *Crematogaster* and *Helixanthera mannii* parasitizing cocoa trees in this orchard in order to use these ants as a means of biological control against these hemiparasites. The myrmecofauna associated with parasitism made it possible to identify 12 species of ants, of which the genus *Crematogaster* was the most frequent and the most active during the flowering and fruiting of *Helixanthera mannii*. The low abundance of the myrmecofauna could be due to the particularity of this Loranthaceae despite the territoriality of *Crematogasters* on it. However, the low rate of parasitism in the orchard could undoubtedly be due to the territoriality of the myrmecofauna of the genus *Crematogaster* associated or combined with the very low practice of mechanical control against *Helixanthera mannii* in this orchard.

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