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Spatial Distribution and Density of Fungus-Growing Termite *Pseudacanthotermes militaris* (Isoptera: Macrotermitinae) In the Congo Republic

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ABSTRACT

Despite the abundance and activity of fungus-growing termites in African ecosystems, few studies deal of their factors of distribution. The aim of this study was to investigate the role of moisture in spatial pattern and concentration of fungus-comb chamber of *Pseudacanthotermes militaris*. This species is exclusive and very widespread in plantations of sugar cane of the Valley of the Niari in the Republic of Congo. The study was conducted in two different plots of sugar cane. These two plots of land, Yokangassi 9 (Yok9) and Moutela 2/3 (M2/3) are 5 km away from each other inside the 22 000 acres sugar area. Contrary to Yok9, M2/3 has a permanent lake nearby. In these two fields, fungus growing is picked into the pits dug. By using the R software package spat stat, the quadrats method, the distance to nearest neighbors method and especially the J spatial regularity indicator; it had been shown that the spatial distribution in both plots is aggregative. Nevertheless, the number and density of fungus growing were different. In the plot M2/3, the density of the fungus-growing chamber is 10.56 m^{-2} at 150 m away from the Lake. In a way Lake, this density gradually decreases to values close to those of Yok9, i.e., 2.68 and 3.87 fungus-growing chamber m^{-2}.

Key words: Fungus-growth, sugar cane plantations, single-crop farming, influence of moisture, spatial patterns, Congo

INTRODUCTION

Termites have an important role in the African sub-Saharan ecosystem (Bodot, 1967; Roy-Noel, 1972; Josens, 1972; Lepage, 1974). They represent the most important animal biomass in the soil (Wood *et al*., 1982). The termites with others macro invertebrate of soil like earthworms and ants are considered like as the soil engineers. Indeed these organisms modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials (Jones *et al*., 1994, 1997). These organisms build organo-mineral structures (galleries, casts, mounds, fungus-comb chambers) usually called biogenic structures that have specific physical, chemical and microbiological properties (Lavelle *et al.*, 1997). It should be noted that in the termites to symbiotic bacteria, there is the Termitidae family which includes the Macrotermitinae sub-family which has acquired an original type of symbiotic relationship with fungi ectosymbiosis of the genus Termitomyces. In fact, workers make fungus comb only from plant fragment wreckages they collect in the ground. After that, these fragments expelled without complete digestion in the form of pellets or mammospheres. Pellets are laid down next to each other and constitute the fungus comb (Josens, 1972) on which a genus of *Termitomyces basidiomycota* fungus who belongs to the Agaricaceae line of descent (Heim, 1942) grows. Yeasts osidasic enzymes, the fungus predigests organic matter which constitutes the fungus comb facilitating the termites own enzyme (Rouland, 1986; Rouland *et al.*, 1991).

The fungus comb is a complete food that is almost used in totality by termites. They are put in cavities called the fungus-comb chambers. These fungus-comb chambers are connected with each other and with the interior by a network of undergrounds galleries. They abound in the African savannah (Josens, 1972) and are located near the binnacle and/or scattered to varying deepness and distances. Laboratory experiments evidenced higher microbial activity and N-mineral production in these fungus-comb chambers (Abbadie and Lepage, 1989).

Macrotermitinae termites are usually considered as belonging to the same functional group when considering their influence on the ecosystem functioning (Lavelle, 1997; Bignell and Eggleton, 2000). Thus, Jouquet *et al.* (2005) showed that fungus-growing termite species (Ancistrotermes and Odontotermes) have approximately the same impact on soil aggregate properties (enrichment in SOM and fine particles). In addition to the bio-ecological interest of the study of the Macrotermitinae, it should be noted that they also have a deleterious action. The *Pseudacanthotermes* genus including *P. militaris* species has economic importance. In fact, Wheatley and Crowe (1967) showed that *P. militaris* causes significant damages on sugar cane crops in Kenya and Taksdal and Nyiira (1971) showed the same thing for Uganda. It attacks the corn in Nigeria (Harris, 1969; Wood *et al.*, 1980), in Zimbabwe (former Rhodesia) (Mitchell, 1972) and in Malawi (Munthali *et al.*, 1989), the tea (*Camellia sinensis)* in Kenya, Tanzania, Uganda and Malawi (Benjamen, 1968a, b); the eucalyptus in Uganda (Nyeko and Olubayo, 2005); rice in Benin (Togola *et al.*, 2012) and cassava root in West Africa (Sands, 1973).

In Congo, *P. militaris* is species which abounds in the sugar cane plantations of the Niari valley and settles them in a nearly exclusive way with *P. spiniger*. *P spiniger* species build a mound that grow on top of the ground and tremendously disturbs mechanical harvest of sugar cane. Contrariwise, *P. militaris* doesn't show a visible deleterious action for the crop yet (Mora *et al.*, 1990; Mora, 1992; Dibangou, 1994). However, it is important to understand its relationship with its environment. So, with the presence of water on the site, the role of moisture is analyzed as a factor that acts on the distribution of the fungus growing-termite as well as on their density.

MATERIAL AND METHODS

Nest of *P. militaris*: *P. militaris* nests are completely hypogean (Grasse and Noirot, 1951), (Josens, 1972). Then, Grasse and Noirot (1951) described them as constituted of one or more calies. One of them contains the royal box and many little chambers with scattered fungus-growing. Josens (1972) finds they are composed of a small number of chambers with isolated fungus-growing and the royal couple is housed in one of them. The comments conducted in the plantations of sugar cane from the Niari Valley, show rather than *P. militaris* nests are composed of two very distinct units:

- An ovoid shaped central part or endoecie which contains the royal box. This box has a horizontal floor and thin walls partitions contrary to the other fungus-growing chambers: It is laminated one
- A scattered fungus-growing chamber network with also horizontal floor which appears to be much more compact around the endoecie. They are located at distances varying from endoecie from a few centimeters to several meters of it and also varying depths

Studied area localization: The ground data's were obtained in the sugar cane plantations of the Niari valley south-east of the Congo Republic. The Niari Valley is framed by the 12°21 and 14°57 west meridians and lies astride the 04°00 south.

Sampling: Samplings were conducted according to the following principle: the soil moisture is higher when it is close of permanent water area and decreases as one moves away. Thus, the counting and the monitoring of the fungus growing chamber spatial distribution have been done in two different plots of land in the sugar area. They are 5 kilometers far from each other:

- **Yok9:** This plot is situated on a plateau and there is no water-based area nearby. Samples are taken from pits dugs two 16 square centimeters, each one dug at random in the n°5 square of the plank 1 (S5P1) and in the n°6 square of the plank 3 (S6P3). These two pits separated from 500 meters, served to study the spatial distribution of the fungus growing chamber and to estimate their density in this plot
- **Moutela 2/3 (M2/3):** This plot is situated near a permanent water-based area which could be fed by an underground spring because there is no river nearby. The study of the spatial distribution of fungus-growing is made from a 16 square meter pit dug at random near the n°5 square of the plank 1 (S5P1). Then, to follow the fungus growing chamber density evolution, 4 pits of 16 square meters each were opened by regular intervals along a transect of a 800 m starting from the non-submerged immediate vicinity of the water

All the measurements/readings have been done during the dry season, between May and June (1991). Pits are 1 m deep. The surface to be inventoried is delimitated by ropes and then the earth is removed by successive layers of 10 cm interns of. The spatial density distribution, fungus growing chamber are localized, counted and identified. The position of each fungus growing chamber is transferred to a graph paper according to x and y-axis.

Statistical analysis: Spatial structure analysis of the fungus growing chamber is realized with the R software spat stat package. The quadrats method is used to test the Ho null hypothesis of an unpredictable and homogeneous distribution (adequacy test to the Poisson law done thanks to the χ^2 test). In case of a rejection of the null hypothesis H0 (risk $\alpha = 0{,}05$) the method of the distances to nearest close is used (Van Lieshout and Baddeley, 1996) and in particular the J spatial regularity indicator to specify if the distribution is more regular ($J>1$) or less regular ($J<1$, aggregation) than a random selective/occasional process ($J = 1$).

RESULTS

Fungus distribution: Spatial distribution is shown in Fig. 1-4, sampling done in M2/3 plot situated at 150 m from the permanent water based area (Fig. 1 and 2). The Chi-squared test of Complete Spatial Randomness (CSR) using quadrat counts shows the following data: $\chi^2 = 128.7811$,

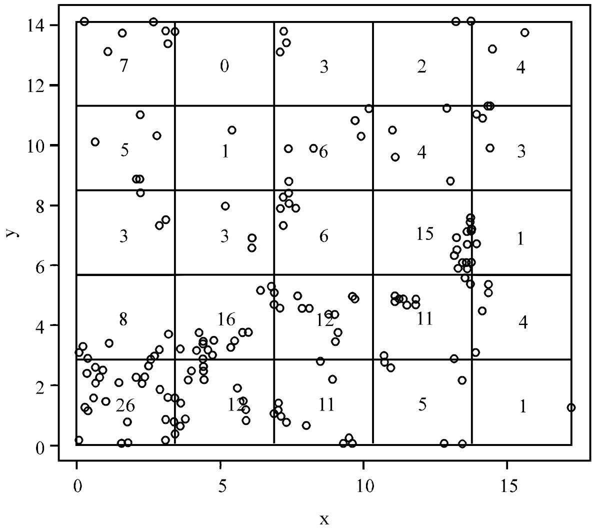

Fig. 1: Fungus-growing spatial distribution in M2/3. Number of fungus-growing: 169, Density: 10.56 fungus-growing m^{-2}, Chi-squared test of CSR using quadrat counts; data: χ^2 = 128.7811, df = 24, p-value = 2.577e-16, x-axis and y-axis indicate the Cartesian coordinates of each fungus-growing in the two dimensions plan representing the sampling area

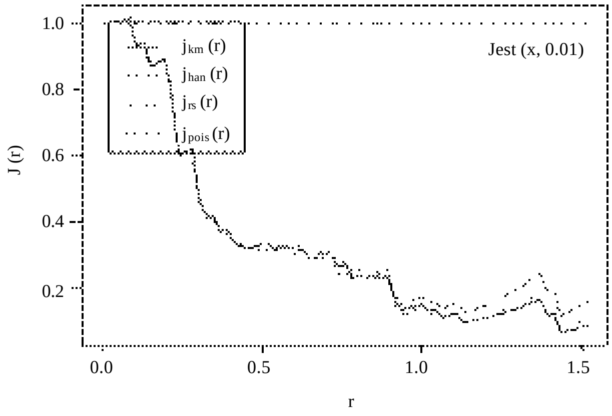

Fig. 2: Method of the distances to nearest close in M2/3: J regularity indicator, J<1, the distribution is made of aggregates, J is function indicating spatial regularity using nearest neighbour distance (r). J(r)<1 or J(r)>1 may suggest spatial clustering or spatial regularity, respectively

df = 24. Critical value = p-value = 2.577e-16 (Fig. 1). It is clear that p-value is smaller than the threshold α = 0.05. The H0 hypothesis is therefore rejected.

Using the method of distances to nearest close, the regularity indicator obtained is smaller than 1 (J<1). These results indicate the distribution is made by aggregates (Fig. 2).

First sampling done in Yokangassi 9 (Fig. 3 and 4): The chi-squared test of Complete Spatial Randomness (CSR) using quadrat counts has given the following results: Chi-squared

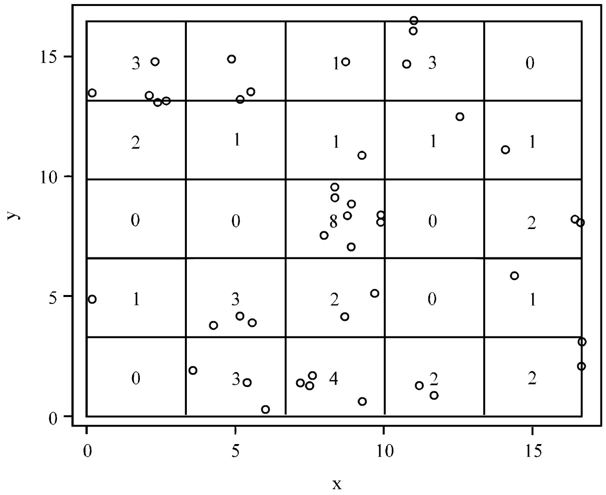

Fig. 3: Fungus-growing horizontal distribution in Yok9 (S5P1). Number of fungus-growing: 43, Density: 2.68 fungus-growing m^{-2}, Chi-squared test of CSR using quadrat counts; Data: $\chi^2 = 42.4651$, df = 24, p-value = 0.01144, x-axis and y-axis indicate the Cartesian coordinates of each fungus-growing in the two dimensions plan representing the sampling area

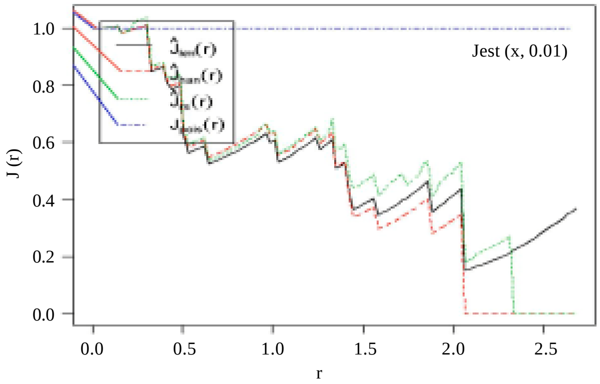

Fig. 4: Method of the distances to nearest close (Yok9 S5P1), J regularity indicator, J>1, the spatial distribution is made of aggregates, J is function indicating spatial regularity using nearest neighbour distance (r). J(r)<1 or J(r)>1 may suggest spatial clustering or spatial regularity, respectively

(χ^2) = 42.4651, df = 24, Critical value = p-value = 0.01144. The obtained critical probability is even smaller than threshold $\alpha = 0.05$ (Fig. 3). The null hypothesis of an unpredictable and homogeneous distribution is rejected.

The method of distances to nearest close shows that the regularity indicator obtained is smaller than 1 (J<1) and enable to confirm that the distribution is made by aggregates (Fig. 4).

Second sampling done in Yokangassi 9 (Fig. 5 and 6): For this 2nd sampling carried out approximately 5 kilometers from the lake, the Chi-squared test of CSR using quadrat counts has

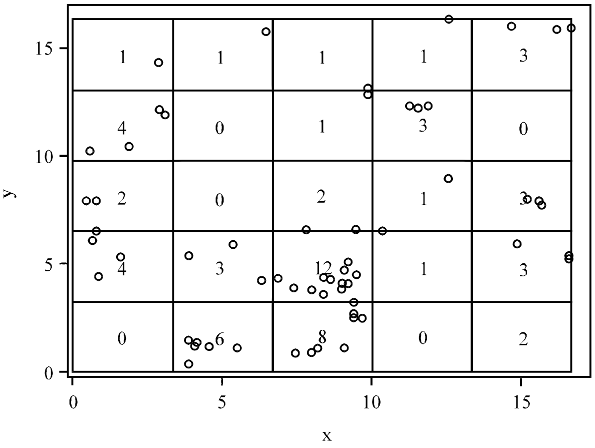

Fig. 5: Fungus-growing spatial distribution in Yok9 (S6P3), Number of fungus-growing: 62, Density: 3.87, fungus-growing m^{-2}, Chi-squared test of CSR using quadrant count, Data: χ^2 = 75.0968, df: = 24, p-value = 3.603e-07, x-axis and y-axis indicate the Cartesian coordinates of each fungus-growing in the two dimensions plan representing the sampling area

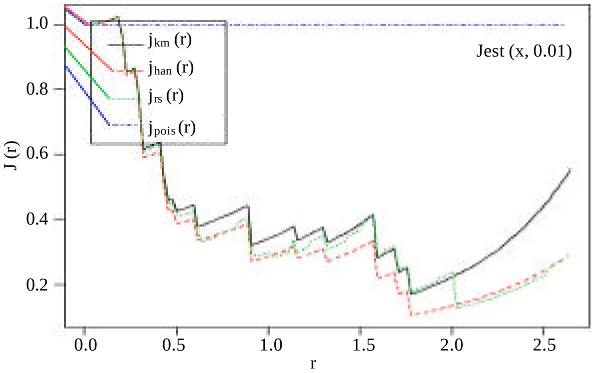

Fig. 6: Method of the distances to nearest close (Yok9 S6P3), J regularity indicator, J>1, the spatial distribution is made of aggregates, J is function indicating spatial regularity using nearest neighbour distance (r). J(r)<1 or J(r)>1 may suggest spatial clustering or spatial regularity, respectively

given Chi-squared X-squared = 75.0968, df = 24, p-value = 3.603e-07. The obtained critical probability is always smaller than threshold α = 0.05. Therefore, the hypothesis H0 of an unpredictable and homogeneous distribution is once again rejected (Fig. 5). The distribution is made by aggregates because the J regularity indicators is smaller than 1 (J<1) (Fig. 6).

Enumeration and density of fungus-growing: The density and numbers of fungus-growing chambers are indicated in the Fig. 1, 3, 5 and 7.

Sampling done in M2/3 plot: The results showed that in the quadrat situated in Moutela 2/3 quadrat, near the lake, the number is important (Fig. 1: N = 169, or 10, 56 fungus-growing m^{-2}).

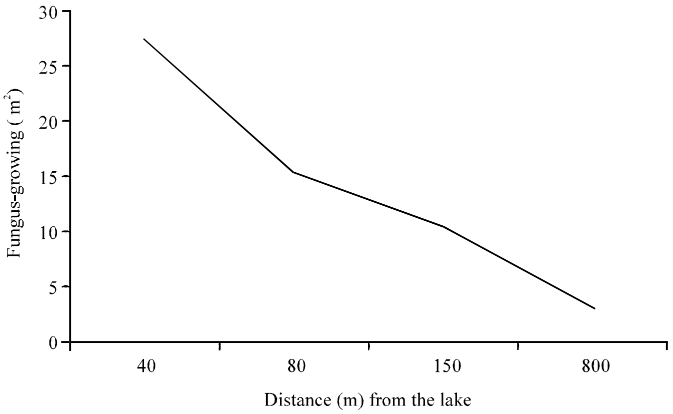

Fig. 7: Humidity influence on *P. militaris* fungus-growing chamber's abundance (M2/3) on 4 sampling done is June

However, there is a density difference in the samplings if there are done near the water based area or not (Fig. 7). That's how the fungus-growing density is of 27 m^{-2} for the first sampling done by 40 m deep and of 15 m^{-2} for the second one done by 80 m deep. In the sampling done at almost 150 m far from the water based area, the average density is 11 fungus-growing m^{-2}.The sampling done far from the marshland, 800 m far from it to be precise, shows a much lower density of 3 fungus-growing m^{-2} (Fig. 7).

Sampling done in Yok9 plot: On the 2 surveys to Yokangassi 9, density is relatively low (Fig. 3: N = 42, or 2,68 fungus-growing m^{-2} and Fig. 5: N = 62, or 3,87 fungus-growing m^{-2}).

DISCUSSION

With respect to the spatial distribution of the fungus growing, the results obtained in the Niari Valley sugar cane plantations are in agreement with those described by Konate (1998), in the Lamto savanna ecosystem and also by Joucquet *et al.* (2004) in *Odontotermes*, in a Guinean savannah situated in Ivory Coast. In this last case, the mounds created by the fungus-growing termite species, *Odontotermes* n. *pauperans*, influence the grass pattern. It was suggested that the spatial distribution trends aggregative are due to the termites' preferential appetence for certain kind of grasses. They think about the *Imperata cylindrica* whose presence is associated with a high fungus-growing concentration. Therefore, to optimize the fungus-growing diet and building efforts, this termite would build these fungus-growing next to alimentary resources. Indeed, food choice experiments made by Konate (1998) showed strong termite preferences in favor of *I. cylindrica*, as compared to *Andropogon chinensis*, the grass very rare on mounds in the Guinean savannah in Ivory Coast. This hypothesis of a food preference is inconsistent with the observations in the sugar cane plantations as these plantations are single-crop farming. The mulch exclusively made of cane leaves which are left on the ground during the harvest, are the only dietary source for the termite (Dibangou, 1994). Moreover, in this same Guinean savannah located in Ivory Coast, it was noted that the presence of other fungus growing termite's species that belong to *Microtermes* and *Ancistromes* genus and whose fungus-growing are not aggregated neither implicated in associations with the vegetation (Jouquet *et al.*, 2004). This would be linked to the fact that these fungus-growing are temporary structures which change according to the season or the local food

(Josens, 1972). These results do not correspond with those obtained in sugar cane plantations where *P. militaris* is practically the only termite hypogenous species which colonizes in an exclusive way the environment. In addition, the presence of this termite is permanent throughout the year in the environment (Mora *et al*., 1990; Dibangou, 1994).

The observations in sugar plantations showed that fungus-growing are more located near the sugar cane lines. It is well known that the cultural practices need sub soiling operations up to almost 75 cm deep and/or simple plugging to air the soil before getting the cuttings buried. Moreover, during the various following operations (harvest and manual or mechanical cares), to not choke the cane growths, the engines avoid to wheel on these lines. Only the lines spaces are strongly compacted by their repeated passing. Tucker *et al.* (2004) found that in the eastern subterranean termite, the number of foraging tunnels was significantly higher in moderately and highly compacted soils. This is an adaptation for better environmental conditions and in this present study, this situation would give a double advantage for the sugarcane: through the soil structures but also through the nutrients available near the fungus comb chamber. Indeed, in the Macrotermitinae, fungus-growing play an important role in the fertility and airing of soils (Abbadie and Lepage, 1989; Holt and Lepage, 2000; Jouquet, 2002; Jouquet *et al.*, 2005). Laboratory experiments indicated that such structures are enriched in SOM and clay and that termite workers can modify, in a more or less irreversible way, the mineralogical properties of silicate clays, creating expandable clay minerals (Jouquet *et al.*, 2002). Their aggregation would mobilize nutrients which the sugarcane roots would benefit. Ultimately, the ecosystem services provided by termites outweigh their pest status (Jouquet *et al.*, 2011).

With regard to the density, (Josens, 1971), without establishing a connection with the moisture, estimated the density of fungus-comb chambers to be more than 10 units m^{-2} in a Guinean savanna. These results are consistent with surveys in the plantations of sugarcane near the surface of the water. However, it should be noted that in these surveys the water would play an important role in the quantity and density of fungus-growing. In general termites are susceptible to desiccation; having a soft cuticle with poor water-retaining properties (Moore, 1969). Laboratory experiences carried by Green *et al.* (2005) in three subterranean termite species belonging to genus Reticulitermes showed that although termites may forage for food resources randomly, their occurrence and harborage location are dependent on environmental factors, particularly moisture. Others studies have demonstrated that subterranean termites preferentially tunnel in soil with a higher moisture content (Evans, 2003; Su and Puche, 2003; Arab and Costa-Leonardo, 2005). The presence of a permanent water area would mean the existence of an underground water sheet. Soils above the water-tables are never saturated with water during the year but would always present a high humidity rate that would promote the termite's activity. These observations are in agreement with those carried out by (Kalshoven, 1941) who had find that termites go down to seek the water at 4 m deep in East India (Marais, 1950) reported that termites go down a depth of 12 m and even 30 during a year of a severe drought in South Africa. In the big sahelian area termitaries, termites can go down to seek water at several meters to maintain humidity in their settlement. In the Macrotermitinae, Bodot (1967) found damps from 15 to 30% (from the dry sampling) in *Bellicositermes*' nests. He valued that a 2 m^3 termitory almost contains 200 L of water. The termitaries of these species are localized near the breaks, copses and forest galleries, above the permanent sheet of water or above the temporary suspended ones.

CONCLUSION

In view of ecological and economic roles played by termites in ecosystems, it is important to know factors that are conducive to their presence. Knowledge of the relationship between termites and their environment can help control their proliferation in case of damage. This study showed the influence of moisture on the density of the fungus growing Chambers of *P. militaris*. According to the proximity of water based in M2/3 and compared to the ones in Yok9 it revealed that the high density of fungus-comb chambers growing are linked to the proximity of a permanent water. These fungus-comb chambers are preferentially located along the side of the sugar cane lines because the soil is aerated. These results showed therefore that P. militaris preferentially colonizes soil with higher moisture to actively collect plant fragments and build fungus comb chamber. Water is indeed an essential element in termites' life.

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