Biological control of soil-dwelling insect pests in cocoa agroforests using CO_2-emitting capsules co-formulated with entomopathogenic fungi

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DECLARATION AND APPROVAL

Declaration by candidate

I, the undersigned, declare that this thesis submitted to the North-West University for the degree of Doctor of Philosophy in Biology in the Faculty of Natural and Agricultural Sciences, in the School of Biological Sciences, and the work contained herein is my original work with exception of the citations and that this work has not been submitted to any other University in part or entirety for the award of any degree.

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DEDICATION

This thesis is dedicated to God Almighty for preserving my life after a ghastly motor accident I was involved in during this research work. It is also dedicated to my entire family, especially my lovely husband Anyame Nelson Ambia and kids, Anyame Shammah Azie and Anyame Shamita Amah for enduring the long period of my absence from home, and for their love and timeless support.

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ACRONYMS AND ABBREVIATIONS

AF	Agroforestry
A&K or A&I	Attract and Kill or Attract and Infect
ANOVA	Analysis of Variance
APU	Arthropod Pathology Unit
a.s.l	Above sea level
ARPPIS	African Regional Programme in Insect Science
CEC	Carbon dioxide emitting capsules
CEC_{EPF}	Carbon dioxide emitting capsules co-formulated with entomopathogenic fungi
Df	Degrees of Freedom
EPF	Entomopathogenic fungi
GLMs	Generalized Linear Models
icipe	International Centre of Insect Physiology and Ecology
IRAD	Institute of Agricultural Research for Development
SNK	Student –Newman Keuls
PDA	Potato Dextrose Agar
SDA	Sabouraud Dextrose Agar
SE	Standard error
SSA	sub-Saharan Africa
RH	Relative Humidity

GENERAL ABSTRACT

Termites have recently gained importance as major pests in cocoa agroforests (AF) because of a loss in overall biodiversity at the transition from shaded agroforestry system to intensively managed unshaded monocultures (full sun) systems. Termite control relied almost exclusively on persistent organochlorine insecticides which are currently under restrictive use due to increasing concern over damage to human health and the environment. Entomopathogenic fungi (EPF) are considered as promising biocontrol agents in inundative augmentative biocontrol strategies against termite pests. However, there are limitations in their application as they do not achieve high control efficacies in the field when applied as conidial suspensions due to repellency, host avoidance, and defense mechanisms against virulent EPF. Subterranean termites use CO_2 to locate plant roots, thus making the use of EPF a promising biocontrol strategy against termites when combined with CO_2 in a strategy known as attract and kill (A&K) or Attract and Infect (A&I). This study was therefore undertaken to explore the potential efficacy of encapsulated CO_2-emitting material co-formulated with a virulent EPF (*Metarhizium brunneum* (Metschnikoff) Sorokin) for biological control of termites in cocoa agroforests. The first objective of this study focused on a review of soil-dwelling insect pests of tree crops in sub-Saharan Africa where termites were identified as the major soil-dwelling insect pests affecting tree crops and have recently gained importance as major pests in cocoa agroforests. The study further compared termite assemblages under five cocoa agroforestry shade types in Cameroon to assess the impact of shade on termite taxonomic and functional group diversity and to identify the termite species causing damage to cocoa. Sixty-nine termite species in 33 genera, 5 subfamilies under 2 families were sampled. Termite species richness decreased significantly from the shaded cocoa AF (92.54% shade cover), dominated with soil feeders or non-pest species to the full sun AF systems (22.5% shade cover), dominated with pest species. Functional group composition was strongly correlated with variation in shade level, with functional group III and IV representing the most abundant in the shaded systems and rare in the low shade and full sun systems. The shaded AF systems maintained all the termite species found in the full sun system and causing damage to cocoa trees. The shaded systems also harboured a diversity of non-pest species, suggesting that the establishment of shade in cocoa AF conserves important part of functional biodiversity. Screening to select virulent EPF fungi to co-formulate with CO_2 generating materials for control of subterranean termite pests in cocoa agroforests was conducted. The results showed that *Metarhizium* isolates were more virulent with lower LT_{50} values than *Beauveria* isolates, *M. brunneum* Cb15-III being the most virulent ($LT_{50} = 1.5$ days). The study further investigated whether calcium alginate beads containing baker's yeast (*Saccharomyces cerevisiae* Meyen ex Hansen) as an encapsulated CO_2 source (CO_2-emitting capsules) could outcompete CO_2 gradients established by other CO_2 generating materials and other attract components to attract subterranean termites (*Microtermes* spp.). The capsules co-formulated with the highly virulent EPF *M. brunneum*: Cb15-III (CEC_{EPF}) were further assessed for their ability to establish CO_2 gradients in the soil that can outcompete CO_2 produced by cocoa seedlings root respiration to attract and consequently kill termites. In addition, infection of the worker termites by the fungal spores growing from the CEC_{EPF} as well as their horizontal transmission was investigated through the autodissemination approach. Significantly more termites were attracted to CEC compared to other attract components. No significant difference was observed in the number of termites attracted by CEC_{EPF} and cocoa seedlings. The capsules were further tested under semi field and field conditions for their attractiveness to termites. Under the semi field condition, no significant differences were observed in the number of termites collected around cocoa seedlings in control and treatment plots when CEC or CEC_{EPF} were introduced into treatment boxes. Similarly, for the field trials, no significant difference was observed in the number of attractive stations found with termites in the control and treatment plots during the study period, as well as in the mortality of seedlings. The "attract and kill" strategy therefore offers a high potential to promote biological termite control in cocoa agroforests as an alternative to insecticides.

Key words: "attract and kill", *Beauveria bassiana*, cocoa, cocoa agroforests, CO_2 emitting capsules, entomopathogenic fungi, *Metarhizium brunneum*, *Metarhizium anisopliae*, termites

LIST OF PUBLICATIONS

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Candidate's Contributions: designed the study, conducted the literature searches, analysed the data, wrote the first draft of the manuscript and revised the paper based on reviewers coments.

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Chapter 5: Establishment of fungal entomopathogens as endophytes in cocoa seedlings for biological control of subterranean termite pests in cocoa agroforests. The manuscript contains additional information to chapter 5 of this thesis and is edited for submission to *Journal of Microbial Pathogenesis*.

Authors: Chaba F Ambele, Olubukola O Babalola, Hervé DB Bisseleua, Komivi S Akutse, Christian TL Djuideu, Odhiambo Levi Ombura, Sunday Ekesi

Candidate's Contributions: managed the literature searches, conducted the laboratory and field experiments, performed all the analyses, interpreted the results and wrote the first draft of the manuscript.

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1 CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background information

Cocoa (*Theobroma cacao* Linn.) is the "engine of economic growth" in many countries of West and Central Africa (Duguma et al., 2001). It is often described as the "Golden Tree", an apt description that is derived from its ripe golden pods that hang on the brown stem, against the green background of leaves, but is indeed worth more than gold to many countries especially West Africa because of its contributions to their economic development (Wessel and Quist-Wessel, 2015). It is a native species of tropical humid forests and is the economic mainstay of countries such as Cameroon, Ivory Coast, Nigerian and Ghana where over 70% of the world cocoa is produced (Nair, 2010). Cocoa is a major export earner in these countries, yet its production is still in the hands of aging smallholder-farmers (Figure 1.1), with over 70% yield losses as a result of numerous challenges including insect pests such as mirids, termites, shield bug, pod and stem borers, and mealybugs which are also vectors of the swollen shoot virus (Boadu, 2001).

Figure 1-1: Smallholder cocoa farmers harvesting (a) and removing the seeds for fermentation (b)

Cocoa cultivation in some countries in Africa like Cameroon and Ghana, was restricted to the forest region where cocoa was planted under the shade of taller trees. This was based on thinning out the natural forest and planting the cocoa under the residual shade (Anglaaere et al., 2011). Over time however, this has given way to a practice where the forest is now clear felled, burnt and the cocoa planted. Agroforests are often assumed to be the best strategy for growing cocoa in terms of environmental protection and ecological services because of biodiversity and in terms of income diversification, especially in West and Central Africa (Gockowski and Sonwa, 2008; Asare and David, 2010). However, since the 1980's, in the major cocoa producing countries, many cocoa smallholders have preferred the full sun or very light shade strategy, close to the concept of monoculture (Kazianga and Sanders, 2006). Monocultures create favourable conditions for specialized pest species, in the presence of abundant supply of their preferred food to multiply rapidly and become notorious pest. Most research has been focused on above-ground insect pests of cocoa mainly mirids, the shield bug, pod and stem borers, and mealybugs (Boadu, 2001). However, damage by soil dwelling insect pests is not well studied. Termites are the major soil dwelling insect pests of tree crops in sub-Saharan Africa (Ambele et al., 2018) and have recently gained importance as major pests in cocoa agroforests. This is because of a loss in overall biodiversity at the transition from shaded agroforestry system to intensively managed unshaded monocultures (full sun) systems. Economically important termite species attacks on cocoa have been reported by extension staff, growers, and during field observations in Ghana (Ackonor, 1997), and recently in Ivory Coast (Tra Bi et al., 2015), and termites have long been associated with cocoa in Cameroon (Eggleton et al., 2002). Assessment of damage caused by termites shows that 20% to 80% of cocoa, especially seedlings, are sometimes damaged to the extent of requiring replacement (Ackonor, 1997). Termites cause direct damage on cocoa by destroying the roots and stems (Fig. 1.2), or by cutting down seedlings mostly in the dry season leading to the death of cocoa plants. Indirectly, they lower cocoa yield through decreased translocation of water and nutrients (Asare and David, 2010). Termites also attack cocoa agroforestry trees causing similar damage as on cocoa, the trees which are important in contributing to the livelihoods of the family (Cerda et al., 2014). The feeding lesions of termites on the cocoa plant also provide entry points for secondary infection by pathogens especially *Aspergillus flavus* which causes yield loss by contamination of cocoa beans with aflatoxins (Osipitan and Oseyemi, 2012). Termite attacks on cocoa seedlings are the main constraint to the development of cocoa in some countries like Vietnam (Le Van and Nguyen, 2009). Termites gain pest status, because as they fulfill their ecological role of recycling plant materials, they come accross and utilize forestry commodities. Of the total of 3,106 termite species, about 371 are considered as pest species (Krishna et al., 2013). The problem of termite damage in cocoa nurseries and young plantations will likely continue as agricultural expansion and intensification of cocoa production lead to higher demand for new forest clearings, expanding into lands and forests of termite habitats. However, the management of termites relies heavily on the abusive use of synthetic chemicals (Logan et al., 1990) with high negative impacts on the environment and human health; and the use of some traditional control methods (Akutse et al., 2012; Ambele et al., 2018). Therefore, there is a need to search for environmentally friendly alternative methods to mitigate termite problems in cocoa agroforests.

Figure 1-2: Termite damage on cocoa (A and B) cocoa stems destroyed by termite feeding, (C) termite gallery on cocoa stem and destruction of a branch, (D) cocoa stem completely destroyed by termites

1.2 Problem statement

Control of termites in food crops and forestry has relied almost exclusively on persistent organochlorine (cyclodiene) insecticides. However, the use of insecticides for termite control is currently under restrictive use due to increasing concerns over damage to human health and the environment. This has resulted in increased public concern over pollution to the environment, risk of safety to human and safety of animal health. A recent key example is the application of insecticide coated seeds which has caused serious non-target effects on bees (Vincenzo et al., 2012). Thus, many countries in the world either have banned or have placed severe restrictions on the use of synthetic chemicals in termite control. Resistance of termites to chemical pesticides has also further complicated the problem. So, because of serious negative impacts and deleterious effects of insecticides, research on the identification of eco-friendly tools for termite control has been a major concern for entomologists. Biological control is generally perceived as providing both long-lasting insect control approach and having less potential for damage to the environment or non-target organisms than chemical interventions. Biological control using entomopathogenic fungi (EPF) is the most promising alternative to synthetic chemical pesticides that is being explored for termite control (Culliney and Grace, 2000; Rath, 2000; Wright et al., 2002; Grace, 2003; Chouvenc et al., 2008; Chouvenc and Su, 2010; Nyeko et al., 2010; Addisu et al., 2013). The use of EPF could provide an opportunity for sustainable control of termites in cocoa agroforests. EPF are also less toxic to humans as compared to chemical pesticides. However, there are limitations in their application due to repellency or avoidance by termites, and the need to apply high doses in order to achieve termite control (Grace, 2003; Mburu et al., 2009).

1.3 Justification of the study

The manipulation of insect behavior makes it possible to utilize biocontrol agents (e.g. fungi) more effectively, by combining them with semiochemicals used in host finding as attractants. Such a combination known as “Attract and Kill” (A&K) or “Attract and Infect” (A&I) has been proven to be a very effective method of control in several subterranean insect pest species (Schumann et al., 2014; Vernon et al., 2015). A&K or A&I mechanisms have the potential to target organisms from their cryptic habitats in complex environments that are normally difficult to reach with ordinary application techniques (El-sayed et al., 2009). The insect pest is lured to an attractant (e.g. semiochemical = attract) and subjected to an insecticide or EPF (= infect/kill) killing off the insect (El-Sayed et al., 2009). All plants roots release respiratory emissions of carbon dioxide (CO_2) in the rhizosphere, and studies have found that soil dwelling insects are attracted to it (Johnson and Gregory, 2006). CO_2 has also been reported as an attractant for termite species (Bernklau et al., 2005). Bernklau et al. (2004) suggested that encapsulating CO_2 would result in a more controlled and continous release of CO_2 over longer period. The integration of a CO_2-emitting material with chemical control in an A&K approach has been evaluated for the control of Western corn rootworm larvae (Schumann et al., 2013) and control of wireworm in potatoes (Stephan et al., unpublished). However, the A&K or A&I approach using EPF in terms of increasing the efficacy for termite control in cocoa agroforests, as well as mitigating the repellence and fungal avoidance has not been assessed. There is therefore a need to develop a strategy that would enhance the effectiveness of virulent entomopathogenic fungi in management of subterranean termite pests in cocoa agroforests.

1.4 Objectives of the study

1.4.1 General objective

The overall objective of this study was to develop an environmentally friendly control strategy against subterranean termite pests in cocoa agroforests.

1.4.2 Specific objectives

The following specific objectives were addressed;

1. Undertake a preliminary desk research on soil-dwelling insect pests of tree crops in sub-Saharan Africa with a focus on problems and management strategies.
2. Determine the taxonomic patterns and functional diversity of termites in cocoa agroforestry systems in Cameroon.
3. Screen and select virulent entomopathogenic fungal strains on key subterranean termite species attacking cocoa.
4. Assess the attractiveness of an encapsulated CO_2-emitting source co-formulated with the most virulent entomopathogenic fungal isolate to key subterranean termite pests attacking cocoa through “attract-and-kill” strategy.
5. Evaluate the “Attract and Kill” strategy using CO_2-emitting capsules co-formulated with virulent entomopathogenic fungal isolate against subterranean termites in cocoa agroforests.

1.5 Hypotheses

The following hypotheses were tested;

- Among the soil-dwelling insect pests of tree crops in sub-Saharan Africa, termites are the major economically important threat in cocoa agroforests.
- Patterns of termite species richness in different cocoa agroforestry types are related to responses by termite functional groups to changes in shade management.
- Virulent entomopathogenic fungi are good biological control candidates against termites when co-formulated with CO_2.
- Co-formulating virulent EPF with CO_2 used by subterranean termites to locate roots can reduce the repellency of EPF and attract termites
- Vectoring of fungal spores by unimpaired termites is the most effective method of infiltrating termite colony with potent EPF.
- Spores of EPF can be horizontally transferred to other individuals by mutual grooming leading to epizootic in termite colonies.

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2 CHAPTER TWO: LITERATURE REVIEW

2.1 Cocoa

2.1.1 Origin of cocoa

Cocoa (*Theobroma cacao* Linn.) belongs to the family of Malvaceae (or Sterculiaceae) and consists of three main cultivar groups, *Criollo*, *Forastero* and *Trinitario*. The genus *Theobroma* originated in the Amazon and Orinoco basins and has been part of human culture in the last 2000 years. It subsequently spread to Central America (particularly Mexico), where it was known and used by the local population. It was considered by the Olmec and Mayas, and later the Toltecs and Aztecs as the "food of the gods" (Pohlan and Perez, 2010). In the 16th century, Spanish explorers were the first to bring cocoa beans to Europe. Nowadays, cocoa is one of the most important cash crops and it is a key ingredient for many sweets and cosmetics. Since the discovery by the Europeans, the tree rapidly spread and has become important throughout the humid tropics (ICCO, 2008). The genus *Theobroma* has twenty-two species, but *T. cacao* is the most widely known.

2.1.2 Cocoa production in Africa

Cocoa was introduced in Africa around the 18th century. Cocoa plantations now cover more than five million ha of land previously covered by forests. It is produced in West Africa by mainly four countries (Ivory Coast, Ghana, Nigeria and Cameroon), with Ivory Coast accounting for approximately 50% of the African continent's production (Wessel and Quist-Wessel, 2015). According to Shahbandeh (2019), the predicted number of tons of cocoa beans for 2018/2019 was 2.15 million, 900 thousand, 250 thousand, and 240 thousand for Ivory Coast, Ghana, Cameroon and Nigeria, respectively. The economies of these countries are highly dependent on cocoa production (Sonwa et al., 2005). Smallholders produce the bulk of cocoa beans in Africa with up to 95% of all production in some countries. The average farm holdings range from 0.4 ha to 4.0 ha (Chamberlin, 2007). In Cameroon, about 80% of the production is in three regions, namely, the South West (35%), the Centre (28%) and the South (16%); and accounts for around 6% of Cameroon's exports, and is of key importance for Cameroon's economy (Sonwa et al., 2012).

2.1.3 Importance of cocoa

Cocoa has been the "engine of economic growth" in many areas of West and Central Africa (Ruf and Zadi, 2003). Some herbal practitioners consider cocoa as the tree of life. The cocoa tree supports the growth of medicinal plants such as mistletoes, and there are claims that diseases like piles, malaria, hypertension, anemia, sexual weakness in men, and a lot more diseases could be treated with almost every plant growing on cocoa plant, and the cocoa plant itself (Appiah, 2004). Cocoa is also used in the food industry to produce chocolate (main product made from cocoa), cocoa butter and liquor, cocoa cake or powder, instant drinking chocolates and chocolate spreads (Fold, 2002). The important role of cocoa as a driver of economic growth has gained overall acceptance in all cocoa growing countries. The United Nations Conference on Trade and Development, UNCTAD, (2004), ranked cocoa as a highly competitive and lucrative economic cash crop. It is also regarded as the highest income generating commodity amongst other agricultural crops in the global market. The cocoa beans or seeds also provide carbohydrate, protein, fat and minerals. Cocoa is grown primarily for chocolate production, but the edible pulp is very delicious and often consumed by many farming households, especially children. Cocoa butter is used medicinally in Brazil for healing bruises and is also used by the cosmetic and pharmaceutical industries (Brunner et al., 2007). The seeds contain about 2% of the alkaloid theobromine, which is a central nervous system stimulant, like caffeine (Brunner et al., 2007). Theobromine is used as a diuretic to lower blood pressure because it dilates the blood vessels (Brunner et al., 2007). Dry cocoa seeds contain as much as 12-18% polyphenols, known as cocoa polyphenols or cocoa flavonoids which have anti-inflammatory and immune modulator activities, and may promote cardiovascular and immune health (Brunner et al., 2007). An estimated 450,000 rural households (more than a third of the total number of rural households) earn the larger part of their cash income from cocoa (Gockowski and Sonwa, 2011).

2.1.4 Cocoa agroforestry and importance of agroforestry products

Cocoa agroforest is a complex shade grown cocoa system in which forest tree species and food crops are integrated with cocoa for their economic, social and environmental benefits (Asare and David, 2010). Agroforests are often considered to be the best strategy for cocoa smallholders because of environmental protection, ecological services, biodiversity and income diversification (Gockowski et al., 2004; Gockowski and Sonwa, 2008; Asare and David, 2010). Research conducted by Bos et al. (2007), showed that cocoa agroforests with high floristic and structural diversity help in regulating pests and disease in cocoa agroforests. In addition, the diversification of agroecosystems increases the availability of habitats, alternative prey or hosts, and shelter for natural enemies (Landis et al., 2000). Diversification with food crops occurs in the early stages of cocoa farms (from year 0 to 3) when farmers plant food crops like beans, cocoyams, plantain, cassava, yam, maize etc.) with cocoa seedlings. These crops provide cocoa seedlings with temporal shade and prevent weeds from growing. The crops also provide income and food for the household in the short term until the cocoa is ready for harvest (Asare and David, 2010). Fruit trees like mango, citrus, avocado, cola nut, plumes are usually planted or retained during the establishment of a cocoa farm for food and for income generation (Asare and David, 2010). Similarly, some farmers also plant or retain timber trees like *Melicia excelsa, Ceiba pentandra, Terminalia ivorensis, T. superba,* etc as permanent shade in cocoa for medium to long-term economic gains (Asare and David, 2010). In a nutshell, cocoa agroforestry products (food crops, fruit trees, timber trees, medicinal plants) are as important in contributing to the livelihoods of the family as cocoa. Cerda et al. (2014) also found out that the main contribution to cash flow in agroforestry system is made by cocoa and little by agroforestry products but in terms of family benefit, agroforestry products are as important as cocoa beans. Some farmers maintain shade trees for various benefits to the agroecosystem. For example, leguminous tree species (e.g., *Erythrina* spp., *Gliricidia* spp. and *Inga* spp.) are widely used for their nitrogen fixation from atmospheric nitrogen (Anhar, 2005). Other shade trees in cocoa agroforests such as *Terminalia superba* have been related to high carbon storage and sequestration, microclimate stabilization and soil protection against heavy rainfall (Sporn et al., 2009).

2.2 Insect pests of cocoa

2.2.1 Above-ground pests of cocoa

Large-scale development of cocoa is limited by insect pests. Of the large number of insects observed on this crop, only a few are of economic importance. These include the shield bug, *Bathycoelia thalassina* (H-S) (Heteroptera: Pentatomidae), the cocoa stem borer, *Eulophonotus myrmeleon* Feld (Lepidoptera: Cossidae), the cocoa pod borer, *Characoma stictigrapta* Hampson (Lepidoptera: Noctuidae), mealybugs, *Planococcoides njalensis* (Laing) (Homoptera: Pseudoccocidae) (vectors) (Afreh-Nuamah, 2007). Apart from these, the cocoa capsids (mirids), *Distantiella theobroma* Distant (black cocoa capsids), *Sahlbergella singularies* Haglund (brown cocoa capsids), *Bryocoropsis laticollis* Schumacher (glossy cocoa capsids), *Helopeltis* spp. (cocoa mosquito-bug) all belonging to Heteroptera: Miridae, are the major above-ground pests of cocoa in the cocoa growing countries in Africa (Afreh-Nuamah, 2007).

2.2.2 Below-ground or soil dwelling insect pests of cocoa

Termites are the major soil dwelling insect pests of cocoa (Ambele et al., 2018). Termites attack young cocoa trees between the ages of 6-36 months during the dry months of the year (December to March) (Asare and David, 2010). They attack young plants in nurseries and seedlings or young trees at the base and without control, the plants will wilt suddenly and die. This type of damage can also happen to suckers or full-grown trees. In full-grown trees, some species of termite attack injured and dead wood. They may enter a wound higher up in the tree and spread downwards. Other species chew into the roots and tunnel up into the branches. Termites can attack living cocoa trunk where they chew the wood, causing openings for disease such as black pod. Termites also attack chupons on the base of mature trees. They are destroyers since the principal food of their caste is cellulose. Several species of termites are incriminated; *Glyptotermes parvulus*, *Coptotermes sjostedti*, *Microcerotermes solidus*, *Nasutitermes* spp. and *Neotermes aburiensis* which feed on bark, branches and, sometimes, cocoa pods and build many galleries on the stem from the collar to the chupons. Other species such as *Microtermes* spp., *Odontotermes* spp. and *Ancistrotermes* spp. build subterranean nests and forage on roots, going sometimes up to the stem (Vos et al., 2003; Tra Bi, 2013; Tra Bi et al., 2015). Termite damage on cocoa trees also provides entry for secondary infection by pathogens especially *Aspergillus flavus*, which cause post-harvest yield loss and contamination of cocoa seeds with aflatoxins (Osipitan and Oseyemi, 2012). Termite attack on cocoa seedlings is the main constraint to the development of cocoa in many countries like in Vietnam (Le Van and Nguyen, 2009).

2.3 Termite ecology

Termites are a highly successful group of insects coevolving for over 300 million years and constituting an integral part of the ecosystem (Pardeshi and Prusty, 2010). Termites can be divided into 3 major categories based on their habitat. These categories are damp wood, dry wood and subterranean termites (Paul and Reuben, 2005). The damp wood termites require more wood moisture than is provided by ambient humidity and are restricted to moist wood in contact with damp soil under natural conditions. The dry wood termites are pests of dry structural wood furniture. They require no contact with soil and live entirely within their food source (Su and Scheffrahn, 2000). The subterranean termites are the most widespread and the most destructive group especially to agricultural crops. They derived their name subterranean termites based on their association with the soil. Although they live entirely in the soil, they construct underground tunnels to move about in search of food. Subterranean termites can be pervasive pests and account for about 80% of losses to wooden structures and agricultural crops (Su and Scheffrahn, 2000).

2.4 Termite taxonomy and diversity

Termites belong to the insect infraorder Isoptera, within the cockroach order Blattodea (Krishna et al., 2013). The Latin name Isoptera means “equal wing” and refers to the fact that the front set of wings on a reproductive termite is similar in size and shape to the hind set. All termite genera (except about 2%) end in the suffix “termes” which is the Latin word for termite. They are closely related to cockroaches (Meyer, 2005) and can be separated taxonomically using different external morphology, internal features, food and nest type, as well as chemical and behavioural differences. According to the recent Treatise on the Isoptera of the world, 3,105 living and fossil species classified into 12 families and 330 genera exist (Krishna et al., 2013). Termites are also divided into two groups, “lower termites” which include the families Kalotermitidae, Termopsidae, Rhinotermitidae and Hodotermitidae and “higher termites”, all belonging to the family Termitidae, based on the composition of the symbiont microbiota in the termite’s gut. The guts of “lower termites” contain flagellate protozoans and bacteria and these termites feed only on wood, while the guts of the “higher termites” contain a variety of prokaryotic microbes that do not have flagellate and show various feeding habits (Ohkuma, 2008). Lower and higher termites also differ in lifestyle. Many lower termite species nest and feed within the same wood resource whereas higher termites usually exhibit foraging behavior that allows feeding in a place different from their nesting site. Among the lower termites, the Kalotermitidae and Rhinotermitidae feed on dry wood, while the Termopsidae feed mainly on decaying wood. The Hodotermitidae feed mainly on grass although they also cause damage to structural timber. The higher termites (the Termitidae) are probably the most notorious for wood and crop damage (Rouland-Lefèvre, 2010).

Termites are found in a wide range of terrestrial environments, distributed throughout the tropical, subtropical and temperate regions of the world. These insects are very diverse in their behaviour and ecology and inhabit about two-thirds of the earth’s land surface (Robert et al., 2007). Africa is by far the richest continent in termite diversity (Eggleton, 2000). Termite numbers, species and nest variety increase from the higher latitudes towards the equator. The Rhinotermitidae are the major pest species in America, Europe and Asia while Macrotermitidae are a major pest in Africa and Asia (Rouland-Lefèvre, 2010). In Africa, the Termitidae are represented by about 601 species (Eggleton, 2000), divided into four subfamilies (Apicotermitinae, Termitinae, Macrotermitinae and Nasutitermitinae). The subfamily Apicotermitinae consist of about 70 species in Africa (Eggleton, 2000), while the subfamily Termitinae consists of about 272 species (Eggleton et al., 2002). The subfamily Nasutitermitinae has about 56 species and all the species feed mainly on grass, leaf litter and wood. The Macrotermitinae, also known as the fungus growing termites, consist of over 165 species found in Africa (Eggleton et al., 1999). The Macrotermitinae are the most destructive wood-feeding insects. Economically important genera in this subfamily include *Macrotermes, Odontotermes* and *Microtermes* (Sileshi et al., 2010).

2.5 Termite life types

Termite life types consist of different nesting and feeding types.

2.5.1 Nesting types

Some termites like the Termopsidae, mostly Kalotermitidae, nest and feed in the same single item of substrate. They are referred to as the single-piece nesters. Some like many species of *Microcerotermes* nest in one or more items of feeding substrate and forage out onto other items of substrate. These are the intermediate nesters. Others nest in one substrate and forage out onto a diffuse substrate. They are the separate-piece nesters, e.g. mould-building *Macrotermes* (König and Varma, 2006).

2.5.2 Trophic groups (feeding groups)

There are generally four major trophic groups recognized, consisting of wood-feeders, soil-feeders, soil-wood interface feeders, litter and grass-feeders (Bignell and Eggleton, 2000). However, some termite species can fit into more than one trophic group especially those under unfavorable conditions. The four major groups are:

i. **Group 1**: These are termites that feed on wood and woody litters, including dead branches that are still attached to the trees. Most lower termites and all the subfamilies of Termitinae except the Apicotermitinae are wood-feeders (Bignell and Eggleton, 2000). In some cases, the wood that termites consume becomes the colony centre. However, some Macrotermitinae have subterranean or epigeal nests in which fungus gardens are cultivated (Donovan et al., 2001).
ii. **Group II:** These are the wood and litter feeders (Termitidae with a range of feeding habits), with many of the species responsible for damage to cocoa (Vos et al., 2003; Tra Bi, 2013).
iii. **Group III:** Members of the family Termitidae feeding in the organic rich upper layers of the soil (humus feeders) (Donovan et al., 2001).
iv. **Group IV:** True soil-feeders (all Termitidae), ingesting apparently mineral soil (Donovan et al., 2001).

2.6 Termite biology

2.6.1 Colony structure and life cycle

Termites are soft-bodied, terrestrial, social insects that live together in a colony. A colony is defined as a group of individuals of the same species sharing an interconnected gallery system (Chouvenc et al., 2011). The biology of subterranean termite has been described by Miller (2010). Winged termites or alates (swarmers) emerge in large numbers following a rain. The winged termites or alates are the new kings and queens. These reproductive forms pair up during their flight, then land and attempt to establish new colonies. Wings break up shortly after landing, and the new king and queen if they have landed in a suitable area, begin their new colony by excavating a small chamber in moist soil. When the chamber is large enough, the pair seals themselves inside and mate. A successful queen lays her first batch of 6-12 eggs within a few days or weeks of mating. The first offspring are usually the workers which feed on regurgitated wood or other plant matter primed with gut symbionts (Gullan and Cranston, 1998). Early in the life of the colony, production is directed towards workers, later soldiers to defend the colony (Gullan and Cranston, 1998). Initially, young termites (nymphs) are tended by the queen and king. As the queen’s egg-laying capacity increases, the older offspring take over the responsibility of tending the young. The colony continues to grow as the number of termites produced each year increases. A termite colony is highly structured and has castes that perform distinctly different duties. There are three castes that vary in form and function:

2.6.1.1 Worker caste

Worker termites are the most numerous and most destructive members. They vary in colour from white to yellowish appearance and navigate with their antennae rather than their eyes because they are blind. This caste is responsible for all the labour in the colony. They care for the young, repair the nest, locate food, feed and groom the other castes and each other. Subterranean worker termites use a mixture of mud, saliva and faeces to create mud tunnels to and from sources of food. The termite workers are both male and female, but they are functionally sterile. Their bodies are soft, but their mouth parts are very hard and adapted for chewing (Miura and Scharf, 2010).

2.6.1.2 Soldier caste

Soldier termites are pale yellow-brown and have enlarged heads and mandibles. The soldier caste’s enlarged jaws prevent them from feeding themselves, and they rely upon workers for feeding. The only function of the soldier termite caste is to defend the colony from attack from enemies. Soldier termites are similar to workers in that they are blind, soft-bodied and wingless. They differ from workers in that they have an enlarged, hard yellowish-brown head that has been modified for defensive purposes. The head has a pair of very large mandibles that are made to puncture, slice and kill enemies (Scholtz et al., 2008).

2.6.1.3 Reproductive castes

Reproductive termites are an integral piece of the colony's structure. There are three types of reproductives in a termite colony: the primary, secondary and tertiary reproductives. Mature subterranean termites at certain times of the year will produce large numbers of winged alates that will eventually become kings and queens. These are the primary reproductives and the only caste with functional eyes. Once they select a mate, they lose their wings. The new king termite remains virtually unchanged after losing its wings. However, as the new queen begins to produce eggs, her abdomen grows larger with the development of her ovaries. The queen becomes an egg laying machine of over 3000 eggs per day through its enlarged abdomen and lives for up to 25 years (Thompson et al., 2000). The king is always by her side to mate and fertilize the eggs. Unlike ants, termites mate periodically, and this (fertilizing the queen every now and then) is the only role of the termite king. The sole queen and king termites are the heart of the colony, although some species may have secondary queens and kings as well. Most species house the queen and king in a special cell called the royal chamber/cell, usually located in the central part of the nest. The royal chamber is a special cell with thicker walls on all sides and has many outlets to allow a steady stream of workers to move in and out to tend to the queen and carry off her eggs the moment they are laid. When a colony gets very large and the queen cannot lay enough eggs to keep up with the colony's demand or when the queen ages, her reproductive capacity declines, the colony may then select supplementary queens called secondary reproductives (neotenic) from the various developmental stages of reproductives to replace the queen and king. These reproductives however never go on a colonising flight. The female secondary reproductives lay fewer eggs than the queen but there can be hundreds of secondary reproductives in an established colony. Because of their numbers, they are the most important source of new eggs, and allow the colony to increase rapidly in size. They are also important because the reproductive needs of the colony can be entirely taken over by these secondary reproductives if the queen dies. They are smaller than the queen but larger than the workers. Both males and females lack membranous wings. When part of the colony is “cut off” from the queen, fully developed workers can become reproductives. The resulting tertiary reproductives are wingless and look like big workers. They can produce two to three times more eggs than the primary queen; however, this accelerated reproductive rate may be short-lived (Korb and Hartfelder, 2008).

2.7 Management practices for termite pests

Termite control has been difficult to achieve because their cryptic feeding habit makes it difficult to assess their behavior and anticipate their management. Persistent organochlorine insecticides were used but are currently under restrictive use due to increasing concern over damage to human health and the environment (Gitahi et al., 2002). Farmers in many parts of Africa use various traditional methods, including queen removal and plant extracts, often with limited success. The management practices used by farmers against termites in Africa are discussed in detail in Chapter 3.

2.7.1 Location of host plants by termites

Unlike above-ground insects that can also exploit visual cues to locate a host plant, subterranean termites must rely solely on chemical or physical cues that indicate the location of a host plant and/or enable them to distinguish it from an unsuitable plant. Chemicals released from roots appear to be the major cues exploited by subterranean termites. Primary plant metabolites are those chemicals that are generally exuded from all plant roots, such as CO_2, amino acids and sugars. Secondary compounds are those that are derived from secondary biochemical pathways and are host-specific (Johnson and Gregory, 2006). All plants roots emit respiratory CO_2 in the rhizosphere, and the earliest studies found that soil insects were frequently “attracted” to it (Johnson and Gregory, 2006). CO_2 is the most abundant gas released from plant roots and has been reported as an attractant for termite species in the United States, with the attraction differing with different concentrations (Bernklau et al., 2005). Broadbent et al. (2006) tested termite bait stations with or without CO_2 generating bait. They found out that termites discovered more monitoring stations faster when the CO_2 generating bait was present.

2.7.2 'Attract and kill'-approach in termite management

Several techniques have been tested for termite control including the use of microbial pathogens. Inundative methods have been used to demonstrate the effectiveness of pathogens to eradicate termite colony, most especially for accessible ones (Jackson et al., 2010). Unfortunately, the inundative method is not realistic for termite species with a diffused nest structure such as subterranean termites because only a fraction of the colony is accessible. A "trap and treat" method was mentioned by Milner et al. (1996), which consists of collecting individuals from a colony, treating them with a virulent entomopathogen, and then releasing them back into their original nest in the hope that they will contaminate the rest of the colony. However, it is difficult to inoculate enough individuals simultaneously to trigger an epizootic outbreak within a colony (Chouvenc et al., 2008). A baiting approach has been considered by Milner (2003), but the development of a stable and non-repellent formulation remains problematic. Despite efforts to screen for virulent strains of EPF, the delivery of enough inocula to a subterranean termite colony remains an unsolved problem (Grace, 2003). Therefore, alternative methods should be considered for introducing pathogens into subterranean termite colony.

ATTRACT is a project that focuses on the development of an innovative technology taking advantage of the fact that subterranean insect pests orientate towards their host plants via detecting root CO_2-exudates (Michael et al., 2014). Providing an alternative CO_2-source ('attract-component) combined with an environmentally friendly insecticidal compound as 'kill'-component may be the key to efficient soil pest control (Vemmer et al., 2013). Several literatures exist in the use of EPF to control termites using different application methods. No literature up to date has mentioned the use of CO_2 in an A&K strategy for the control of termite pests. Experiments have shown that 'attract-and-kill'-formulations affected wireworm (larva of clickbeetles) in corn (Michael et al., 2014), where the 'kill-component was *M.*

brunneum and yeast acting as the ‘attract’-component, generating CO_2. This method has also recorded positive results in controlling wireworm in potatoes (Stephan et al., unpublished), with the ‘attract’component being CO_2 emitting capsules impregnated with *M. brunneun* as the ‘kill’-component, and in the control of Western corn rootworm larvae (Schumann et al., 2013), with CO_2 emitting capsules as ‘attract’component and the insecticide tefluthrin as ‘kill-component. All these experiments showed some potential to kill pests both in the laboratory and in the field. Using this approach to control termites not only as stand-alone control strategy, but also as a component of IPM approaches, insecticide applications or other control strategies can be replaced, and the environment and health of farmers and consumers can be protected.

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3 CHAPTER THREE: SOIL-DWELLING INSECT PESTS OF TREE CROPS IN SUB-SAHARAN AFRICA, PROBLEMS AND MANAGEMENT STRATEGIES - A REVIEW

Abstract

This review aims to draw the attention of researchers, ecologists and farmers to the threats of soil-dwelling insect pests on important tree crops in sub-Saharan Africa, with a special focus on termites. It synthesizes the information on the effects of various factors affecting soil pest occurrence and damage, suggesting that the resultant undesirable effects of soil pests in this region are largely because of indiscriminate tree cutting, slash-and-burn agriculture and indiscriminate use of pesticides. Major insect orders, their host ranges and the nature of damage on selected tree crops are described. This study further critiques existing soil pest management practices, showing that most soil pest management practices are ineffective. Thus, management strategies like "attract and kill" approach based on entomopathogenic fungi need to be studied, developed and emphasized for the management of soil insect pests in sub-Saharan Africa. A conclusion section attempts to offer suggestions for ways in which future work on soil pests in sub-Saharan Africa could proceed.

KEYWORDS: control strategies, ecological factors, plantation crops, subterranean pests, termites

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3.1 Introduction

Africa faces challenges in feeding its population and has changed from being an exporter of agricultural commodities to an importer of these commodities (Manitra et al., 2011). Human populations more than tripled in the second half of the twentieth century (FAO and WFP, 2010). Despite this increase, average growth in food production is stagnant, with reports indicating decline in crop yields over the last few decades (Garrity et al., 2017). Hunger is projected to worsen in sub-Saharan Africa (SSA) in the next two decades unless some measures are taken to reverse the current trend (FAO and WFP, 2010). The main cause of food insecurity is poor crop yields, poor soil fertility, no access to improved planting materials and recurrent outbreaks of insect pests due to climate change (Bisseleua et al., 2017). Increasing crop and fruit production is thus important in alleviating poverty and hunger in SSA.

Tree crops are any edible fruits, nuts or legumes that can serve as food (Smith, 2013). Tree crop commodities have been the fastest growing land uses over the past 30 years, directly impacting more than 500 million rural people in developing and developed countries (African Development Bank, 2016). At least 10 countries in SSA are dependent on a single tree crop commodity in terms of GDP (Nair, 2010; Nyambo et al., 2003). A further 10 agriculture-based economies derive 50% of all agricultural export value from at least two to three commodities. Tree crops can thus play a key role in addressing food insecurity in SSA through income generation for smallholder farmers, commercial farmers, traders and processors and the countries as a whole. Major tree crops in SSA include cocoa (*Theobroma cacao*), oil palm (*Elaeis guineensis*), coffee (*Coffea arabica*), cashew (*Anacardium occidentale*) and fruit trees like citrus (*Citrus sinensis*) and mango (*Mangifera indica*). Production constraints mainly include challenges posed by above-ground insect pests (Nyambo et al., 2003; Obeng- Ofori, 2007) and below-ground pests (Venter, 2015). Below-ground (soil) insect pests are becoming more important as pests due to climate change. These are those insects whose damaging stages of the life cycle live in the soil or within the crop at or below ground level. Among these pests, termites, cutworms and mole crickets are the most predominant (Venter, 2015). Some are responsible for up to 80% losses specifically on seedlings after transplanting in the field, causing destruction to roots, stems and whole plants (Ackonor, 1997; Asogwa et al., 2008).

Effective control measures for these pests have been difficult to achieve. The reason is that their cryptic feeding habit makes it difficult to assess their behaviour and anticipate their management. The management of termites, for example, was achieved with persistent organochloride insecticides (Logan et al., 1990), but is currently under restrictive use due to increasing concern over damage to human health and the environment (Gitahi et al., 2002; Ma et al., 2009) as higher concentrations of active ingredients need to be applied for their control as compared to above-ground pests (Blackshaw and Kerry, 2008). Sex pheromone trapping has been tested and proved less effective in plantation tree crops (Bedford, 2014). The use of nematodes and inhibitors of termite symbiotic fungi showed very little response in managing termites (Lanier, 1990). Recently, various virulent strains of entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria fumosorosea* have been used against these subterranean pests with limited success. Soil insect pests can deploy a diversity of defence mechanisms ranging from mutual grooming behaviour to remove conidia from cuticles of their nestmates (Yanagawa et al., 2008), to walling-off of infected areas of a colony, or to release pathogen alarm behaviour which warns nestmates about the presence of lethal fungi causing them to leave the area or the removal of fungal infected termites (Hussain et al., 2010). However, Lozano-Tovar et al. (2013) as well as Grace and Zoberi (1992) were able to demonstrate the mortality effects of the endophytic fungi *B. bassiana* on termite workers. In many parts of Africa, farmers use various traditional methods, including queen removal and plant extracts, often with limited success as well (Nyeko and Olubayo, 2005; Sileshi et al., 2009). Gould (1991) suggested that a combination of an active ingredient with an attractant semiochemicals used by the pest for host plant location could increase the chances for a contact between the target and the toxic substance (Huang and Mack, 2001). Such a combination known as attract and kill strategy (A&K) is shown to improve efficacies resulting in superior control levels as compared to other control methods (Schumann et al., 2013). Additionally, the A&K mechanism is shown to be more effective against pest species with cryptic habitats and complex environments that are normally difficult to reach with ordinary application techniques (El-Sayed et al., 2009). Johnson and Gregory (2006) suggested that carbon dioxide (CO_2) could be used as an attractant for several soil-dwelling organisms such as subterranean termites and numerous insect larvae, adult insects, mites, chilopods, nematodes and bacteria. Zimmerman et al. (1986) demonstrated that CO_2 concentration in the immediate environment of termites may affect the sensory information being perceived by an individual termite. They showed that termites associate higher CO_2 levels with nest vicinity or food and that they may follow a CO_2 gradient to locate the source. Bernklau et al. (2004) revealed that CO_2-releasing materials that are degraded by soil micro-organisms can attract termites. Patel et al. (2013) showed that encapsulated baker's yeast can generate CO_2 gradients in soil that attract wireworms and western corn rootworm larvae. These authors also demonstrated that an adjacent station/farm in proximity to a CO_2-baited station does not need a CO_2 bait to be located by termites, therefore enhancing the performance of a CO_2-baiting system. Schumann et al. (2013) tested the combination of encapsulated CO_2-emitting capsules with an insecticide in an A&K approach for the biological control of the western corn rootworm *Diabrotica virgifera virgifera* and they have demonstrated the efficacy of this strategy against subterranean insect pests. Encapsulated CO_2-emitting capsules in combination with entomopathogenic fungi and multifunction shade tree management (Tscharntke et al., 2011) offer great prospect in the management of subterranean pests in tree crops.

3.2 Objectives

This chapter provides all (to the extent of our knowledge) information published between 1990 and 2016 on the economic importance of key subterranean insect pests of tree crops in SSA and management implications. More specifically, it looks at most soil-dwelling insect pests of plantation tree crops in Africa and the nature and damage of their feeding. Recommendations for the successful management of soil-dwelling termites using an integrated approach based on biological control practices using semiochemicals combined with entomopathogenic fungi and the appropriate management of companion plant species in plantation crop agroecosystems are provided.

3.3 Data and methods

Academic publications related to soil insect pest research in SSA were gattered. Bibliographical searches with search terms, such as “soil insect pests,” “soil-dwelling insects in SSA,” “soil insect pests of fruit trees,” “soil insect pests damage of tree crops,” “indigenous control methods of termites in SSA,” “factors influencing soil pests occurrence,” “termite attack on fruit trees” and “attract and kill approach in soil insect management”, were used to locate publications that contained these words in publications’ titles, abstracts or keyword lists. Individual document information, including author name(s), journal name(s), publication title(s) and publication year(s) were then retrieved. A bibliometric analysis to reveal the trends in major soil-dwelling insect pests of tree crops in sub-Saharan Africa from pest bioecology and management studies to the following perspectives: most encountered species and factors causing their outbreaks, damages inflicted to plantation crops and management practices were performed.

3.4 Results and discussion

3.4.1 Frequently encountered species of soil-dwelling insect pests of tree crops in sub-Saharan Africa

Important soil insects associated with damage in SSA include termites (which are the major soil pests), root weevils, white grubs, cutworms, mole crickets, the cashew trunk and root borer as well as the cashew weevil (Alford, 2014; Asogwa et al., 2008; Hill, 2008; Venter, 2015). Table 3.1 summarizes important soil-inhabiting insect pest orders and genera in SSA.

3.4.1.1 Isoptera

Termites are an important component of African ecosystems as bioindicators (Norgrove et al., 2009). They can be serious pests of agriculture. Of the approximately 3,106 termite species, about 371 are considered as pests (Krishna et al., 2013). Although some species play major roles in processes such as decomposition, nutrient and carbon cycling (Jones and Eggleton, 2000), in certain ecosystems, their harmful effects outweigh their beneficial roles to the extent that they have been considered as the primary cause of ecosystem deterioration (Mugerwa et al., 2012). In Uganda, losses of coffee tree stands ranging from 50% to 100% have been attributed to termite attack (Sekamatte, 2001). In a participatory assessment of farmers' experiences of termites, farmers rated termites as their most important constraint to tree cultivation (Nyeko and Olubayo, 2005). In a survey conducted in Cameroon, termites were considered by farmers as some of the main constraints to tree crop production (Kekeunou et al., 2006). Termites are probably the most serious soil pests causing mortality of seedlings after transplanting in the field (Ackonor et al., 2001; Tra Bi, 2013). Important termite pest genera in SSA include *Macrotermes*, *Odontotermes*, *Coptotermes*, *Nasutitermes*, *Microtermes* and *Ancistrotermes* (Uys, 2002).

3.4.1.1.1 *Macrotermes* spp.

They construct large epigeal mounds that occupy significant portions of the soil surface and thus reduce total farmland available for cultivation (Picker et al., 2007) (Figure 3.1a). The mounds are unsuitable for establishment of plants as the soils are hardened and contain only small quantities of nitrogen and phosphorous as they bring up soil from deep underground (López- Hernández et al., 2006). *Macrotermes* can cause up to 100% loss to tree crops and various domestic products (Shiday and French, 2011), and 90% of tree mortality (Mitchell, 2002). Their success as pests has been attributed to their ability to feed on dead as well as living plant materials, to survive cultivation and to use fungi combs as food stores through the dry season (Hyodo et al., 2003). Some species like *M. bellicosus* have gained pest status on cocoa trees in recent times. Previous research on *M. bellicosus* showed that although present in cocoa agroforests, this species did not cause damage on cocoa (Ackonor, 1997). However, recent reports have shown that this species causes significant damage on cocoa seedlings and even old cocoa trees (Ogedegbe and Ogwu, 2015). Some *Macrotermes* species have diverse food habits (Ackonor, 1997). For example, species such as *M. bellicosus* and *M. subhyalinus* are considered the most abundant termite species in

Uganda and were observed to attack a wide range of trees as well caused the most damage to tree crops (Nyeko and Olubayo, 2005).

3.4.1.1.2 *Odontotermes* spp.

Damage by *Odontotermes* spp. may occur through the natural cracks or wounds on the stems. They also feed directly on the underground portion of plants and roots and kill plants. Some species construct narrow runway on the stem and plastering with earthen sheet. Below ground, their attack usually begins on the root and then spreads to the upper parts of the plant. In older plants, the bark and the underlying tissue are eaten up, exposing the pith and hollowing out the stem, resulting in the death of the plant. *Odontotermes* species are polyphagous, causing damage to many tree crops including mango (Kwasi, 2009), coconut and oil palm (Materu et al., 2013).

3.4.1.1.3 *Coptotermes* spp.

Coptotermes contains the largest number of termite pests worldwide and is the most widely distributed, economically important and highly destructive in nature (Su and Scheffrahn, 2000). Their damage frequently occurs in mature trees, although it can occur at earlier stages of growth. *Coptotermes* usually invade trees via the soil and bore into the tree through the roots. Once a tree is infested, they hollow out the heartwood of the trunk (Hillocks et al., 1996). Severely damaged trees can be so weakened that they are prone to be blown over by strong winds. They are opportunistic feeders of any material containing cellulose, causing damage to plants such as citrus trees, eucalyptus, sugar cane and cocoa (Ackonor, 1997).

3.4.1.1.4 *Ancistrotermes* spp.

Ancistrotermes spp. have been reported as pests of shade trees in cocoa agroforests in Ghana as well as tree crops like coffee (Ackonor, 1997). They can cause up to 80% mortality in 4-6 months following seedling planting (Wood and Pearce, 1991). They eat the outside of the roots, removing the cortex and cambium, and destroying the below-ground plant entirely (Logan and El- Bakri, 1990). *Ancistrotermes* spp. have the tendency to displace other termite species in their habitats (Konate, 1998).

3.4.1.1.5 *Nasutitermes* spp.

Many nasutes construct spherical carton nests on tree trunks, while others build small dome-shaped mounds on trees (Figure 3.1b) (Schabel, 2006). In Cameroon, many species build nests up cocoa trees, maintaining contact with galleries extending down the trunk. These nests could lower the yield through decreased translocation of water and nutrients (Asare and David, 2010). Closely related genera to *Nasutitermes* in terms of nest-building behaviour are the *Microcerotermes*, which also causes damage to cocoa and companion trees in Ghana and Nigeria (Vos et al., 2003).

Figure 3-1: *Macrotermes* sp. mound (a) and *Nasutitermes* sp. Mound (b)

3.4.1.2 Other important group of soil-dwellings insect pests

3.4.1.2.1 Coleoptera

The order Coleoptera is the most diverse among the insects (Caterino et al., 2002). Despite their diversity, very few have been recorded as soil pests causing damage to tree crops in SSA. These include root weevils and white grubs. White grubs (Family: Scarabaeidae) are serious pests of young oil palms, feeding at night on the root systems (Harrison and Wingfield, 2016). White grubs uproot young plants as they move about in and on the soil. Their feeding on root systems causes plants to become stunted and lodge easily. Adult (chafers) damage to young plants is easily noticeable as the growing point of the shoot dies, caused by gnawing just below soil level reaching and destroying the shoot meristem (Conlong and Mugalula, 2003).

3.4.1.2.2 Lepidoptera

The order Lepidoptera is the second largest order of insects (Arnett, 2000). The severe pests of seedlings in nurseries are the larvae of Lepidoptera (Noctuidae), called cutworms. Cutworms (*Agrotis* spp.) are moth larvae that hide under litter or soil during the day, coming out in the dark to feed on plants. A larva typically attacks the first part of the plant it encounters like the stem, often of a seedling at the base, and consequently cuts it down and hence the name cutworm. They can cause more damage in no time. Some climb and feed on all plant parts. Cutworms vary in their feeding behaviour, and many species constitute serious pests to gardeners in general, but to tree crops at the seedling stage.

3.4.1.2.3 Orthoptera

This order includes grasshoppers, crickets, katydids and locusts, but the mole crickets (*Gryllotalpa* spp.) are among the most damaging soil pests of tree crops. Although they feed on the roots of grasses, they do not usually directly damage tree crops. As they tunnel through the soil, they destroy roots and cause the soil to dry out. As a result, crops on infested soils wilt from lack of water. Mole crickets browse indiscriminately on roots and the basal parts of stems, and injured plants in nursery beds may subsequently wilt and die (Alford, 2014).

Table 3.1: Economically important soil dwelling insect pest genera in sub-Saharan Africa

Order	Genus	Location	Crop affected	References
Isoptera (termites)	*Macrotermes*	Tanzania	Coconut/Citrus	Nyambo *et al*. (2003) Materu et al. (2013)
		Nigeria	Coconut/Cocoa	Ogedegbe and Ogwu (2015), Vos et al. (2003)
		Uganda	Citrus	Nyeko and Olubayo (2005)
		Ghana	Cocoa	Ackonor (1997)
	Nasutitermes	Ivory Coast	Cocoa	Vos et al., 2003, Tra Bi (2013)
		Ghana	Cocoa	Ackonor (1997)
	Microcerotermes	Ghana	Cocoa	Vos et al. (2003)
	Microtermes	Kenya	Eucalyptus	Mutitu et al. (2008)
		Ivory Coast	Cocoa	Tra Bi (2013)
	Odontotermes	Ghana	Mango	Kwasi (2009)
		Benin	Mango	Vayssieres et al. (2009)
	Ancistrotermes	Ghana	Cocoa/Coffee	Ackonor (1997)
		Ivory Coast	Mango	Ténon et al. (2014)
	Coptotermes	Ghana	Cocoa	Ackonor (1997)
		Ivory Coast	Cocoa	Tra Bi (2013)
Coleoptera (Beetles)	*Alcidodes*	East Africa	Young plants	Schabel (2006).
	Plocaederus	Nigeria	Cashew	Asogwa et al. (2008)

3.4.2 Damages caused by termites on plantation tree crops

3.4.2.1 Cocoa

Cocoa is the most important export crop for countries in the forest zone of West Africa and is the main source of foreign exchange for countries such as Ghana, Cameroon, Ivory Coast and Nigeria (Falola et al., 2014; Horesh and Joekes, 2015; Tchokote et al., 2015). Despite the economic importance of cocoa, yields continue to be low. The main reason for the low yield is the ravages caused by soil insect pests such as termites specifically under poor soil fertility condition and the use of poor-quality unimproved planting materials are not used (Bisseleua et al., 2017). Termites cause direct damage by destroying the roots and stems or by cutting down seedlings mostly in the dry season leading to the death of plants (Figure 3.2 a) or indirectly by lowering the yield through decreased translocation of water and nutrients (Asare and David, 2010). Attacks are mainly carried out by workers and often start on the taproot and sometimes on the trunks through sites of previous injuries (Ackonor, 1997; Tra Bi, 2013). In the plant, termites will feed on the woody parts, thus hollowing out the plant and filling it with moistened soil. The soil eventually dries up and hardens, thus keeping the damaged plants or seedlings upright (Figure 3.2 b). Termites that move back and forth on the surface of the bark also carry spores, particularly those of a fungus *Phytophthora* spp. that causes black pod disease on pods and cankers on stems (Vos et al., 2003). The gnawing activity of termites also opens the door to other fungi causing the decomposition of wood. In addition to cocoa, termites cause damage to crops associated with cocoa as well as shade trees and agroforestry fruit tree species, negatively affecting smallholder cocoa livelihoods (Vos et al., 2003).

3.4.2.2 Coffee

Coffee is an important cash crop in Uganda, Kenya, Rwanda and Ethiopia. In Uganda, it is the biggest export commodity, produced mainly by an estimated one million smallholder farmers (Wang et al., 2015). Termite attack can cause economic damage to coffee plantations as they feed on the roots and bark region of seedlings and plants resulting in wilting of the plant. The attack is usually noticed mainly on older coffee plants and shade trees with dead wood.

3.4.2.3 Cashew

Cashew nut is the most consumed nut in the world. Its cultivation is spread all over SSA. The cashew trunk and root borer, *Plocaederus ferrugineus* is the most devastating soil pest of cashew because its infestation can result in the sudden death of the tree within weeks. It has been implicated with economic losses of up to 75% in Nigeria (Asogwa et al., 2008). Cashew weevil (*Mecocorynus* sp.) tunnels the sapwood of cashew, causing substantial economic damage to the crop (Hill, 2008). The tiny grub of cashew shoot and root borer bores into the fresh tissue and feeds on the phloem and xylem tissues of the trunk and root, making irregular tunnels, resulting in exudation of gum and extrusion of fibrous frass from damaged portion. Due to extensive feeding damage caused by the grubs, the flow of sap is arrested, and the leaves become yellow and are shed prematurely (Sahu and Sharma, 2008). Heavy infestations lead to the death of mature trees. Termites are also minor pests of cashew and have been reported to cause damage to roots and shoots in Nigeria (Asogwa et al., 2008).

3.4.2.4 Oil palm and coconut

Oil palm production in SSA provides products and income to millions of people, particularly women involved in harvesting, processing and trading palm oil, palm kernels and palm wine (Carrere, 2010). On oil palm and coconut, termites can feed just under the bark or under leaf bases. Large cavities can be eaten out of trees (Figure 3.3), leading to significant damage to young nuts. For example, in coconut and oil palm farms in Tanzania and Nigeria termites can be very destructive on seed nuts in nurseries, on sprouts and on newly transplanted seedlings (Nyambo et al., 2003; Ogedegbe and Ogwu 2015). Palm trees and coconuts are relatively hardy, but they are prone to termite attacks as they have aggressive root systems and hold more moisture, which makes them an ideal environment for termites. Termites normally start at the root of the palm tree or coconut and feed up to the base. They can cause damage to the tree for years before their presence is noticed.

3.4.2.5 Mango

In SSA, mango contributes to farmer family diets as well as serves as cash crop (Fi, 2011). Termites are some of mango's worst enemies (Vannière et al., 2007), and their attacks have been reported in Benin (Vayssieres et al., 2009), Ghana (Kwasi, 2009), Uganda (Nyeko and Olubayo, 2005) and most parts of French- speaking West Africa (Vannière et al., 2007). Some termite species cover mango stems with galleries and chew away the bark underneath these galleries (Figure 3.4). Others damage the roots and underground stems of the plant. Some gain access through the dead ends of pruned branch stumps, from which they may invade the living tissues. On the above-ground section, the tree gradually dries out from the extremities downwards, until the entire tree dies. The damage to the root system is scarcely visible and only concerns the farmers when the drying out has reached an advanced stage (Vannière et al., 2007).

3.4.2.6 Citrus

Citrus is one of the most widely grown fruit trees in subtropical Africa and is also one of the most important economic fruit crops (Olife et al., 2015). Seedlings and young saplings of citrus trees are cut off below the ground level, while some of the mature trees may be damaged by piping by termites. They also destroy fruits that fall under trees (Ogedegbe and Ogwu, 2015). In some parts of Nigeria, termites damage the exposed parts of roots and the woody structure of some citrus trees and are suspected to have positively influence the spread of *Phytophthora*-induced diseases (Umeh et al., 2002). Some studies have shown that termites kill young citrus by removing the bark from the trunk and cambium in a neat ring between soil line and crown roots (Stansly, 1993). After the tree has been girdled and killed, the termites leave the tree but may return to consume the wood after it has dried, the result being the creation of a suitable food source (Stansly, 1993).

Figure 3-2: Termite damage on old cocoa tree (a) and cocoa seedling (b)

Figure 3-3: A coconut tree (left) being tunneled through and damaged by termites and Soil-covered tunnels built by termites on a mango tree (right)

3.4.3 Factors influencing the damage of termites to plantation tree crops

Termite distribution and damages in SSA are affected by indigenous, climatic and ecological factors. The extent of termite damage to plantation tree crops is determined by plant species and the interactions between the pest and environmental factors (Chakraborty et al., 2000). Thus, insect pests and their status can change according to the local conditions.

3.4.3.1 Climatic factors

Soil moisture, temperature and altitude affect soil pest distribution in SSA. Soil moisture, and indirectly rainfall, is an important determinant of distribution of soil pests. Termites, for example, are widespread in Africa, while beetles have a much more limited distribution and, and areas within which they occur, and the severities of the damage they cause varies (Hillocks et al., 1996). Termites affect plants especially in water-stressed conditions (Logan et al., 1990; Nyeko and Olubayo, 2005). In Uganda and Kenya, farmers consider termite damage to be more severe in the dry months compared to the wet months (Sileshi et al., 2005). Temperature and moisture are key factors affecting termite survival, activity and geographical distribution because their cuticles are soft, and they possess poor water-retaining properties, and therefore, termites are likely to be found in warm, humid areas (Green et al., 2005; Su and Puche, 2003; Wiltz, 2015). The success of soil insect pests is in part due to their ability to survive the dry season (Hillocks et al., 1996). Some termite species move their populations deeper in the soil where they are protected from desiccation (Dibog et al., 1998). Some bring water to the nest from considerable depths, while the fungus-growing termites benefit from water produced as a metabolic by-product by the fungi (Cornelius and Osbrink, 2010). Soil-inhabiting beetle larvae construct cells of mud from which they emerge at the start of the rains (Hillocks et al., 1996). Some soil larvae like scarab grubs curl within their cells, and this chamber reduces moisture loss from evaporation (Villani and Wright, 1990). Mole crickets are widely distributed in the warmer parts of Africa (Alford, 2014).

3.4.3.2 Anthropogenic factors and deforestation

Human-induced habitat disturbance has been demonstrated to alter termite assemblage structure and extinction of some termite species and favour the pestiferous behaviour of others, hence contributing to the present termite problem in SSA (Mugerwa, 2015). Farmers, especially in southern African countries (Palm et al., 2013), practise slash-and-burn agriculture where they clear forests to produce food and make a living for their families (Ando, 2014; Kanmegne, 2004). Almost all tropical forests are cleared by similar methods that start with slashing the forest with chainsaws, axes and machetes and burning the felled vegetation after it has dried. This has negative effects on termite natural enemies like ants, leading to the proliferation of termites (Castaño- Meneses and Palacios- Vargas, 2003). Other human activities such as deforestation (Dawes, 2010) and fires (Neoh et al., 2015) alter relative abundance of termite species and composition of termite assemblages. When termite food resources such as dry logs, litter, stumps and grass are consumed by fires, the surviving termite species switch their feeding on living trees, thereby constituting pests (Bandeira et al., 2003). The disappearances or reduction of certain feeding groups from the ecosystem often gives way to new feeding groups that are adapted to feeding on living trees, thus constituting pests (Attignon et al., 2005). Many ant species that are natural enemies of soil pests, particularly *Pheidole* species, are sensitive to perturbations promoted by tree cutting leading to low species diversity in open areas (Vasconcelos, 1999). Many farmers in SSA also rely on chemical pesticides to control above-ground pests. The indiscriminate use of broad-spectrum pesticides, such as pyrethroids (deltamethrin) used for mirids control in cocoa-growing regions of SSA (Bateman, 2008), depletes the natural enemy populations of soil pests such as ants leading to the concomitant rise in the pest status of soil insect (Prabhuraj et al., 2016).

3.4.3.3 Ecological factors

Tropical forests are being replaced at an increasing rate with plantation crops, and the forest conversion alters the composition of soil fauna such as termites. Soil-feeding forest termites are vulnerable to clearance and cultivation, thus decreasing species richness and abundance (Bandeira et al., 2003; Eggleton et al., 2002); whereas wood and litter feeders increase species richness and abundance due to the accumulation of litter or crop residues (Davies, 2002). Conversion of tropical forest and agricultural intensification are the most important drivers of tropical biodiversity loss and associated ecosystem services (Foley et al., 2005; Matson et al., 1997). Agricultural intensification usually leads to reduction in plant species richness with corresponding changes in the community composition of the pest complex herbivorous insects and their natural enemies (Orians et al., 2012). Ecosystem deterioration also resultsin the loss of suitable habitats and the disappearance of key natural vertebrate enemies of termites such as aardvark (*Orycteropus afer*), pangolin (*Manis* sp.), aardwolf (*Proteles cristatus*) and hedgehog (Pomeroy et al., 1991), hence escalating termite activity and proliferation. The monoculture agricultural system that is mostly practised in tree crop production systems in SSA typically results in greater crop losses from an insect pest complex that is less diverse but more abundant like termites (Tonhasca and Byrne, 1994). The trend for higher insect pest densities in monocultures is especially seen for insect pests with a narrow host range such as the cashew shoot and root borer (Asogwa et al., 2008). Even though moderate shade levels have little effect on yields of some tree crops such as cocoa and coffee, many farmers in SSA prefer unshaded monocultures to shaded systems to increase short-term income (Rice and Greenberg, 2000). Monocultures create conditions favourable for specialized pest species, in the presence of abundant supply of their preferred food, to multiply rapidly and become successful pests.

3.4.4 Management practices against termites

The management of termites in plantation crop systems is hindered in part by the difficulties in detection and access. Some management approaches generally being practised are discussed below. Table 3.2 summarizes the most commonly used methods as well as their constraints.

3.4.4.1 Biopesticides

Farmers in SSA exploit the toxic and deterrent properties of various plants to control soil insects. Neem (*Azadirachta indica*) oil and leaf extracts have been reported to control termites in Nigeria and Ghana (Akutse et al., 2011; Nwilene et al., 2008). Cocoa farmers in Cameroon use moringa (*Moringa stenopetala*) extracts to manage termites in cocoa farms (Djuideu et al., 2019). Plant parts of *Euphorbia tirucalli* are used as termite deterrents in Malawi and Zambia (Sileshi, Akinnifesi, et al., 2008) as well as in Uganda (Mugerwa et al., 2011). Plant parts and/or extracts of *Tithonia diversifolia* (Nyeko and Olubayo, 2005), *Cassia spectabilis*, *Tephrosia vogelii* (Kiwuso et al., 2004), red pepper and tobacco (Kiwuso et al., 2004; Nyeko and Olubayo, 2005), *Maesa lanceolata* (Tasisa and Gobena, 2013), *Millettia ferruginea* (Shiberu et al., 2013) and *Bobgunnia madagascariensis* (Sileshi et al., 2008) are also used in different parts of SSA to manage termites. Raw seed extracts of *Parkia biglobosa* were investigated in Nigeria and were shown to exhibit varying degrees of termiticidal activity on the workers of *C. intermedius* (Olugbemi, 2012). The efficiency of biopesticides in the control of termites is questionable because most biopesticides are unstable in the presence of ultraviolet radiation in sunlight (Isman, 2006), as such, break down rapidly in the soil and do not give prolonged protection (Logan et al., 1990). The concentration of the toxic substance by weight in biopesticides is usually lower compared to the concentration required for toxicity (Isman, 2006), consequently, enormous quantities are thus required to achieve the required concentration. Also, some substances that deter feeding by one species can serve as attractants/stimulants for another (Isman, 2006). The hazards biopesticides present to humans and the environment are often not studied very well and as such, limits their adoption for pest control. Heaping wood ash around the base of tree trunks is reported to reduce termite attack in Ghana (Maayiem et al., 2012), Nigeria, Uganda (Mugerwa et al., 2011; Nyeko and Olubayo, 2005), Zambia (Sileshi et al., 2008) and Cameroon (Djuideu et al., 2019). However, the mechanism by which wood ash provides protection against termites is unclear.

3.4.4.2 Physical methods

Farmers use several methods aimed at destroying termite mounds to reduce termite densities and subsequently mitigate termite damage to tree crops. Physical destruction mainly involves digging out the entire mound using locally available farm tools until the queen and king are reached and removed (Akutse et al., 2011; Kiwuso et al., 2004; Ogedegbe and Ogwu, 2015). As the mound is dug out, the workers and soldiers are exposed to direct sunlight leading to desiccation. However, this method is labour-intensive, and the practice simply provides temporary relief to foraging activity of termites after which their activity is fully restored (Nyeko and Olubayo, 2005). The practice is also only directed towards mature colonies of the mound-building species, and species that do not build epigeal mounds but cause serious damage to tree crops are often overlooked (Sileshi et al., 2008). Heat treatment as a means of mound destruction involves inserting dry wood, grass or car tyres into the mound, setting it on fire and sealing to confine the smoke (Mugerwa et al., 2011; Nyeko and Olubayo, 2005). Others burn plant residues on top of termites' mound to suffocate them (Tasisa and Gobena, 2013). Burning plant residues in or on termites' mound does not give long- lasting results, as it does not kill the entire colony.

3.4.4.3 Excreta

Cattle excreta is used to reduce termite damage in agricultural fields in Kenya and Uganda (Kiwuso et al., 2004), while farmers in Nigeria and Ghana use goat excreta as well as human excreta and urine for termite control in plantation crops (Akutse et al., 2011; Ogedegbe and Ogwu, 2015). In Cameroon, cocoa farmers use dung, and some defecate on holes dug on termite mounds, with the belief that human excreta repel termites (Djuideu et al., 2019). Some researchers have suggested that the mechanism through which dung reduces termite damage is by providing alternative food resources to termites, hence relieving crops from termite attack or by enhancing proliferation of termite predators that eventually reduces termite foraging activity and by enhancing soil fertility and thus boosting plants' vigour, making them less vulnerable to termites (Mugerwa, 2015). The efficacy of this method as a termite management intervention is also highly questionable.

3.4.4.4 Chemical control

Farmers use different chemicals in SSA to control soil pests on tree crops. In Cameroon, cocoa farmers use chemicals such as Actara® (thiamethoxam), Cryptogil® (propiconazole, Xamox, carbendazim, bifenthrin), Furadan® (carbamate) and Bastion™ (fluroxypyr- meptyl, florasulam, propylene glycol) to manage termites in cocoa agroforests (Djuideu et al., 2019). In some parts of Nigeria, “insecticidal powders” are sprinkled on termite mound and the black material of used batteries (carbon) is also used as a control measure against termites in cocoa farms (Adesiji et al., 2007; Owolabi and Okunlola, 2015). Some farmers in Uganda mix chemicals such as Ambush, dimethoate, diazole and thiodan in water and make a hole on top of the mound where the mixture is poured down (Nyeko and Olubayo, 2005). In Chad, fungicides targeted at the *Termitomyces* spp. have been demonstrated (Rouland- Lefevre and Mora, 2002). Chemical control of soil pests in tree crops can cause enormous management problems and is also expensive and requires skilled labour (Logan et al., 1990). In addition, chemical control has many negative impacts on human welfare and the environment. First, direct exposure of farmers to pesticides could occur because people who apply pesticides usually do not take precautions or wear protective clothing. Also, people who consume termites and mushrooms from treated termite mounds could be exposed to pesticide residues. Geophagy has been observed in diverse number of species including gorillas, elephants and even humans (Mahaney et al., 1999). Animals and humans can thus be exposed to pesticides through consumption of soil from treated termite mounds. During swarming, the alates form a source of food for many amphibians, reptiles, birds and mammals (Deblauwe and Janssens, 2008). In addition, many animals nest in termite mounds (Pomeroy et al., 1991). Chemical control has also been employed in the control of white grubs in Uganda, but none of the many formulations tested offered prolonged control of the pest (Conlong and Mugalula, 2003). Therefore, the use of pesticides to control soil insect pests like white grubs is not feasible and in termite mounds may put the health of many species of animals and humans at risk.

Table 3.2: Commonly used methods for soil pest control in sub-Saharan Africa

Control practices	Country	References	Limitations
Termite mound destruction	Malawi Ghana Zambia Uganda	Sileshi et al. (2008) Akutse et al. (2012) Sileshi et al. (2008) Nyeko and Olubayo (2005)	• Colony usually rejuvenates because many termite species can produce substitute queens to restore the colony. • Targets only mound building termites and overlook species with subterranean moulds and those that do not build moulds, Labor intensive.
Plant extracts	Nigeria Malawi Ghana Mozambique Zambia	Nwilene et al. (2008) Sileshi et al. (2008) Akutse et al. (2012) Sileshi et al. (2008) Sileshi et al. (2008), Nkunika (1998)	• Unstable in ultraviolet radiation and break down rapidly in the soil without giving prolonged protection from termite attack. • Enormous quantities required. • Some may act as attractants for other pest species.
Application of wood ash	Zambia Ghana Uganda	Sileshi et al. (2008), Nkunika (1998) Akutse et al. (2012) Nyeko and Olubayo (2005)	• Less applicable in ecosystems with high termite densities. • Practically limited to small plantations • May not be environmentally friendly as the practice may promote deforestation
Use of animal and human excreta	Zambia Ghana	Nkunika (1998) Akutse et al. (2012)	• Highly questionable • Unethical and/or unacceptable in many cultures. • Presents health hazards if not treated before application in the field
Chemical control	Nigeria Ghana Chad	Ogedegbe and Ogwu, (2015) Akutse et al. (2012) Rouland-Lefevre and Mora, (2002)	• Expensive and requires skilled labor. • Have negative impacts on the ecosystem and humans.
Biological control (application of pork, meat, intestines, sugar, fat to attract ants)	Mozambique Ghana Nigeria	Sileshi et al. (2008) Akutse et al. (2012) Logan et al. (1990)	• Cryptic habit of termites limits their susceptibility to ants. • Not applicable in ecosystems with high densities of termites.

Biological control (entomopathogenic fungi)	Kenya Uganda Ethiopia	Maniania et al. (2001) Nyeko and Olubayo (2005) Sileshi et al. (2013)	• Most virulent entomopathogenic fungal strains are repellent • Its mainly suitable for the aboveground and unhidden pests

3.4.4.5 Biological control of termites

Control methods with sugar-based products to attract termite predators in termite-infested fields have been utilized in some parts of Africa to increase the populations of predatory ants associated with the reduction in termite population (Sekæamatte, 2001). Farmers in Mozambique use leftover pork or beef to control termites in agricultural fields (Nwilene et al., 2008), while Nigerian farmers bury dead animals or fish viscera to attract ants which in turn reduce termite attack on cocoa trees (Adesiji et al., 2007; Owolabi and Okunlola, 2015). These protein products are aimed at attracting ants which in turn prey on termites. A few parasitoids of termites are known, but their potential for regulating termite population seems negligible because of the protected, underground location of many termite species (Culliney and Grace, 2000). Thus, there seems little potential for use of these agents for termite control.

3.4.4.5.1 The use of entomopathogenic fungi

Research has been conducted to develop biological technologies for use against economically important termite species, focusing on *Metarhizium anisopliae* and *Beauveria bassiana* in Kenya, Uganda and Ethiopia (Maniania et al., 2002; Nyeko and Olubayo, 2005; Sileshi et al., 2013). Although both fungi have been shown to be effective against various termite species in the laboratory (Hussain et al., 2010), their success in the field has been extremely limited because of the ability of termites to detect and avoid other infected termites (Yanagawa et al., 2008) and because virulent entomopathogenic fungi are repellent to termites (Mburu et al., 2009). Therefore, alternative methods must be deemed necessary to introduce entomopathogenic fungi into termite colony.

3.4.4.5.2 The use of semiochemicals in an “attract and kill” approach-Learning from success stories in the management of soil insect pests

Unlike above-ground insects that can also exploit visual cues to locate host plants (Bernays and Chapman, 1994), soil insects rely solely on chemical or physical cues that indicate the location of a host plant and/or enable them to distinguish it from an unsuitable plant (Barsics et al., 2016). Carbon dioxide (CO_2) is the most abundant gas released from plant roots, and many studies have shown that it is the primary plant metabolite that allows insects to locate and orientate towards roots (Johnson and Gregory, 2006). This type of orientation is common to soil-dwelling larvae across numerous insect orders where CO_2 acts as a general non-specific semiochemical and a “search trigger” for larvae to locate roots by triggering a more directional response and intensifying the search for roots (Schumann et al., 2013). It has also been reported as an attractant for different termite species, with the attraction differing with different concentrations (Broadbent et al., 2006). Bernklau et al. (2004) tested various CO_2-producing compounds that diverted western corn rootworm larvae away from their host (maize). These authors proposed the encapsulation of CO_2- emitting products to extend the time of CO_2 production. Other researchers have encapsulated CO_2 with various killing agents for the control of various soil- dwelling pests in what is referred to as “attract and kill” approach (Schumann et al., 2013; Przyklenk et al., 2015). The insect pests are attracted by the CO_2-emitting formulations (beads, granules) and subsequently stimulated to feed on the formulations due to incorporated phagostimulants. The attracting formulations additionally contain a kill component, such as insecticides, neem or entomopathogenic fungi (Przyklenk et al., 2015; Schumann et al., 2013). By making use of this strategy, the amount of insecticides can be minimized, entomopathogenic fungi successfully introduced into termite colonies and the environment and health of farmers and consumers protected. This strategy has been successfully applied in Europe for the control of wireworms, western corn rootworm larva and black vine weevil in potato, maize and strawberry, respectively (Schumann et al., 2013; Przyklenk et al., 2015), which can be adopted in SSA for the control of soil pests especially termites, as they also use similar mechanisms to locate host plants. However, the common termite behaviour of isolating and avoiding infected colony members could be a significant impediment to the transfer of pathogens within colony. As avoidance of infected individual depends on recognition of the infection, the A&K strategy eases vectoring of pathogen inoculum by unimpaired termites and is the most effective method of infiltrating the colony as the spores of entomopathogenic fungi are vectored passively on the termite cuticle and transferred to other individuals by mutual grooming behaviour before infection is apparent resulting in the spread of the fungi in the colony (Przyklenk et al., 2015).

3.5 Conclusions

This review clearly shows that some soil-dwelling insects, especially termites, are pests of young plants including tree seedlings and saplings in tree nurseries and young plantations. This group of insects is likely to become more important especially with the increasing conversion of forest ecosystems to farmlands. While some soil insect pest orders like Isoptera have been well researched, information on the others such as Coleoptera, Lepidoptera and Orthoptera has not been described in detail in SSA. Although our knowledge is somewhat limited for these pests, there is good evidence that these insects have a large impact on tree crops. Appropriate management of soil-dwelling pests will include a combination of IPM packages around “A&K” with encapsulated CO_2 in combination with entomopathogenic fungi, considered as the best bet for management. of biological control agents such as ants and the maintenance of species-rich agroecosystem (Bisseleua et al., 2017). However, more research on these insects is needed to curb food insecurity in SSA. There are several areas of study that would appear to require considerable research: firstly, detailed studies to quantify losses by soil insect pests on tree crops; secondly, the need to explore the genetic variation in the tolerance and resistance of plant roots to insect pests; and thirdly, the exploitation of entomopathogenic nematodes and fungi in combination with cues such as CO_2 which soil insects use to locate host plants (“attract and kill” approach), in sub-Saharan African tree crop soil pest management. In addition, the effectiveness of the various traditional control strategies practised in different parts of SSA for termite management needs to be verified, developed and emphasized for the management of termites. The importance of farmer information literacy and awareness towards soil pests and food security cannot be overemphasized.

Efforts should be devoted to the dissemination of information and modern knowledge on soil pests and their management to farmers.

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4 CHAPTER FOUR: TAXONOMIC PATTERNS AND FUNCTIONAL DIVERSITY OF TERMITES (BLATTODEA: TERMITOIDAE) IN COCOA AGROFORESTRY SYSTEMS

Abstract

Termites have gained importance as major pests in cocoa agroforests. Proper identification of termite species and knowledge on their functional diversity are the first steps in developing environmentally compatible management strategies. We tested the hypothesis that patterns of termite species richness in different cocoa agroforests is related to responses of termite functional groups to changes in shade management. We compared termite assemblages under five cocoa agroforestry systems in Cameroon to assess the impact of shade on termite taxonomic and functional group diversity. Sampling was done using a modified standardized transect method. Two 30 × 30 m quadrats each divided into three transects were laid on four farms at each site. Termites sampled were identified and grouped according to habitats, functional groups, and feeding habits. Sixty-nine termite species in 33 genera and five subfamilies under two families were sampled. The Termitidae was the most dominant family and Rhinotermitidae the least with just one species. Termite species richness decreased significantly from the heavy shaded cocoa agroforests (44 species) to the full sun (11 species). Functional group pattern differed significantly in all the cocoa agroforests and within each agroforestry system while all systems were dominated by wood and litter feeder species. Many species belonging to this group were responsible for most of the damage on cocoa trees. Both the richness of termite pests and marketable yield followed a quadratic curve and were found to be lowest and highest in plots with shade cover above 40%. The simulated optimal shade levels for low termite infestations and marketable yield overlapped between 45% and 65% indicating that cocoa agroforestry systems with around 55% shade cover may be optimal to balance termite infestations and marketable yield. Shade maintenance in cocoa agroforests is valuable in reducing termite pest species and conserving soil feeding termites which provide beneficial ecosystem services.

Keywords: Agricultural intensification, Cameroon, cocoa, cocoa agroforests, shade management, termites.

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4.1 Introduction

Cocoa (*Theobroma cacao*) is shade tolerant and traditionally grown under shade trees in complex agroforestry systems, thereby providing a refuge for biodiversity and sustaining other ecosystem services (Rice and Greenberg, 2000; Bisseleua et al., 2009). Despite its ecological benefits, recent decades have seen a transformation of cocoa farming in southern Cameroon to a more intensified systems by eliminating shade trees to increase short-term income. This has resulted in a broad range of cocoa plantation management, ranging from low input shaded plantations to high-input full sun plantations (Bisseleua et al., 2013). It is assumed that shaded plantations are less profitable, but this assumption is often based on incomplete cost-benefit calculations (Tondoh et al., 2015). This is because cocoa productivity is often used as an indicator for profitability, which is assumed to be lower for shaded systems. For example, there have been recent studies demonstrating the biodiversity benefits of shaded cocoa systems, with foci on ants, wasps, spiders and plant diversity and how to manage farms to optimize diversity (Bisseleua and Vidal, 2011; Bisseleua et al., 2013). However, these studies documented a loss of biodiversity at the transition from shaded agroforestry systems to unshaded monocultures (Bisseleua et al., 2009, 2013) with the likeliness changing in ecosystem services resulting in higher incidences of pest outbreaks, such as termites (Bisseleua and Vidal, 2011).

In cocoa agroforestry systems of West Africa, termites have long been associated with cocoa (Eggleton et al., 2002; Tra Bi et al., 2015; Ambele et al., 2018). They are major soil-dwelling ecosystem providers that influence ecosystem functions by changing their biotic and abiotic surroundings (Jouquet et al., 2014). They contain species that may become pests where cocoa is gradually grown in less-shaded plantations or in full sun. However, they are far more beneficial through soil turnover during their foraging and nesting activities; facilitate soil aeration, enhancing absorption and storage of water including carbon fluxes and storage. An assessment of their damage in cocoa plantations of West Africa revealed that 20 to 80% of cocoa, specifically seedlings, were sometimes damaged to the extent of requiring replacement (Tra Bi et al., 2015). They are known to feed on bark, branches and, sometimes, cocoa pods and to build many galleries on the stem from the base to the branches (Tra Bi et al., 2015). Termite species vary in their basic biology and ecology, including colony size, nesting, and feeding, swarming, and reproductive behavior. Understanding termite functional and taxonomic diversity in cocoa agroforests is a very important initial step to developing realistic compatible management strategies against the pest species as not all termite species are pestiferous.

Although termites have long been associated with cocoa in Southern Cameroon (Eggleton et al., 2002), and the effect of fungicide application on soil fauna in shaded cocoa agro-forests was assessed (Norgrove et al., 2009), no research has documented the diversity of termites in cocoa agroforests in Cameroon. In other parts of Africa, few studies have looked at termite species associated with cocoa farms in Ghana (Ackonor, 1997) and Ivory Coast (Tra Bi, 2013). Other studies on termites have focused on termite ecology, on changes in the diversity of termite species along gradients of human disturbance (Eggleton et al., 1996; Jones and Eggleton, 2000; Jones et al., 2003). Others have looked at the variations of termite assemblages across forests subject to various degrees of fragmentation (De Souza and Brown, 1994; Davies, 2002; Kaiser et al., 2015), or along a gradient of forest disturbance (Davies et al., 1999; Eggleton et al., 2002; Bandeira et al., 2003; Jones et al., 2003; Luke et al., 2014), or on spatial scale and habitat heterogeneity (Isra et al., 2007), and the effects of wet and dry seasons on soil termites within a humid tropical West African forest (Dibog et al., 1998). Previous studies have shown that termite diversity usually decreases following habitat conversion, but it is not known how many termite species or termite functional groups persist when farmland is cleared for cocoa plantation or the effect of the different shade management systems on termite diversity in cocoa agroforests; especially considering the fact that a change of land use is the most significant factor that affects changes in biodiversity (Chapin et al., 2000). In an agroforestry trial in southern Cameroon, Dibog et al. (1999) demonstrated that tree canopy cover was positively correlated with termite abundance. This implies that agroforestry systems with more shade trees are habitats for more species, many of which help to increase the yield of cocoa like termites, ants and other pollinators.

Although cocoa is important for national macroeconomic balances and provides livelihoods to millions of people in developing and developed countries, no studies have looked at the link between the biodiversity of termites and the functioning of cocoa agroforestry systems. Understanding termite functional diversity in cocoa agroforests is thus a very important initial step to developing realistic compatible management strategies against the pest species in cocoa agroforests. However, since species richness is also commonly used as the main measure of biodiversity, biological diversity concept that also includes taxonomic and functional diversity needs to be explored (Pavoine and Bonsall, 2011; Nunes et al., 2016). It is therefore paramount to understand both termite taxonomic and functional diversity in cocoa agroforestry systems that can also improve our knowledge on biodiversity patterns and how different land use intensifications influence termite species diversity and their traits, since they capture different aspects of termite ecological roles. It is very important to recognize termite functional groups in cocoa agroforests to develop specific and effective control measures to the target group (s) causing damage on cocoa as functional diversity is commonly assumed to be a better predictor of ecosystem productivity and vulnerability than species diversity (Heemsbergen et al., 2004). The aim of this study was therefore to compare the termite taxonomic and functional diversity of five different cocoa agroforestry shade systems in the central region of Cameroon. It was hypothesized that the degree of shade cover and management in cocoa agroforests are the main determinants of termite diversity and functional group patterns, and that termite diversity and functional groups would vary according to the different shade systems.

4.1.1 Definitions, terms and concepts used in this chapter

There are several terms and concepts which will appear throughout this chapter. Simple definitions of these key terms are presented below.

A **cocoa growing area** is the area where farms having cocoa plantations are located. **A cocoa plantation or farm** is a single block of land, of variable size and form, dedicated to the cultivation of cocoa with or without other associated plants. A cocoa plantation can have two components: cocoa and shade canopy. The **cocoa component** includes all cocoa plants while the **shade canopy component** includes all non-cocoa plants taller than cocoa trees. Cocoa planted under thinned natural forest can either be very heavily or heavily shaded and are referred to as r**ustic cocoa agroforests** (Figure 4.1 a). A cocoa plantation with moderate shade is referred to here as **intermediate shade** (Figure 4.1 b). Cocoa farms with very low shade or no shade at all are **full sun cocoa agroforests** (Figure 4.1 c). **Agroforestry** is a land use management system in which trees or shrubs are grown around or among crops or pastureland. It is broadly defined as growing an agricultural crop and a forest crop on the same land at the same time. **Agroforestry systems** include both traditional and modern land-use systems where trees are managed together with crops and/or animal production systems in agricultural settings.

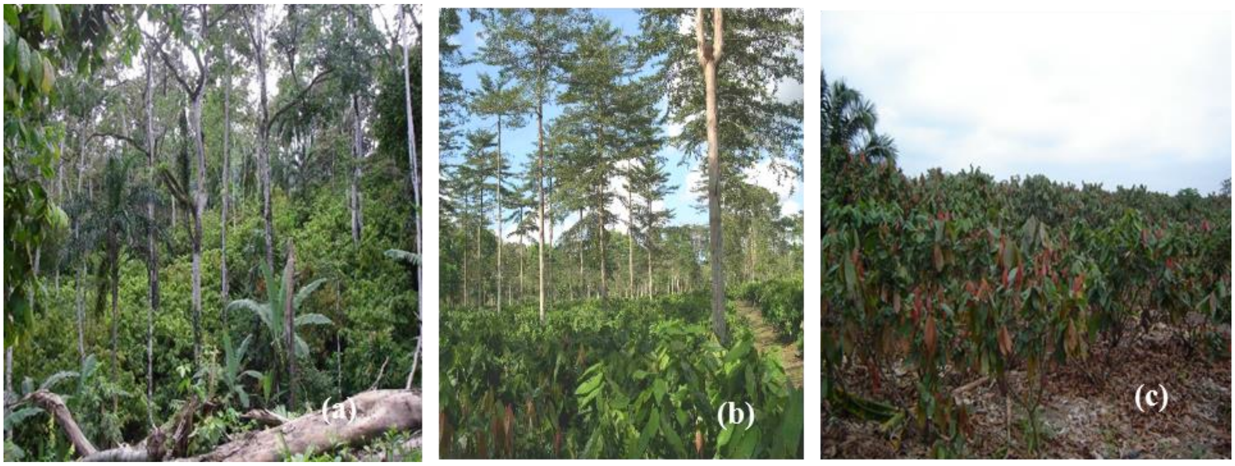

Figure 4-1: Photographs of representative areas on the shade-intensity: (a) rustic, (b) moderately shaded and (c) full sun cocoa agroforest.

4.2 Materials and methods

4.2.1 Study sites

The study took place in five major cocoa-growing areas in the Central Region of Cameroon located between 03°53′01″N and 4° 56'42" N and 10°50′56″ E and 11° 16'47″ E (Figure 4.2). The choice of plantations was guided by: (i) contrasting cocoa agroforestry systems that follow the trend of the different cocoa agroforestry systems; (ii) the geographical location of the plantations with a view to covering contrasting agroecological conditions; and (iii) the existence of recent termite damage. The altitude varies between 402 m and 557 m above sea level (a.s.l.), characterized by a sub-equatorial climate with a dry season lasting over 3 months (December–March) during which the monthly rainfall is less than 70 mm and a rainy season lasting over 9 months (March-December), where the mean annual rainfall is about 1600 mm. The mean annual temperature is about 25 °C with a relatively small thermal variation (Vidal, 2008). Three main soil types are encountered in these sites: a), Ferric Acrisols, mostly seen in Kedia, Bakoa and Talba, where soils are less profound, well drained with good chemicals properties, with top soil containing over 60% sand and less than 2% organic matter; b), oxisols with fairly unsaturated acid rocks, localized in Obala, with a well-drained system and good chemicals properties; c), oxisols with highly unsaturated acid rocks in Boumnyebel, with low agricultural yield because of the high acid content (Kanmegne et al., 2006; Santoir and Bopda 1995). The pH of the soils varies from 4.29 to 5.43 (Kotto-Same et al., 1997; Kanmegne et al., 2006). Table 4.1 lists the main geographical and agroecological characteristics of the chosen sites.

The five selected cocoa agroforestry (AF) systems are characterized as follows: (a) Boumnyebel (03°53′01″N 10°50′56″E, 402 m a.s.l.), where cocoa is grown under a dense cover of many forest tree species near pristine forests with very old cocoa plantation (30 years) on oxisols highly unsaturated acid rocks; (b) Obala (04°15′82″N 11°53′62″E, 557 m a.s.l.) located in semideciduous forest near houses, where cocoa is grown together with a wide variety of fruit tree species, with no remnant forests because of very high human population density with relatively old cocoa plantations (20 years) on oxisols with fairly unsaturated acid rocks; (c) Talba (04°34'421"N 11°28'33"E, 462 m a.s.l.) where cocoa is grown on larger farms with mature cocoa plantations (30–35 years) in or near forests on ferric oxisols; (d) Kedia (4°50'0.46"N, 11°07'87"E, 459 m a.s.l.) where young cocoa plantations (20–25 years) are grown under very low shade with young cocoa plantation in the savanna on ferric acrisols; (e) Bakoa (4°56'42"N 11°16'47"E, 469 m a.s.l.), where young cocoa plantations (20–25 years) are grown under full sun on modified savanna agroecosystems on ferric acrisols. The shade levels in the five AF systems were described by Bisseleua et al. (2013) with a little modification that more farms are gradually grown under full sun in Bakoa, and range from very low shade in Kedia, intermediate shade in Talba, moderate shade in Obala, and very heavy shade in Boumnyebel. The geographical coordinates of the selected AF systems were taken using a GPS (GPSMAP 60CSx). Termites were sampled in four cocoa plantations per system (20 farms in total). At each AF system, the selected plantations were distant by at least 500 m away from each other. Permission for assessing the species richness and to conduct field studies within the cocoa plots were provided by the cocoa grower's associations from the selected regions. The field locations were privately owned by the farmers and did not harbour endangered or protected species. All cocoa farmers in the study area manage their farms in the same way despite differences in shade cover. Insecticides are directly sprayed on the cocoa pods, and weeding operations were applied once before flowering in all selected cocoa plantations.

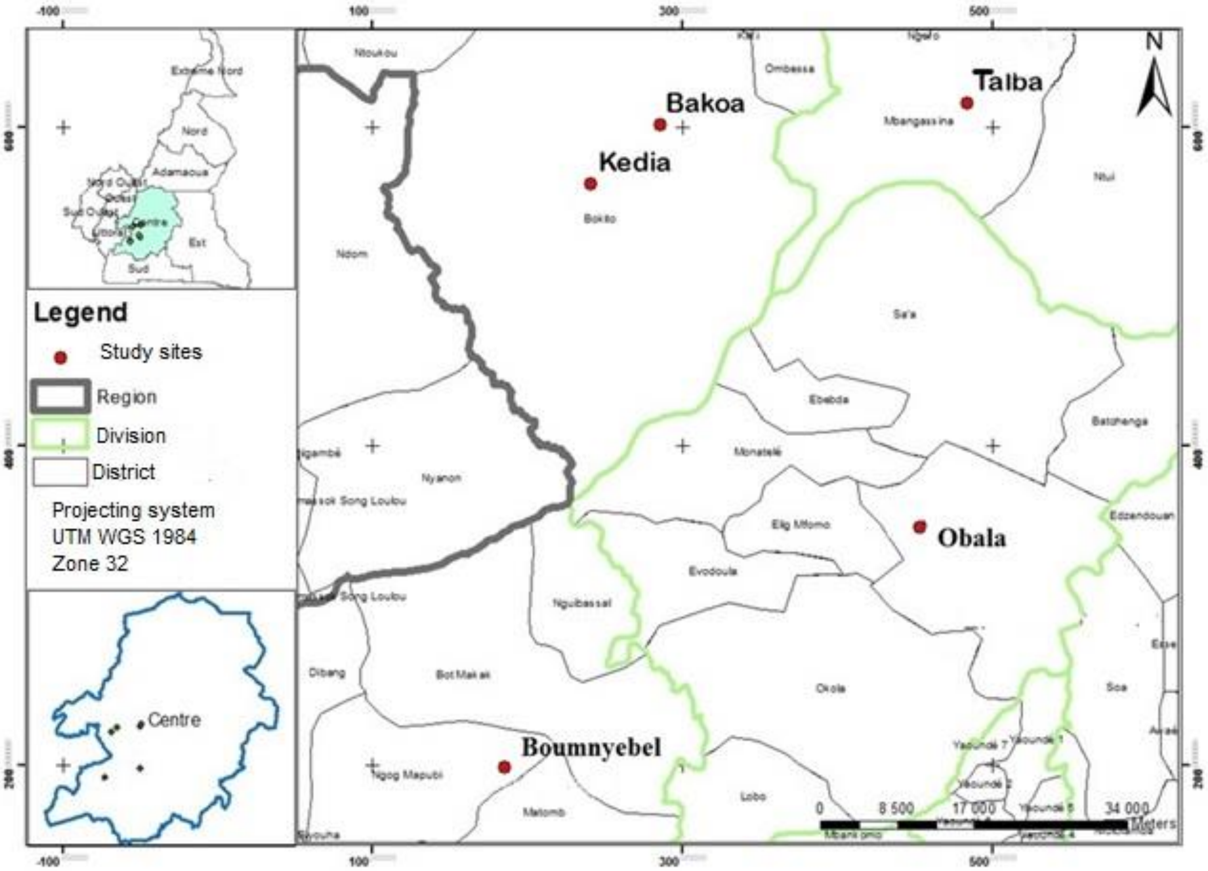

Figure 4-2: Map of the Central Region of Cameroon showing the study sites

4.2.2 Sampling methods

4.2.2.1 Termite sampling

The termite species composition was assessed using a modified transect method to obtain a representative sample of both the taxonomic and functional group compositions of the local termite assemblage (Jones and Eggleton, 2000). Two 30 m × 30 m quadrats were established in each cocoa plantation, with a minimum distance of 50 m between the two quadrats. The quadrats were placed at least 30 m away from the edges to avoid edge effect. Each quadrat was divided into three transects of 30 m × 2 m at the right, middle and left of the quadrat (Figure 4.3). Each transect was further divided into 15 contiguous sections of 2 m × 2 m each; making a total of 45 sections in each 30 m × 30 m quadrate. In each section, all fallen dead wood of less than 1 m in diameter were broken and opened to remove termites. In addition, the leaf litter was collected, and 3 soil samples of 12 cm × 12 cm and 10 cm deep dug with the aid of a machete and mixed to form one composite soil sample for each section were searched for termites. The soil was sieved and hand-searched for termites with searching independent of time. Dead wood (diameter > 5 cm) within each of the quadrates (up to a height of 2 m) as well as galleries on cocoa trees were also searched for termites' collection. Soldier and worker castes were collected and stored in 75% ethanol in 1.5 ml Eppendorf tubes and labelled accordingly with the site, quadrat and section numbers. The micro-habitats (dead wood, litter, soil and tree) where termites were encountered were also recorded simultaneously during the collections. Every species, but not every termite specimen was collected for identification in the laboratory. Sampling was done only during the rainy season (June to October 2016) because climatic seasons have been found not to significantly affect the termites' fauna (Dibog et al., 1998; Couto et al., 2015).

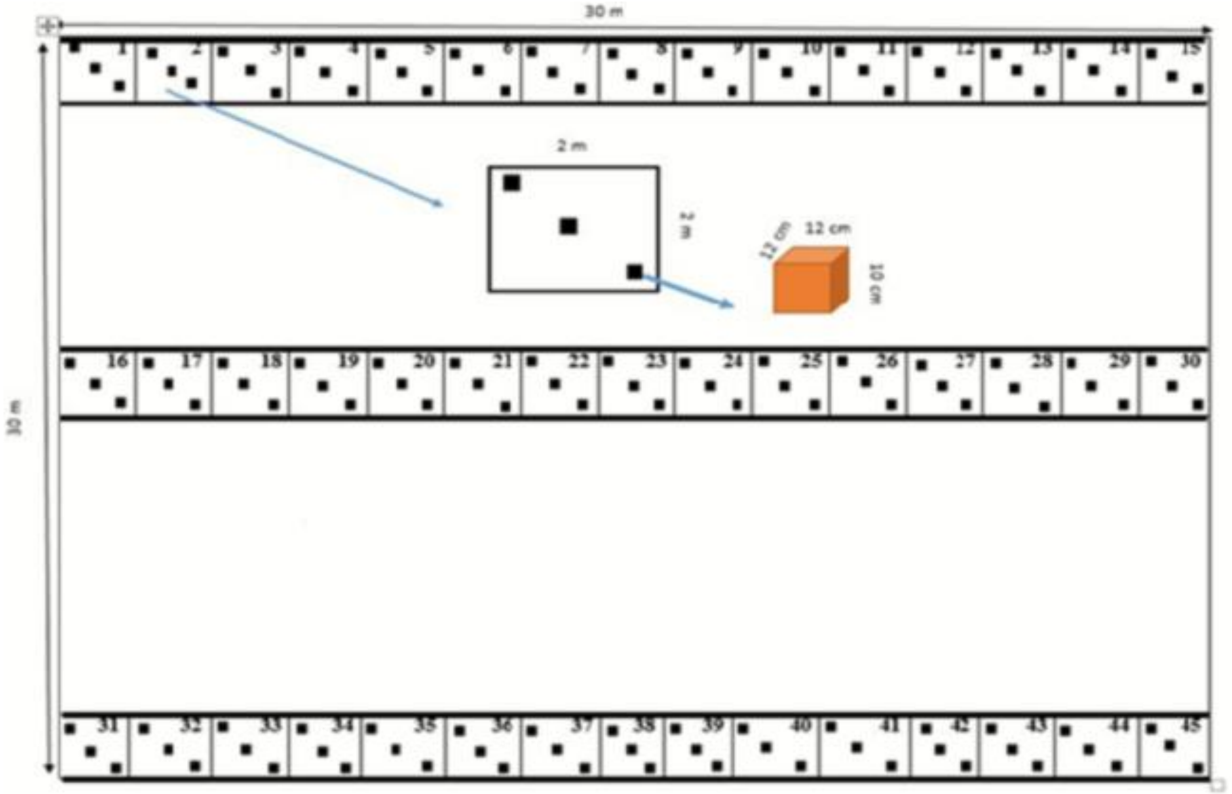

Figure 4-3: Schematic representation of the sampling method

4.2.2.2 Sampling of vegetation and yield

In each selected plantation, data on the vegetation characteristics were collected. The number of shade trees species was recorded, with unknown tree species given a unique morphospecies number. Shade cover was measured at 10 subpoints for each of the four cardinal directions within a 30 m radius using a convex spherical densitometer (R. E. Lemmon Forest Densiometers, USA) and the mean shade cover per circle calculated. Scientific and vernacular names (the latter given by local stakeholders) were recorded. Species that could not be identified in the field were identified at the National Herbarium of Cameroon (Yaoundé).

The marketable yield in each quadrat was assessed by recording the number of flowers and young and mature cocoa pods (classified by size). Ripe pods were harvested, beans were pooled from the pods of one subplot, and dry weight of marketable beans was recorded. To account for yield, defective beans were separated and weighed separately from marketable dry beans.

4.2.3 Termite incidence/damage on cocoa trees

To assess termite damage on cocoa tree, in each transect, 15 trees with galleries on them and 15 without galleries (Figure 4.4) were randomly selected. Termites were collected from the infested cocoa trees from the trunk and branches and preserved in 70% ethanol in eppendorf tubes. The infested trees were carefully examined to categorize the damage into 4 levels (no damage, primary, secondary and tertiary damage). Primary was assigned to galleries when present on the tree trunks but without signs of feeding on the trees; while secondary damage, represented termite damage that was accompanied by deep bites into the trunk and; tertiary damage is where termites were feeding within the tree trunks accompanied with dying of the cocoa trees.

Figure 4-4: (a) Termite infested cocoa tree, (b) healthy cocoa tree

4.2.4 Termite identifications

4.2.4.1 Identification of termites with soldier castes

Collections with soldier castes were identified to genus and sometimes to species or morphospecies using dichotomous keys of Emerson (1928), Bouillon and Mathot (1965) and Sands (1998) with the assistance of Dr. Luc Dibog of the Entomology Laboratory of the Institut de Recherche Agricole pour le Développement (IRAD) Nkolbisson, Yaounde (Cameroon). Whenever possible specimens were identified to species, and when this proved impossible letters (for example *Microtermes* sp. A.) were used to refer to unidentified species because the taxonomy of Macrotermitinae is notoriously difficult, and many species are not easy to identify with certainty (Darlington et al., 2008). The identified species were placed in 75% ethanol in 1.5 ml Eppendorf tubes and labels (name of species, site, name of identifier, date identified) inserted into each tube. The tubes are deposited in the Zoological Collections of the Entomology Laboratory of IRAD, Nkolbisson, Yaounde (Cameroon).

4.2.4.2 Identification of soldierless termites (Apicotermitinae)

The part of the termite that was used in identifying soldierless termites was the digestive tube. The character of the tube was the enteric valve, a reduced proctodeal segment (P2 homolog) that is located between the first proctodeal segment (P1) and the third proctodeal segment (P3) of the digestive tube. The specimens were emersed in 75% ethanol and dissected under a Huvitz microscope using entomological mandrins. The abdominal integument was removed from each specimen and the mandrin was used to cut out the section of the gut containing part of the P1, P2 (containing the enteric valve) and P3. The gut section was placed on a microscopic slide in a drop of Berlese mountant and the organic matter from inside the gut section and muscle tissue from the outer wall of the gut was carefully removed using the entomological mandrins. Once the tissue and soil were removed, or as much of it that could be removed without destruction of the enteric valve, the gut section was cut in half, and splayed out on another microscope slide in another drop of Berlese mountant. After re-orientation on the slide so that the enteric pads and longitudinal ridges were facing upwards, a drop of Berlese mountant was added and a cover slip carefully placed on the slide. The slides were then observed under an Olympus microscope at a magnification of 100xs for a clearer picture of the pad structure. The enteric valve structures were identified by comparisons with specimens from the Isoptera to previous structures by Sands (1998) and Sands (1972) or to reference slide preparations of enteric valve structures of the termite collections from Mbalmayo forest reserve in Cameroon at IRAD.

4.2.5 Functional and Nesting Groups

After being identified, the termite species were assigned to feeding guilds or functional groups based on known feeding habits (De Souza and Brown, 1994; Eggleton et al., 1996; Constantino, 1999; Donovan et al., 2001; Isra et al., 2008). These groups were:

Group I: Wood and grass feeders (lower termites that feed on dead wood and grass).
Group II: Wood and litter feeders (Termitidae with a range of feeding habits including dead wood, grass, leaf litter, and micro-epiphytes). In addition, many termite species belonging to this group feed and cause damage to live plants including cocoa (Ackonor, 1997; Constantino, 2002; Vos et al., 2003; Tra Bi, 2013).
Group III: Members of the family Termitidae feeding on the organic rich upper layers of the soil

Group IV: True soil-feeders (all Termitidae), ingesting apparently mineral soil.

In addition, termites were also classified or grouped into one of the five nesting groups (Bignell and Eggleton 2000): (i) Hypogeal nesters that have their colony centers below the ground; (ii) Epigeal nesters which have colony centres above the ground (excluding arboreal nests); (iii) Arboreal nesters that are found at different heights fixed to trees; (iv) log nesters normally found within dead logs or standing trees; (v) and the hypo-epigeal nesters which have their colony centres partly below and partly above ground level (Isra et al., 2008).

4.2.6 Data analyses

The presence of a species in a section was used as a surrogate for relative abundance (Davies et al., 2003). To confirm sufficient sampling efforts for each site, the first-order jackknife estimator was utilized, using EstimateS (Version 9.1.0) (Colwell, 2013), with 500 randomizations without replacing the samples. The first-order jackknife estimator is the best estimator of nonparametric species richness (Basualdo, 2011). To compare species richness between localities, rarefaction curves were constructed by randomly simulating 500 curves based on the initial data from each transect. The overall spatial autocorrelation between environmental and response variables (species richness and occurrence) of the cocoa farms was determined by generating spatial correlograms. In so doing, similarity (Moran's I) of data points was plotted (i and j) as a function of the distance between cocoa farms (dij) to generate the Moran's I correlograms (Legendre and Legendre, 1998) using PASSaGE (Pattern Analysis, Spatial Statistics and Geographic Exegesis, version 2; Rosenberg and Anderson, 2011). The analysis also included a Mantel test with 999 permutations to establish significance ($\alpha = 0.05$) for the pairwise determination of spatial autocorrelation between the study sites (Legendre and Legendre, 1998). Generalized least squares (GLS) model was used to correct for spatial autocorrelation and analyze for the differences between species richness in the selected cocoa agroforestry types (Dormann et al., 2007). This analysis was performed using the R software, version 3.2.3 (R Development Core Team, 2015). The termite species sampled were also categorized into two groups: the pest and non-pest species, and the differences observed between AF systems analyzed using one-way ANOVA. The percent shade cover between sites was also analyzed using ANOVA. Prior to performing ANOVA, the assumption of homogeneity of variance was tested and satisfied using Bartlett's test. In case of significance differences, means were separated with Student-Newman-Keuls (SNK) post hoc test. In data cases where the assumptions of ANOVA were violated (variances not homogenous), the nonparametric Kruskal-Wallis tests was conducted with a post hoc Dunn test performed, where p-values were adjusted using the Benjamini-Hochberg method. Replicate transects were pooled for these analyses. The Shannon-Wiener diversity index (Ḱ) was further used to compare the species richness and evenness between the five cocoa agroforestry types.

To describe beta-diversity or spatial turnover, a pairwise comparison between the five shade types was computed using the Jaccard's index (JI) of similarity. The functional group occurrence between the different localities was compared using nonparametric Kruskal-Wallis tests with a post hoc Dunn test pairwise comparison in R software. This is because functional group occurrence data were not normally distributed and could not be normalized by transformation. The functional evenness was also processed with the conventional species diversity index (Shannon-Wiener index), since the information on species' assignment to functional groups was available and easy to obtain and needed a low level of detail in contrasting species traits (Schleuter et al., 2003).

Based on the feeding group classification, a weighted humification score (HS) was used to compute the community weighted mean (CWM) for each functional group. The HS depicts the position of termite species along a gradient of increasing humification of their food substrate (Donovan et al., 2001; Davies et al., 2003), and the weighted HS depicts the position of the functional groups along this gradient. The CWM was therefore calculated following Garnier et al. (2004):

$$\mathrm{CWM} = \sum_{i=1}^{N} \mathrm{pifi}$$

Where pi is the relative abundance of functional group i, fi the corresponding termite feeding group score, ranging from f = 1 for wood and grass feeders (Group I) to f = 4 for true soil-feeders (Group IV), N is the total number of termite encounters per functional group per locality.

4.3 Results

4.3.1 Taxonomic diversity

The differences in shade between the five sites is summarised in Table 4.1 below**.**

Table 4.1: Cocoa agroforestry system features (± SE of the mean) of the five study sites

	Boumnyebel	**Obala**	**Talba**	**Kedia**	**Bakoa**
Number of tree species (n)	77	19	18	13	13
Tree species richness	10.81 ± 1.0a	5.81 ± 0.61b	3.5 ± 0.49c	4.0 ± 1.24bcd	5.0 ± 0.46bd
Tree density (ha)	196.5 ± 15.5a	158.3 ± 21.6ac	65.9 ± 9.15b	98.6 ± 31.8bc	140.3 ± 31.4c
Total tree height (m)	12.67 ± 0.47a	12.92 ± 0.62a	17.91 ± 0.96b	8.6 ± 0.73c	12.58 ± 0.63a
Shade cover (%)	92.54 ± 2.43a	83.21 ± 1.42b	67.63 ± 5.28c	55.00 ± 5.71d	22.5 ± 2.08e

Values within a row followed by the same letter are not significantly different ($p < 0.05$, SNK test)

Analysis of Moran's I indicated significant autocorrelation for both species' richness and species abundance (Figure 4.5). The pairwise comparison of sites with Mantel test indicated spatial autocorrelation between Boumnyebel and Obala ($r = -0.57$, $p = 0.22$), Boumnyebel and Kedia ($r = 0.11$, $p = 0.8$), Boumnyebel and Bakoa ($r = -0.37$, $p = 0.43$), Obala and Talba ($r = -0.44$, $p = 0.34$), Obala and Kedia ($r = 0.02$, $p = 0.95$), Obala and Bakoa ($r = -0.49$, $p = 0.36$), Talba and Kedia ($r = 0.09$, $p = 0.82$), Talba and Bakoa ($r = -0.49$, $p = 0.29$), Kedia and Bakoa ($r = -0.28$, $p = 0.34$) but no evidence of spatial autocorrelation was shown between Boumnyebel and Talba ($r = 0.94$, $p = 0.04$).

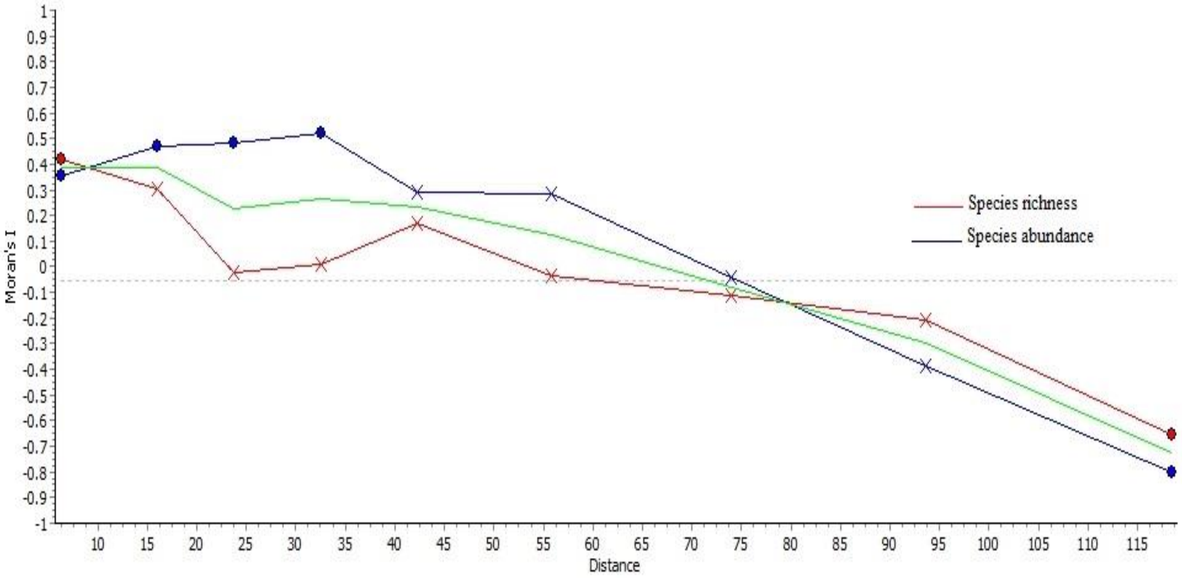

Figure 4-5: Moran's I correlograms of species richness and abundance between the five sampling sites

A total of 69 termite species belonging to two families; Termitidae and Rhinotermitidae (sub family: Coptotermitinae) were recorded. In all the localities, the family Termitidae comprising 4 subfamilies (Macrotermitinae, Termitinae, Nasutitermitinae and Apicotermitinae) was dominant in terms of richness. Rhinotermitidae was represented by only a single species (*Coptotermes sjoestedti*) recorded at one farm in one locality (Kedia). The rarefaction curves (Figure 4.6), the Shannon index, and the ANOVA result showed significant differences (p = 0.0005, df = 4) between the species richness of termites in the five AF systems.

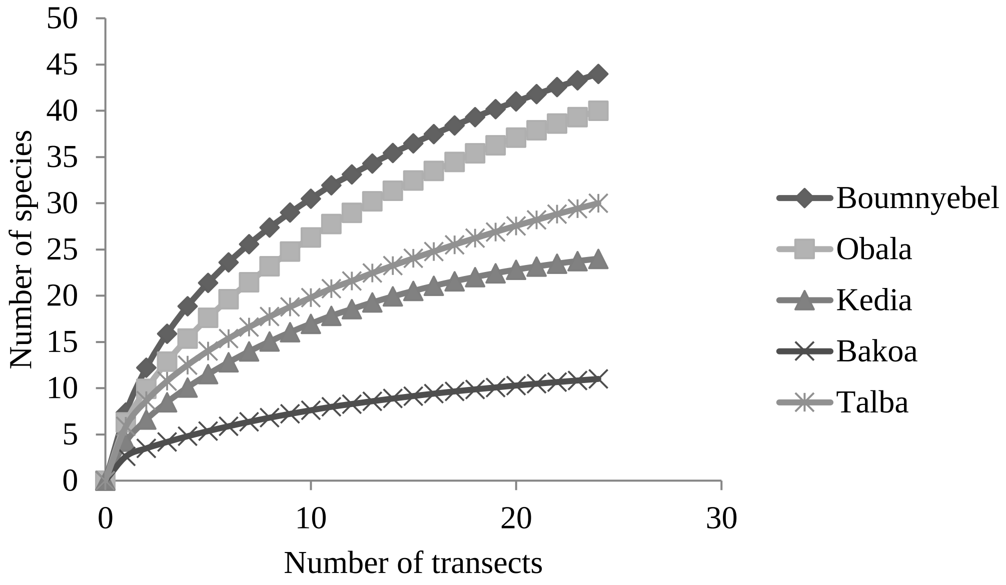

Figure 4-6: Termite species rarefaction curves of the five cocoa agroforestry shade systems

More species were recorded in Boumnyebel, followed by Obala, Talba, Kedia and the least in Bakoa (Figure 4.7). Pair-wise comparison with SNK post-hoc test showed no significant differences in species richness of termites between Boumnyebel (20.25 ± 2.68; mean ± S.E., F = 9.39, df = 4) and Obala (17 ± 2.68), Talba (12.75 ± 2.68) and Kedia (10.50 ± 2.68) or Kedia (10.50 ± 2.68) and Bakoa (5.25 ± 2.68) but significant differences in species richness were however observed between Boumnyebel (20.25 ± 2.68) and Talba (12.75 ± 2.68), Boumnyebel (20.25 ± 2.68) and Kedia (10.5 ± 2.68), Boumnyebel (20.25 ± 2.68) and Bakoa (5.25 ± 2.68), and Talba (12.75 ± 2.68) and Bakoa (5.25 ± 2.68).

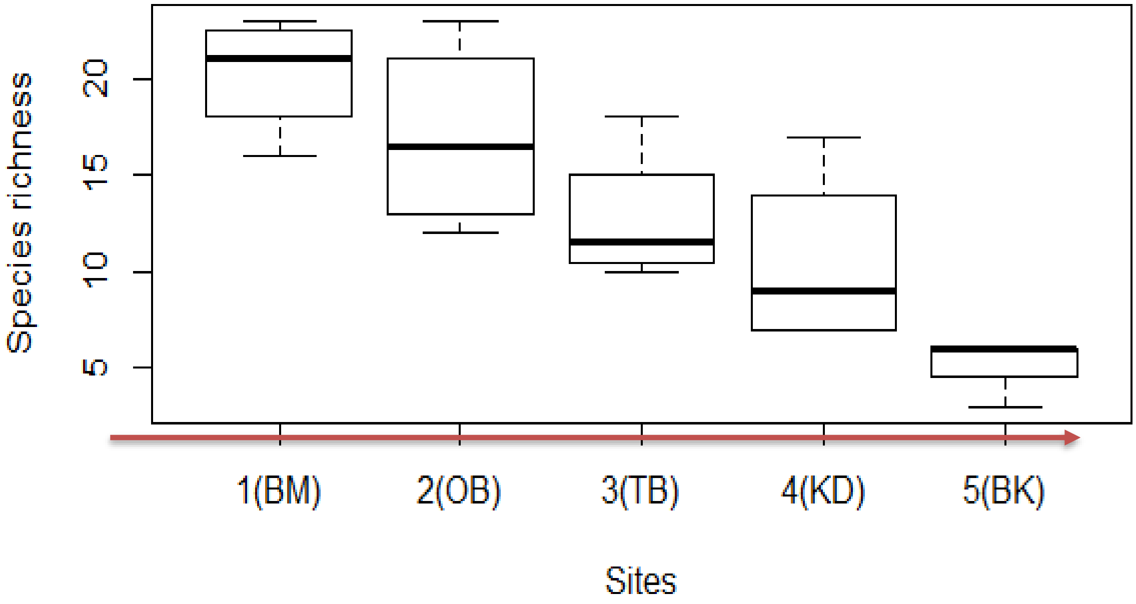

Figure 4-7: Species richness of the five cocoa agroforestry shade systems with the arrow below the x-axis showing the direction of decreased levels of shade (1(BM) = Boumnyebel, 2(OB) = Obala, 3(TB) = Talba, 4(KD) = Kedia, 5(BK) = Bakoa)

It was noted that from the 44-termite species recorded in Boumnyebel, eleven (11) species (16.18%) were endemic to this locality. In Obala, 40 species were recorded, of which 5 (7.35%) were endemic, while in Talba, 4 (5.88%) species out of the 30 species recorded were endemic to this AF system. In Kedia, 24 species were recorded, with only 2 (2.94%) endemic species. All the 11-termite species recorded in Bakoa were also found in other AF systems. It was observed that five termite species (*Ancistrotermes crucifer*, *Anenteotermes polyscolus*, *Microcerotermes parvus*, *Microtermes* sp. 1, and *Microtermes* sp. 2) from the 69 identified were recorded across all the five AF systems with *Microtermes* sp.1 scoring the highest number of specimens (211.4 ± 50.05), followed by *Ancistrotermes crucifer* (101 ± 50.05) and *Microtermes* sp. 2 (41.2 ± 50.05).

An overall Jaccard index (*JI*) between the five AF systems of 7.35% was recorded. The highest species similarity was between Boumnyebel and Obala (47%), and the lowest between Boumnyebel and Bakoa (15.91%) (Figure 4.8). *Adaiphrotermes, Astratotermes,* and *Ateuchotermes* (all soil termites) were only found in Boumnyebel and Obala, whereas *Nasutitermes diabolus* was only found in Kedia and Bakoa.

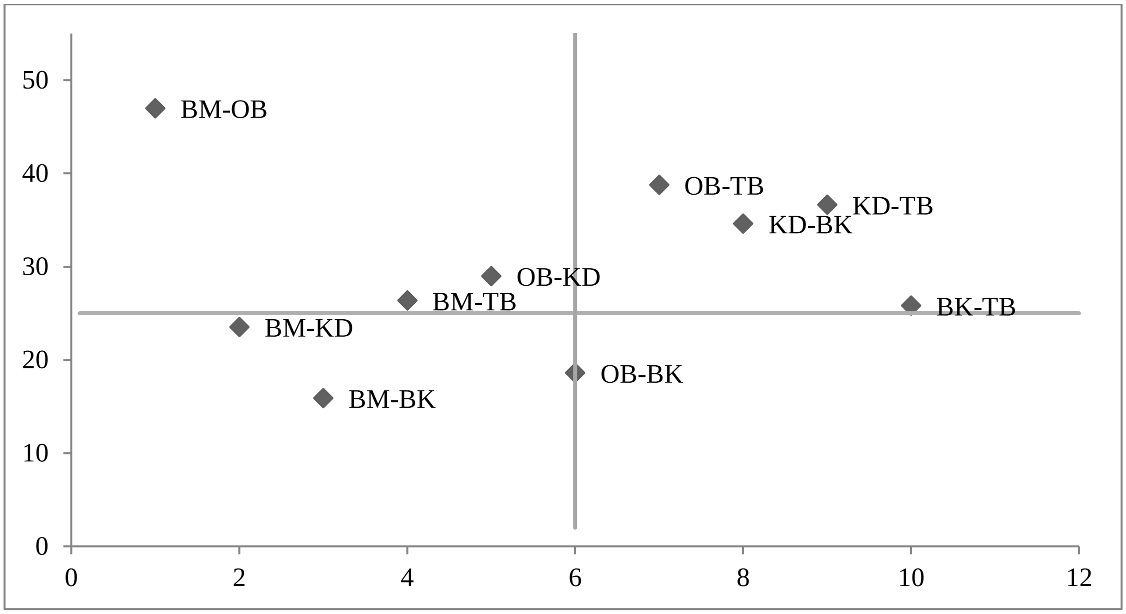

Figure 4-8: Similarity between the five cocoa agroforest types (BM = Boumnyebel, OB = Obala, TB = Talba, KD = Kedia, BK = Bakoa)

The soldier castes of some of the commonly found termite species are shown in (Figure 4.9).

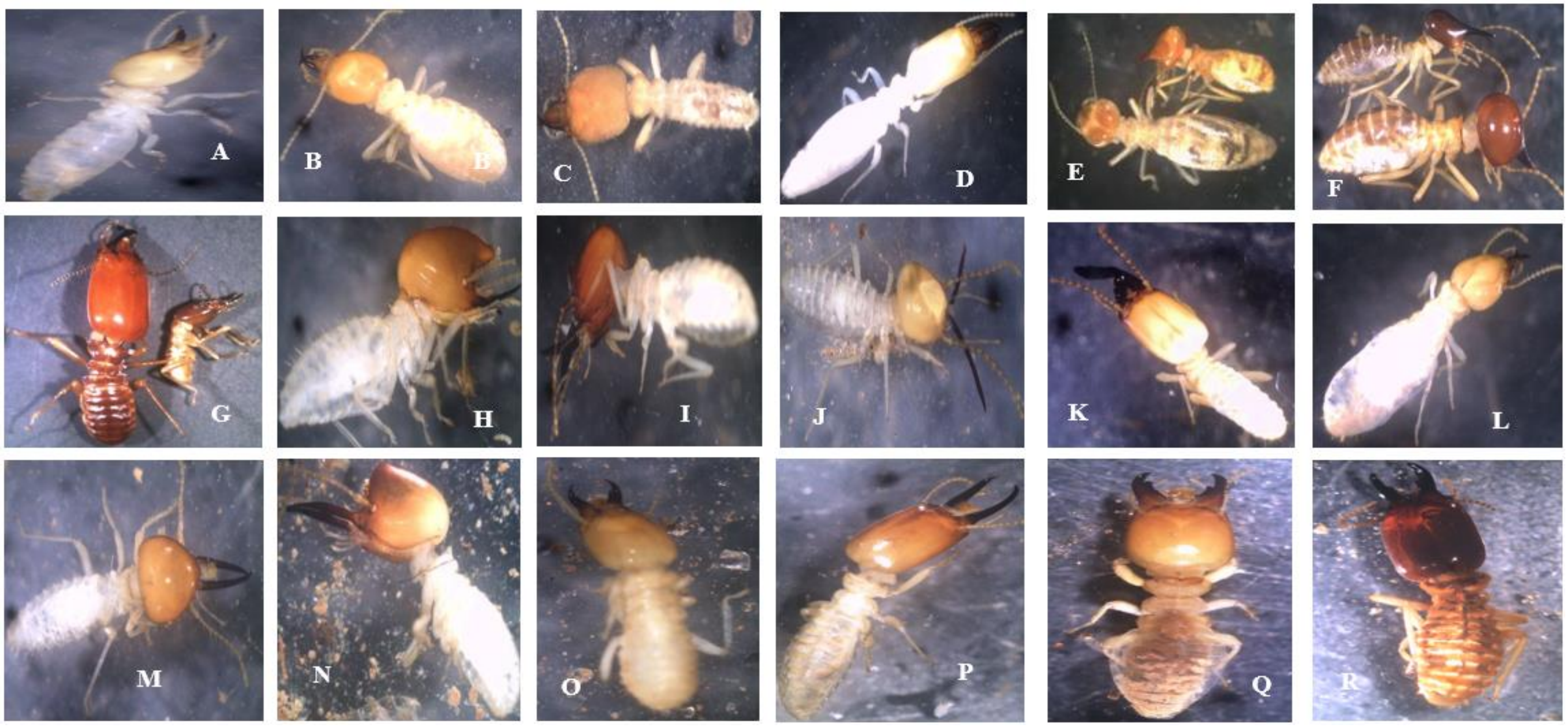

Figure 4-9: Some of the commonly sampled soldier castes of termite species at the various study sites: (A) *Microtermes* (B) *Ancistrotermes* (C) *Sphaerotermes* (D) *Microcerotermes* (E) *Nasutitermes* (F) *Trinervitermes* (G) *Pseudocanthotermes* (H) *Proboscitermes* (I) *Fastigitermes* (J) *Promirotermes* (K) *Pericapritermes* (L) *Coptotermes* (M) *Basidentitermes* (N) *Profastigitermes* (O) *Protermes* (P) *Mucrotermes* (Q) *Duplidentitermes* (R) *Odontotermes*

4.3.2 Functional group diversity

The species composition per functional group in the different AF systems varied significantly (H = 57.28, $p < 0.001$). Group II termites recorded the highest number of termites (90%), followed by Group IV (5%), Group III (4%) and Group I (1%). Group I termites were represented by only one genus (*Trinevitermes*) while Group II were represented by 11 genera with 4 genera (*Ancistrotermes*, *Microtermes*, *Microcerotermes*, *Macrotermes*) present in all the AF systems. Termites from Group II were the most frequently found functional group in Boumnyebel recorded 325 times (77.57%) out of the 419 total times recorded. This group in Boumnyebel was dominated by the genus *Microtermes* collected at 137 points, followed by *Microcerotermes* (93 points) and then *Ancistrotermes* (82 points). Termites from Group IV were the second most abundant recorded in 47 points of excavation and *Anenteotermes* the most dominant genus recorded 13 times. The remaining genera were sparsely represented. Termites in Group III from Boumnyebel were mainly dominated by the genera *Adaiphrotermes* and *Astallotermes*. Group I was represented only by one genus (*Trinervitermes*). Termites from Group II were dominant in Obala recorded 474 times out of the total 542 times recorded. *Microtermes* was also identified as dominant, with 364 times out of the 474 times recorded, followed by *Microcerotermes* recorded 47 times. Group III and IV were recorded at 33 points of excavation each with the dominant species belonging to the genera *Amicotermes* and *Anenteotermes* respectively. Functional Group I (*Trinervitermes*) was recorded only twice in Obala. Group II also dominated termites found in Talba with 591 (94.11%) out of the 628 times recorded. Again, *Microtermes* collected in 426 points was the dominant genus followed by *Ancistrotermes* (134) and *Microcerotermes* (40). Group III and IV were dominated by *Amalotermes* and *Anenteotermes* respectively, while *Trinervitermes* was collected only at one point. Group II also dominated the functional group diversity patterns in Kedia and Bakoa. In these AF systems Group III and IV were less represented while Group I was not recorded in Bakoa but represented only by a single genus *Trinervitermes* in Kedia. Like for species evenness, the functional groups in Boumnyebel were more even, followed by Obala, Kedia, Talba and then Bakoa.

The number of termites collected in the different microhabitats per AF system varied significantly ($F = 6.7$, $df = 2$, $p = 0.01$). Post hoc comparison with the SNK test showed that the mean number score of termites collected from soil (235.6 ± 38.48) was significantly higher than termites obtained in wood (132 ± 38.48) and litter (mean: 101.6 6 ± 38.48). From the 69 identified species, only 26 species were wood and litter feeders (Group I and II), while 43 species were soil feeders (Group III and VI). Functional Groups I and II had weaker correlations ($r = 0.60$ and 0.42 respectively) with reduction in shade than Groups III and IV ($r = 0.94$ and 0.99 respectively). For the nesting guilds, most of the termite species sampled in the different sites were hypogeal, followed by hypo-epigeal, while few species were arboreal termites (Figure 4.10).

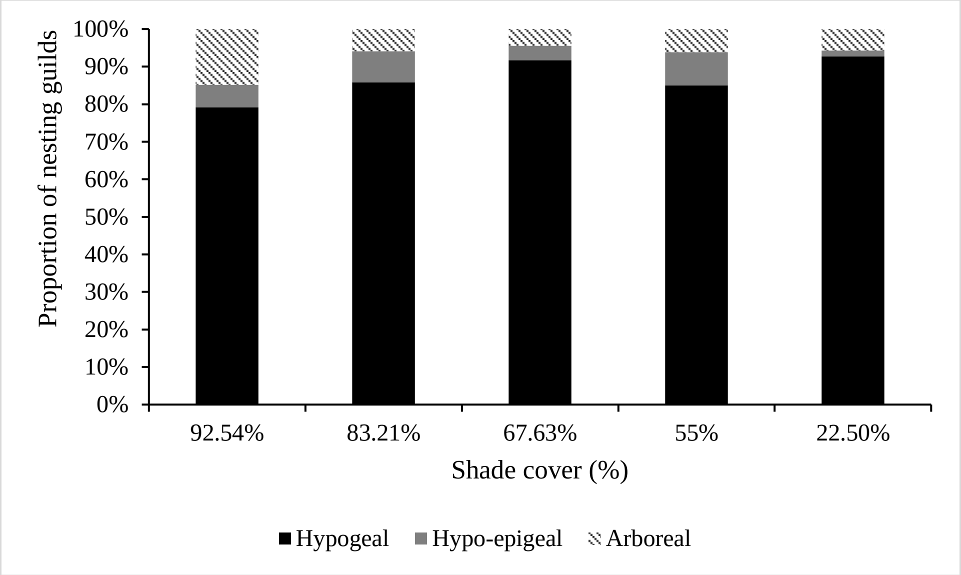

Figure 4-10: Nesting guilds of the four functional groups of termite species sampled from five cocoa agroforestry systems in Southern Cameroon

4.3.3 Effect of shade management on termites and yield

Of a total of 69 termite species sampled, 26 were pest species while 43 were non-pest species. Significant differences (F = 6.4, df = 4, p = 0.003) were observed in the species richness of pest species sampled from the five different AF systems. However, no significant differences (F = 1.22, df = 4, p = 0.34) were observed in the occurrence of the pest species in the five AF systems (Table 4.2). For the non-pest species, significant differences (F = 10.04, df = 4, p = 0.0004) were observed in the species richness and in the occurrence (F = 3.76, df = 4, p = 0.02) in the five AF systems (Table 4.2).

Richness of termite pest species was significantly lower under shade cover between 20% and 40%. The quadratic model showed a significant increase in the richness of pest species above 40% shade cover (F = 18.56, $R^2 = 0.68$; $p < 0.0001$). A significant and positive relationship between shade cover and richness of beneficial termite species (e.g., non-pest species) at shade cover >60% (F = 19.60, R2 = 0.69; $p < 0.0001$) was noted (Table 4.2).

The community weighted mean (CWM) results showed that functional Group II (pest species) dominated in all the AF systems but with a decrease with increase in shade cover, while the functional Group III and IV (soil termites) increased with increase in shade cover (Figure 4.11). The functional Groups I and II (pest species) therefore had weaker correlation (r = 0.76) with percent shade cover than Groups III and IV (soil termites) (r = 0.84). However, there was a weaker correlation (r = 0.2) of pest species occurrence and percent shade cover as compared to non-pest species occurrence and percent shade cover (r = 0.71).

Table 4.2: Termite pest and non-pest species richness and occurrence (± SE of the mean) sampled in five cocoa agroforestry systems in Southern Cameroon in 2016

Cocoa AF system	Shade cover (%)	Pest termite species richness	Pest termite species occurrence	Non-pest termite species richness	Non-pest termite species occurrence
Very heavy shade	92.54 ± 2.43 a	8.5 ± 1.7 a	82.25 ± 60.28 a	12.0 ± 1.82 a	23.00 ± 10.39 a
Heavy shade	83.21 ± 1.42 b	8.75 ± 0.5 a	119 ± 60.61 a	8.50 ± 4.43 a	16.50 ± 14.06 ab
Intermediate shade	67.63 ± 5.28 c	9.5 ± 1.7 a	148 ± 49.91 a	3.75 ± 2.63 b	9.00 ± 7.62 ab
Light shade	55 ± 5.71 d	7.75 ± 2.8 a	86 ± 22.30 a	2.75 ± 3.09 b	5.25 ± 5.5 ab
Full sun	22.5 ± 2.08 e	3.75 ± 1.2 b	90 ± 50.97 a	1.25 ± 0.50 b	1.75 ± 0.95 b

Values within a column followed by the same letter are not significantly different ($p < 0.05$, SNK test)

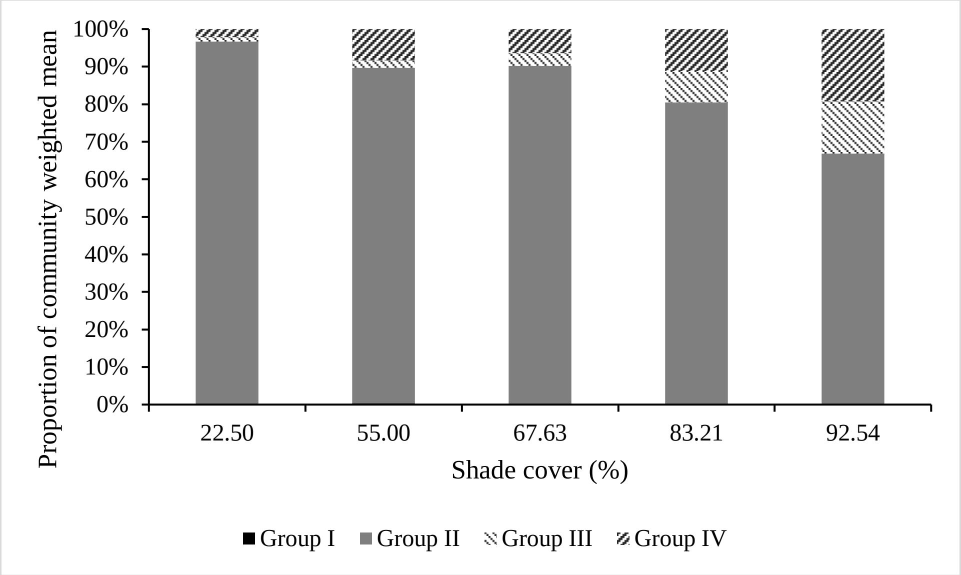

Figure 4-11: Community weighted means of the four functional groups of termite species sampled from five cocoa agroforestry systems in Southern Cameroon

Cocoa yield per AF system was significantly higher at optimal shade level between 45% and 65% after which yield decreased significantly ($F = 42.14$, $R^2 = 0.83$; $p < 0.0001$). The optimal shade levels to maintain a balance between the richness of termite pests and that of beneficial termites with cocoa yield overlapped between 45% and 65% (Figure 4.12).

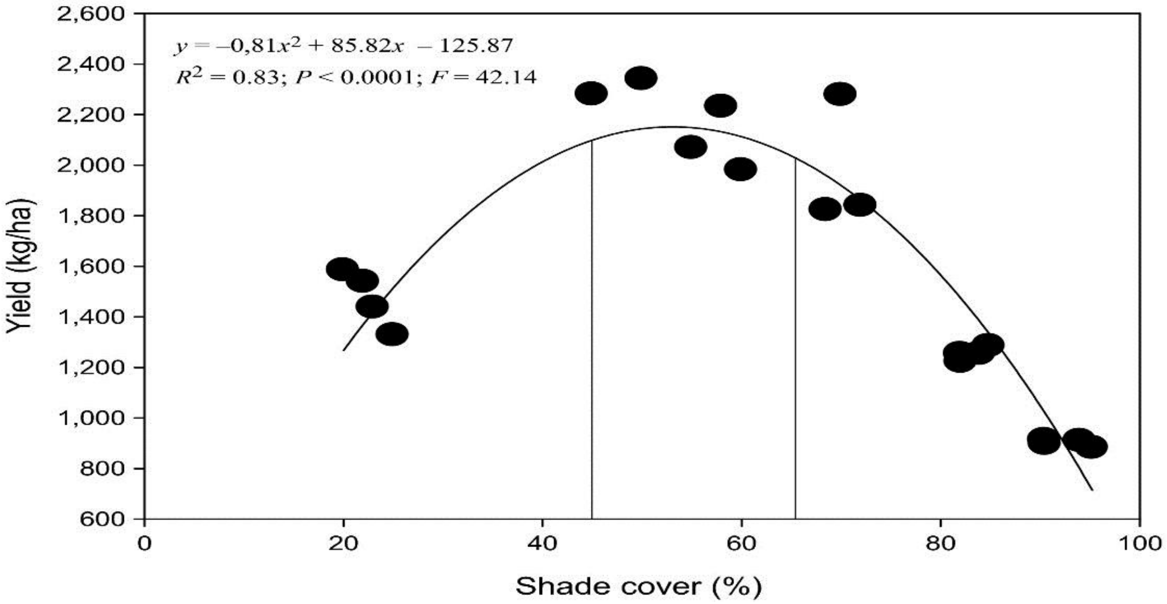

Figure 4-12: Relationship between yield and shade cover. Each point represents the mean value of all observation of 30 individual cocoa trees per plot over a 2-year period. Vertical lines indicate optimal shade levels (solid)

Table 4.3 lists the 69-termite species collected from the five cocoa agroforestry systems in the centre region of Cameroon.

Table 4.3: List of the 69-termite species collected from the five cocoa agroforestry systems in Cameroon and classified according to their functional, nesting and feeding groups. Feeding groups: W= wood feed, W/L/P = wood and litter feeding including feeding on cocoa live plant, W/S = wood/soil feeding, (F) = fungus growing, S = soil-feeders

Species	Functional Group	Nesting Group	Feeding Group	Species occurrence at the various sites				
				Boumnyebel	Obala	Kedia	Bakoa	Talba
Rhinotermitidae: Coptotermitinae								
Coptotermes sjostedti Holmgren	II	Hypogeal	W/P			2		
Termitidae: Macrotermitinae								
Microtermes sp. 1	II	Hypogeal	W/L/P (F)	82	256	144	184	341
Microtermes sp. 2	II	Hypogeal	W/L/P (F)	52	106	12	3	35
Microtermes sp. 3	II	Hypogeal	W/L/P (F)	3	2			
Ancistrotermes crucifer (Sjoestedt)	II	Hypogeal	W/L/P	81	18	111	161	134
Ancistrotermes sp. 1	II	Hypogeal	W/L/P		1			
Macrotermes bellicosus (Smeathman)	II	Hypo-epigeal	W/L/P		12	1		2
Macrotermes lilljeborgi (Sjoestedt)	II	Hypogeal	W/L/P		1	1	2	2
Odontotermes culturarum Sjoestedt	II	Hypogeal	W/L/P			6		6

Odontotermes silvaticus Harris	II	Hypogeal	W/L/P					1
Odontotermes bequaerti Emerson	II	Hypogeal	W/L/P			2		3
Odontotermes schmitzi	II	Hypogeal	W/L/P	9		9		3
Pseudacanthotermes militaris (Hagen)	II	Hypo-epigeal	W/L/P (F)		27	31	6	
Sphaerotermes sphaerothorax Holmgren	II	Hypogeal	W	5	2			9
Protermes prorepens	II	Hypogeal	W		2			
Synacanthotermes heterodon	II							2
Termitidae: Termitinae								
Termes-group								
Pericapritermes nigerianus	III	Hypogeal	S	5		3		
Pericapritermes sp. nov. 1	III	Hypogeal	S	1	2			
Promirotermes orthoceps	III	Hypogeal	S				1	2
Profastigitermes putnami Emerson	IV	Hypogeal	S	5	3	3	2	
Fastigitermes jucundus (Sjoestedt)	IV	Hypogeal	S	3	3	5		5
Proboscitermes tubuliferus	IV	Hypogeal	S	3	1	1		2
Cubitermes-group								
Apilitermes longiceps Holmgren	IV	Hypogeal	S	1				
Apilitermes sp. nov. 1	IV	Hypogeal	S	2				
Basidentitermes malelaensis (Emerson)	IV	Hypogeal	S	4				

Cubitermes severus	IV	Hypogeal	S	7				
Furculitermes winifredi Emerson	IV	Hypogeal	S	1				
Amitermes-group								
Microcerotermes fuscotibialis (Sjoestedt)	II	Arboreal	W	26	6			19
Microcerotermes parvus (Haviland)	II	Arboreal	W	32	30		1	1
Microcerotermes edentatus Wasmann	II	Hypogeal	W	18	3			2
Microcerotermes progrediens	II		W	13	8			23
Microcerotermes sylvestrianus	II		W	4				
Termitidae: Nasutitermitinae								
Nasutitermes arborum (Smeathman)	II	Arboreal	W/P			7		8
Nasutitermes eleganthulus (Sjoestedt)	II	Arboreal	W/P	1		7		
Nasutitermes diabolus ((Sjöstedt)	II	Arboreal	W/P			5	1	
Trinervitermes carbonarius Sjöstedt	I		G	1		3		
Trinervitermes roseni (Holmgren)	I			2	2			1
Termitidae: Apicotermitinae								

Anoplotermes-group

Adaiphrotermes choanensis (Fuller)	III	Hypogeal	S	12	1			
Adaiphrotermes cuniculator Sands	III	Hypogeal	S	1				
Aderitotermes fossor Sands	III	Hypogeal	S	3			2	2
Aderitotermes cavator Sands	III	Hypogeal	S					1
Aderitotermes sp. nov. 1	III	Hypogeal	S	2	4			
Alyscotermes trestus Sands	III	Hypogeal	S		1			
Alyscotermes kilimandjaricus (Sjoestedt)	III	Hypogeal	S		2	2		1
Alyscotermes sp. nov. 1	III	Hypogeal	S	1				
Amalotermes phaeocephalus Sands	III	Hypogeal	W/S	1	2			6
Amalotermes sp nov. 4								1
Amicotermes galenus Sands	IV	Hypogeal	S		1			1
Amicotermes sp. nov. 1	IV	Hypogeal	S		3			
Amicotermes sp. nov. 2	IV	Hypogeal	S	2	1			
Amicotermes sp. nov. 3	IV	Hypogeal	S		1			2
Amicotermes sp. nov. 4	IV	Hypogeal	S		1			4
Anenteotermes polyscolus Sands	IV	Hypogeal	S	9	16	1	4	10
Anenteotermes sp. nov. 1	IV	Hypogeal	S	2				
Anenteotermes ateuchestes Sands	IV	Hypogeal		1				
Astalotermes amicus Sands	III	Hypogeal	S	4	2			1

Astalotermes quietus (Silvestri)	III	Hypo-epigeal	S	8	6			
Astalotermes empodius Sands	III	Hypogeal	S	1	1			
Astalotermes sp. nov. 1	III	Hypogeal	S	1	4			
Astalotermes sp. nov. 2	III	Hypogeal	S	1				
Astratotermes aneristus Sands	III	Hypogeal	S		1			
Astratotermes pacatus (Silvestri)	III	Hypogeal	S	1	1			
Astratotermes prosenus Sands	III	Hypogeal	S	2	1			
Ateuchotermes ctenopher (Sands)	IV	Hypogeal	S	1	5			
Apicotermes-group								
Duplidentitermes furcatidens (Sjoestedt)	IV	Hypogeal	S		2	2		
Eburinitermes sp.	IV	Hypogeal	S	3				
Apicotermitinae new genus	IV	Hypogeal	S			2		
Total number of encounters				416	542	365	367	628
Observed number of species (S_{obs})				44	40	24	11	30
Expected richness (Jackknife 1(mean ± S.E))				60.29 ± 0.46	55.33 ± 0.52	29.75 ± 0.30	14.83 ± 0.22	43.42 ± 0.45
Sampling effort				73%	72%	81%	74%	70%
Shannon-Wiener index (*H*')				2.76	2.03	1.87	1.00	1.65
Functional evenness (*H*')				0.71	0.48	0.30	0.11	0.27

4.3.4 Termite incidence/damage on cocoa trees

A total of 1654 cocoa trees were sampled and 10 species of termites building galleries on cocoa were identified, all belonging to the family Termitidae; subfamilies Macrotermitinae (*Microtermes* sp1 and *Microtermes* sp2), Nasutitermitinae (*Nasutitermes arborum*, *Trinervitermes trinervius* and *Trinervitermes rhodesiensis*), Apicotermitinae (*Amalotermes phaeocephalus*) and Termitinae (*Microcerotermes edentus*, *Microcerotermes parvus*, *Microcerotermes progrediens* and *Microcerotermes fuscotibialis*). These species are all wood feeders' group except *Amalotermes phaecephalus* which is a soil-wood feeder. The very heavy shaded, shaded and intermediate shaded systems of Boumnyebel, Obala, and Talba respectively recorded more species richness of termites on cocoa trees, with 9 species in Boumnyebel, 7 in Obala, and 6 in Talba while only 1 species was recorded in the full sun and low shade systems of Bakoa and Kedia. The most frequent species recorded on trees were *Microcerotermes edentus*, *Microcerotermes parvus*, *Microtermes* sp2 and *Nasutitermes arborum*.

The heavy shaded systems had more galleries on cocoa trees than the low shaded systems. According to plot infestation, all plots presented galleries on cocoa trees at Boumnyebel (100% prevalence) and this infestation decreases with shade tree removal, with lowest infestation at Bakoa with a prevalence of 12.5%. The number of infested trees was also very high in heavy shaded systems with a prevalence of 69.77% at Boumnyebel followed by Obala (36.78%). The prevalence in the heavy shaded system (Boumnyebel) was significantly higher than that of the full sun and low shaded systems of Bakoa and Kedia respectively.

Overall, the highest damage level in this study was the primary level. However, the heavy shaded systems had more damage on cocoa trees compared to low shaded systems. Boumnyebel presented higher level of damage reaching sometimes the tertiary level and the most observed level in this agrosystem was the secondary level. Obala and Talba also had some damages of termites on cocoa trees but the primary level was the most frequent. Very little damage was observed on cocoa trees in Bakoa and Kedia.

4.4 Discussion

This study shows that shade management in cocoa agroforestry systems strongly impacts termite species richness and composition. It also documents that rustic cocoa agroforestry systems with heavy shade such as practiced in Boumnyebel revealed higher termite species richness and functional group diversity than in low shaded or full sun cocoa agroforestry systems. Soil-feeding termites are strongly affected by shade removal than wood and litter feeders which are adapted to drier conditions. This suggests that when shade cover drops below a threshold the soil-feeder assemblages will diminish.

Analysis from the species accumulation curves corroborated with the results from diversity indices, with Boumnyebel having more termite species than Obala, Talba, Kedia and Bakoa. However, Boumnyebel, Obala and Talba curves showed no signs of reaching a plateau, although the rate of increase for Boumnyebel was higher than that of Obala, Talba and Bakoa and the sampling effort was more than 70% for both AF systems. Previous ecological studies on the species richness of termites using the same approach obtained similar curves, showing the difficulty to stabilize accumulation curves when sampling termites in places with high diversity (Constantino, 1992; Carrijo et al., 2009). According to Chao et al. (2009), species accumulation curves have a disadvantage when large numbers of individuals must be sampled to reach the asymptote as high richness and rarity of many species makes estimation of sampling effort with accumulation curves difficult to predict. The sequence in species richness in the selected AF systems was (in decreasing order) Boumnyebel (rustic shaded system)-Obala (moderate shade)-Talba (intermediate shade) - Kedia (light shade) and Bakoa (full sun). Previous studies on the effect of habitat fragmentation on the diversity of termites across forest landscape concluded that termite diversity decreases along the sequence from primary forest to secondary forest and food crop fields (de Souza and

Brown, 1994; Davies, 2002). Other studies have shown that termite richness is higher in mature forests, intermediate in reforestation areas, and lower in secondary forest and pastures (de Paula et al., 2016). Since the rustic shade system of Boumnyebel and the moderate shade of Obala could be compared to secondary forest, and Kedia and Bakoa systems of very light shade and full sun cocoa to food crop fields, we can clearly see that the pattern of termite species richness observed in this study corroborates with previously reported results. Our results showed that removal of shade trees in cocoa plantations usually lead to hotter and drier microclimates that are not suitable for the survival of many soil-feeding termites which are subterranean and soft bodied and therefore more sensitive to variation in microclimate (de Souza and Brown, 1994; Davies et al., 2012). This implies that differences in shade management in cocoa agroforestry systems strongly impact termite species richness and composition.

The 11-termite species that were exclusively sampled in Boumnyebel and the 4 species that were exclusively found in Obala were all soil-feeding termites. *Anenteotermes polyscolus* was the only soil termite recorded in all the AF systems. Apart from *A. polyscolus*, three other soil termite species *Promirotermes orthoceps* (recorded in Cameroon for the first time), *Profastigitermes putnami,* and *Aderitotermes fossor* were encountered in Bakoa under full sun cocoa production. Besides being the lowest locality in species richness, no species was endemic to Bakoa. This implies that apart from the wood and litter feeders (Jones et al., 2003), many species are lost as shade cover decreases, but are not displaced by others. Contrary to our findings, Norgrove et al (2009) in their study of termite fauna in shaded cocoa plantations of southern Cameroon did not record *Apilitermes*, *Cubitermes*, *Microcerotermes* and *Nasutitermes.* This could be due to the fact that the sampling methods differ in their emphasis on particular substrates, whereas in this study, the transect method used thoroughly sampled soil as well as leaf litter.

Termite species from the Termitidae family represented more than 95% of the total species recorded. Similar conclusions were made by Couto et al. (2015). These termite species easily adapt to disturbance because they can utilize other sources of cellulose in addition to wood (Inward et al., 2007), hence their distribution in all the AF systems. The most common species of Termitidae encountered in all the AF systems included *Microtermes* spp., *Ancistrotermes* spp. and *Microcerotermes* spp. *Microtermes* and *Microcerotermes* were sampled on the truck and galleries on cocoa trees in the rustic shaded system (Boumnyebel), moderate shade (Obala) and intermediate shade (Talbal) cocoa agroforests, as well as on broken branches of cocoa hanging from the main branches, while *Ancistrotermes* were sampled on roots and soil around cocoa trees in all the AF systems. These species are known to be responsible for a lot of damage on cocoa and companion trees (Pomeroy et al., 1991; Mitchell, 2002). *Ancistrotermes* spp. feed on the root systems of young and mature cocoa trees while *Microtermes* and *Microcerotermes*, by building their galleries on cocoa trunk, contribute to reduced flowers and pod production negatively affecting yield and productivity (Vos et al., 2003; Ackonor, 1997). Damage from *Ancistrotermes* and *Microtermes* species was more severe in cocoa farms at the forest-savanna transition zone (e.g. Kedia and Bakoa). These species have been reported to tolerate semi-arid and even arid environmental conditions such as the one of the full sun and light shade systems where they are responsible for up to 80% seedling mortality following their transplantation (Rouland-Lefevre & Mora, 2002). Therefore, their high abundance in cocoa agroforests should be a great concern to cocoa stakeholders, the cocoa industry and the cocoa producing countries in particular.

Functional group composition was strongly correlated with variation in shade level, with members of functional Groups III and IV (soil feeders) being most abundant in the shaded systems and rare in the low shade and full sun systems. This result is in line with previous findings from southern Cameroon, where tree canopy cover was positively correlated with termite abundance and species richness, with a decline in soil faunal population when trees were removed from land use systems (Dibog et al., 1999).

Soil-feeding termites have been shown to be vulnerable to habitat clearance, decreasing in species richness over wood feeders (Bandeira et al., 2003; Eggleton et al., 2002). Soil feeders are strongly affected by low shade, as they need stable conditions to fill their energetic limitation gaps (Isra et al., 2007). Functional Groups I and II had weaker correlations with reduction in shade than Groups III and IV. This observation could be attributed to the fact that they possess higher exoskeleton sclerotisation which provides resistance to desiccation in open habitats like Bakoa and Kedia (Luke et al., 2014). Other researchers have also found that wood feeders are more resilient to habitat conversion than soil feeders (Eggleton et al., 1995, 2002; Jones et al., 2003). Therefore, the significant decreases in occurrence of the soil feeders in Bakoa and Kedia could also be because these termite groups are prone to desiccation (Gathorne-Hardy et al., 2002). In Kedia and Bakoa, leaf litter and crop residues may also provide a year-round available and abundant food resource for wood and litter feeders (Davies, 2002). This explains the abundance of *Ancistrotermes* and *Microtermes* species accounting for the dramatic increase in wood feeding termites in Bakoa and Kedia as compared to Bounmyebel, Obala and Talba.

Significantly more termite species and galleries were found on cocoa trees in the heavily shaded systems. This can be explained by the high humidity and hygrometry conditions favorable for arboreal termites to build galleries on trees while in low shaded systems, the high temperatures caused by direct sunlight on the cocoa trees prevent termites from building galleries on cocoa trees. Most of the termite species dwell in soil and feed on dry wood and roots, causing damage to the roots. No significant difference was also observed in the number of pods counted on infested and non-infested cocoa trees. This clearly shows that the presence of galleries on trees does not necessarily imply damage on cocoa trees that could lead to a reduction of yield, but the damage caused by subterranean termites which feed on the roots of cocoa trees does bring about damage to the trees.

4.5 Conclusions

The results of our study conclusively showed that richness and evenness of termite species are driven by cocoa shade systems, with emphasis that: (i) the number of termite species in the shaded systems was more diversified than that of the unshaded systems, demonstrating that shade management characteristics dictate termite faunal differences; (ii) the shaded systems maintained all the termite species found in the full sun systems, and harboured a diversity of species, suggesting that the establishment of shade in cocoa agroforestry systems conserves important part of functional biodiversity of termites; and, (iii) most damages in cocoa agroforests is associated with members of the sub-families Macrotermitinae and Termitinae. The high number of occurrence of members of these sub-families across all the localities call for a need to develop adequate management strategies against these species, and to implement appropriate agricultural practices and extension programs targeting shade management and emerging pests such as termites in cocoa agroforestry systems. Furthermore, soil-feeding termites which create and maintain favourable soil conditions for plant growth and nutrient intake are more vulnerable to reduced shade cover over wood feeders which are becoming main pests of cocoa trees. Shade reduction is also affecting wood feeders which are gradually shifting their food sources from leaf litter and crop residues to becoming main pest of cocoa trees feeding mainly on the root system. Our results indicate that cocoa agroforestry systems with around 55% to be optimal to balance termite infestations and marketable yield, should therefore be encouraged in cocoa production systems to create favorable conditions for the survival of soil-feeding termites. Shaded cocoa agroforestry systems will maintain beneficial ecosystem services and minimize potential ecosystem disservices through trophic and non-trophic interactions. Investment is also needed to encourage and educate local farmers about the diverse ecosystem services provided by shaded systems.

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5 CHAPTER FIVE: SCREENING AND SELECTION OF VIRULENT ENTOMOPATHOGENIC FUNGAL ISOLATES FOR CO-FORMULATION WITH CO_2 GENERATING MATERIAL FOR CONTROL OF SUBTERRANEAN TERMITE PESTS IN COCOA AGROFORESTS

ABSTRACT

Subterranean termites are significant pests of cocoa and cocoa agroforestry crops. Efforts to control termite infestation depend heavily on termiticide applications, with harmful effects to humans and the environment. Natural associated pathogens to termites such as entomopathogenic fungi (EPF) are promising alternatives to chemical control. However, there are limitations in their application because highly virulent EPF are also found to be repellent to termites. Subterranean termites use CO_2 to locate cocoa roots, thus making the use of EPF a promising biocontrol strategy against termites when combined with CO_2. This study was conducted to screen for virulent EPF isolates to co-formulate with a CO_2 generating material for subterranean termite pest control in cocoa agroforests. Termites (*Odontotermes* spp.) were collected from infested wood around *icipe* Duduville campus and tested for susceptibility to 14 entomopathogenic fungal isolates. The fungal isolates were evaluated by direct spraying of conidia suspensions at 1×10^8 conidia/ml concentration on termite workers. In general, all the isolates screened were pathogenic to the termites, achieving 100% mortality 4-7 days post-inoculation. However, *Metarhizium* isolates were more virulent with lower LT_{50} values (1.5–2.84 days) than *Beauveria* isolates (LT_{50} ranging from 2.23-4.41 days). The most virulent isolate was *M. brunneum* Cb15-III, followed by *M. anisopliae* isolates ICIPE 30 and ICIPE 60, while the least virulent were *B. bassiana* isolates ICIPE 273, followed by ICIPE 603 and 660. *Metarhizium* isolates are thus suitable for co-formulation with CO_2 generating materials for control of subterranean termite pests in cocoa agroforests.

Keywords: *Beauveria bassiana*, Entomopathogenic fungi, *Metarhizium anisopliae*, *Metarhizium brunneum*, subterranean termites, relative pathogenicity, virulence

5.1 Introduction

Cocoa (*Theobroma cacao* Linn.) is the "engine of economic growth" in many areas of West and Central Africa (Duguma et al., 2001). It is an important export crop and the main source of foreign exchange for Ghana, Cameroon, Ivory Coast, and Nigeria (Padi and Owusu, 1998; Falola et al., 2013). However, despite the economic importance of cocoa and farmers' sustained interest in production, its production continues to decline over the years (Dormon et al., 2007). The main reasons for this low productivity are the ravages caused by disease and insect pests including termites which harm the cocoa crop, especially the roots of young seedlings after transplanting (Ambele et al., 2018a, b). Although some termite species are decomposers and have a positive impact on numerous ecological functions, others become serious pests when they attack agricultural crops. In the past, termites were considered as minor pests of cocoa, but they are now becoming major pests in cocoa agroforests because of intensification by eliminating shade trees to increase short-term income, climate change and increase pesticide use to control above-ground pests, thereby destroying their natural enemies (Bisseleua et al., 2013; Ambele et al., 2018a, b). Termites cause direct damage on cocoa by destroying the roots and stems or by cutting down seedlings mostly in the dry season. They also attack cocoa plants in the field by attacking the trunks and pods of cocoa, causing the plant and the pods to dry up after severe infestations (Anikwe et al., 2009). In addition to cocoa, termites also damage crops associated with cocoa as well as shade trees and agroforestry fruit trees.

In a current study conducted in cocoa agroforests in Cameroon, Ambele et al. (2018b) reported the *Microtermes*, *Ancistrotermes*, *Odontotermes* and *Microcerotermes* species as the major termite pests of cocoa. Other studies have also shown that these species are responsible for most of the crop damage and 90% of tree mortality in forestry (Ackonor, 1997; Mitchel, 2002; Tra Bi et al., 2015;). *Ancistrotermes* and *Microtermes* species have been shown to cause up to 80% mortality in 4-6 months old plants after transplanting seedlings (Wood and Pearce, 1991). The high abundance of these termite species in cocoa agroforests calls for concern and a need for effective and sustainable control strategies to be developed against them.

Termite control has relied exclusively on persistent organochlorine (cyclodiene) insecticides (Logan et al., 1990), which are not only expensive for farmers but are also under restrictive use due to increasing concern over risk posed to human health and the environment (Su and Scheffrahn, 1998; Blackshaw and Kerry, 2008). Farmers in many parts of Africa use various traditional termite control methods to avoid chemicals and their unwanted side effects but with limited success (Ambele et al., 2018a). There is therefore a need for identification of eco-friendly alternative tools for termite control. Biological control is generally perceived as providing both long-lasting insect control and having less potential for damage to the environment or non-target organisms than chemical interventions. EPF provide an opportunity for sustainable control of various agricultural pests including termites in cocoa agro-ecosystems. Among these EPF, *Metarhizium* and *Beauveria* are potentially the most useful genera in termite biocontrol because of their self-replicating nature and safety to non target animals (Maniania et al., 2002; Chouvenc et al., 2011). *Metarhizium anisopliae* are especially recommended for practical control of termites due to their easy mass production, formulation and conidial persistence in termite nests (Milner et al., 1996). Another advantage of EPF is that to date no reports of resistance development has been reported. However, there are limitations (repellency and host avoidance and defense mechanisms) in their application against termites in the field when applied as a conidial suspension (Yanagawa et al., 2008; Mburu et al., 2009).

A combination of an active ingredient with an attractant semiochemical used by the pest for host plant location could increase the chances for a contact between the target and the toxic substance (Huang and Mack, 2001). Such a combination of “attract and kill” (A&K) or attract and infect (A&I) strategies have been shown to improve efficacies resulting in superior control levels as compared to other control methods (Schumann et al., 2013). Additionally, A&K mechanism can be more effective against pest species with cryptic habitats and complex environments that are normally difficult to reach with ordinary application techniques (El-Sayed et al., 2009). For soil pests like subterranean termites feeding on tree roots, such an attractant is CO_2 - a volatile released by respiring plant roots (Bernklau et al., 2005; Schumann et al., 2013). This study was designed to screen different isolates of *Metarhizium* and *Beauveria* for their time-mortality effects against termite species in order to select virulent ones that could be co-formulated with CO_2 generating materials for control of subterranean termite pests under the umbrella of termite-IPM in cocoa agroforests.

5.2 Materials and methods

5.2.1 Description of the experimental site

These experiments were carried out at the Arthropod Pathology Unit (APU) of the International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus in Nairobi, Kenya (S 03.35517°, E 037.33861°, and 1616 m.a.s.l.).

5.2.2 Termites collection and maintenance

Termites (*Odontotermes* spp.) were collected from infested wood around *icipe* campus and placed on sterilized soil in plastic containers. They were then transferred into an incubator (26 ± 2 °C and 75 ± 5% RH in the dark) and kept for 30 minutes for acclimatization before being used in the bioassays. The relative humidity in the incubator was controlled using stable saturated solution of K_2SO_4 (Supelco, Sigma-Aldrich, United Kingdom). Active and no injured worker castes with uniform size were selected for the various treatments.

5.2.3 Fungal culture and suspension preparation

The origin and source of the fungal isolates used in this study are summerised in Table 5.1. Six fungal isolates of *Metarhizium anisopliae* and seven of *Beauveria bassiana* were obtained from the *icipe* APU germplasm, while *M. brunneum* Cb15-III was obtained from Bielefeld University, Germany. *Metarhizium* isolates were cultured on Sabouraud Dextrose Agar (SDA) while *Beauveria* isolates were cultured on Potatoes Dextrose Agar (PDA) medium in complete darkness at 25 ± 1°C. Conidia were harvested from 2 to 3-week-old surface cultures by scraping with a sterile spatula and suspending in 10 ml distilled water containing 0.05% Triton X-100 (Fluka, Sigma-Aldrich, UK) and five glass beads (ϕ = 3 mm) in 30 ml universal bottles. The suspensions were filtered through cheese-cloth to separate the conidia from the hyphae and remove mycelia debris. Bottles were stoppered and then vortexed in a Vortex (Genie 2 Scientific Industries, Bohemia, New York) for 5 min at about 700 rpm to break the conidial clumps and ensure a homogeneous conidial suspension. Conidial concentrations were quantified using an Improved Neubauer haemocytometer (Weder Scientific International Ltd, Teddington, UK) under a light microscope. The conidial suspensions were adjusted to 1×10^8 conidia/ml through dilution in distilled water prior to bioassays.

Table 5.1: Identity of fungal isolates screened against the termite *Odontotermes* spp. for virulence under laboratory conditions.

Fungal species	Isolates	Source	Locality/Country	Year of isolation
Metarhizium anisopliae	ICIPE 20	Soil	Migori (Kenya)	1989
	ICIPE 30	*Busseola fusca*	Kendubay (Kenya)	1989
	ICIPE 60	Soil	Kakelo-Seme (Kenya)	1990
	ICIPE 18	Soil	Mbita (Kenya)	1989
	ICIPE 69	Soil	Matete (DRC)	1990
	ICIPE 78	*Temnoschoita quadripustulata*	Ungoe (Kenya)	1990
Metarhizium brunneum	Cb15-III	-	Germany	-
Beauveria bassiana	ICIPE 273	Soil	Mbita (Kenya)	2006
	ICIPE 279	Coleopteran larva	Kericho (Kenya)	2005
	ICIPE 284	Coleopteran larva	Mauritus	2005
	ICIPE 603	Hymenoptera	Taita (Kenya)	2007
	ICIPE 706	Monocots	Kenya	2008
	ICIPE 660	Soil	Chemokock (Kenya)	2008
	ICIPE 662	Soil	Mariakani (Kenya)	2008

5.2.3.1 Conidial germination test

For viability test, a concentration of 3 × 10^6 conidia/ml was prepared, and 0.1 ml of the suspension was evenly spread on SDA or PDA plate and three sterile microscope cover slips (22 mm × 22 mm) were placed randomly on the surface of each inoculated plate. The plates were sealed with Parafilm and incubated under complete darkness at 25 ± 2°C. At 18 hr post-inoculation, lactophenol cotton blue was added to terminate germination and stain the spores for ease of counting. The percentage germination of conidia was determined from 100 spore counts under the cover slips at 400× magnification under a light microscope using the method described by Goettel and Inglis (1997). Conidia were considered to have germinated when the length of the germ tube was at least twice the diameter of the conidium (Goettel and Inglis, 1997; Inglis et al., 2012) (Figure 5.1). Four replicate plates were used per isolate, and viability of each isolate was determined, where more than 95% germination was obtained for all the isolates (Table 5.1).

Figure 5-1: Germinated conidia of *Metarhizium anisopliae* at x 400 manification

5.2.3.2 Screening of fungal isolates for time-mortality responses

Fourteen fungal isolates (7 *Metarhizium* and 7 *Beauveria*) were screened against the worker termites of *Odontotermes* spp. The termites used were larger workers involved in foraging and therefore the most vulnerable to pathogens and mostly act as carriers of pathogens. Twenty (20) termites per treatment were counted and placed in round plastic containers of 12 cm diameter × 6 cm. To avoid contamination, the control samples were sprayed with 10 ml sterile distilled water containing 0.05% Triton X-100 (Fluka, Sigma Aldrich, UK) (without conidia) prior to fungal treatments inoculation using a Potter spray tower. For the fungal treatment groups, each plastic bowl containing the test termites was sprayed with 10 ml of a standard concentration of 1×10^8 conidia ml^{-1}. The spray tower was cleaned with 90% alcohol and sterile distilled water after each treatment. To maintain social cohesion within the group (Sun et al., 2003), two soldier termites were also added into each plastic bowls after conidial inoculation. A piece of wet cotton wool was used to maintain high humidity in each plastic bowl throughout the experiment. The lids of the plastic bowls had aeration holes to ensure free flow of air. About five pieces of sterile wood were added as food and sterilized soil (sterilized by autoclaving at 121°C for 1 hr) was provided as shelter after applications of conidia on the termites. The various treatment bowls were placed on moist cotton wool spread on a metal stand. The metal stand was immersed in a stable saturated solution of K_2SO_4 to maintain moisture in bigger plastic containers (length = 34 cm, width = 24 cm and height = 20 cm). The plastic containers were then placed in an incubator maintained at 20 ± 2° and 75 ± 5% RH (Figure 5.2). Mortality was recorded daily for seven days for the calculation of LT_{50} values (time needed to cause 50% mortality to the exposed termite population) for each of the fungal isolates and controls.

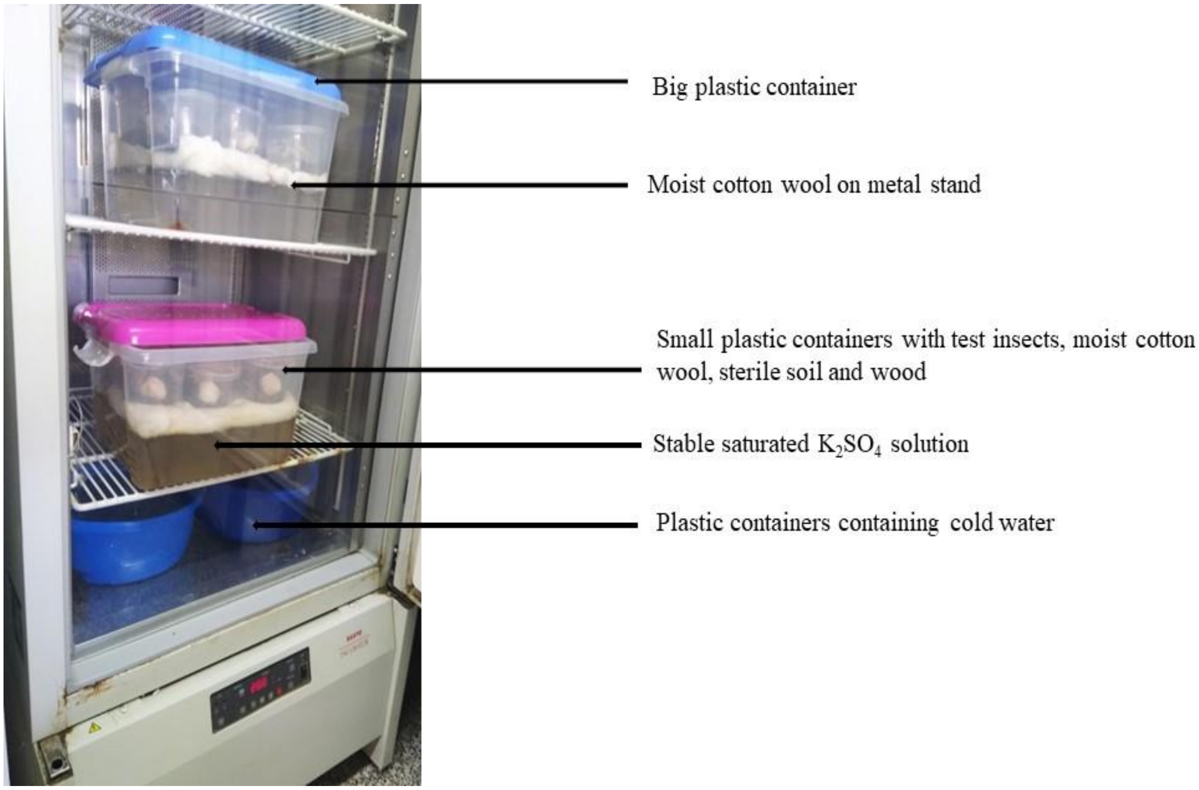

Figure 5-2: Set up of bioassay experiment to assess virulence of the various entomopathogenic fungal isolates to termites in incubator

5.2.3.3 Mycosis test

To confirm that mortality was due to fungal infection, the cadavers were removed from treatments, surface-sterilized in 1% sodium hypochlorite solution and then in 70% alcohol for 3 s in each solution and rinsed for 3 min in sterile distilled water. They were then placed into Petri dishes lined with filter paper moistened with sterile distilled water. The Petri dishes were covered with their lids, the edge sealed with Parafilm and placed in an incubator. Mycosis was confirmed by daily microscopic examination of hyphae and spores at a magnification of 400×. Petri dishes containing cadavers were observed for fungal growth for at least one week.

5.2.4 Data analysis

Mortality data was corrected for natural mortality in controls using Abbott’s formula (Abbott, 1925) as follows:

$$\mathrm{CM\%} = \frac{(\mathrm{T(\%)} - \mathrm{C(\%)})}{(100 - \mathrm{C(\%)}} \times 100$$

where , CM is corrected mortality, T is mortality in treated insects and C is mortality in untreated (control) insects. For each isolate, lethal time for 50% mortality (LT50) was analyzed by Generalized Linear Model (GLM), using the function ‘dose.p’ from the MASS library, to estimate the lethal time to 50% mortality (LT_{50}) for each isolate. Corrected percent mortalities of the worker termites at 3 days post inoculation were calculated using a binomial generalised linear model with logit link (McCullagh and Nelder, 1989). Mycosis data was also analyzed using one-way ANOVA. Prior to performing ANOVA, the assumption of homogeneity of variance was tested and satisfied using Bartlett's test. Means were separated with Student–Newman–Keuls (SNK) post hoc test. All analyses were performed using R version 3.5.1 (R Development Core Team, 2018).

5.3 Results

Conidia viability test showed that the germination of the different isolates used in this study exceeded 95% after 18 hr incubation at 25 ± 2˚C (Table 5.1). The screening for pathogenicity of the 14 isolates at a concentration of 1×10^8 conidia/ml indicated that all the isolates were pathogenic to termites, causing significantly (provide statistics) higher mortality level (100%) between 4-7 days. Calculated LT_{50} values for the isolates (Table 5.2) and the corrected percent mortality of the worker termites 3 days post inoculation (Figure 5.3) show that, in general, the *Metarhizium* isolates elicited quicker mortality with lower LT_{50} values compared to *Beauveria* isolates.

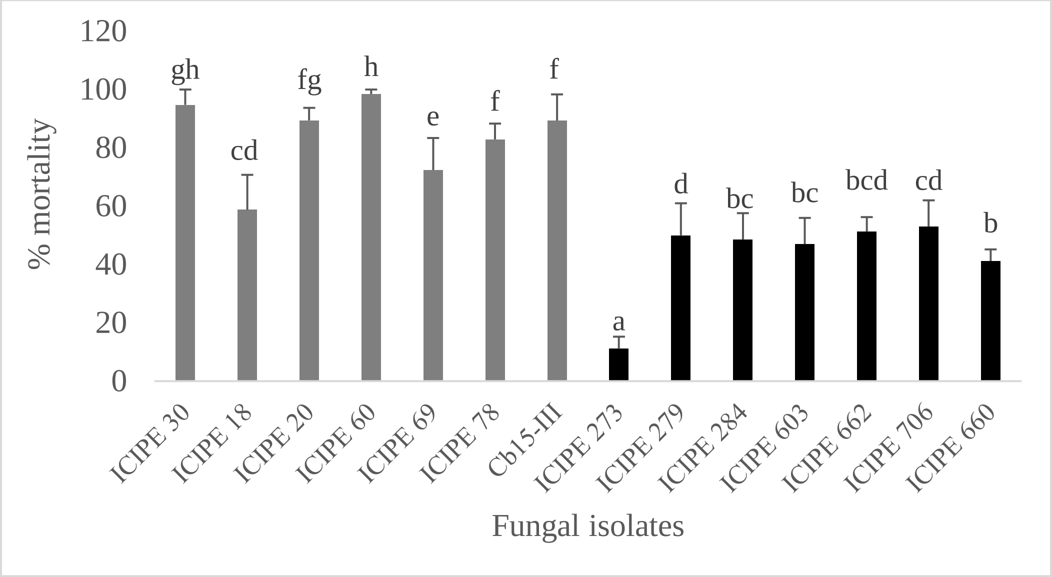

Figure 5-3: Mean mortality after 3 days post-innoculation of worker caste of *Odontotermes* spp. with *Metarhizium anisopliae*, *M. brunneum* and *Beauveria bassiana* isolates

The most virulent isolates with LT_{50} less than 2 days were *Metarhizium* isolates (*M. brunneum* Cb15-III and two *M. anisopliae* isolates (ICIPE 60 and ICIPE 30), followed by four isolates with LT_{50} between 2-3 days and these included four *M. anisopliae* isolates (ICIPE 20, ICIPE 18, ICIPE 78 and ICIPE 69) and five *B. bassiana* isolates (ICIPE 279, ICIPE 284, ICIPE 706, ICIPE 662, ICIPE 660). Two *B. bassiana* isolates (ICIPE 603 and ICIPE 273) were the least virulent with LT_{50} of more than 3 days (Table 5.2). There were significant differences ($p = 0.001$, df = 13) in mycoses among the isolates (Table 5.2).

Table 5.2: Median lethal time (LT_{50}) 7 days post treatment of worker termites of *Odontotermes* spp. inoculated with different isolates of entomopathogenic fungi

Fungal species	Isolates	LT_{50} (Days) (± S.E.)	% Mycosis of termite cadavers	% Germination
Beauveria bassiana	ICIPE 279	2.23 ± 0.04	66.25 ± 5.91 a	98.25 ± 0.85 bcd
	ICIPE 603	3.09 ± 0.05	47.50 ± 5.20 e	97.26 ± 0.48 de
	ICIPE 284	3.00 ± 0.04	61.25 ± 9.44 ab	99.22 ± 0.38 ab
	ICIPE 706	2.85 ± 0.03	61.25 ± 4.27 ab	99.51 ± 0.01 a
	ICIPE 662	2.85 ± 0.03	70.00 ± 4.56 a	99.33 ± 0.14 ab
	ICIPE 660	3.00 ± 0.04	62.50 ± 3.23 ab	98.70 ± 0.06 abc
	ICIPE 273	4.41 ± 0.04	46.25 ± 5.54 bcd	96.96 ± 0.27 e
Metarhizium anisopliae	ICIPE 20	2.23 ± 0.04	48.75 ± 9.66 de	98.25 ± 0.47 bcd
	ICIPE 60	1.51 ± 0.02	52.50 ± 7.77 abcd	99.10 ± 0.29 abc
	ICIPE 30	1.72 ± 0.03	41.25 ± 3.15 cde	98.06 ± 0.47 cde
	ICIPE 18	2.84 ± 0.04	53.75 ± 2.39 abcd	99.77 ± 0.01 a
	ICIPE 78	2.38 ± 0.03	47.50 ± 4.33 bcd	99.09 ± 0.03 abc
	ICIPE 69	2.41 ± 0.03	67.50 ± 6.61 abc	99.34 ± 0.28 ab
Metarhizium brunneum	Cb15-III	1.50 ± 0.06	57.50 ± 10.9 bcd	95.25 ± 0.48 f

Means within a column followed by same letter are not significantly different by Student-Newman-Keuls (SKN) test ($p < 0.05$).

Generally, the sign of penetration of conidia on the termite cuticle treated with *Metarhizium* isolates appeared faster than on those treated with *Beauveria* isolates.

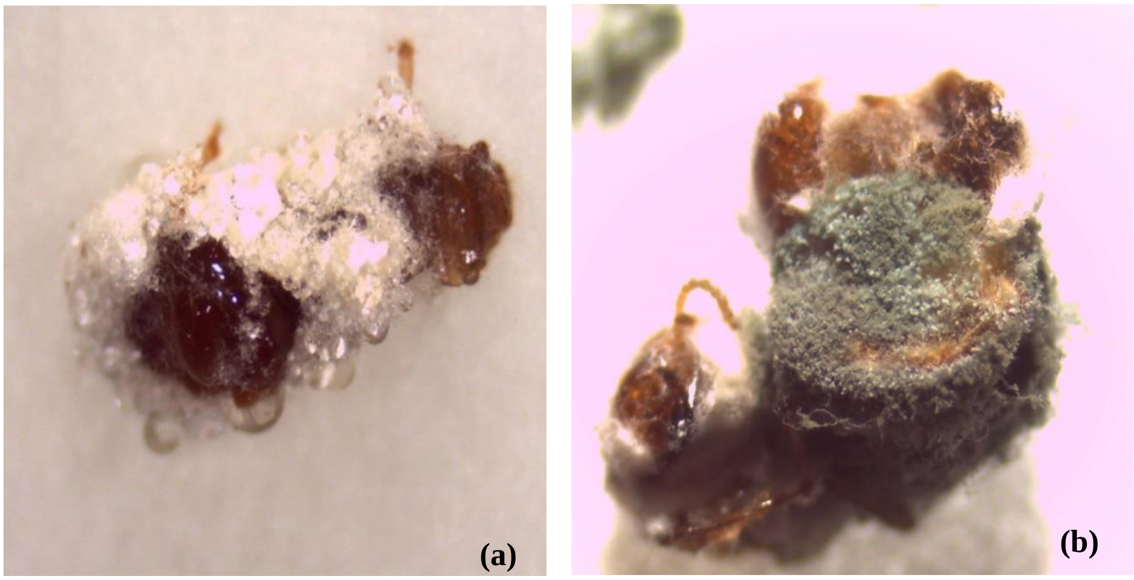

Figure 5-4: Mycosed cadavers of *Odontotermes* spp. workers by fungi (a) *Beauveria bassiana* (b) *Metarhizium anisopliae* under laboratory conditions.

After the penetration, the conidia emerged on the surfaces of the cadavers (Figure 5.4). Termites started to change in colour after few days of infection (became darker) and became more fragile when touched.

5.4 Discussion

All the fungal isolates screened were pathogenic to the termites, causing 100% mortality 4-7 days post-innoculation. The results obtained in this study corroborate other laboratory studies, which showed that many fungal isolates are virulent to termites (Rath, 2000). For example, Zoberi and Grace (1990) showed that 100% mortality of *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae) workers was attained at 1-3 days post-exposure to a strain of *B. bassiana* isolated from *R. flavipes* workers. Similarly, under laboratory conditions, a strain of *M. anisopliae* has been reported to cause 100% mortality to *Odontotermes formosanus* Shiraki (Isoptera: Termitidae) 3 days post-inoculation at the concentration of 3×10^8 conidia/ml (Dong et al., 2007). Hoe et al. (2009) reported that isolates of *M. anisopliae* were pathogenic against subterranean termite, *Coptotermes curvignathus*, causing 100% mortality at $1\text{x}10^7$ conidia/ml within 3 days post-inoculation. Kramm and West (1982) also reported that 100% mortality occurred within one day after exposure of termites to *M. anisopliae*, while similar mortality rates were obtained within five days in the case of *B. bassiana*. Following this high susceptibility of termites to EPF, researchers suspect that the rapid kill by EPF such as *M. anisopliae* of its host could be caused not only through direct physical invasion of the hyphae, but also possibly due to some enzymatic mechanisms or toxic metabolites produced by the EPF (Sun et al., 2003). In addition, the soft nature of the termite cuticle also contributes a lot to the rapid kill by EPF.

Although all the isolates screened were pathogenic, there was a variation in their virulence. Generally, *Metarhizium* isolates elicited quicker mortality with lower LT_{50} values than did *Beauveria* isolates. This higher virulence effect of *Metarhizium* isolates compared to *Beauveria* isolates confirms earlier reports

(Kramm and West, 1982; Wang and Powell, 2003; Cherry et al., 2005; Singha et al., 2006; Yanagawa et al., 2008; Singha et al., 2011; Sileshi et al., 2013), showing a similar pattern of activity with isolates of these two fungal pathogen species. It was also observed that after termites were sprayed with the fungal spores, the mortality rate was lowest after the first day (in some cases 0%) and increased rapidly in subsequent days, especially with isolates of *Metarhizium*. A research conducted by Brogden et al. (2005) showed that mortality increased after the second day of exposure of termites to EPF because of specific toxins (destruxin) released by the fungi, which penetrates rapidly into the termites' haemocoel.

Metarhizium brunneum Cb15-III, *M. anisopliae* ICIPE 30 and 60 were the most virulent against the tested termite species. Other studies have reported that *Metarhizium*, especially *M. anisopliae* and *M. brunneum*, is one of the EPF genera that has high potential in controlling subterranean termites (Denier et al., 2015; Kuswanto et al., 2015). Research conducted by Milner et al. (1998) also revealed *M. anisopliae* to be the most effective fungal pathogen for termite control. However, highly virulent isolates may not be ideal candidates for biological control programs, especially in the case of social insects like termites because other reports have shown that the conidia of highly virulent strains of EPF are very repellent to termites (Staples and Milner, 2000; Myles, 2002; Mburu et al., 2009). However, the manipulation of insect behavior can make it possible to utilize virulent EPF more effectively by combining them with semiochemicals used in host finding as attractants. Some studies have suggested the possibility of reducing the repellency or avoidance of conidia and overcome behavioral defenses of termites by formulating the conidia with attractants (Lenz, 2005). Research conducted by Wang and Powel (2004) showed that the use of palatable baits greatly increased the effectiveness of a virulent strain of *M. anisopliae* against *R. flavipes* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae), where cellulose bait treated with *M. anisopliae* caused 100% mortality to *R. flavipes* and *C. formosanus* groups without causing any repellency effects to the termites. Therefore, if these highly virulent EPF isolates (*M. brunneum* Cb15-III, *M. anisopliae* ICIPE 30 and 60) are co-formulated with attractants such as CO_2

used by termites to locate plant roots, the repellent effect can be mitigated. The co-formulation can further be tested for the attractiveness of subterranean termite pests for their control in cocoa agroforests.

5.5 Conclusion

In conclusion, this study suggests that *M. brunneum* Cb15-III, *M. anisopliae* isolate ICIPE 60 and ICIPE 30 have the potential to be developed as microbial agents for controlling subterranean termite pests in cocoa agroforests. These isolates can be coformulated with termite attractants for effective management of subterranean termite pests.

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6 CHAPTER SIX: TESTING A CO-FORMULATION OF CO_2 RELEASING MATERIAL WITH AN ENTOMOPATHOGENIC FUNGUS FOR THE MANAGEMENT OF SUBTERRANEAN TERMITE PESTS (BLATTODEA: TERMITOIDAE) IN COCOA AGROFORESTS

Abstract

Termites cause significant damage to cocoa trees. Entomopathogenic fungi (EPF) such as *Metarhizium brunneum* have been considered as a sustainable alternative to the unenvironmentally sound use of insecticides against termites. However, potent EPF application is limited by their repellency effects, host avoidance, and termite defense mechanisms such as grooming among nest mates. Termites use CO_2 to locate plant roots. This study investigated whether CO_2-emitting capsules could attract termites. Capsules formulated without fungus (CEC) as well as those formulated with *M. brunneum* Cb15-III (CEC_{EPF}) were tested for attractiveness to termites with other attract components using modified four arm olfactometers. Worker termites' infection by the fungus growing from the capsules as well as its horizontal transmission were assessed through the autodissemination approach. Significantly more termites were attracted to CEC compared to other attract components. Higher number of termites were attracted by CEC_{EPF} and cocoa seedlings than dry wood and yeast in a choice test. When termites were directly exposed to sporulating capsules, 100% mortality was obtained within 4-5 days. However, in the horizontal transmission experiment, no significant difference was observed with regards to termite mortality when the treatment group was compared with controls. CEC_{EPF} did not cause any apparent repellency to termites as compared to CEC. This strategy offers some good potential to promote biological termite control using CEC_{EPF} as an alternative to insecticides. Further studies are warranted to evaluate the efficacy of these capsules under field conditions and explore its commercialization for termite management.

Keywords: Attract and kill. Carbon dioxide. Cocoa. Encapsulation. *Metarhizium brunneum*. Termites.

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6.1 Introduction

Cocoa is the most important export crop for countries in the forest zone of West Africa and the main source of foreign exchange for Ghana, Cameroon, Ivory Coast, and Nigeria (Falola et al., 2013; Tchokote et al., 2015). Despite the economic importance of cocoa, its production continues to decline over the years due to low productivity (Dormon et al., 2007). The main reason for this low productivity is the ravages caused by diseases and insect pests that specifically harm cocoa pods. Among these are black pod disease caused by different *Phytophthora* spp. and mirids including subterranean termites, which harm the cocoa trees especially the roots of young seedlings after transplanting (Ambele et al., 2018a). Subterranean termites are the most widespread and the most destructive termite species especially to agricultural crops including cocoa (Ambele et al. 2018a; Ambele et al. 2018b). They derived their name subterranean termites because of their association with the soil. Although they live entirely in the soil, they construct underground tunnels or mud tubes to move about in search for food (Su and Scheffrahn, 1998). These mud tubes are like highways that connect them to their food source and their main home or colony. A subterranean termite colony is highly structured and has castes that perform distinctly different duties (Mao et al., 2005). There are three castes that are different morphologically and with specific function. The worker caste (the most numerous and destructive) is responsible for all the labor in the colony like creating tunnels and gathering food. The soldier caste is responsible for defending the colony against possible invaders, while the reproductive caste includes a king (male) and a queen (female) who are the parents of the subterranean termite family and founders of the colony. The queen lays large numbers of eggs which develop into more workers and soldiers as the family grows. Subterranean termites can cause up to 80% mortality in 4-6 months old cocoa plants after seedling transplant in the field (Wood and Pearce, 1991).

Termite control in the past relied on persistent organochlorine insecticides (Su and Scheffrahn, 1998) but is now under restrictive use due to increasing concern over potential harm to humans and negative effects on the environment. Furthermore, the cryptic lifestyle of termites, especially subterranean termites makes the direct application of insecticides difficult. Farmers in many parts of Africa use various traditional termite control methods often with limited success (Ambele et al., 2018a). Most termite control practices are ineffective and ecologically unsustainable, and above all, do not address the root attacks of termite infestation, but merely providing a temporary relief to the problem (Ambele et al., 2018a). There is therefore a need to identify eco-friendly alternative methods for termite control in cocoa agroforests.

Biological control using viruses, entomopathogenic nematodes, fungi and bacteria have been reported for control of termites (Bishwajeet et al., 2018). However, nematodes appear to have a limited impact on subterranean termites due to behavioral defense mechanism (Wilson-Rich et al., 2007). The potential of bacteria to reduce termite populations has not yet been demonstrated under field conditions because of the poor survival of bacteria in the soil (Fierer, 2017). The use of viruses remains limited in termite biological control literature. Accessibility of the pest to be controlled is the prime factor affecting the efficacy of viral pathogens and thus insects living in concealed habitats such as subterranean termites are more difficult to control with viruses (Culliney and Grace, 2000). Biological control using entomopathogenic fungi (EPF) is therefore the most promising alternative to synthetic chemical pesticides (Rath, 2000; Grace, 2003; Chouvenc and Su, 2010). Several genera of EPF have been reported to be pathogenic agents in controlling termites. Among these genera, *Metarhizium*, especially *M. anisopliae* and *M. brunneum* have high potential in controlling subterranean termites (Denier and Bulmer, 2015; Kuswanto et al., 2015). EPF can provide an opportunity for sustainable control of termites due to easy mass production and application (Rath, 2000). However, there are limitations in their application because they do not achieve high control efficacies in the field when applied as conidia suspensions. This is due to direct repellency or host avoidance of conidia (Mburu et al., 2009) and also because termites are able to deploy a diversity of defense mechanisms ranging from mutual grooming behaviour to removing conidia from cuticles of their nest mates, to walling-off of infected areas of a colony, or to release pathogen alarm kairomones which warn nest mates about the presence of lethal fungi causing them to leave the area or to quickly remove infected conspecifics (Yanagawa et al., 2008).

Several mechanisms of introducting EPF for termite management have been used in the past but all these methods have been unable to trigger an epizootic in termite mounds (Chouvenc et al., 2011). So, despite efforts to screen for virulent strains of EPF, the delivery of sufficient inocula to a subterranean termite colony remains an unsolved problem (Grace, 2003; Chouvenc et al., 2011). The manipulation of insect behavior may allow the utilization of biocontrol agents more effectively by combining them with semiochemicals used as attractants during foraging. Such a combination is known as “Attract and Kill” (A&K) or “Attract and Infect” (A&I) and has been proven to be very effective method of control for western corn rootworm larvae (Schumann et al., 2013). The A&K strategy has the potential to target organisms from their cryptic habitats in complex environments that are normally difficult to reach with ordinary application techniques (El-Sayed et al., 2009). The insect pest is lured to an attractant (e.g. semiochemical = attract) and thus coming into contact with an insecticidal compound or entomopathogenic fungi (= infect/kill) killing off the insect (El-Sayed et al., 2009). For soil pests like subterranean termites feeding on roots, such an attractant is CO_2, a volatile released by respiring plant roots (Bernklau et al., 2005; Schumann et al., 2013). Ziesmann (1996) demonstrated that a sensillum on the antennae of the termite *Schedorhinotermes lamanianus* Kolbe contains a neuron that responds specifically to CO_2 and that the sensitivity of this neuron to other odors is inhibited by exposure to CO_2. This implies that the CO_2 concentration in the immediate environment may affect the sensory information being perceived by an individual termite. Bernklau et al. (2004) tested various CO_2 producing compounds that diverted western corn rootworm larvae away from their host (maize). These authors proposed the encapsulation of CO_2-emitting products in order to extend the time period of CO_2 production. Other researchers have encapsulated CO_2 releasing baker's yeast with various kill agents for the control of various soil dwelling pests (Schumann et al., 2013; Przyklenk et al., 2015; Vemmer et al., 2016; Brandl et al., 2017; Humbert et al., 2017a, b; Humbert et al., 2018). However, the A&K approach using EPF co-formulated with CO_2 in terms of increasing the efficacy of the EPF for the control of termites and mitigate their repellence and fungal grooming behavior of conidia has not been assessed. We hypothesize that virulent entomopathogenic fungi are good biological control candidates against termites when formulated with CO_2.

In this study, we tested whether calcium alginate beads containing baker's yeast (*Saccharomyces cerevisiae* Meyen ex Hansen) as an encapsulated CO_2 source (CO_2-emitting capsules) could outcompete CO_2 gradients established by other CO_2 generating materials and other attractants aiming at attracting subterranean termites (*Microtermes* spp.). The capsules formulated with the EPF *Metarhizium brunneum* (Metschnikoff) Sorokin (strain: Cb15-III) (CEC_{EPF}) were further assessed for the growth of the fungus out of the capsules and for their ability to establish CO_2 gradients in the soil that could outcompete CO_2 produced by root respiration of cocoa seedlings to attract termites. In addition, infection of the worker termites by the fungal spores growing from the CEC_{EPF} as well as their horizontal transmission was investigated through an autodissemination approach.

6.2 Materials and methods

6.2.1 Collection and maintenance of termites

Termites (Isoptera: Termitidae: *Microtermes* spp.) were collected from infested fallen wood and by digging subterranean mounds in full sun cocoa farms at Kedia (4°50.46N, 11°07.87E) in the central region of Cameroon. The infested wood and the termites dug out from the nests together with fungus combs were placed on soil collected from the cocoa farms in plastic containers with the top covered with muslin cloth to allow air exchange between the termites and the outside environment. Fungus combs are complex convoluted structures cultivated by termite species of the sub-family Macrotermitinae and serve as nutritious sources of food for the termites (Kuja et al., 2014). The collections were made in the evenings or early in the mornings, and the termites were transported to the Institute of Agricultural Research for Development (IRAD), Yaoundé insectary, where termites were kept for at least 2 weeks before use. At the insectary, the infested woods were cut into small blocks and termites dislodged from their galleries by gently tapping the wood. The collected termites were placed on moist autoclaved soil in plastic containers (50 × 38 × 30 cm) connected to each other using two water supply tubes (50 cm in length and 2 cm in diameter), with tops of each container covered with 38 by 15 cm muslin cloths to allow air ventilation and preven the escape of the termites. The 50 × 38 × 30 cm plastic containers were connected to two smaller plastic containers using 30 cm water supply tubes. The setup (Figure 6.1) was designed in this way to provide space for movement and gallery building as part of termites' natural behaviour. The plastic containers were provided with small pieces of the dried cocoa wood from which the termites were collected. A moist wad of cotton was placed inside the boxes and sprayed with distilled water after every two days to maintain the required moisture level. Fungus combs and moist soil were placed in each of the smaller plastic containers. The plastic containers were maintained in complete darkness at 26 ± 1°C and 85 ± 5% relative humidity. Adult workers (the most numerous and destructive) were separated from the rearing unit and used for the various bioassays, because they are in charge of gathering food and feeding other members of the colony. The workers are therefore the ones that can mainly transport the EPFs from the capsules to other colony members.

Figure 6-1: Artificial system for maintaining termites in the laboratory

6.2.2 Soil preparation

Black soil was obtained from IRAD Nkolbisson, Yaoundé (Cameroon). The soil was sieved to remove all debris and roots, then autoclaved at 121°C for 1 hr. It was allowed to cool for at least 72 hours and preserved in plastic bags for some time before use the bioassays. Black soil was chosen to enable the effective observation of worker termites and because it contains high organic matter.

6.2.3 Preparation of four choice test apparatus

The experimental arena was composed of 5 DANAplast plastic containers of similar dimensions (height = 19 cm, diameter = 17 cm). The central chamber constituted the release chamber, interconnected horizontally by transparent flexible PVC tubes (1.6 cm outer diameter and 1.4 inner diameters) to four attractive chambers equidistant from one another (Figure 6.2). The tubes and plastic containers were cleaned with detergent and dried before each experiment. The lengths of the tubes were varied (10, 15 and 20 cm) in different experimental setups.

Figure 6-2: Experimental set-up for testing the attractiveness of CO_2 emitting capsules to termites

6.2.4 Experiment 1: Do CO_2-emitting alginate capsules attract termites?

This experiment tested the attractiveness of CO_2-emitting capsules (CEC) formulated without a killing agent in the choice test apparatus. The attract components used were either commercial baker's yeast, pieces of dry wood from where the termites were collected, fungus comb and the CEC. The CEC were obtained from Bielefeld University of Applied Science and were formulated by encapsulating commercially available yeast (as an artificial source of CO_2) together with starch as a substrate in calcium alginate (Humbert et al., 2017a, b). Each capsule has a size of 3 mm with an optimal concentration of 1800 ppm of CO_2 and can release CO_2 for about 105 days after application. After absorbing water in the soil, the production of the CO_2 starts from the capsules. Five treatments of the CEC (5, 10, 15, 20 and 25 capsules) (each treatment replicated four times) together with the other attract components (dry wood, yeast and fungus comb) were tested for attractiveness to termites in different experimental set ups. The CEC (5, 10, 15, 20 and 25 capsules) were counted and mixed with moist soil and placed in one chamber of the choice apparatus at three different depths (5, 10 and 15 cm) in different experimental set ups and covered with soil. Dry wood, baker's yeast and parts of fungus combs were also placed in moist soil in the other three compartments, respectively. The experiment was established 48 hours before worker termites were introduced into the release chamber to enable the formation of CO_2 gradients produced by the CEC (Schumann et al., 2013). Before the start of the experiments, a batch of 100 adult worker termites each were removed from the rearing containers and placed on moist autoclaved soil in Petri dishes sprayed only with distilled water over night. The lids of the Petri dishes had aeration holes made with an electric soldering iron to ensure free flow of air. To begin the test, 100 adult worker termites were released at the central plastic container (release chamber). The chambers were covered with their lids also provided with aeration holes to avoid the escape of the termites. Termites were allowed to crawl around the container and to make a choice between the chambers where CEC, baker's yeast, dry wood or fungus combs were located. The experiments were maintained in the dark to avoid exposure of the termites to light. At every 1 hr post-exposure, and subsequently for 6 hrs, the number of termites in all the test chambers was removed and recorded giving six counts for each replicate. To reduce perturbation effects on worker termites during removing and counting, a spotlight was used to illuminate only the compartment to be counted, to avoid extended exposure of the termites to light. The experiments were terminated after 6 hrs because preliminary experiments have shown that almost all the termites moved away from the release chamber after this time. Some termites did not move from the release chamber, most likely due to stress or damage from transfer into the chamber. Termites that did not enter any of the chambers at the end of each experiment (> 5% in all the tests) were treated as non-responders and not included in data analysis. The treatment chambers were swapped after every replicate to eliminate any asymmetric bias of the surroundings and individual termites were not used for more than one replicate. The experiments were maintained at 25 ± 2° C and 85 ± 5% RH.

6.2.5 Experiment 2: Attractiveness of CO_2-emitting capsules co-formulated with *Metarhizium brunneum*

In this set of experiments, CO_2-emitting capsules co-formulated with an entomopathogenic fungus *M. brunneum* (CEC_{EPF}) were tested for their attractiveness to termites as described above. The capsules consist of encapsulated yeast cells and the fungus *M. brunneum* (Cb15-III). The initial spore concentration was 1.6 × 10^7 spores/g/capsules. Conidial viability and germination from the capsules were assessed before testing the attractiveness of the capsules to termites by placing some of the capsules on sterilized moist black soil and on SDA medium in Petri dishes. The Petri dishes with soil were placed under environmental conditions while those with SDA medium were placed in an incubator at 25 ± 1°C and examined daily. After 5-7 days, white mycelia had developed, and green conidia appeared around the capsules (Figure 6.3).

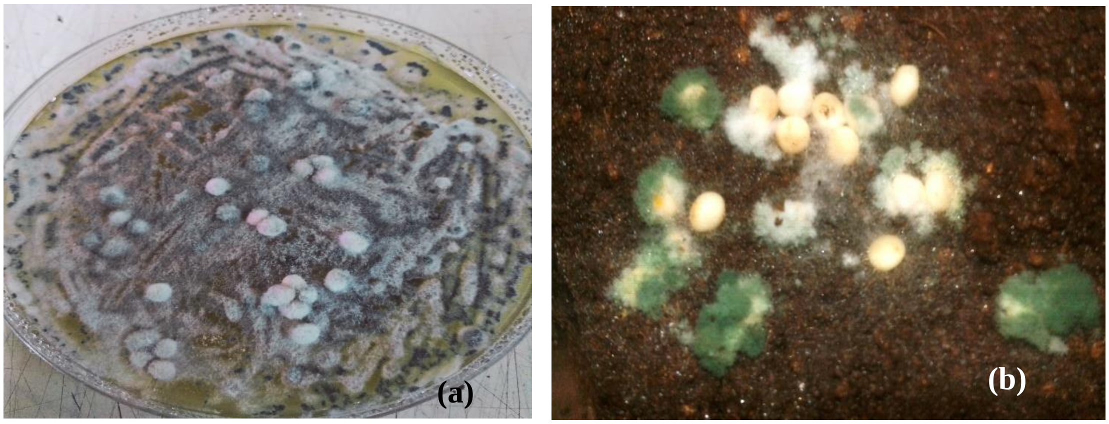

Figure 6-3: Fungal growth from capsules in (a) SDA media plate (b) soil

The viability of the spores growing out from the capsules was assessed. A concentration of 3 × 10^6 conidia/ml was prepared from conidia harvested from SDA media plates, and 2 to 3-week-old surface cultures by scraping with a sterile spatula and suspending in 10 ml sterile distilled water containing 0.05% Triton X-100 (Fluka, Sigma-Aldrich, UK) and five glass beads (ϕ = 3 mm) in 30 ml universal bottles. The suspensions were filtered through cheese-cloth to separate the conidia from the hyphae, remove conidial clumps and mycelia debris. Bottles were stoppered and then vortexed in a Vortex (Genie 2 Scientific Industries, Bohemia, New York) for 5 min at about 700 rpm to break the conidial clumps and ensure a homogeneous conidial suspension. Conidial concentrations were quantified using an improved Neubauer haemocytometer (Weder Scientific International Ltd, Teddington, UK) under a light microscope. The conidial suspensions were adjusted to 3 × 10^6 conidia/ml through dilution with distilled water and 0.1 ml of the suspension was evenly spread on SDA and four sterile microscope cover slips (22 mm × 22 mm) were placed randomly on the surface of each inoculated plate. The plates were sealed with Parafilm and incubated under complete darkness at 25 ± 2°C. At 18 hours. post-inoculation, lactophenol cotton blue was added to terminate germination and stain the spores for ease of counting. The percentage germination of conidia was determined from 100 spore counts under the cover slips at ×400 magnification under a light microscope using the method described by Goettel and Inglis (1997). Conidia were considered to have germinated when the length of the germ tube was at least twice the diameter of the conidium (Inglis et al., 2012). Four replicate plates were used, and viability was determined, where 95% germination was obtained.

After ascertaining the viability of spores in the capsules, 10 CEC_{EPF} were counted and mixed with moist soil and placed in one chamber of the choice apparatus at 10 cm depth in moist soil. Dry wood, baker’s yeast and cocoa seedling were placed in the other three compartments respectively in moist soil (Figure 6.4). The CEC_{EPF} were placed 48 hours before worker termites were placed in the release chamber (Schumann et al., 2013). The growing cocoa seedlings were transferred from the nursery into the test chambers (one seedling per test chamber) two weeks before the start of the experiment. The cocoa seedlings were 4-6 months old as they are reported to be very attractive to termites (Ackonor et al., 2001). Before the experiment, a batch of 100 worker termites each were removed from the maintenance containers and placed on moist sterilized soil in Petri dishes sprayed only with sterile distilled water over night, with the lids of the Petri dishes having aeration holes to ensure free air flow. For this test, 100 termites were released at the central container (release chamber). Termites were allowed to crawl around the container and made a choice between CEC_{EPF}, cocoa seedling, dry wood or baker’s yeast chambers. The experiment was replicated 20 times. The number of termites in all the test chambers were removed and recorded at every 1-hour interval for 6 hours for each replicate.

To evaluate the infection of attracted termites, the experiments were conducted using round plastic containers of 15 cm diameter × 5 cm in two-container choice devices. The bowls were filled with sterilized black soil moistened with distilled water. The central bowl (the release chamber) was connected to two test chambers using 10 cm transparent tubes (1.6 cm outer diameter, 1.4 cm inner diameter). Fifteen CEC_{EFP} were placed in one of the test chambers on moist soil for 14 days to allow the growth and sporulation of *M. brunneum* from the capsules. A 4-6 monthsold cocoa seedling was placed in the other test chamber. Hundred worker termites were released in the central chamber and movement of the termites in both test chambers were observed with a torch light, the number of termites in each test chamber was counted. Termites collected from the chambers containing the capsules were placed on moist soil in round plastic bowls as treatments and corresponding numbers of termites that were not used in the experiments were placed in other bowls as controls. Pieces of the wood from where the termites were collected were placed in each bowl to serve as food. Soaked cotton was placed in each bowl to maintain moisture. The experiment was replicated 10 times. Mortality was recorded daily for 5 days.

Figure 6-4: Experimental set-up for testing the attractiveness of CO_2 emitting capsules co-formulated with *M. brunneum* to termites

6.2.6 Experiment 3: Infection of attracted termites

To evaluate the infection of attracted termites, the experiments were conducted using round plastic containers of 15 cm diameter × 5 cm high in two-container choice devices. The bowls were filled with sterilized black soil moistened with distilled water. The central bowl (the release chamber) was connected to two test chambers using 10 cm transparent tubes (1.6 cm outer diameter, 1.4 cm inner diameter) (Figure 6.5). Fifteen $\mathrm{CEC_{EFP}}$ were placed in one of the test chambers on moist soil for 14 days to allow the growth and sporulation of *M. brunneum* from the capsules. A 4-6 months old cocoa seedling was placed in the other test chamber. Hundred worker termites were released in the central chamber and movement of the termites in both test chambers were observed with a torch light, the number of termites in each test chamber was counted. Termites collected from the chambers containing the capsules were placed on moist soil in round plastic bowls as treatments and corresponding numbers of termites that were not used in the experiments were placed in other bowls as controls. Pieces of the wood from where the termites were collected were placed in each bowl to serve as food. Soaked cotton was placed in each bowl to maintain moisture. The experiment was replicated 10 times. Mortality was recorded daily for 5 days.

Figure 6-5: Set up to determine the attractiveness of $\mathrm{CEC_{EPF}}$ and mortality of attracted termites

6.2.7 Experiment 4: Direct exposure of termites to sporulating $\mathrm{CEC_{EPF}}$

Ten $\mathrm{CEC_{EPF}}$ were placed on sterilized soil moistened with distilled water in round transparent plastic bowls (15 cm diameter × 5 cm high) for 14 days to allow the growth and sporulation of the fungus from the capsules. The plastic bowls were then connected to other plastic bowls, also filled with moist soil using 10 cm transparent tubes to provide space for movement of the termites (Figure 6.6). Batches of 100 adult worker termites each were then released into the bowls containing the sporulated capsules. Only sterilized moist soil was placed in the control bowls without the capsules with the same number of termites. The experiment was replicated 6 times. Both the control and treatment groups were provided with dry wood to serve as food for the termites. Mortality was recorded daily for 5 days and thereafter the experiment terminated because most of the termites in the treatment group attended 100% mortality by the 4th day post-treatment. The experimental conditions were maintained at 25 ± 2° C and 85 ± 5% RH. To confirm that mortality was due to fungal infection, the cadavers were removed from treatments, surface-sterilized in 70% alcohol for 3 s and rinsed three times for 3 mins in sterile distilled water. They were then placed into Petri dishes lined with filter paper moistened with sterile distilled water. The Petri dishes were covered with their lids, sealed with parafilm and placed in an incubator. Mycosis was confirmed by daily microscopic examination of hyphae under the microscope.

Figure 6-6: Set up for direct exposure of termites to sporulating capsules

6.2.8 Experiment 5: Horizontal transmission of *Metarhizium brunneum* spores

This experiment was conducted to assess the possibility of transmission of spores growing from the capsules from infected termites to healthy conspecifics. Twenty CEC_{EPF} were placed on sterilized moist soil in plastic containers (15 cm diameter × 5 cm high) for 14 days prior to conducting the experiments to enable the growth and sporulation of *M. brunneum* from the capsules. Fifty adult worker termites acting as "donors" were then placed in the plastic containers for 6 hrs for fungal infection. The donors were later transferred into clay pots containing 1,000 untreated ("receivers" or "recipients") termites. The treated termites could easily be distinguished from the untreated termites as they had dark green cuticle from the adherent conidia. Fifty unexposed termites were also transferred to other clay pots containing 1,000 termites as controls. The termites were placed on moist soil to provide shelter and fed with dry wood. Soaked cotton was placed in each clay pot to maintain moisture and the cotton was sprayed daily with distilled water. Treatment and control groups were replicated 4 times. The clay pots were placed in the dark and mortality monitored daily. Dead termites were not removed from the containers and final mortality was recorded after 10 days.

6.2.9 Experiment 6: Attractiveness of CO_2 emitting capsules together with cocoa seedlings to termites

Six months old cocoa seedlings were used to evaluate the attractiveness of CEC_{EPF} buried around cocoa seedlings as compared to cocoa seedlings without the capsules in the laboratory. The experiments were conducted using transparent DANAplast plastic containers of similar dimensions (height = 19 cm, diameter = 17 cm). The central chamber constituted the release chamber, interconnected horizontally by transparent flexible PVC tubes (1.6 cm outer diameter and 1.4 inner diameters), to two chambers equidistant from one another to constitute two-container choice devices. The containers were filled with black soil moistened with distilled water. The central container (the release chamber) was connected to two test chambers using 10 cm transparent tubes. Fourteen days before experiment commencement, 20 CEC_{EFP} were spread around the cocoa seedling in one of the test chambers to promotethe growth and sporulation of spores from the capsules. Cocoa seedlings without the capsules were planted in the other test chamber. The seedlings were transferred from the nursery into the test chambersa week earlier and kept in the sreen house before the start of the experiment. Hundred worker termites were released in the central chamber and movement of the termites in both test chambers was observed with a torch light, the number of termites in each removed and counted. The experiment was replicated 6 times.

6.2.10 Data analysis

Prior to the analyses, the assumption of homogeneity of variance was tested using Bartlett's test. The data for the number of worker termites attracted by the different number of capsules (5, 10, 15, 20 and 25 capsules) and the other attract components at the different arm lengths (20 cm, 15 cm and 10 cm) and capsule placement depth(5 cm, 10 cm and 15 cm), did not follow a normal distribution, and were thus analyzed using the generalized linear models (GLMs) with a quasi-poisson error distribution and a log link function to account for overdispersion (Ver Hoef and Boveng, 2007). The p-values were adjusted using Tukey's method. The two choice tests for direct exposure of termites to sporulating capsules and horizontal transmission experiments data were analyzed using the two-sample t-test (unpaired). All models and analyses were done using the R software, version 3.2.3 (R Development Core Team, 2015). All data were given as means ± SE.

6.3 Results

6.3.1 Attractiveness of CO_2-emitting alginate capsules to termites

Neither the depth of the capsules in the soil, nor the amount of capsules had a significant influence on termite attraction, as significant differences ($F_{14} = 2.57$, $p = 0.008$) were observed in the number of termites attracted by the different number of capsules (5, 10, 15, 20, 25), placed at different depths (5, 10, 15 cm) in the soil only when arm lengths of the olfactometer was 20 cm (Figure 6.7). However, when 15 cm length of arms was used, no significant differences ($F_{14} = 0.686$, $p = 0.78$), were observed in the number of termites attracted by the capsules placed at different depths in the soil. No significant differences ($F_{14} = 0.276$, $p = 0.99$) were also observed in the number of termites attracted by the different number of capsules, placed at the different depths when the lengths of arms of the modified olfactometers were 10 cm.

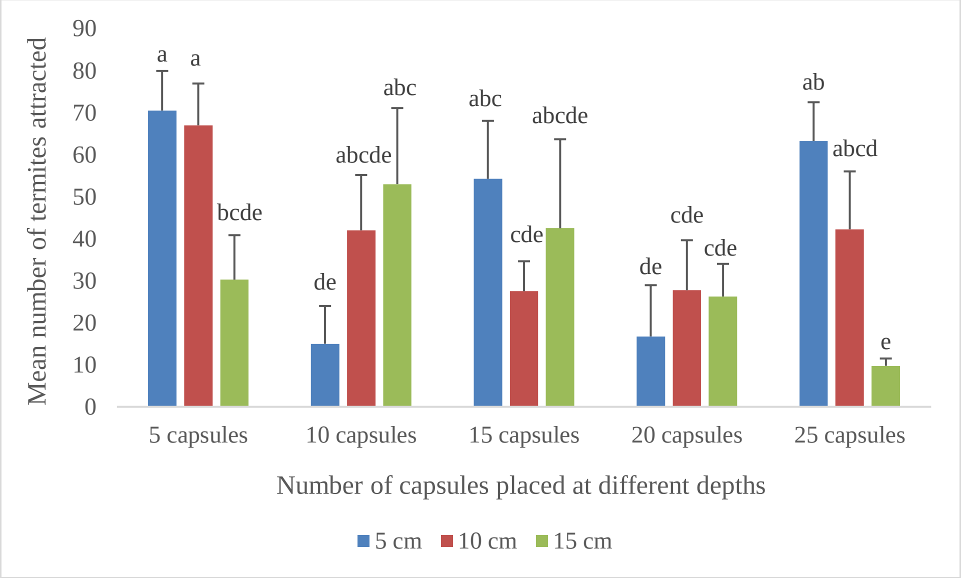

Figure 6-7*:* Number of worker termites (means + SEs, $F_{14} = 2.25$, $p = 0.008$) attracted by varying number of capsules (5, 10, 15, 20, 25) placed at different depths in the soil (5, 10, 15 cm) in 4-choice tests with olfactometer arm length = 20 cm

For the number of termites attracted by CEC at the different time periods, significant differences ($p < 0.05$, df = 5) were observed in the number of termites attracted by the capsules (Figure 6.8) when the capsules were placed at 5 cm and 10 cm depths, but no significant difference was observed at 15 cm.

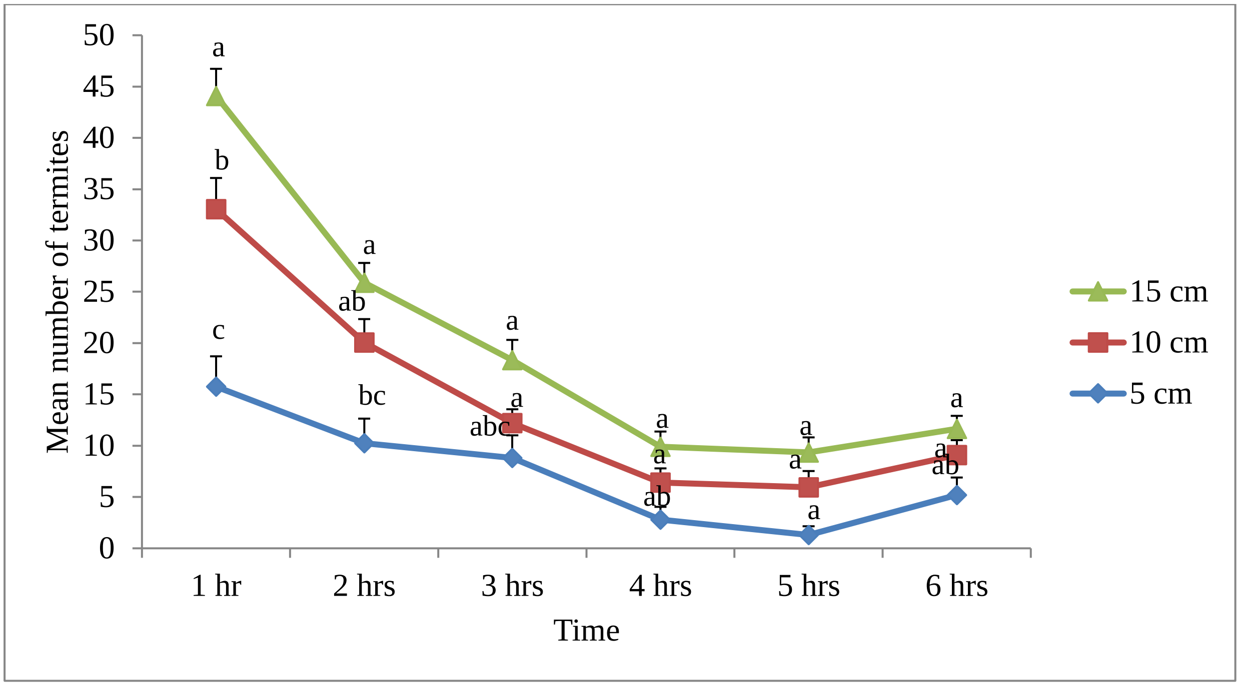

Figure 6-8: Number of termites attracted by CEC at different time periods at lengths of arms 20 cm and placement of CEC at 3 different depths

6.3.2 Termites attracted by other attractants

Termites significantly discriminated (p = 0.02, df = 8) among the attract components (yeast, wood and fungus comb) offered when tested in the set-up with 20 cm arm length (Figure 6.9). However, no significant differences were observed in the number of termites attracted by the attract components when 15 cm length of arm (p = 0.17, df = 8) and 10 cm length of arm (p = 0.98, df = 8) were used.

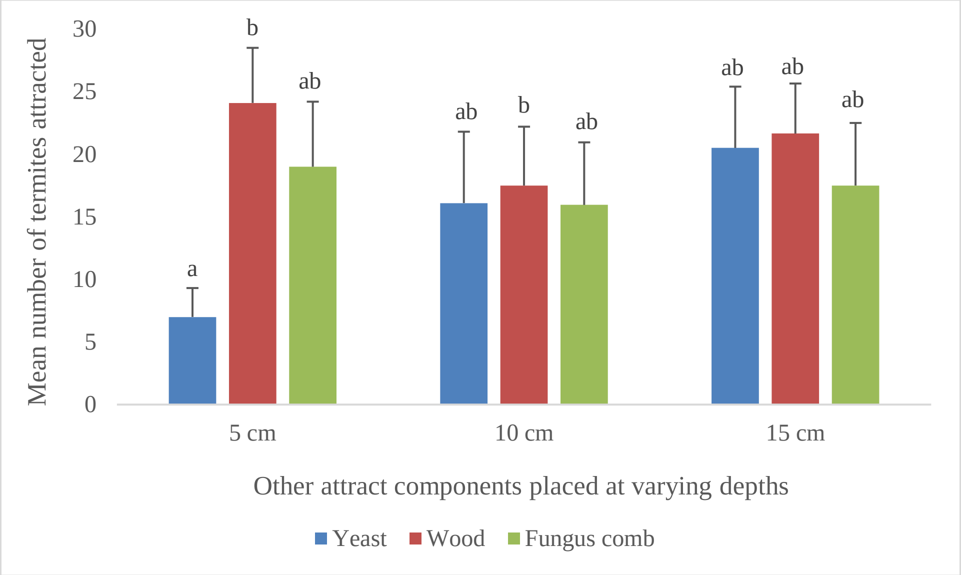

Figure 6-9: Number of worker termites (means + SEs, p = 0.02, df = 8) attracted by other attract components, placed at different depths in the soil (5, 10, 15 cm) in 4-choice tests with olfactometer arm length = 20 cm

Considering all the attract components (CEC, yeast, wood, fungus combs), significant differences were observed in the number of termites attracted by the different attract components (p = 0.004, df = 11) only when the 20 cm arm lengths were used, with more termites attracted by CEC (Figure 6.10). No significant differences were observed in the number of termites attracted by the different attract components in the 4-choice test when 15 cm length of arm (p = 0.06, df = 11), and 10 cm arm lengths (p = 0.15, df = 11) were used.

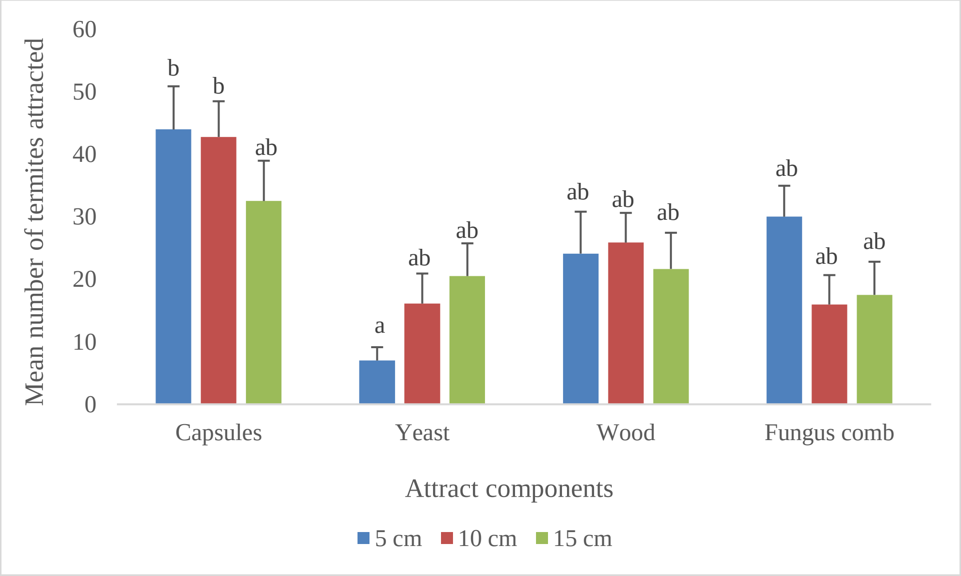

Figure 6-10: Number of worker termites (means + SEs, p = 0.004, df = 8) attracted by different attract components (CO_2-emitting capsules (CEC), yeast, wood and fungus comb) in 4-choice tests with olfactometer arm length = 20 cm. The CEC were placed at different depths in the soil (5, 10, 15 cm).

6.3.3 Attractiveness of CO_2 emitting capsules co-formulated with *Metarhizium brunneum* and infection of attracted termites

Based on the results from the experiments above, the 4-arm modified olfactometer with 10 CEC co-formulated with *M. brunneum* (CEC_{EPF}) at 10 cm depth in the soil at one arm and cocoa seedlings, dry wood, baker's yeast in the other three arms showed a significant difference (p = 0.005, df = 3) in the number of termites attracted by the cocoa seedling, CEC_{EPF}, dry wood and yeast. However, we recorded no significant difference between the number of termites attracted by the CEC_{EPF} and cocoa seedlings, as well as between the cocoa seedling, dry wood and yeast (Figure 6.11). In addition, we also found no significant differences in the mean number of termites attracted by the cocoa seedlings and the CEC_{EPF} (t = 0.85, p = 0.41) in the two-choice experiment between cocoa seedling and CEC_{EPF}.

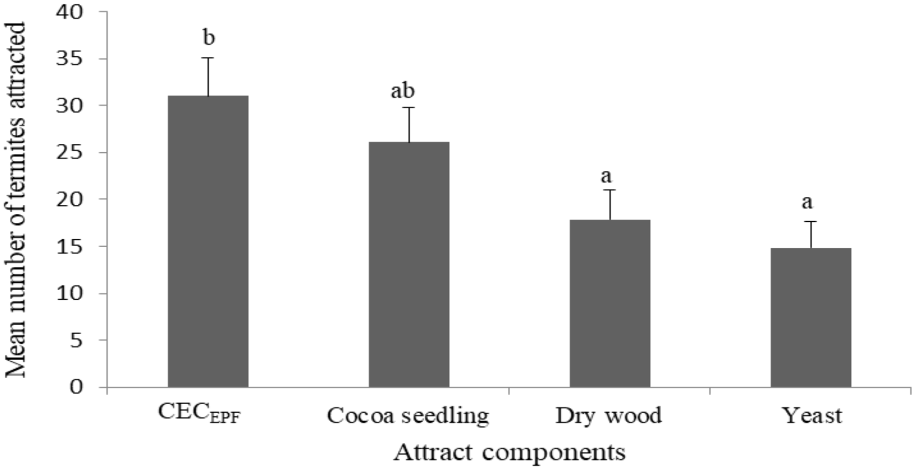

Figure 6-11: Mean number of termites attracted by different attract components

The two-choice experiment (cocoa seedling and CEC_{EPF}) showed no significant difference in the mean number of termites attracted by the cocoa seedlings and the CEC_{EPF} (t = 0.85, p = 0.41, df = 15.21). It was observed that although the capsules attracted termites, these termites initially avoided close contact with the sporulated capsules within about 15 minutes. However, after this time, termites were observed moving around the sporulated capsules (Figure 6.12).

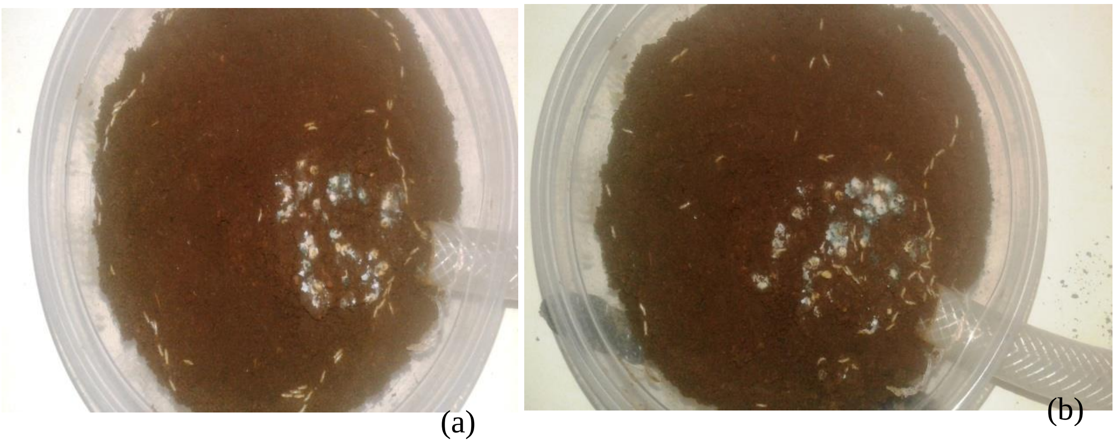
(a) (b)

Figure 6-12: (a) Termites initial avoidance of sporulated capsules (b) Termites movement within sporulated capsules

6.3.4 Direct exposure of termites to sporulating CEC$_{EPF}$ and horizontal transmission of *Metarhizium brunneum* spores

A significantly higher mortality (100%) of termites exposed to sporulating capsules was found compared to the mortality in the controls (15.5%), 5 days post-exposure (t = -8.5, p = 0.003) (Figure 6.13). Termites in the treatment arena showed symptom of sluggishness (slow movement) when compared with control specimens a day after exposure. We observed the appearance of white mycelia 4 to 7 days after the death of some of the termites that were exposed to the CEC$_{EPF}$. The mycelia changed from white to light green and became progressively darker after 7 days. A total of 86% of the cadavers showed signs of mycelial growth.

The horizontal transmission of EPF from infected to non-infected termites (treatments) and the controls (non-infected to non-infected termites) revealed higher percent mortality (3.2 ± 0.5%) in the treatment groups than the control (2.6 ± 0.5%). However, the differences were not statistically significant.

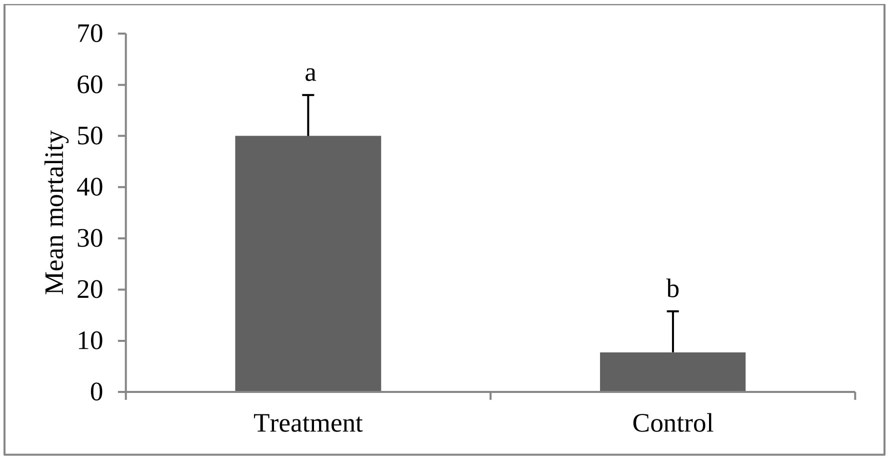

Figure 6-13: Mortality of termites exposed to sporulating capsules after 5 days

6.3.5 Attractiveness of CO_2 emitting capsules together with cocoa seedlings to termites

No significant difference (t = 0.45, p = 0.64, df = 9.99) was observed in the number of termites attracted by the cocoa seedlings together with the CEC_{EPF} and the cocoa seedlings without the CEC_{EPF} in the two choice container devices.

6.4 Discussion

The termites were able to distinguish between different offered attractive materials at a certain distance. Significantly more termites were attracted to the CO_2-emitting capsules (CEC) when 20 cm arm lengths were used than 15 and 10 cm. This implies that the capsules can produce CO_2 gradient that can outcompete the CO_2 gradient produced by the other attract components (dry wood, fungus comb and yeast) at longer distances. Although the CO_2 producing component in the capsules is baker's yeast, more termites were attracted by the capsules than the baker's yeast that was not encapsulated. This is because encapsulation of these components results in a prolonged and steady release of CO_2 (Bernklau et al., 2004; Schumann et al., 2013; Vemmer et al., 2016). Several CO_2-emitting products have previously been tested by Bernklau et al. (2004) under laboratory and field conditions for the attraction of western corn root (WCR) (*Diabrotica virgifera virgifera*) larvae. However, the baker's yeast tested in their study did not attract the larvae, which was explained by potential repellent effects from secondary metabolites produced by the test compound. In contrary to Bernklau et al. (2004) research findings, Schumann et al. (2013) demonstrated that formulated baker's yeast does not have any repellent effect on WCR larvae. According to these authors, using encapsulated baker's yeast might have changed or lowered the production of secondary metabolites, thus reducing the repellent effect. Additionally, encapsulated baker's yeast stays viable for a longer time compared with an application of pure baker's yeast into the soil. The length of CO_2 release is further increased when substrates are incorporated into the beads like starch in this system (Vemmer et al., 2016; Humbert et al., 2017a, b) In addition, Bernklau et al. (2004) also tested dried yeast granules mixed with maize-based products which diverted WCR larvae away from maize roots. This therefore implies that the use of baker's yeast as an attractant for soil insects depends on its formulation.

Although no significant differences were observed in the number of termites attracted by CEC_{EPF} and cocoa seedlings, there is a high potential of this strategy to control termites in cocoa agroforests. Previous researchers have reported that termites completely avoid virulent EPF (Rath and Tidbury, 1996; Staples and Milner, 2000; Mburu et al., 2009). However, in the present study, termites were attracted to sporulated CEC_{EPF} and it was observed that they even made attempts to feed on the capsules. Another study also suggested the possibility of reducing the repellency or avoidance of conidia and overcome behavioral defenses of termites by formulating the conidia with attractants (Lenz, 2005). Wang and Powell (2004) showed that the use of palatable baits significantly increased the effectiveness of virulence of *M. anisopliae* against *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) and *Coptotermes formosanus* (Isoptera: Rhinotermitidae), where cellulose bait treated with *M. anisopliae* caused 100% mortality to *R. flavipes* and *C. formosanus* groups without causing any repellent effects to the termites. Chouvenc et al. (2011) reviewed 50 years of research findings on the microbial control of termites to understand the underlying factors that led to bioproducts being developed using fungal pathogens, but not being successful against termites' colonies under field conditions. These long-lasting experiences have prevented EPFs from becoming a commercialized termite control measure. Co-formulating virulent EPF with CO_2 releasing material in an A&K or A&I strategy could therefore be key to improving the fungal efficacy at the field level.

No significant differences were observed in the attractiveness of the number of capsules used as well as the depth of placement of the capsules. We therefore suggest that during field release of the capsules, it will be more economical and appropriate to place between 5 and 10 capsules (rather than 20 and 25) at a minimum depth of 10 cm (or between 5 to 15 cm) to keep the capsules moist and efficient. Placing the capsules in the upper layer of the soil will not be appropriate since the capsules will need to absorb moisture from the soil and thus to rehydrate before starting to emit CO_2 which in turn attracts termites.

Termites that were exposed to the sporulating capsules started dying a day after exposure, resulting in 100% mortality 4-5 days post-exposure. Kramm and West (1982) reported that 100% mortality occurred within one day after exposure of *Reticulitermes flavipes* to *M. anisopliae*, while similar mortality rates were obtained within five days in the case of *Beauveria bassiana*. Many researchers speculate that the rapid kill of termites by some EPFs such as *M. anisopliae* could be due to not only through direct physical invasion of the hyphae, but also possibly due to some enzymatic interactions or toxic metabolites produced by the fungi (Rath, 2000; Sun et al., 2003). In addition, the delicate nature of termite cuticle might also contribute to the rapid speed of kill by EPFs.

Previous studies have shown that highly virulent isolates may not be ideal candidates for biological control programs, especially in the case of social insects like termites (Staples and Milner, 2000; Santos et al., 2007). This is because a rapid development of disease symptoms may be more easily detected by other nest mates in the colony, stimulating some defense reactions such as grooming or exclusion of the infected termites from the colony (Staples and Milner, 2000). Assuming this hypothesis is correct, screening for moderately virulent EPF isolates (slow killing speed) co-formulated with CO_2 might mitigate the defensive behaviour of termites, allowing the fungus to establish in termite mounds before the infection becomes apparent.

No significant differences were observed regarding the mortality of termites in clay pots containing mixed infected termites (“donors”) with the healthy ones (“receivers”) and the controls (healthy termites without infected ones) in the horizontal transmission experiment. Similar mortality rates were therefore obtained in the donors and receivers’ groups. Previous studies have shown that mutual grooming behavior by termite workers is highly effective in protecting them against a *M. anisopliae* infection (Yanagawa and Shimizu, 2007). Termites reared individually were highly susceptible to *M. anisopliae*, in contrast to those reared in groups which were highly resistant. This implies that the differences observed in the mortality of the termites directly exposed to sporulating capsules (direct exposure) and those transferred to the untreated group after exposure (horizontal transmission) might be due to spores on the infected termites transferred to the group that would be removed by mutual grooming behavior in the termites. The insect cuticle is the first barrier that EPFs must overcome. However, when termite workers remove foreign organisms, such as fungal conidia from the cuticle of their nest mates by grooming behavior, fungal infection is prevented at the first stage of infection (Yanagawa and Shimizu, 2007). According to Staples and Milner (2000), mutual grooming is probably an effective defense strategy of termites because the groomed conidia end up in the gut of the termites where they are unable to infect the insect. Kramm and West (1982) showed that termites exposed to *M. anisopliae* groomed more extensively than unexposed termites did, suggesting that mutual grooming behavior was initiated by the detection of foreign microbial organisms on their body surfaces. Other studies have noted that the larger the groups, the more likely it is that an individual termite carrying spores on its integument will be freed from adhering spores, and thus will have increased chances of survival (Rosengaus and Traniello, 2001). Termites also exhibit additional behavioral responses towards conspecifics such as avoidance of dead colony members (Neoh et al., 2012). Healthy termites are also able to prevent potentially harmful microorganism from proliferating by physically isolating dead individuals (Chouvenc et al., 2008; Rath, 2000). Termite corpses release a complex mixture of fatty acids (including oleic acid), indol, and phenol that act in combination to trigger burial behavior (Chouvenc et al., 2011). The burial results in the physical isolation of corpses, thus reducing the chances for opportunistic pathogens to spread among the remaining individuals. *Reticulitermes flavipes* workers were individually inoculated with conidia of *M. anisopliae* and after being kept in groups, most of the inoculated conidia were groomed from the surface of the cuticle by nest mates and many conidia were subsequently found in different parts of the gut of the groomers (Chouvenc et al., 2009). Among the thousands of conidia found in the termite's gut, conidial germination never occurred in all inspected specimens, even when the conidia had the chance to bind to the surface of the cuticular lining of the gut, implying that gut activity provides a defense mechanism against fungal epizootics at a colony level. Therefore, for the A&K or A&I strategy to become effective against subterranean termites, termites need to carry rather pre-sporulated mycelia and not consume or digest them but deposit them within the nest where they can produce spores. This way, the pathogen could be introduced to the nest before it becomes repellent to termites or removed by mutual grooming by healthy conspecifics.

Green conidia could be seen on the capsules after 4-7 days when the capsules were placed in moist soil. This implies that the fungal strain (*M. brunneum* Cb15-III) that was co-formulated in the capsules in this study sporulate rapidly, probably in the presence of the emitted CO_2. A study by Coghlan (2004) even demonstrated that the mycelium of *M. anisopliae* in the state of pre-sporulation can be 'irresistible' to termites which carry it back to the nest where it will then produce viable spores. This way, the pathogen is introduced to the nest before it becomes repellent or avoided by thetermites. One approach based on these observations is to select fungal strains with a longer pre-sporolytic phase (Coghlan, 2004), to increase the chances for successful CEC_{EPF} infection. Further screening for EPFs with a longer pre-sporolytic phase to be co-formulated in the capsules is therefore warranted.

6.5 Conclusion

In this study, both the CO_2-emitting capsules formulated without the EPF (CEC) and those formulated with *M. brunneum* spores (CEC_{EPF}) significantly attracted subterranean termites. A combination of a CO_2 attractant with *M. brunneum* in an A&K strategy could therefore represent a new method to control subterranean termite pests where CO_2-emitting capsules offer new opportunities to increase not only the efficacy of the EPFs as biocontrol agents, but also limit the repellency effects. Since the results of this study are laboratory-based, field efficacy trials should be conducted as the attractiveness of these capsules between laboratory and field conditions could differ. Hence, the effectiveness of CO_2-emitting capsules co-formulated with EPF could be proven and marketed for the development of eco-friendly and sustainable bio-insecticide for future use of termite control in cocoa agroforests.

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7 CHAPTER SEVEN: SEMI-FIELD AND FIELD EVALUATIONS OF THE ATTRACTIVENESS OF CO_2-EMITTING CAPSULES TO SUBTERRANEAN TERMITE PESTS

ABSTRACT

Subterranean termites search for host roots following CO_2 gradients. Laboratory experiments demonstrated that CO_2 as a cue could be used as an attractant in combination with entomopathogenic fungi (EPF), limiting the repellent and avoidance effects of virulent EPF in termite management. This study assessed the efficacy of calcium alginate beads containing baker's yeast (*Saccharomyces cerevisiae* Meyen ex Hansen) as an encapsulated CO_2 source (CO_2-emitting capsules) in attracting subterranean termites in semi-field and field conditions, aimed at boosting subterranean termite infection by EPF infection and preventing cocoa plants damage ("attract and kill" strategy). The attractiveness of CO_2-emitting capsules co-formulated with *Metarhizium brunneum* (CEC_{EPF}) was first assessed in the laboratory in a two-choice experiment with cocoa seedlings, and then in semi-field plots in greenhouse and lastly, under field conditions. The laboratory results showed no significant differences in the number of termites attracted to CEC_{EPF} and cocoa seedlings in the choice test. Similarly, no significant differences were observed in the number of termites recorded under cocoa seedlings in control and treatment glass cages where CO_2 emitting capsules formulated without fungi (CEC) and CEC_{EPF} were introduced in greenhouse trials. At the field, no significant differences were observed in the number of attractive stations found with termites in the control and treatment plots during the study period, as well as in the mortality of seedlings in the treatments and control plots. The "attrack and kill" strategy offers a high potential to promote biological termite control in cocoa agroforests as an alternative to insecticides.

Keywords: cocoa agroforests; carbon dioxide; *Metarhizium brunneum*; attract and kill; below ground interaction; termites

7.1 Introduction

Termites are the most dominant arthropod decomposers in the tropical forests (Culliney, 2013) and show high diversity and abundance (Bignell and Eggleton, 2000). Within tropical ecosystems, termites play a key role in modifying the biotic and abiotic environment (Bhavana et al., 2015). Unfortunately, they become serious economic pests when their appetite for wood extends to agricultural products. Termites cause damage to cocoa by destroying the roots and stems or by cutting down seedlings mostly during the dry season leading to the death of the plants (Ambele et al., 2018). They attack the plants by travelling up through the roots into the trunk and branches and eventually disrupt the movement of nutrients and water through the vascular system, resulting in plant death (Asare and David, 2010). Attacks are mainly carried out by workers and start from the taproot and sometimes on the trunks through sites of previous injuries (Tra Bi, 2013). In the plant, termites feed on the woody parts, thus hollowing out the plant and filling it with moistened soil. The soil eventually dries up and hardens, thus keeping the damaged plants or seedlings upright. Heavy termite attacks on cocoa have been reported by extension staff, growers, and during field observations in Ghana and Côte d'Ivoire where 20 to 80% of cocoa, especially seedlings, are sometimes damaged to the extent of requiring replacement (Ackonor et al., 2001; Tra Bi, 2013). In addition to cocoa, termites also cause damage to crops associated with cocoa (plantain, maize and cassava, etc.) as well as shade trees and agroforestry fruit tree species (Vos et al., 2003).

Termite control in the past relied on persistent organochlorine insecticides (Nyeko and Olubayo, 2005), which pose a significant threat to the environment and human health (Ma et al., 2009). Control of especially subterranean termites is more difficult because of unknown distribution, rapid disintegration of control compounds in the soil and difficult application techniques. Biological control using entomopathogenic fungi (EPF) has long been considered a promising technology for future termite control options (Grace, 1997; Rath, 2000). The study of pathogens for termite control started as early as 1965 (Smythe and Coppel, 1965), and mainly focused on the inundative use of *Beauveria bassiana*

(Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Grace, 1997; Culliney and Grace, 2000; Rath, 2000). However, although they have generally been proveneffective against termites in laboratory studies, they have little success in field trials due to avoidance and sealing off the diseased termites, grooming among nest mates (Rath, 2000), as well as repellency of virulent EPF (Yanagawa et al., 2008, Mburu et al., 2009; Chovenc et al., 2011). Because of these reasons, biological control of termites using these pathogens has been generally regarded as unfeasible (Culliney and Grace, 2000; Grace, 2003; Chovenc et al., 2011).

The manipulation of biological factors such as the orientation behaviour of the target organism may lead to more effective uses of EPF by increasing the probability that the insect gets into contact with the toxic substances through formulations that combine an attractant and a killing agent (El-Sayed et al., 2006). For soil pests like subterranean termites feeding on roots, such an attractant is carbon dioxide (CO_2). CO_2 is an ubiquitous volatile released by respiring plant roots (Kuzyakov and Larionova, 2005) and commonly used by many soil dwelling insect species as a cue to locate their host plants (Johnson and Gregory, 2006). It is regarded as a reliable cue for orientation because plants are unable to switch off CO_2 production (Johnson et al., 2006) and CO_2 diffuses well in the soil (>10 cm) (Hinsinger et al., 2005). Termites have also been shown to orient towards plant roots following CO_2 gradients (Bernklau et al., 2005).

Previous studies showed that termites avoid virulent EPF (Mburu et al., 2009). However, when the attract-and-kill strategy was evaluated under laboratory conditions, CO_2-emitting capsules co-formulated with a virulent EPF (*Metarhizium brunneum*) were seen to be attractive to termites (Ambele et al., unpublished data-Chapter 6). To validate these laboratory findings, semi-field and field trials were conducted to assess the attractiveness of these capsules under these conditions. The aim of this study was therefore to evaluate the attractiveness of the CO_2-emitting capsules in comparison with cocoa seedlings coupled with CO_2-emitting capsules coformulated with *M. brunneum* (CEC_{EPF}) to subterranean termites in greenhouse and field conditions. This study investigated whether CO_2-emitting capsules will increase CO_2 levels in the soil and interfere with the ability of subterranean termites to locate cocoa seedlings' roots as well as an attract-and-kill strategy, i.e., the use of CEC_{EPF} for potential control of subterranean termites. This management option is aimed at developing a more sustainable strategy for control of subterranean termites in cocoa agroforests.

7.2 Materials and methods

7.2.1 Laboratory bioassays

7.2.1.1 Collection and maintenance of termites

Termite collection and maintenance was done as previously described in chapter 6, section 6.2.1.

7.2.1.2 Soil preparation

Soil preparation was done as described in chapter 6, section 6.2.2.

7.2.1.3 Attractiveness of CO_2-emitting capsules co-formulated with *Metarhizium brunneum* and infection of attracted termites

In this set of experiments, the attractiveness of CO_2-emitting capsules co-formulated with an entomopathogenic fungus *M. brunneum* (CEC_{EPF}) was assessed in comparison with that of cocoa seedling in a two-choice test. The experimental arena was composed of 3 DANAplast plastic containers of similar dimensions (height = 19 cm, diameter = 17 cm). The central chamber constituted the release chamber, interconnected horizontally by 10 cm transparent flexible PVC tubes (1.6 cm outer diameter and 1.4 inner diameters) to two attractive chambers equidistant from one another. The tubes and plastic containers were cleaned with detergent and dried before each experiment.

The capsules consist of encapsulated yeast cells and the fungus, *M. brunneum* strain Cb15-III. Conidial viability and germination from the capsules were assessed before testing the attractiveness of the capsules to termites as previously described in chapter 6. A 95% germination of the spores was obtained.

After ascertaining the viability of spores in the capsules, 10 CEC_{EPF} were mixed with moist soil and placed in one chamber of the two-choice apparatus at 10 cm depth in moist soil. Ten CEC_{EPF} were used because preliminary studies showed no significant difference in the number of termites attracted by the different numbers of CEC used (pretesting of CO_2-emitting capsules formulated without *M. brunneum* for termite attraction, Chapter 6). A cocoa seedling was placed in the other compartment in moist soil to assess the number of subterranean termites attracted to CEC_{EPF} or cocoa seedlings. The CEC_{EPF} were placed 48 hrs before worker termites were placed in the release chamber. The growing cocoa seedlings were transferred from the nursery into the test chambers (one seedling per test chamber) two weeks before the start of the experiment. The cocoa seedlings were 4-6 months old because at this growth stage, cocoa seedlings are reported to be very attractive to termites (Ackonor et al., 2001). Before the experiment, a batch of 100 worker termites were removed from the maintenance containers and placed on moist sterilized soil in Petri dishes overnight, with the lids of the Petri dishes having aeration holes to ensure free air flow. For this test, 100 termites were released at the central container (release chamber). Termites were allowed to crawl around the container and made a choice between CEC_{EPF} or cocoa seedling. The experiment was replicated 20 times. The number of termites in all the test chambers was recorded and removed at every 1 hr interval for 6 hrs, for each replicate.

To evaluate the infection of attracted termites, the experiments were conducted using round plastic containers of 15 cm diameter × 5 cm in two-container choice devices. The bowls were filled with sterilized black soil moistened with distilled water. The central bowl (the release chamber) was connected to two test chambers using 10 cm transparent tubes (1.6 cm outer diameter, 1.4 cm inner diameter). Fifteen CEC_{EFP} were placed in one of the test chambers on moist soil for 14 days to allow the growth and sporulation of *M. brunneum* from the capsules. Four to six months old cocoa seedling was placed in the other test chamber. Hundred worker termites were released in the central chamber and movement of the termites in both test chambers was observed with a torch light, where the number of termites that were attracted into each test chamber was counted. Termites collected from the chambers containing the capsules were placed on moist soil in round plastic bowls as treatments and corresponding numbers of termites that were not used in the experiments were placed in other bowls as controls. Pieces of the wood from where the termites were collected were placed in each bowl to serve as food. Soaked cotton was placed in each bowl to maintain moisture and mortality was recorded daily for 5 days. The experiment was replicated 10 times.

Mycosis test also was conducted, where the dead insects were surface sterilized with 70 % alcohol and then rinsed thrice in distilled water. The surface sterilized cadavers were kept separated in Petri dishes lined with sterile moistened filter paper to record fungal outgrowth and verify if mortality could be attributed to the capsulated *M. brunneum*. Mortality due to fungal infection was confirmed by the presence of hyphae and conidia on the surface of the cadaver.

7.2.2 Semi-field evaluation of the attractiveness of CO_2-emitting capsules (Disruption of host finding)

Termites were collected from the field and maintained in the laboratory as described above. The disruption of host finding experiments were conducted to test the attractiveness of CO_2 emitting capsules formulated without the entomopathogenic fungi (CEC) as well as the attractiveness of CO_2 emitting capsules formulated with *M. brunneum* (CEC_{EPF}) to termites in 1.5 m × 1 m × 0.5 m high cages designed with 5-mm-thick transparent glass sheets (semi-field plots) (Figure 7.1). The capsules were prepared by encapsulating commercially available yeast (as an artificial source of CO_2) in calcium alginate beads, and the CEC_{EPF} co-formulation included *M. brunneum* Cb15-III spores and baker´s yeast, co-encapsulated in one bead (Patel et al., 2014). The CEC and CEC_{EPF} were obtained from the University of Applied Science Bielefeld, Germany. The glass cages were kept in a greenhouse at 25 ± 2° C, 70 ± 5% relative humidity and ambient light (semi-field experiment), and were filled with black soil, obtained from a local soil supply company in Yaoundé (Cameroon). The soil was sieved to remove all debris and roots. Black soil was chosen to enable effective observation of worker termites and because it contains high organic matter which is very important for plant growth. Each semi-field glass cage was placed on a 73 cm metal stand with each leg of the metal stand emerged in a bowl of water to prevent the entry of ants into the boxes. The cages were then filled with the sieved black soil to the depth of 20 cm. Six months old cocoa seedlings were transferred from the nursery and transplanted into the glass cages and kept for at least two weeks before starting the bioassays. Each glass cage had two rows of cocoa seedlings with three cocoa seedlings per row, with 1 m between rows and 0.5 m within row spacing (Figure 7.1). The seedlings were watered once every week. Holes of 10 cm deep were made half way (25 cm) between seedlings in the treatment plots where 20 capsules (CEC or CEC_{EPF} in different set of experiments) were applied by digging up the soil, inserting the capsules, and then covering them with soil. Two hundred termites collected from the termites kept in the laboratory (as described above) were released in a single hole at the middle of each cage at 10 cm depth, 48 hrs after the CEC was applied and 8 days after CEC_{EPF} was applied respectively. The number of termites attracted to each cocoa seedling in a cage was determined 1 hr post-release by removing a cube of soil around the base of each seedling in the semi-field plot. The soil was searched for termites and the number of termites per cube counted and recorded. Each treatment was replicated four times.

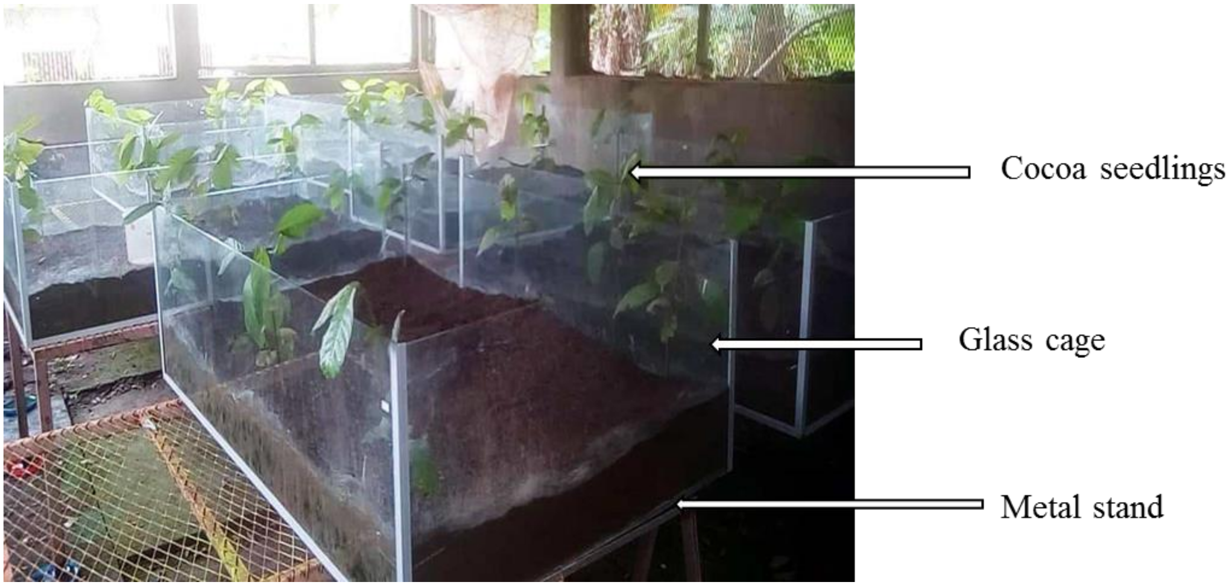

Figure 7-1*:*Set-up for semi-field trial of the attractiveness of CO_2-emitting capsules to subterranean termites

7.2.3 Field trial of the attractiveness of CO_2 emitting capsules formulated with *Metarhizium brunneum* for "attract-and-kill" strategy of subterranean termite pests

7.2.3.1 Experimental site

This study was conducted at the Institute of Agricultural Research for Development (IRAD) Regional Center, Nkolbisson, Yaoundé (11° 46 'E; 3° 86 'N and 740 m a.s.l). At Nkolbisson, the climate is equatorial with a bimodal rainfall pattern. The average annual rainfall is 1560 mm with rains occurring mainly during two rainy seasons (April to June, and September to November), alternate with two dry seasons (November to March, and June to August). Daily temperature is constant throughout the year with an average annual temperature of about 23.5 °C. The soil is of clayey loam texture (Manet et al., 2017).

7.2.3.2 Experimental design

Eight plots (4 treatments and 4 controls) of 5 m × 5 m each were established with 5 m distance apart. A random block design was used for the placement of plots. In each plot, 16 six-month-old cocoa seedlings from the nursery were transplanted, with 1.67 m between and within rows. The seedlings were transplanted in the experimental plots in June (the peak of the rainy season). Twenty-five (25) CEC_{EPF} were placed in soil dug from the plots in round transparent plastic bowls (15 cm diameter by 7 cm high), with lids to constitute attractive stations. The aim here was to determine whether subterranean termites will be attracted away from cocoa seedlings to bowls (stations) that were actively generating CO_2 and if sporulated CEC_{EPF} could also infect the attracted subterranean termites, thus offering protection to cocoa seedlings against subterranean termite pest damage. Each plastic bowl was perforated with 14-16 holes (1-2 cm in diameter) around the circumference using an electric soldering iron and then filled to within 5 cm with soil. The plastic bowls together with the CEC_{EPF} (treatments), and those without CEC_{EPF} but dry wood (controls) were buried 10 cm deep in the soil and with 0.84 m between and within each row of the treatment and control plots respectively. A total of 24 stations were installed in each plot. The treatments were applied two weeks after the seedlings were transplanted to ensure that all the seedlings were in good condition and had started growing. The seedlings that had withered because of poor handling were replaced prior to the treatment applications. The stations were checked once every week for five weeks. To check a station, the soil was removed over the bowl, the lid was removed, and the soil searched for termites. The lids were replaced, and the bowls covered again with soil as it had been found. A station was reported as positive if termites were attracted into the bowl. The bowls were removed, and the capsules applied directly in the soil after five weeks to avoid the possible interference of ants with the bowls. The capsules were replaced after every 20 days for five months. This is because previous studies have shown that the application of CO_2-emitting capsules in the soil increased CO_2 levels around the capsules for up to 20 days (Schumann et al., 2014). The number of cocoa seedlings dislodged or wilted because of termites feeding from the treatment plots was also compared to those dislodged from the control plots after five months. To confirm that the dislodged seedlings were because of termite feeding, the presence of live termites was confirmed by pulling out the affected plants, and the roots and lower stem examined for termite attack. Also, the wilted plants were counted as wilting is usually considered as the first sign of termite attacking roots on seedlings or older plants (Bishwajeet et al., 2018).

7.2.4 Data analyses

The data for the number of termites attracted to the CEC_{EPF} and cocoa seedling was tested for normality using the Shapiro-Wilk test. Often the datasetswere not normally distributed and was therefore analysed using the Wilcoxon test. The data for the number of termites collected around cocoa seedlings in the control and treatment plots when the CEC or CEC_{EPF} were applied in the semi-field boxes were normality distributed when tested for normality with the Shapiro-Wilk test and thus compared using the two-sample t-test (unpaired). The total number of stations where termites were found at least once up to that week of the study in the treatment and control plots in the field trial as well as the mortality of seedlings in the control and treatment plots were also compared using the two-sample t-test (unpaired). All analyses were performed using R software, version 3.2.3 (R Development Core Team, 2015).

7.3 Results

When the capsules co-formulated with *M. brunneum* (CEC_{EPF}) were placed at 10 cm depth in the soil and tested for the attractiveness to termites, no significant differences (W = 202, P = 0.97) were observed in the number of termites attracted by the cocoa seedling and CEC_{EPF}. However, in assessing the infection of attracted termites, no significant difference (t = -1.5, P = 0.15) was observed in termite mortality collected from the bowls with sporulated capsules and controls after five days post-exposure in the experiment, even though the percent mycosis in the cadavers collected from the treatment boxes was 83%.

No significant differences (t = 0.67, df = 5.25, P = 0.53) were observed in the number of termites collected around seedlings in the control (mean = 95.25), and treatment plots (mean = 79.25) when CEC were introduced into the glass cages in the semi-field experiment. Also, no significant difference (t = -1.4, df = 6, P = 0.21) was observed in the number of termites collected around seedlings in the control (mean = 110.2) and treatment (mean = 129.5) boxes when CEC_{EPF} was placed in the boxes.

For the field trials, no significant differences (t = 1.90, P = 0.11) were observed in the number of attractive stations found with termites in the control plots (mean = 12.25) and treatment plots (mean = 10) for five weeks. Also, no significant differences (t = 2.61, P = 0.07) were observed in the mortality of seedlings in the treatment and control plots. However, no seedling mortality was observed in the treatment plot (0%) out of the 64 compared to 5 out of the 64 seedlings in the control plots (7.81%) that were destroyed by termites.

7.4 Discussion

When the capsules co-formulated with *M. brunneum* (CEC_{EPF}) were tested for the attractiveness to termites in the choice test with cocoa seedlings, no significant differences were observed in the number of termites attracted by the cocoa seedling and CEC_{EPF}. It can therefore be deducted that both cocoa seedling and CEC_{EPF} have equal attraction capacity and termites could not identify the difference between the capsules and the seedlings. This result shows a high potential of the “attract and kill” strategy to control termites in cocoa agroforests. Previous researchers have reported that termites completely avoid virulent EPF (Rath and Tidbury 1996; Staples and Milner 2000; Mburu et al. 2009). However, although *M. brunneum* was found to be very virulent, when co-formulated with CO_2 producing material in the present study, termites were attracted to sporulated CEC_{EPF} and even found making attempt to feed on the capsules. Some studies have also suggested the possibility of reducing the repellency or avoidance of conidia and overcome behavioral defenses of termites by formulating the conidia with attractants (Lenz, 2005). Research conducted by Wang and Powel (2004) showed that the use of palatable baits greatly increased the effectiveness of virulence of *M. anisopliae* against *Reticulitermes flavipes* (Isoptera:

Rhinotermitidae) and *Coptotermes formosanus* (Isoptera: Rhinotermitidae) whereby cellulose bait treated with a virulent EPF caused 100% mortality to *R. flavipes* and *C. formosanus* groups without causing any repellency effects to the termites. Chouvenc et al. (2011) examined 50 years of research outputs on the microbial control of termites to understand why commercial bio-products have been well developed, but most field studies failed to eliminate termite colonies using fungal pathogens. The authors concluded that these failed experiences have prevented EPF from becoming a stand-alone termite control measure. Co-formulating virulent EPF with CO_2 releasing material in “attract and kill” (A&K) or “attract and infect” (A&I) strategy could therefore be the key to improve the fungal efficacy at the field level and overcome the repellency and avoidance effects.

No significant differences were observed in the number of termites collected around seedlings in the control and treatment plots when CEC were introduced into the glass cages in the semi-field experiment. Also, no significant differences were observed in the number of termites collected around seedlings in the control and treatment boxes when CEC_{EPF} was placed in the boxes. Therefore, application of CEC or CEC_{EPF} failed to disrupt the worker termites in finding cocoa roots in the semi field trial. The capsules were placed between the cocoa seedlings (25 cm from each plant base) to reduce the chance of termite encountering cocoa roots as much as possible. However, based on the laboratory results which showed that the capsules were attractive to termites, it can be stipulated that the termites might have been initially attracted to the capsules in the boxes but were later able to locate cocoa seedling roots. This becausethe capsules were not formulated with phagostimulants or host specific compounds that could cause the termites to feed on the capsules, making them spend more time around the capsules. This further explains why no significant differences were observed in termite mortality collected from the bowls with sporulated capsules and controls after five days post-exposure in the experiment conducted in the laboratory. Thus, an improved formulation of the capsules with an enhanced attractiveness for termites needs to be developed for future application.

No significant differences were observed in the number of attractive stations found with termites in the control and treatment plots. This strategy, however, offers a high potential to promote biological termite control in cocoa agroforests as termites did not avoid the virulent EPF (*M. brunneum*) co-fomulated with a CO_2 generating material as observed in previous studies (Mburu et al., 2009). Also, no significant differences were observed in the mortality of seedlings in the treatment and control plots. However, no seedling mortality was observed in the treatment plots compared to five seedlings in the control plots that were destroyed by termites. This could be because even though termites damage crops from sowing till harvest; it is usually difficult to detect damage in the field as it is oftentoo late when the symptoms are noticed (Bishwajeet et al., 2018). This investigation should therefore be considered as a preliminary study for further large-scale field trials on the attractiveness of the capsules to termites in cocoa farms with few ants' presence and for longer duration, since the five months duration used for observing termite damage on seedlings in this study may have been insufficient. However, the termites in the plots treated with CEC_{EPF} might have died from the infection of the spores from the capsules, which explains why no seedling mortality was observed in these plots.

Foraging by insects has been described as a three-stage process (Judd, 2018): (i) search patterns, (ii) response to food location cues, and (iii) assessing a food source (Bernays and Chapman 1994). The first stage usually occurs in the absence of cues that indicate the location of a food source while the forager identifies a potential food source at the second stage. Generally, cues will allow foragers to determine the location of a food source and provide some indication of its quality. The third stage occurs at the food source itself. It is at this final stage that phagostimulants and other cues allow the forager to determine the palatability of the food source because once the forager reaches a food source, the individual insect must determine if the food is palatable or not. These cues should indicate the nutrients available to the termites and the presence of secondary metabolites that would affect survival. Subterranean termites are central-place foragers and based on a research conducted by Judd et al. (2018), it appears that they apply strategies based on these three stages when foraging for food. However, phagostimulants were not incorporated in the capsules. As suggested by Sattar et al. (2017), the success of slow acting toxicant bait depends not only on its attraction, but also on its palatability and should be introduced into the colony's gallery system and transferred to unexposed nest-mates by social grooming or trophallaxis. Studies have shown that subterranean termites prefer foods that contain nutrients (Oi et al., 1996), and it is therefore possible to associate a nutrient, or group of nutrients in addition to the CO_2 source which could enhance its palatability for termites, thus aiding in the transfer of the spores to the unexposed nest mates. Studies conducted by Judd (2009; 2018) have shown that high levels of cellulose and other phagostimulants like glucose, yeast, popular sawdust extract and urea can increase termite's food consumption. Various carbohydrates have also been suggested to act as termite phagostimulants (Zhou et al., 2008). Therefore, if these phagostimulants and nutrients are incorporated with the capsules, the effectiveness of CO_2 emitting capsules co-formulated with entomopathogenic fungi could be proven and marketed for the development of eco-friendly bioinsecticide for future use in termite control in cocoa agroforests. Other types of formulations to increase *M. brunneum* outgrowth or the delivery of conidia to subterranean termite pests such as endophytic colonization should also be considered to fully exploit the control of termite pest species in cocoa agroforests with EPF.

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8 CHAPTER EIGHT: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

8.1 General discussion

Termites are the major soil dwelling insect pests in cocoa agroforests. Currently, termite management heavily rely on the use of persistent organochlorine insecticides (Su and Scheffrahn, 1998). However, following the banning of the organochlorine insecticides in many countries due to their negative associated impacts in recent years, there has been renewed interest in the development of alternative termite control measures. Farmers in many parts of Africa use various traditional methods, including queen removal and application of plant extracts, which are often with limited success (Nyeko and Olubayo, 2005; Sileshi et al., 2009; Ambele et al., 2018). Biological control using entomopathogenic fungi (EPF) is the most promising alternative to synthetic chemical pesticides (Rath, 2000; Grace, 2003; Chouvenc and Su, 2010). However, there are limitations in their application because they do not achieve high control efficacies in the field when applied as conidial suspensions. This is mainly due to repellency or host avoidance behavior towards these biocontrol agents (Yanagawa et al., 2008; Mburu et al., 2009). To overcome these limitations, manipulation of termites' behavior could offer a potential solution in effectively managing the pest and enable a more effective use of EPF by combining them with semiochemicals used in host finding as attractants. Such combination known as "Attract and Kill" (A&K) or "Attract and Infect" (A&I) has been proven to be a very effective method of controlling the Western corn root worm larvae (Schumann et al., 2013). With this strategy, the insect pest is lured to an attractant used by the insect for locating its host (e.g. semiochemical = attract) and subjected to an insecticide or EPF (= infect/kill) killing off the pest (El-Sayed et al., 2009). For subterranean termites feeding on roots, such an attractant is CO_2, released by respiring plant roots (Bernklau et al., 2005; Schumann et al., 2013). This study, therefore focused on developing an environmentally friendly control strategy (A&K or A&I) against these major soil dwelling insect pests in cocoa agroforests. A critical review was conducted on soil-dwelling insect pests of tree crops in sub-Saharan Africa to determine the major destructive soil insect pests of tree crops. The outcome of this review (Chapter three) showed that termites are not only major pests of cocoa but are also pests of other tree crops in sub-Saharan Africa. Although some species of termites play major roles in processes such as decomposition, as well as nutrient and carbon cycling (Jones and Eggleton, 2000). In certain ecosystems, their harmful effects are greater than their beneficial roles to the extent that they have been considered as the primary cause of ecosystem deterioration (Mugerwa et al., 2012). It was also established that termites are the most serious soil insect pests causing mortality of seedlings after transplanting in the field. In cocoa agroforests, termites cause direct damage by destroying the roots and stems or by cutting down seedlings mostly in the dry season leading to the death of cocoa seedlings and plants. Termites also cause indirect yield loss to cocoa by decreasing translocation of water and nutrients (Asare and David, 2010). In addition, some termite species like *Macrotermes* spp. attack cocoa pods causing the pods to dry up after severe infestations (Anikwe et al., 2009). Attacks are mainly from the workers and will start from tap root and sometimes from the trunks through sites of previous injuries (Ackonor, 1997). Once in the plant tissues, termites feed on the woody parts, thus hollowing out the plant and filling it with moistened soil. The soil eventually dries up and hardens thus keeping the damaged plants or seedlings upright. The gnawing activity of termites also opens the door to other fungal pathogens causing the decomposition of wood (Chapter three). In addition to cocoa, termites cause damage to crops associated with cocoa as well as shade trees and agroforestry fruit tree species, negatively affecting smallholder cocoa livelihoods (Vos et al., 2003).

The study further underlined the taxonomic patterns and functional diversity of termites in cocoa agroforestry systems, where the major termite species attacking cocoa were identified, and the consequences of different types of shade management on the taxonomic patterns and functional diversity of termites in cocoa agroforestry systems determined (Chapter four). Termite sampling in the different shade management systems of cocoa plantations provided evidence that shade reduction in cocoa agroforests is the main cause of termites becoming major pests in cocoa agroforests, and that most damage in cocoa agroforests is associated with termite species from sub-families Macrotermitinae and Termitinae. Also, it was observed that soil-feeding termites which create and maintain favourable soil conditions for plant growth and nutrient intake are more vulnerable to reduced shade cover over wood feeders and as such are becoming main pests of cocoa trees. Other studies have also shown that with increasing shade, herbivorous pests of cocoa such as mirid bugs and cocoa pod borers are fewer when compared to full sun systems where the rate of herbivory on cocoa pods is less (Bisseleua et al., 2013). Shade removal in cocoa agroforests has also been proven to be a determining factor of other major insect pests of cocoa like cocoa mirid infestation and damage in West Africa, where unshaded plantations are usually more damaged by mirids than shaded ones (Babin et al., 2010). In addition, the severity of attack by the cocoa beetle (*Steirastoma breve*) has also been observed to increase with shade level reduction (Liendo-Barandiaran et al., 2010), while mealybug vectors of cocoa swollen shoot virus disease (CSSVD) were found to be more abundant under full-sun conditions than under shade. On the other hand, the number of mealybug predators and parasitoids are often higher on shaded plots (Andres et al., 2018). Other studies have also shown that infection with mistletoes, which indirectly encourage the spread of CSSVD were found to be higher under full-sun conditions in West Africa (Schroth et al., 2000). Investment is therefore needed to encourage and educate cocoa farmers about the diverse ecosystem services provided by shaded systems. Production of cocoa in agroforestry systems, with shade tree species should also be encouraged because shade trees reduce soil temperature, wind speed, excessive vegetative growth, nutritional imbalance and dieback of cocoa trees, and fruit abortion. Shade trees also protect cocoa pods against windborne spores of fungal diseases, improve soil moisture availability, increase soil fertility, prevent soil erosion, suppress weed growth, and increase the overall insect biodiversity which improves yield through natural control of pest population and increased pollination services (Vanhove et al., 2016). Apart from these agroecological benefits, cocoa farmers also get additional income from selling fruits from some shade trees (Bisseleua et al., 2009).

In chapter 5, fourteen entomopathogenic fungal isolates were screened against a key subterranean termite pest species that causes significant damage to cocoa to select the most virulent isolate for effective biopesticide development. Althhough all the tested fungal isolates were found to be pathogenic to termites, the *Metarhizium* isolates were more virulent than the *Beauveria* isoates, with the three most virulent being *M. brunneum* Cb15-III, *M. anisopliae* ICIPE 30 and 60. These isolates outperformed all the other isolates screened against the termite species, achieving LT_{50} in less than two days (Chapter five). This higher virulence effect of *Metarhizium* isolates compared to *Beauveria* isolates confirms earlier reports (Wang and Powell, 2003; Cherry et al., 2005; Singha et al. 2006; Singha et al., 2011; Sileshi et al., 2013) showing a similar pattern of activity with isolates of these two fungal pathogenic species. However, highly virulent isolates may not be ideal candidates for biological control programs, especially in the case of social insects like termites because most of them have been found to be repel termites (Mburu et al., 2009). The study therefore tested whether calcium alginate beads containing baker's yeast (*Saccharomyces cerevisiae* Meyen ex Hansen) as an encapsulated CO_2 source (CO_2-emitting capsules) co-formulated with the EPF *M. brunneum* Cb15-III (CEC_{EPF}) could establish CO_2 gradients in the soil that could outcompete CO_2 produced by respiring cocoa root seedlings to attract and consequently kill subterranean termite pests both in the laboratory and under field conditions. Since CO_2 is known to attract termites and used by termites to locate cocoa plant roots and plants, CO_2-emitting capsules were used to mimic the natural scenario. The laboratory evaluation of the attractiveness of CO_2 emitting capsules co-formulated with *M. brunneum* Cb15-III for A&K strategy against the major subterranean termite pest species (*Microtermes* sp.) sampled in cocoa agroforests (Chapter six) showed no significant differences in the number of termites attracted by cocoa seedlings and the capsules. When termites were directly exposed to sporulating capsules, 100% mortality was obtained within 5 days post-exposure. Although no significant differences were observed in the number of termites attracted by CEC_{EPF} and cocoa seedlings, there is a high potential of this strategy to control termites in cocoa agroforests. This is because the tested termite species were equally attracted to the capsules co-formulated with the EPF, and consequently could not differenciate between CO_2 emission from capsules and/or from cocoa roots. Previous researchers have reported that termites completely avoid virulent EPF (Rath and Tidbury, 1996; Staples and Milner, 2000; Mburu et al., 2009), which was not the case in this study where the virulent EPF was coformulated with a CO_2 producing material (attract component). This finding confirms the report of Lenz (2005), suggesting the possibility of reducing the repellency or avoidance of conidia to overcome behavioral defenses of termites by formulating the conidia with attractants.

The semi-field evaluation of the attractiveness of CO_2 emmiting capsules to termites did not give satisfactory results, as application of CEC or CEC_{EPF} failed to substantially disrupt the finding of cocoa roots by worker termites in the semi field trial. However, based on the laboratory results which showed that the capsules were attractive to termites, it can be stated that the termites might have been initially attracted to the capsules in the boxes but were later able to locate cocoa seedlings roots since the capsules were not formulated with particular phagostimulants or host specific compounds that could cause the termites to feed on the capsules, making them to spend more time around the capsules. Thus, an improved formulation of the capsules with an enhanced attractiveness for termites needs to be developed for future application. For the field trials, no significant differences were observed in the number of attractive stations found with termites in the control and treatment plots. This confirms the laboratory results where termites were equally attracted to the CEC_{EPF} and cocoa seedling roots. This “attract and infect” or “attract and kill” strategy offers a high potential to promote biological termite control in cocoa agroforests as termites could not avoid the virulent EPF (*M. brunneum*) as observed in previous studies (Mburu et al., 2009). Also, no seedling mortality was observed in the treatment plots compared to 5 plants in the control plots that were destroyed by termites. This implies that the termites in the plots treated with CEC_{EPF} might have died from the infection of the spores from the capsules. CO_2-emitting capsules co-formulated with virulent EPF therefore offer new opportunities to increase the efficacy of biocontrol agents not only in cocoa plantations, but also could be extended to other termites' susceptible crops.

7.3 Conclusion

From this study, it can be concluded that soil-dwelling insects, especially termites, are pests of young plants including tree seedlings and saplings in tree nurseries oryoung plantations and are likely to become more important especially with the increasing conversion of forest ecosystems to farmlands. More research on these insects is therefore needed to curb food insecurity in SSA.

In addition, richness and evenness of termite species are driven by cocoa shade systems, where soil-feeding termites which create and maintain favourable soil conditions for plant growth and nutrient intake are more vulnerable to reduced shade cover than wood feeders which are becoming main pest of cocoa trees. Shade reduction is also affecting wood feeders which are gradually shifting their food sources from leaf litter and crop residues to becoming main pests of cocoa trees. Therefore, shaded cocoa agroforestry systems should be encouraged in cocoa production systems to create favourable conditions for the survival of soil-feeding termites and reduction of wood feeding ones.

The screening of entomopathogenic fungi to select potent ones for termite pest control suggests that *Metarhizium brunneum* Cb15-III and two *M. anisopliae* isolates (ICIPE 60 and ICIPE 30) have the potential to be developed as microbial agents for controlling subterranean termite pests in cocoa agroforests. The CO_2-emitting capsules formulated without the EPF (CEC) and those co-formulated with *M. brunneum* spores (CEC_{EPF}) significantly attracted subterranean termites. A combination of a CO_2 attractant with *M. brunneum* in an A&K strategy could therefore represent a new method to control subterranean termite pests where CO_2-emitting capsules offer new opportunities to increase not only the efficacy of the EPF as biocontrol agents, but also limit the repellent effects of virulent EPF. The A&K

strategy therefore offers a high potential to promote biological termite control. There is however a need for further large-scale field trials of the A&K strategy in cocoa farms.

7.4 Recommendations and future research routes

Although the results of this research work proposed an approach to mitigate the avoidance or repellency effect of termites towards entomopathogenic fungi, the study also opens new research routes for further investigations.

1. Increased exploitation of entomopathogenic fungi in combination with cues such as carbon dioxide which soil insects use to locate host plants ("attract and kill" approach) could be recommended as potential biocontrol strategy of termites in sub-Saharan African cocoa plantations. The approach could also be extended by exploring its efficacy with entomopathogenic nematodes.
2. Cocoa agroforestry systems with around 55% shade should be optimal to balance termite infestations and marketable yield and should therefore be encouraged in cocoa production systems in order to create favorable conditions for the survival of soil-feeding termites which have beneficial ecosystem functions in cocoa agroforests.
3. Cocoa farmers should be trained on the importance of shade canopy in reducing not only termite damage but also damage caused by other major insect pests of cocoa.
4. Detailed studies and quantification of losses by soil dwelling insect pests on tree crops in sub-Saharan Africa are needed.
5. There is a need to explore genetic variation in the tolerance and resistance of plant roots to soil insect pests, with focus on termites in cocoa production.
6. The effectiveness of the various traditional control strategies practiced in different parts of sub-Saharan Africa for termite management needs to be empirically verified, improved and promoted for the control of termites as well as other soil dwelling insect pests.

7. The importance of farmer information literacy and awareness towards soil pests and food security cannot be over emphasized. Efforts should be devoted to the dissemination of information and modern knowledge on soil pests and their management to farmers.
8. Further research on belowground orientation and movement of termites is needed to potentially improve and increase the efficacy of attract and kill strategy as a management option against subterranean termite pests.
9. New formulation development of moderately virulent fungal isolates with CO_2 generating materials (CO_2 emitting capsules) are warranted to improve on its efficacy and application techniques, as well as assessement of the attractiveness to termites in both laboratory and field conditions.
10. *Metarhizium brunneum* and the other fungal isolates such as *M. anisopliae* ICIPE 30 and 60 which have been proven to be very virulent against termites, could also be evaluated for their colonization of cocoa plant tissues as endophytes that could confer systemic resistance against termites and protect the seedlings against termites' damage.
11. It is highly recommended to also study the attractiveness of the CO_2-emitting capsules co-formulated with virulent EPF to other ecologically beneficial soil dwelling non-target insects and termites associated natural enemies before adopting this technology in integrated termite management in cocoa agroforests.
12. It is important to assess effective horizontal transmission of the fungi from the capsules by termites to conspecifics, ultimate colony death and survival under large scale field conditions.

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