EVALUATING THE POTENTIAL OF LURING *Pheidole megacephala* USING FOOD BAITS TO MANAGE *Xylosandrus compactus* IN UGANDA

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EVALUATING THE POTENTIAL OF LURING *Pheidole megacephala* USING FOOD BAITS TO MANAGE *Xylosandrus compactus* IN UGANDA

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DEDICATION

To my dear wife Grace and our children; Glennis, Gloria and Jabes.

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LIST OF ABBREVIATIONS AND ACRONYMS

BCTB	Black Coffee Twig Borer
CABI	Centre for Agricultural Biosciences International
FAO	Food and Agricultural Organization
IACO	Inter African Coffee Organisation
ICO	International Coffee Organisation
IPM	Integrated pest management
NaCORI	National Coffee Research Institute
SEM	Standard error of the mean
UCDA	Uganda Coffee Development Authority
UCF	Uganda Coffee Federation

ABSTRACT

The black coffee twig borer (BCTB) *Xylosandrus compactus* (Eichhoff) is an economically important pest of Robusta coffee in Uganda. In this study, the predatory ant, *Pheidole megacephala*, was evaluated for potential use as a biological control agent against *X. compactus*. The research tested hypotheses that (1) *P. megacephala* feeds on all stages of *X. compactus*, (2) *P. megacephala* can enter galleries of BCTB inside coffee twigs in search for the prey and (3) presence of *P. megacephala* on infested twigs reduces populations of BCTB in the galleries (4) *P. megacephala* prefers certain food baits and (5) *P. megacephala* presence on infested Robusta coffee reduces incidence and abundance of BCTB in the galleries.

In a petri dish feeding bioassay, between 6th and 22nd July 2014, *P. megacephala* preyed upon all the stages of BCTB without any indication of preference for any stage. In a bioassay from 4th to 6th August, 2015 to determine if *P. megacephala* can enter galleries of *X. compactus* inside Robusta coffee twigs, the researcher found that the predator was unable to enter the galleries. In a field cage bioassay between 16th September and 16th October 2014, *P. megacephala* reduced the population of all life stages of BCTB on Robusta coffee twigs by almost 22 fold compared to untreated control. In screening baits for attractiveness between 30th and 31st July 2015, the results revealed that *P. megacephala* is attracted to honey, fish, beef and Royco but 25, 6.5 and 4.4 times more to honey than to Royco, fish and beef respectively. In determination if *P. megacephala* presence on infested Robusta coffee reduces incidence and abundance of BCTB in the galleries between 3rd August and 3rd September 2015, the findings indicate that *P. megacephala* significantly reduces *X. compactus* incidence in the Robusta coffee field and also reduces *X. compactus* abundance though not significantly. In view of these findings, the study concludes that *P. megacephala* is an indiscriminate predator of all growth *X. compactus* stages, and, though unable to enter BCTB galleries, the predator reduces *X. compactus* population on infested twigs. Exploitation of *P. megacephala* in the biological control of BCTB on coffee and other crops would require additional studies on how to enhance presence of the predator on the infested crop and to establish if blending sugar and protein baits would produce synergistic attractiveness to predatory ants.

CHAPTER ONE

GENERAL INTRODUCTION

Introduction

This chapter provides a detailed description of the back ground to the study, the problem statement and high lights the major objective, specific objectives and research hypotheses. It also describes the significance and justification of the study. This chapter also provides the definition to key terms.

1.0 Back ground

Black coffee twig borer (*Xylosandrus compactus)* (Eichhoff) (Coleoptera: Curculionidea) is a polyphagous pest of over 225 known host plants and forest tree species(CABI, 2005, Ngoan et al., 1976). The pest's native distribution is reported to be tropical and subtropical Southeast Asia from where it has been introduced into Africa, Indian Ocean islands, United States of America, Latin America, Caribbean Island, New Caledonia, New Zealand, Hawaii, Samoa and Fiji (Pennacchio et al., 2012). Pennacchio et al. (2012) reported spread of the pest in African countries including Mauritania, Sierra Leone, Liberia, Ivory Coast, Ghana, Nigeria, Cameroon, Equatorial Guinea, Gabon, Republic of Congo, Democratic Republic of Congo, Uganda, Kenya, Tanzania and South Africa.

In Uganda, *X. compactus* was first reported in 1993 on Robusta coffee in Bundibugyo district western Uganda (Egonyu et al., 2009). The second outbreak was in Rukungiri, Kanungu and Bushenyi in 2002, another in Nabbale subcounty in Mukono district in 2007 and Kayunga and Mukono districts in 2008. The most recent report indicates an enormous spread of *X. compactus* to most Robusta coffee growing regions with central, southern and south western regions registering the highest infestation (Kagezi et al., 2013). Only adult females of the pest initiate infestation of host plants by forming galleries on the twigs. Males are flightless and remain in the gallery throughout their life (Dixon et al., 2003). Flagging of infested branches and shoots usually occurs 5-7 days after gallery formation and wilting within weeks of infestation (Hara and

Beardsley, 1976). Entrance holes are approximately 0.8 mm in diameter and are located on the underside of the branches (Egonyu et al., 2009). While in the twigs, BCTB brood does not feed on the host plant material, but uses it as a medium for growing ambrosia fungus for food, hence the name ambrosia beetles. Absence of a suitable host is therefore not a limiting factor, since any woody material of suitable moisture content and size may be all that is required for the survival of these insects. The life cycle of BCTB is completed in about one month (Ngoan et al., 1976). *Xylosandrus compactus* is an economic pest of coffee which causes death of primary branches leading to considerable decline in the coffee yield (Egonyu et al., 2009).

Fig 1 Damage by BCTB

Several control options for *X. compactus* with varying effectiveness and sustainability have been recommended. Monocrotophos, an insecticide, is reported to have been used effectively against the pest in India (Meshram et al., 1993). Chlorpyrifos is reported to kill between 77 and 92% of all stages of *X. compactus* (Mangold et al., 1977, Shuping et al., 2001). Also Permethrin or bifenthrin, quinalphos or chlorypyrifos plus cypermethrin are reported to give good control (Bambara, 2003). In Florida, a test of Zeta cypermethrine, bifenthrine,Lambda-cyhalothrin and thiamethoxam gave good results as contact insecticides against BCTB (Peña et al., 2011). Malathion is reported by farmers in Mukono and Kayunga districts in Uganda to give good results in the control of the pest (Egonyu et al., 2009). Imidacloprid, a synthetic insecticide, mixed with Tebuconazal a fungicide for the concurrent management of the insect and the ambrosia fungus a management practice which is currently recommended in Uganda (Egonyu, 2013). However, the use of synthetic chemical insecticides normally encounters limitations due to environmental, ecological and regulatory concerns (Burbano et al., 2012, Pennacchio et al., 2012). It is also difficult to apply chemicals to the concealed habitats in which *X. compactus* feeds; and these chemicals can be unaffordable by most farmers. The effective cultural control practice is pruning and burning of infested twigs (Egonyu, 2013). This however,is labor intensive and subsequently, reduces the berry bearing branches and eventually coffee yields. In Africa, there are no known effective bio control agents for *X. compactus*. The entomopathogenic fungus, *Beauveria bassiana* is reported to cause some mortality in *X. compactus* (Balakrishnan et al., 1994, Brader, 1964), although there are no known reports of its application in managing the pest in the field. Ethanol-baited traps have been demonstrated to attract *X. compactus*; while verbenone and limonene repel the pest (Burbano, 2006; Dudley et al., 2007).

Because effective methods of managing BCTB are limited, there is an urgent need for new strategies to reduce the devastating effects of this pest. The Uganda National Coffee Research Institute (NaCORI) Kituza had come up with a candidate natural enemy of this pest. This predatory ant (*Pheidole megacephala*) had commonly been recovered from active coffee twigs and branches infested by *X. compactus*. Preliminary laboratory tests indicated that *P. megacephala* preys on *X. compactus* eggs, larvae, pupae and adults. However, for this predator to be used in the management of *X. compactus,* sufficient research on it was required. This study was therefore designed to evaluate the potential of luring *Pheidole megacephala* for the management of *X. compactus*.

1.1 Problem Statement

There is currently high infestation of coffee by *X. compactus* whose outbreak has been confirmed in a number of districts in Uganda with high infestation in coffee fields in Mukono, Kayunga,Bundibugyo and Luwero (Egonyu et al., 2009, Kagezi et al., 2013) and that it had spread to 96.2% of Robusta coffee growing regions in Uganda by 2012 . The pest affects coffee by killing the twigs which are berry bearing thereby affecting yield (Dixon et al., 2003, Egonyu et al., 2009, Kagezi et al., 2013) and it is estimated to be causing 8.6% loss of coffee yield, equivalent to approximately US$ 40 million annually (Egonyu, 2013, Kagezi et al., 2013) . Predatory ants have a great potential as biological control agents(Hölldobler and Wilson, 1990, Vandermeer et al., 2002), since they are natural enemies to most insect pests by virtue of their being predators, scavengers and generalist foragers (Hölldobler and Wilson, 1990). The invasive African big-headed ant, *P. megacephala*, was more effective at capturing termite prey than 13 native ant species in Mexico. *P. megacephala* has also been used against weevils in an IPM strategy adopted for the sweet potato weevil, in Cuba (Lagnaoui et al., 2000).

The potential of *P. megacephala* in managing the notorious *X. compactus* therefore needed to be explored. The Uganda National Coffee Research Institute (NaCORI) had come up with *P. megacephala* a candidate predator with preliminary laboratory tests indicating that it preys on the pest. For this candidate predator to be used in the management of *X. compactus*, its efficacy against *X. compactus* needed to be empirically tested. This study therefore explored the potential of luring *P. megacephala* discovered at NaCORI for the management of *X. compactus.* The findings of this study are hoped to contribute to the reduction of *X. compactus* infestation on coffee hence increased yield.

1.2 Objectives

1.2.1 Main objective

To reduce infestation of coffee by *X. compactus* through enhanced management using *P. megacephala.*

1.2.2 The Specific objectives

Specific objectives of this study include:

1 To determine if *P. megacephala* feeds on all stages of *X. compactus*,

2 To determine if *P. megacephala* can enter galleries of BCTB inside Robusta coffee twigs in search for the prey,

3 To determine if caging *P. megacephala* on infested twigs reduces populations of BCTB in the galleries.

4 To determine if *P. megacephala* prefers certain food baits and

5 To determine if *P. megacephala* presence in infested Robusta coffee field reduces incidence and abundance of BCTB in the galleries.

1.4 Research hypotheses

This study was designed to test the following hypotheses:

1 *P. megacephala* feeds on all stages of *X. compactus*,

2 *P. megacephala* can enter galleries of BCTB inside Robusta coffee twigs in search for the prey,

3 Caging *P. megacephala* on infested twigs reduces populations of BCTB in the galleries.

4 *P. megacephala* prefers certain food baits and

5 *P. megacephala* presence on infested Robusta coffee reduces incidence and abundance of BCTB in the galleries.

1.5 Scope of the study

This study was conducted at the National Coffee Research Institute, Mukono, Uganda (1209 m above sea level; 0°15′26.33″N and 32°47′26.67″E). The experimental garden was purely coffee without intercrop. Routine farm management practices on this coffee garden included weed management by monthly slashing and application of the herbicide glyphosate 48% at 5 l ha^{-1} twice a year; de-suckering to maintain 3-4 stems per tree and elimination of secondary branches; and soil fertilization twice a year with NPK (25:5:5) at approximately 280 Kg ha^{-1}. However, none of these activities were carried out during the experiment save for the monthly slashing. The study was done between July 2014 and September 2015.The major focus was to determine the most attractive baits for *P. megacephala*, stages of *X. compactus* which are preyed upon by *P. megacephala,* if *P. megacephala* presence on twigs can reduce BCTB population inside galleries, the ability of *P. megacephala* to enter BCTB galleries and if *P. megacephala* can reduce *X. compactus* incidence on Robusta coffee.

1.6 Significance of the study

This study identified the most attractive bait of *P. megacephala* which can now be used by farmers to lure the predator into their farms thereby reducing *X. compactus* infestation in the coffee fields and in turn reduce the losses caused by this pest hence increased yields and incomes to the farmers.

1.7 Justification

Coffee is an important export crop for Uganda contributing to a tune of US$449 and US $393 million in the coffee year 2010/11 and 2011/12 respectively in foreign exchange earnings. It provides livelihood to about 1.32million households out of the 3.95 million Agricultural households (UCF, 2012).. Unfortunately the coffee export earnings are declining due to losses to pests and diseases. Among which are; *X. compactus,* Coffee wilt disease, coffee leaf rust and coffee berry borer with *X. compactus* emerging as a major threat affecting 8.6% of the twigs costing Uganda US$ 40 million in foreign exchange earnings (Egonyu et al., 2009, Kagezi et al., 2013, Ponte, 2002). The losses due to *X. compactus* are impacting negatively to over 1.32 million households obtaining livelihood from coffee (UCF, 2012).This pest causes extensive economic damage to coffee by readily killing seedlings and twigs after initiation of a single gallery formation by an adult female (Dixon et al., 2003). The management of BCTB using the synthetic chemical insecticides normally encounters limitations due to environmental, ecological and regulatory concerns (Burbano et al., 2012, Pennacchio et al., 2012), leaving farmers with the option of cultural strategies such as pruning and burning of the infested twigs ((Egonyu, 2013, Jansen, 2005). However this management strategy affects the number of berry bearing branches thereby impacting on the yield. Environmentally and ecologically sound management options for this pest are therefore highly desired. This study explored the potential of luring *P.megacephala* discovered at NACORI for the management of this notorious pest.

1.8 Operational definitions

Baits: These are substances used to lure or attract the predatory ant (*Pheidole megacephala).*

Xylosandrus compactus*:* This is a black beetle which infests coffee twigs by boring into them hence the name the Black Coffee Twig borer (BCTB).

Coffee: This is a commercial crop whose berries are harvested after ripening and sold or roasted for home consumption. It’s an important export crop in Uganda.

Pheidole megacephala**:** This is a predatory ant

Predator: An organism that feeds on other organisms

Biological control: Use of living organisms to reduce the populations of other living organisms

CHAPTER TWO

LITERATURE REVIEW

2.1 Predatory ants as a pest management tool

Predatory ants are often used as biological control agents of insect pests and fungal pathogens (Hölldobler and Wilson, 1990, Vandermeer et al., 2002) reported that ants (*Azteca* sp) deterred larvae of *Pieris rapae* by making them fall off the coffee bushes in Chiapas Mexico. (Jaffe et al., 1990) also reported that some ant species are possible control agents for *Diaprepes* sp. in Martinique and Guadeloupe. These ants according to the authors, include *Azteca delpini* which preys on all life stages of the curculionid; *Pheidole fallax* Mayr and *Solenopsis qeminata*, which prey on all stages except eggs. *Monomorium* sp and *Solenopsis* sp, which prey mainly on eggs with or without their protecting shield. Ant species are also known to control herbivores that spread fungal diseases (Philpott and Armbrecht, 2006). (Armbrecht and Gallego, 2007) reported 18 ant species which were attracted to adult borers in spiral traps and carried the borers away. This literature suggests that ants have a potential of biological control of phytophagous pests including *X. compactus*. Ants have been reported to remove herbivorous lepidopteran larvae from plants (Bach, 1991) they have also been used against weevils in an IPM strategy adopted for the sweet potato weevil, in Cuba. Two species of predatory ants, Pheidole megacephala and Tetramorium guineense, are common inhabitants of banana plantations and have been transported using rolled banana leaves as “temporary nests" from their natural reservoir to sweet potato fields, where they prey upon weevils and other insects. Setting up colonies in the field 30 days after planting with 60-110 nests/ha is reported to keep weevil infestations at low levels (3-5%) and reduced sweet potato weevil damage from an average of 45% to less than 6% with resultant yield increase from 6 mt/ha to 15 mt/ha nationally (Lagnaoui et al., 2000). Important attributes of useful ant species are listed by (Risch and Carroll, 1982) as follows: (a) they are very responsive to prey density; they can remain abundant even when prey is scarce because they can cannibalize their brood and, most importantly, use honeydew-producing Homoptera as a stable source of energy; (c) they can store tbod and hence continue to capture prey even if it is not immediately needed; (d) besides killing pests, they can deter many others including some too large to be successfully captured; (e) they can be managed to enhance their abundance, distribution, and contacts with prey.

The stability, social organization, and foraging behaviour of some predatory ants enable them to react quickly to increasing prey density, and also make them uniquely able to protect crops from low-density pest (Way and Khoo, 1992). Biological-control attributes of many relatively inconspicuous non dominant ants have been inadequately studied. Some species may be valuable in their own right, but many also make a significant contribution to overall natural mortality, which needs to be understood much better than it is at present.

2.2 Coffee production

Coffee is grown in more than 50 countries around the world and, although utilised in a number of ways, coffe is produced primarily for consumption as a beverage by more than one third of the world's population (CABI, 2006). Of the many species of coffee that exist, commercial production is based principally on two species, namely *Coffea arabica* and *Coffea canephora*. These are often referred to as arabica coffee and robusta coffee, respectively. The global coffee production is based on *C. Arabica* which takes over 60% of the global production. This is because it's considered to produce beans of higher quality and therefore demands a higher market value. However, *C. canephora* is better suited to warmer and more humid tropical environments than *C. arabica* and, are able to withstand more adverse conditions, and is often grown at lower altitudes. Furthermore, *C. canephora* is generally more resistant to coffee pests and diseases (CABI, 2006). Coffee production levels have gradually increased over the last two decades largely due to liberalisation of markets, while the price of coffee on the world market has declined and has become more prone to fluctuations. This has implications for those involved in the coffee commodity chain, including coffee farmers who still endeavour to produce a crop of acceptable quantity and quality but for reduced economic returns. Under such conditions farmers find it increasingly difficult to acquire those resources required for good crop management. It becomes difficult to achieve satisfactory control of prevailing pests and diseases, one of many factors that producers must take into consideration (CABI, 2006). As in other parts of the world, Uganda coffee production is mainly by smallholder farmer families that in most cases have little in the way of resources to manage their farms. In such situations, the burden of the day-to-day running of the farm is often a responsibility of women. In terms of tackling pest and disease problems, resource-limited smallholder farmers are heavily reliant on the use of cultural management practices which are labour intensive.

2.3 Importance of coffee

Coffee is a major commodity on the global market and provides a source of revenue for many millions of people concerned with cultivation, processing, marketing and export of the crop. Globally, Brazil is the biggest exporter of coffee, providing 25 million bags (each 60 kg) in 2003, which accounted for more than 30% of world coffee exports (CABI, 2006). It is the second largest global export commodity after oil, with a value of US$ 9 billion in 1999-2000 (Fitter, 2001). Accordingly the crop employs more than 25 million people on 5 million farms globally and for several countries growing the crop, export earnings from coffee exceeded 10% in the decade 2000-2010. In Africa, among the countries that registered significant dependence on coffee export earnings during this period were Burundi, Ethiopia, Rwanda and Uganda with average shares of coffee exports in total export earnings of 59%, 33%, 27% and 18%, respectively. In Uganda, coffee contributed to a tune of US$ 449 million and US$ 393 million in coffee years 2010/11 and 2011/12 respectively. It also provides livelihood to about 1.32 households out of the 3.95 million agricultural households in Uganda (UCF, 2012).

2.4 Constraints to coffee production

In Africa, as elsewhere, coffee farmers are continuously threatened by a range of pest and disease problems. Many of these are minor in terms of the damage they cause and their effect on yield and quality. However some, such as coffee berry disease, coffee twig borer, coffee leaf rust, and coffee wilt disease *(tracheomycosis*), can be very serious indeed and can have a major impact not only on individual farmers but on the economy of countries or regions heavily dependent on coffee for foreign exchange earnings like Uganda. Given the perennial nature of coffee, some pests and diseases are able to survive and multiply throughout the cropping seasons are always present on the coffee crop, however the populations and effect on the crop may vary through the year. Others are occassional and attack coffee during periods when conditions are favourable. Either way, the damage caused or impact on crop yield and quality can be considerable (CABI, 2006).Where possible, an integrated approach to pest and disease management (IPM), involving use of a combination of cultural, biological and/or chemical measures should is recommended. This approach has advantages in terms of, for example: avoiding or minimising use of chemical pesticides that are often costly and damaging to other organisms, human and the environment. Promoting crop growth and vigour, thereby helping plants to tolerate pest damage and fight off infestations; and maintaining biodiversity and utilise natural enemies against those organisms responsible for pest and disease outbreaks is highly encouraged. This study aimed at contributing to desired approaches of pest and disease management. The coffee pests and diseases if not well managed can affect the entire production chain stakeholders. In Uganda for example, the coffee wilt substantially impacted on coffee exports by lowering it to 2.5 million bags of 60kgs in 1999 below the range of between 2.8-4.2 million bags between 1990/1991 and1996/1997 financial years (Ponte, 2002). This disease first broke out in 1993 in Bundibugyo district, western Uganda (Adipala et al., 2001). By 2002 coffee wilt had destroyed almost 50% of the Robusta coffee trees in Uganda (CABI, 2003). However, the incidence of this disease currently seems to be low following the introduction of Coffee wilt disease resistant clones and use of phytosanitary practices. As a result of the management strategies developed, coffee export volume increased to approximately 3.1 million 60kg bags in the coffee year 2010/2011 (UCDA, 2012). Today, a majority of farmers do not consider the Coffee wilt disease as a major threat as it has been replaced by *X. compactus* whose outbreak has been confirmed in a number of districts in Uganda (Egonyu et al., 2009, Kagezi et al., 2013). The black coffee twig borer is estimated to be causing 8.6% loss of coffee yield, equivalent to approximately US$ 40 million annually (Egonyu, 2013, Kagezi et al., 2013). The black coffee twig borer affects coffee by killing the twigs which are berry bearing thereby affecting yield (Dixon et al., 2003, Egonyu et al., 2009, Kagezi et al., 2013).

2.5 Prospects of utilizing the predatory ant *Pheidole megacephala* (Hymenoptera: Formicidae) for management of the polyphagus scolytid pest *Xylosandrus compactus* (Coleoptera: Curculionidea)

Several insecticides have been recommended for controlling BCTB in many parts of the world (Kagezi et al., 2014, Mangold et al., 1977, Meshram et al., 1993, Peña et al., 2011, Shuping et al., 2001). However, the use of synthetic chemical insecticides normally encounters limitations due to environmental, ecological and regulatory concerns (Burbano et al., 2012; Pennacchio et al., 2012). It is also difficult to apply chemicals to the concealed habitats in which BCTB feeds; and these chemicals can be expensive to most farmers. Although the cultural practice of pruning and burning of infested twigs is commonly practiced for the management of BCTB, this practice can be labor intensive and reduces the berry bearing branches hence reducing coffee berry yields(CABI, 2015; Kagezi et al., 2014). The entomopathogenic fungus, (*Beauveria bassiana)* is reported to cause some mortality in *X. compactus* (Balakrishnan et al., 1994), however, there are no known reports of its application in managing the pest in the field. Ethanol-baited traps have been applied for monitoring this pest in the USA (Burbano, 2006, Dudley et al., 2007; Miller et al., 2011; Miller and Rabaglia, 2009). The reports of their use in other parts of the world are uncommon. *Plagiolepis sp.* was discovered as an indigenous predator of *X. compactus* in Uganda (Egonyu et al., 2015). However more research on the biology of the predator, factors influencing its proliferation and development of mass rearing protocols for exploitation in biological control of BCTB is required. Clearly, effective environmentally friendly and adaptable strategies against BCTBin Uganda and Africa are highly desired.

P. megacephala is notorious for its symbiotic interaction with hemipteran insects in which it feeds on honeydew excreted by hemipterans such as mealybugs, scales and aphids, which in turn benefit by being transported by *P. megacephala* to resource-rich host plants/plant parts (CABI, 2016, Wetterer, 2007). This interaction may facilitate direct crop damage and transmission of plant diseases by the hemipterans. Additionally, *P. megacephala* interferes with natural enemies of crop pests, although the populations of the pests may still remain below damaging levels (Cudjoe et al., 1993). On a positive note, *P. megacephala* preys on several insect pests such as lepidopteran larvae on *Pluchea indica* (L.) (Bach, 1991), sweetpotato weevil (*Cylas* *formicarius*)(Fabricius) (Lagnaoui et al., 2000), the lilly pilly psyllid (*Trioza euginiae*) Froggatt (Young, 2003) and termites (Dejean et al., 2007). It is therefore worthwhile exploring the possibility of utilizing *P. megacephala* in management of *X. compactus*.

2.6 Baiting *Pheidole megacephala* could help reduce incidence and abundance of *Xylosandrus compactus* on Robusta coffee

The prevalence of ant species in any areas frequently been associated with the ability to utilize native food sources, including attending large numbers of hemipterans for their honeydew (Holway, 1998; Le Breton et al., 2005). However with the decline in arthropod diversity and abundance in areas invaded, invasive ants have been assumed to be good predators (Holway, 1998). Behavioral manipulation of the predators using food sprays (simple sugar solutions or more complex concoctions) and semiochemicals such as conspecific aggregation pheromones, pest kairomones and synomones released to prey–plant complex attract naturally occurring predators or retain released animals (Symondson et al., 2002).

Baits have been used mainly in the control of ants, by impregnating them with substances that are poisonous to ants such as fipronil, methoprene and hydromethylnon among others (Boland et al., 2011; Pei et al., 2003). The baits are usually constituted individually or as a mixture of fish, chicken feed pellets, shrimp powder, oil, sugar, honey, peach jam, ground barley seeds, cookies, crushed dog food and pea nut butter among others (Boland et al., 2011; Brühl and Eltz, 2010; Colby and Prowell, 2006; Davidson, 1977; Gusmao et al., 2011, Pei et al., 2003). Underwood and Fisher(2006) and Ward, 2008) reported that arboreal ants preferred protein based baits whereas terrestrial ants preferred Carbohydrate based baits.

CHAPTER THREE:
RESEARCH METHODOLOGY

3.0 Introduction

This Chapter describes the methods and materials used to test the hypotheses (1)*P. megacephala* feeds on all stages of *X. compactus*, (2) *P. megacephala* can enter galleries of BCTB inside Robusta coffee twigs in search for the prey, (3) Caging *P. megacephala* on infested twigs reduces populations of BCTB in the galleries, (4) *P. megacephala* prefers certain food baits and (5) *P. megacephala* presence on infested Robusta coffee reduces incidence and abundance of BCTB in the galleries. It high lights the period when each experiment was conducted and how data collected for each objective was analyzed. The research design, study sample, sampling procedure, sample size, data collection methods and instruments, data management and processing.

3.1 Study Area

This study was conducted at the national coffee research institute(NaCORI), Kituza in Mukono, Uganda (1209 m above sea level; 0°15′26.33″N and 32°47′26.67″E). The experimental garden was a pure stand of coffee. Routine farm management practices on this coffee garden included weed management by monthly slashing and application of the herbicide glyphosate 48% at 5 l ha^{-1} twice a year; de-suckering to maintain 3-4 stems per tree and elimination of secondary branches; and soil fertilization twice a year with NPK (25:5:5) at approximately 280 Kg ha^{-1}. However, none of these activities were carried out during the experiment save for the monthly slashing. The study was done between July 2014 and September 2015.

3.2 Determination of P. megacephala predation and preference on different stages of *X. compactus* by *P. megacephala* in a petri dish bioassay

This experiment was conducted on station at NaCORI, Mukono, Uganda. *P. megacephala* was lured using honey into a glass petri dish (measuring 15 cm diameter) lined with filter paper (Macherey-Nagel, 61.5 diameter 12.5cm, Kobian (Kenya Ltd) in an on station Robusta coffee field. The honey was formulated in the ratio of 1:6, honey: distilled water, and a thin layer applied just to cover the base of the petri dish (Boland et al., 2011; Pei et al., 2003). The petri dish was exposed for 1hr in a mature Robusta coffee garden infested by BCTB to lure approximately 70-80 *P. megacephala* workers. However, none of these activities were carried out during the experiment. Sets of 10 eggs, 10 larvae, 10 pupae and 10 adults of BCTB were placed together in the petri dish containing *P. megacephala* and then covered with muslin cloth for ventilation and to contain the insects. A second petri dish with same numbers of different stages of BCTB but without *P. megacephala* was similarly prepared to serve as an untreated control.

The trial was replicated eight times, each replicate on a separate day between 6th and 22nd July 2014. The treated and untreated petri dishes were randomly arranged in the coffee garden at a spacing of 1 m apart and checked after 24hr to establish if the predator had fed on the respective stages of BCTB. The number of intact eggs, larvae, pupae and adult *X. compactus* in the treated and untreated petri dishes were recorded at the end of the trial and subtracted from the initial number to determine the number of individuals preyed upon by *P. megacephala*.

3.3 Determination of ability of *P. megacephala* to enter galleries of BCTB inside Robusta coffee twigs

Twenty BCTB infested coffee twigs with guard mother beetles at the gallery entrances (to ensure use of only active galleries) were sampled at random from the experimental coffee field above. Each twig was placed in a separate plastic container measuring 30 x 14 x 8 cm. Only twigs with guard mother beetles at the gallery entrance were considered to ensure that active BCTB broods were used for the study. *P. megacephala* was then lured using honey as described earlier into ten of these containers with BCTB infested coffee twigs; whereas the other 10 containers were left as an untreated controls. The treated and untreated containers were randomly arranged in the coffee garden at a spacing of 1 m apart. The set up was left for 48hr to allow the predator ample time to pick up the guarding mother beetles and enter inside to search for the brood (if it does) and was conducted from 4th to 6th August, 2015. The treated and untreated twigs were brushed using a camel hair brush to remove any *P. megacephala* on the surfaces of the twigs. The twigs were then dissected to record *P. megacephala* (if any) inside galleries.

3.4 Determination of ability of *P. megacephala* to reduce populations of BCTB inside galleries of Robusta coffee twigs

P. megacephala was baited using honey as described above. An intact BCTB infested coffee twig was identified (by the presence of guard mother beetle) in *C. canephora* garden described in 2.1 above, and caged up using muslin cloth sleeves (8cm i. d. and 15cm long) (Egonyu et al., 2015). Approximate 70-80 freshly trapped *P. megacephala* workers were carefully introduced into the caged coffee twig. Intact *X. compactus* infested coffee twigs without *P. megacephala* were similarly caged up and left to serve as controls. The trial was replicated seven times. The set up was then monitored for one month from 16th September to 16th October 2014 to allow BCTB complete its life cycle of about one month (CABI, 2015, Ngoan et al., 1976). Monitoring was done by checking daily to ensure that no unnecessary damages occurred in the muslin sleeve cages. The treated and untreated coffee twigs were harvested after one month and dissected. The number of the various stages of *X. compactus,* dead or alive recovered from both inside the twigs and on the sleeve cages was recorded.

3.5 Determination of whether *P. megacephala* prefers certain food baits

3.5.1 Ant baits

Four baits were used in this study. The first bait, A, was formulated by boiling fresh beef for 30 minutes and grinding it in a blender locally fabricated in Katwe, Kampala, Uganda by local artisans. The beef paste was then mixed with tap water in the ratio of 1:6 respectively(Boland et al., 2011; Pei et al., 2003). Bait B was formulated using powder silver cyprinid fish (*Rastrineobola argentea).* This fish is popularly consumed and known as omena/mukene across Uganda. The fish powder was similarly mixed with water in the ratio of 1:6, respectively. Bait C comprised of pure honey which was similarly mixed with water in the ratio of 1:6(Boland et al., 2011; Pei et al., 2003). The honey was procured from the supermarket in Mukono town. The fourth bait, D, was a branded beef flavoured spice Royco muchuzi mix which was also mixed with water in the ratio of 1:6. The ratio of 1:6 was maintained the ratio of across the baits as a means of ensuring that no bait was disadvantaged or takes advantage over the other as a result of disparity in ratios. Royco Muchuzi mix was used because I wanted to compare its performance with that of beef since its beef flavored. I had the hope that if there is no difference in attractiveness between beef and Royco, then Royco would be recommended since it's cheaper and has a longer shelf life compared to beef in most Ugandan markets(Boland et al., 2011; Pei et al., 2003).

3.5.2 Screening of baits for attractiveness to *P. megacephala* in a petri dish bioassay

About 10mls of each of the baits (A, B, C and D) was placed in a petri dish lined with filter paper (Macherey-Nagel, 61.5 diameter 12.5cm, Kobian (Kenya) Ltd). The same volume of tap water only was similarly placed in a petri dish as an untreated control. The treatments were replicated 4 times and the petri dishes were randomly and linearly arranged in a coffee garden at a spacing of 1 m apart. The experiment was conducted in an on-station coffee field at NaCORI, Kituza, Mukono between 30th and 31st July 2015. The experiment lasted 2 hr after which each petri dish was covered and the number of ants inside, recorded(Boland et al., 2011; Pei et al., 2003).

3.6 Determination of effect of applying *P. megacephala* baits on *X. compactus* incidence and abundance on Robusta coffee in a field bioassay

Forty two experimental coffee trees in coffee garden spaced 3 x 3 m were selected on-station at the National Coffee Research Institute (NaCORI). A line of coffee trees was left out around the field and between selected trees as untreated guard rows. A layout of 7 x 6 trees was randomly assigned to different treatments of honey, fish, and untreated control. The honey and fish treatments were formulated according to pre-tested ratios. Each treatment was randomly assigned 14 trees, as replicates. The treatments were applied weekly for one month by thoroughly spraying on the assigned trees while the control treated with water only. The total number and the number of infested twigs per sampled tree were determined before and after treatments to estimate the proportion of infested twigs per tree. Ten infested twigs were randomly sampled from each treatment before and after treatments and dissected under a microscope to enumerate different stages of BCTB per twig. Honey was selected for this field bioassay owing to its excellent attractiveness to *P. megacephala* in the petri dish bioassay, (see results). Additionally, fish, though not as attractive to *P. megacephala* as honey, was selected for field testing, because it is readily available and more affordable to farmers than both honey and beef.

3.7 Data analysis

All the eggs, larvae, pupae and adults of *X. compactus* in the petri dishes which were not treated with *P. megacephala* were found intact after 24 hr, therefore the treatment and untreated control were not compared; but some individuals of all the stages of the pest in the treated petri dishes were fully and/or partially eaten up by *P. megacephala*. Data on number of different stages of *X. compactus* eaten up by *P. megacephala* were subjected to generalized linear modelling with Poisson distribution error and logit link to determine the stage of BCTB which was most preferred by *P. megacephala*(Knoblauch and Maloney, 2012; Zuur et al., 2009). To cater for over dispersion (deviance: 85.4 on 28 degrees of freedom), a negative binomial generalized linear model was fitted to the data (Osgood, 2000; Zuur et al., 2009). Mean proportions of the number of individuals in each stage of BCTB eaten up were separated using Tukey's test ($\alpha = 0.05$).

In determination of the ability of *P. megacephala* to enter BCTB galleries on Robusta coffee twigs, no *P. megacephala* was recovered inside galleries in both treated and untreated experimental set up and therefore no statistical analysis was carried out.

In determination of the ability of *P. megacephala* to reduce BCTB populations in the galleries in a field cage bioassay, data on the population recovered from both the treated and untreated experimental set ups were subjected to Kruskal-Wallis χ^2 test to determine whether there were significant differences between BCTB population in the treated and untreated control.

To determine the most attractive lure, no *P. megacephala* was attracted to the control, therefore it was not included in the analysis. Data on the number of *P. megacephala* attracted to various baits were subjected to generalized linear modeling with poisson family and logit link to determine variations in the numbers among the different baits(Knoblauch and Maloney, 2012; Zuur et al., 2009). To cater for over dispersion (deviance: 2500.4 on 28 degrees of freedom), a negative binomial generalized linear model was fitted to the data. Mean numbers of *P.*

megacephala attracted to each lure were separated using Tukey's test (α = 0.05). The analyses were carried out in R-statistical computer software version 3.2.1 (Team R Core, 2015).

Data on the number of infested twigs collected before and after treatment were separately subjected to generalized linear modeling with binomial distribution error and logit link and the population of *X. compactus* recovered from each treatment for both before and after were equally separately subjected to generalized linear modeling with Poisson distribution error and logit link to determine effect of application of *P. megacephala* baits on incidence and number of each stage of BCTB (Knoblauch and Maloney, 2012, Zuur et al., 2009). To cater for over dispersion (deviance: 1401.3 and 1103.6 on 2583 and 2808 degrees of freedom for infested twigs before and after treatment respectively and deviance: 118.18 and 37.120 on 27 degrees of freedom for the population before and after treatment respectively), a negative binomial generalized linear model was fitted to the data. Mean proportions of the twigs infested in each treatment and population of BCTB before and after treatment were separated using Tukey's test (α = 0.05). The analyses were carried out in R-statistical computer software version 3.3.1 (Team R Core, 2016).

CHAPTER FOUR

RESULTS PRESENTATION, ANALYSIS AND DISCUSSION OF FINDINGS

4.0 Introduction

This study investigated the potential of luring *P. megacephala* to reduce infestation of coffee by *X. compactus.* This was in light of the high infestation of coffee by *X. compactus* whose outbreak had been confirmed in a number of districts in Uganda with high infestation in coffee fields in Mukono, Kayunga, Bundibugyo and Luwero (Egonyu et al., 2009, Kagezi et al., 2013) and that it had spread to 96.2% of Robusta coffee growing regions in Uganda by 2012 (Kagezi et al., 2013). The pest affects coffee by killing the twigs which are berry bearing thereby affecting yield (Dixon et al., 2003, Egonyu et al., 2009, Kagezi et al., 2013) and it is estimated to be causing 8.6% loss of coffee yield, equivalent to approximately US$ 40 million annually (Egonyu JP, 2013, Kagezi et al., 2013). The data collected was analyzed in R-statistical computer software version 3.2.1(Team R Core, 2015) and R-statistical computer software version 3.3.1 (Team R Core, 2016). This chapter presents the results of the analyses.

4.1 To determine if *P. megacephala* feeds on all stages of BCTB

The purpose of this first objective was to determine predation and preference of different stages of BCTB by *P. megacephala* in a petri dish bioassay. To achieve this objective, the number of intact eggs, larvae, pupae and adult BCTB in the treated and untreated petri dishes were recorded at the end of the trial and subtracted from the initial number to determine the number of individuals preyed upon by *P. megacephala*. The data on number of different stages of *X. compactus* eaten up by *P. megacephala* were then subjected to generalized linear modelling with Poisson distribution error and logit link (Knoblauch and Maloney, 2012, Zuur et al., 2009), to determine the stage of BCTB which was most preferred by *P. megacephala.* Evidence of predation of different stages of BCTB was detected in only petri dishes treated with *P. megacephala*, but none in all the 8 untreated controls. There were no significant differences in the number of different stages of *X. compactus* preyed upon by *P. megacephala* per petri dish in

24 hr (χ^2=0.423, d.f.=3, P=0.936) (Table 1). This study revealed that, *P. megacephala* preyed upon all the stages of BCTB without any indication of preference for any stage. To the researchers knowledge, this is the first report of predator-prey interaction between *P. megacephala* and BCTB. This adds to the spectrum of known insect prey of this polyphagus ant predator (Bach, 1991; Dejean et al., 2007; Lagnaoui et al., 2000; Young, 2003).

Table 1 Mean (±SE) number of various BCTB stages eaten up during 4th to 6th August, 2015 petri dish bioassay.

Stage	**N**	**Mean (±SEM) number eaten up**
Eggs	10	4.4 ± 1.0a
Larvae	10	3.8 ± 0.9a
Pupae	10	4.9 ± 1.4a
Adults	10	4.4 ± 1.5a

Lower case letters ‘a’ besides values indicate no significant differences in the means (Tukey’s test; α = 0.05)

4.2To determine if *P. megacephala* can enter galleries of BCTB inside Robusta coffee twigs in search for the prey.

The purpose of this objective was to determine ability of *P. megacephala* to enter galleries of BCTB inside Robusta coffee twigs. To achieve this objective, the treated and untreated twigs were brushed using a camel hair brush to remove any *P. megacephala* on the surfaces of the twigs. The twigs were then dissected to record *P. megacephala* (if any) inside galleries. No *P. megacephala* was recovered inside galleries in both treated and untreated experimental set ups and therefore no statistical analysis was carried out. The findings revealed that there were no *P. megacephala* recovered inside galleries in both the treatment and the control. Since BCTB brood resides inside galleries except sexually mature adults that migrate to establish new colonies (CABI, 2015; Ngoan et al., 1976). The ability of a predator to enter the gallery would be very critical for effective biological control of BCTB. However, the bioassay on the ability of *P. megacephala* to enter galleries of BCTB indicated that the predator is unable to enter the galleries. The gallery hole of BCTB which is approximately 0.8 mm in diameter (CABI, 2015), is wider than head diameter of minor worker *P. megacephala* (0.5 mm) but narrower than that of the major worker (1.2 mm) (Wetterer, 2007; CABI, 2015). This indicates that heads of minor workers of *P. megacephala* may enter BCTB gallery but the entire body may be prevented from fully entering the gallery due to other appendages such as legs.

4.3 **To determine if caging *P. megacephala* on infested twigs reduces populations of BCTB in the galleries.**

There were significantly higher populations of BCTB per twig in untreated twigs than twigs treated with *P. megacephala* ($\chi^2 = 8.353$; df. =1, P = 0.004) (Table 2). This result indicated that *P. megacephala* reduces BCTB populations on Robusta coffee twigs by almost 22 fold. This result the findings from the Petri dish experiment that *P. megacephala* is a predator of all stages of BCTB. Since there was no evidence found of *P. megacephala* entering BCTB galleries, this study speculates that the ants eat-up adult mother BCTB which commonly guard the entrances to the gallery (CABI, 2015), hence reducing the size of the progeny of BCTB due to premature death of the reproductive female. It is also possible that under natural conditions, the possible removal of guard mother beetles by *P. megacephala* may predispose the brood to other natural enemies which are able to enter BCTB galleries such as *Plagiolepis sp.* (Egonyu et al., 2015).

Table 2 Mean (±SE) Number of population between treated twigs and control in one month bioassay during 16 September 2014 and 16 October 2014 in a farm at NaCORI.

Treatment	**Mean(±SEM) Number of population**
Control	5.4±1.54a
P. megacephala	0.25±0.25b

Lower case letters ‘a’ besides values indicate no significant differences in the means (Tukey’s test; α = 0.05)

4.4 **To determine if *P. megacephala* prefers certain food baits**

There were significant differences in the number of *P. megacephala* attracted to different baits (χ^2=23.497, df=28, P=3.181 x 10^{-5}) Fig 2. These findings revealed that *P. megacephala* was not attracted to tap water only but it was most attracted to honey than fish, beef and Royco. Since fish and beef are sources of protein, while honey is a source of carbohydrates, *P. megacephala* appeared to prefer carbohydrate to protein sources. These findings however, contradictory to Pei et al., (2003) who reported that *Pheidole sp*. and other ant species preferred peanut butter (a protein source) to honey (a sugar source), but the same author reported that *Tapinoma melanocephalum* and *Paratrechina longicornis* preferred honey to the peanut butter Hölldobler and Wilson, (1990) and Hahn and Wheeler, (2002) explained that ants choose baits based on the needs of the colony and will prefer certain type of baits when the nutrients represented in that bait are most limiting in the environment.

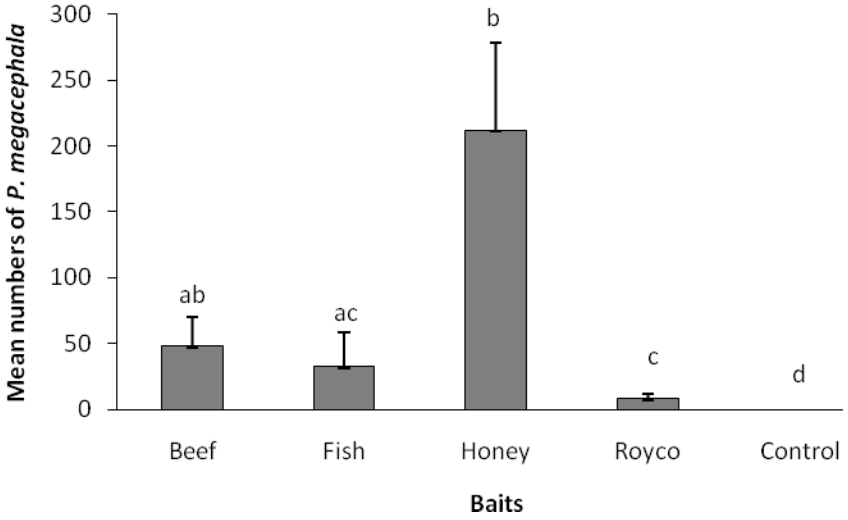

Fig. 2 Mean (±SE) number of *P. megacephala* attracted to different baits in a 2 hr petri dish bioassay in an on station farm at NaCORI between 30th and 31st July 2015. (Columns bearing the same letters were not significantly different at (Tukey test, $\alpha = 0.05$)).

4.5 To determine if *P. megacephala* presence in infested Robusta coffee field reduces incidence and abundance of BCTB in the galleries.

There were no significant differences in the proportions of infested twigs across the various treatments before application of the treatments (χ^2=1.5548, df=2583 P=0.4596). However, after the application of treatments, there were significant differences in the proportions of infested twigs across the various treatments. (χ^2=32.68, df=2808 P=8.012 x 10^{-8}) Fig.3 On abundance, there were no significant differences in the population of BCTB across the various treatments before and after application of the treatments (χ^2=4.6802 and 4.4996, df= 27 P=0.09632 and 0.1054 before and after treatment respectively) Fig.4. Results from this study show that weekly application of both honey and fish to lure *P. megacephala* to Robusta coffee field for 1 month significantly reduced the proportions of BCTB infested twigs in honey and fish treated plants, compared to the untreated control. Before application of the treatments, there were no significant differences in the proportions of infested twigs between the treatments and the control. Also the abundance of BCTB inside the Robusta coffee galleries, though not significantly different, reduced in fish and honey treated coffee plants with *P. megacephala* than in the control without *P. megacephala* after the application of the treatments Fig.4 compared to before the application of the treatments. Though section 4.3 three of this thesis reports that *P. megacephala* significantly reduced the population of BCTB in a field cage bioassay, the results of this section contradict those findings. It appears therefore that the period of one month for a non cage bioassay may not have been sufficient enough for *P. megacephala* to significantly reduce abundance of BCTB as it did in a cage bioassay. However the reduction in the proportions of infested twigs suggests that there is less new infestation taking place as a result of *P. megacephala* presence in the field. This seems to promise that the abundance would eventually reduce as the time goes by with *P. megacephala* presence in the field. These results however, agree with (Symondson et al., 2002) that generalist ants like *P. megacephala* can be effective control agents and their assemblages can reduce pests and yield loss to a significant degree. *P. megacephala* is thought to be an effective predator as arthropod abundance declines in areas it has invaded (Hoffmann et al., 1999). Dejean et al. (2007) as earlier mentioned that the invasive African big-headed ant, *P. megacephala*, was more effective at capturing termite prey than 13 native ant species in Mexico. *P. megacephala* has also been used against weevils in an IPM strategy adopted for the sweet potato weevil in Cuba (Lagnaoui et al., 2000). We presume that the presence of *P. megacephala* in a Robusta coffee field helped reduce BCTB incidence and abundance by removing guard adult females which in turn exposed the brood to other predators that are able to enter galleries since *P. megacephala* does not enter galleries as earlier reported in chapter three. *Plagiolepis sp.* is one such a predator as reported by (Egonyu et al., 2015) that can enter BCTB galleries and prey on all its life stages. Also, the high presence of *P. megacephala* in the field could have helped reduce the number of galleries formed by picking the adult twig borers as they emerged from galleries and before or in the process of initiating galleries. The other observation worth noting is that much as honey attracted more *P.*

megacephala than fish in the petri dish bioassay, there were no significant difference between honey and fish in luring *P. megacephala* to reduce incidence and abundance of BCTB in the Robusta coffee field (Fig 3&4).

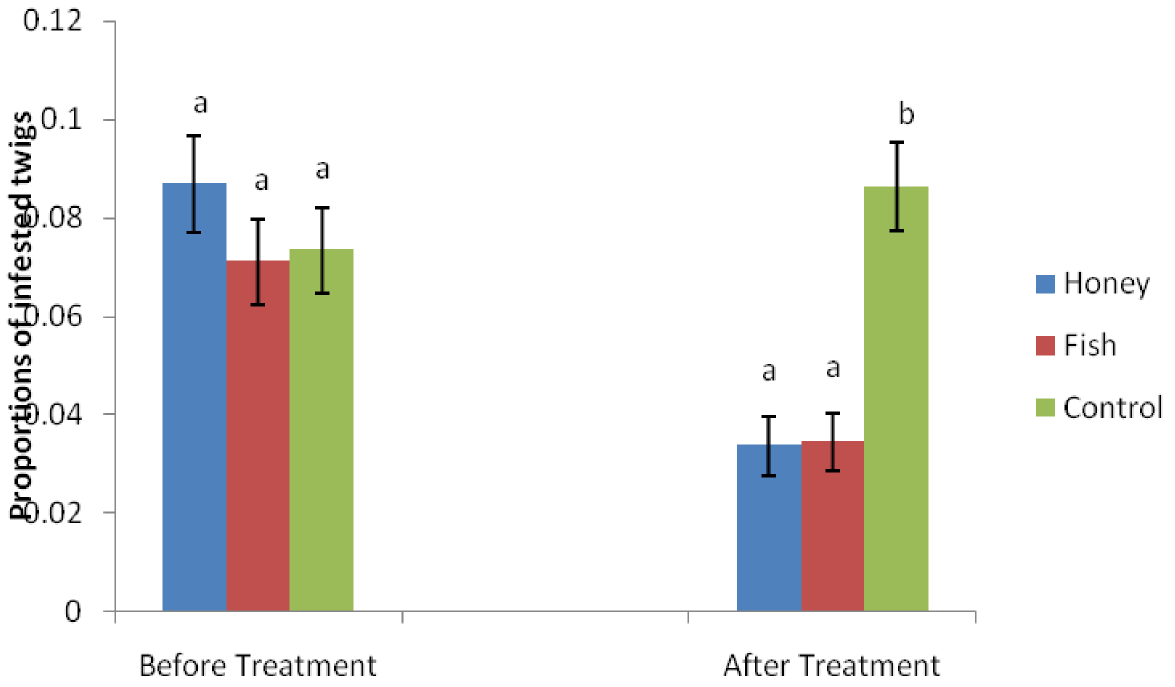

Fig. 3 Mean (±SE) proportion of BCTB infested Robusta coffee twigs before and after weekly treatment with *P .megacephala* baits for one month compared to the untreated control, in an on station farm at NaCORI Mukono during 3rd August and 3rd September 2015. (Columns bearing the same letters were not significantly different at (Tukey test, α = 0.05)).

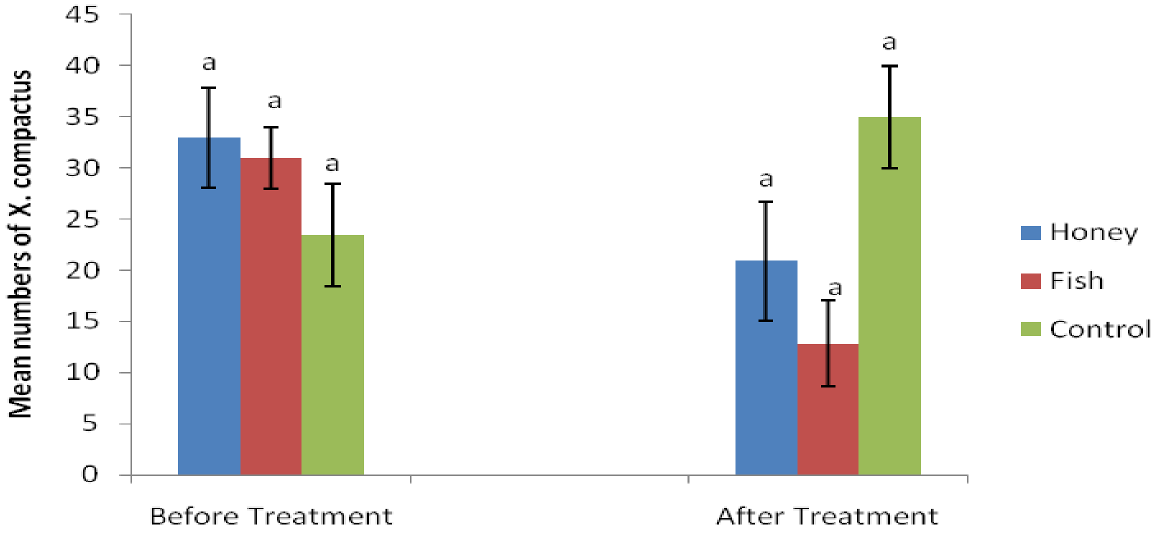

Fig.4. Mean (±SE) Number of population across the various treatments in one month.(Columns bearing the same letters within a stage were not significantly different at (Tukeys test, α = 0.05)).

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.0 Introduction

This chapter presents the summary, conclusion and recommendations of this study. It also proposes areas for further research.

5.1 Summary

This study has generally demonstrated that *P. megacephala* is a predator of BCTB in both petri dish and field bioassays, where it indiscriminately preyed upon all stages of BCTB in a petri dish bioassay and reduced incidence and abundance in the field bioassays. This is the first report of predator-prey interaction between *P. megacephala* and BCTB in Uganda and adds to the spectrum of known insect prey of this polyphagus ant predator (Bach, 1991; Dejean et al., 2007, Lagnaoui et al., 2000, Young, 2003). *P. megacephala* is not able to enter galleries where BCTB brood resides except sexually mature adults that migrate to establish new colonies (CABI, 2015; Ngoan et al., 1976). Yet the ability of a predator to enter the gallery would be very critical for effective biological control of BCTB. This limitation is occasioned by the gallery hole of BCTB which is approximately 0.8 mm in diameter (CABI, 2015), which is wider than head diameter of minor worker *P. megacephala* (0.5 mm) but narrower than that of the major worker (1.2 mm) (CABI, 2015; Wetterer, 2007). Though heads of minor workers of *P. megacephala* would enter BCTB gallery given their size, the entire body may be prevented from fully entering the gallery due to other appendages such as legs.

This study speculates that the ants eat-up adult mother BCTB which commonly guard the entrances to the gallery (CABI, 2015), hence reducing abundance of BCTB due to premature death of the reproductive female. In a nutshell, these results indicated that *P. megacephala* is an indiscriminate predator of all BCTB growth stages, and, though unable to enter BCTB galleries, the predator's presence reduces BCTB population on infested twigs by almost 22 fold. The study also demonstrated that *P. megacephala* is attracted to honey, fish, beef and Royco by 25, 6.5 and 4.4 times more to honey than to Royco, fish and beef respectively hence prefers honey to fish, beef and Royco. *P. megacephala* reduces BCTB incidence and abundance in the Robusta coffee field though not significantly. There is no significant difference between honey and fish in luring *P. megacephala* to reduce incidence and abundance of BCTB in the Robusta coffee field. *P. megacephala* could be exploited for the biological control of BCTB. However for this to be done, additional studies will be needed on blending sugar and protein baits which would produce synergistic attractiveness to predatory ants. Also the most effective concentration of the most preferred bait needs to be determined. There is also need to do cost analysis of the baits and the commonly used chemical pesticides.

5.2 Conclusions

The researcher has argued and shown in this report that: (1) *Pheidole megacephala* is an indiscriminate predator of all BCTB stages, (2) *P. megacephala* is unable to enter BCTB galleries (3) Though unable to enter BCTB galleries, *P. megacephala's* presence reduces BCTB population on infested twigs by almost 22 fold when caged, (4) *P. megacephala* is attracted to honey, fish, beef and Royco by 25, 6.5 and 4.4 times more to honey than to Royco, fish and beef respectively (5) *P. megacephala* significantly reduces BCTB incidence in the Robusta coffee field and also reduces BCTB abundance though not significantly. The researcher also noted that though honey attracted more *P. megacephala* in a petri dish bioassay, there were no significant differences between honey and fish in luring *P. megacephala* to reduce incidence and abundance of BCTB in the Robusta coffee field.

5.3 Recommendations

Despite its limitations, this study could be used to exploit *P. megacephala* for the biological control of BCTB because of its indiscriminate predation of all BCTB stages which enabled it to reduce the population and incidence of BCTB in both the cage and non cage field bioassays.

The baits could be used to lure *P. megacephala* to the coffee fields to help reduce infestation. Since there were no significant differences between honey and fish in attracting *P. megacephala* to reduce incidence and abundance in the Robusta coffee field, the researcher recommends that famers would be encouraged to use fish which is more available than honey.

5.4 Suggestions for further research

The following are suggestions for further research:

1. To determine the strategies to enhance presence of the predator on the infested crop.
2. Further research to establish if blending sugar and protein baits would produce synergistic attractiveness to predatory ants are needed.
3. To determine the most effective concentration of the most preferred bait.
4. There is also need to do cost benefit analysis of the baits and the commonly used chemical pesticides.

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LIST OF APPENDICES

Appendix I: Anova on the Number of various *X. compactus* stages eaten up during 4th to 6th August, 2015 petri dish bioassay.

```
anova(Mod2, test="Chisq")
Analysis of Deviance Table
Model: Negative Binomial(2.3865), link: log
Response: eaten
Terms added sequentially (first to last)

     Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                  31     35.423
Stage  3  0.42322       28     35.000   0.9354

> mean1<-effect("Stage", Mod2, se=TRUE,confidence.level=0.95)
> summary(mean1)
 Stage effect
Stage
 Adult   Eggs Larvae  Pupae
 4.375  4.375  3.750  4.875
 Lower 95 Percent Confidence Limits
Stage
   Adult     Eggs   Larvae    Pupae
2.442703 2.442703 2.058665 2.751002

 Upper 95 Percent Confidence Limits
Stage
   Adult     Eggs   Larvae    Pupae
7.835837 7.835837 6.830884 8.638898

> modmult<-glht(Mod2, linfct = mcp(Stage = "Tukey"))

> summary(modmult)
         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm.nb(formula = eaten ~ Stage, data = Data6, init.theta = 2.38654566,
    link = log)
Linear Hypotheses:
                    Estimate Std. Error z value Pr(>|z|)
Eggs - Adult == 0   -2.146e-16  4.024e-01   0.000    1.000
Larvae - Adult == 0 -1.542e-01  4.082e-01  -0.378    0.982
Pupae - Adult == 0   1.082e-01  3.987e-01   0.271    0.993
```

```
Larvae - Eggs == 0  -1.542e-01  4.082e-01  -0.378    0.982
Pupae - Eggs == 0    1.082e-01  3.987e-01   0.271    0.993
Pupae - Larvae == 0  2.624e-01  4.046e-01   0.648    0.916
(Adjusted p values reported -- single-step method)
```

Appendix II: Chi-square on the population between treated twigs and control in one month bioassay during 16 September 2014 and 16 October 2014 in a farm at NaCORI.

```
kruskal.test(Popn ~ Traetment, data=Dataset)


        Kruskal-Wallis rank sum test


data:  Popn by Traetment

Kruskal-Wallis chi-squared = 8.3526, df = 1, p-value = 0.003851




>library(abind, pos=14)


>library(e1071, pos=15)


>numSummary(Dataset[,"Popn"], groups=Dataset$Traetment, statistics=c("mean",

+   "se(mean)"), quantiles=c(0,.25,.5,.75,1))

mean se(mean) data:n

Treated   0.250000 0.250000     8

Untreated 5.428571 1.540828     7
```

Appendix III: Anova on the number of *P. megacephala* attracted to different baits in a 2 hr petri dish bioassay in an on station farm at NaCORI between 30th and 31st July 2015.

```
> anova(Mod2, test="Chisq")
Analysis of Deviance Table

Model: Negative Binomial(0.6104), link: log

Response: attracted

Terms added sequentially (first to last)


     Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                    31     61.700
Lure  3   23.497        28     38.203 3.181e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> anova(Mod2, test="Chisq")
Analysis of Deviance Table

Model: Negative Binomial(0.6104), link: log

Response: attracted

Terms added sequentially (first to last)

     Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                    31     61.700
Lure  3   23.497        28     38.203 3.181e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> mean1<-effect("Lure", Mod2, se=TRUE,confidence.level=0.95)

> summary(mean1)

 Lure effect
Lure
      A       B       C       D 
 48.250  32.625 211.875   8.375 

 Lower 95 Percent Confidence Limits
Lure
```

```
       A          B          C          D
18.984396 12.800880 83.741014  3.206341

 Upper 95 Percent Confidence Limits
Lure
      A        B        C        D
122.6303  83.1498 536.0696  21.8756

> modmult<-glht(Mod2, linfct = mcp(Lure = "Tukey"))

> summary(modmult)

          Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts


Fit: glm.nb(formula = attracted ~ Lure, data = Data5, init.theta = 0.6104437467,
    link = log)

Linear Hypotheses:
           Estimate Std. Error z value Pr(>|z|)
B - A == 0  -0.3913     0.6449  -0.607   0.9300
C - A == 0   1.4796     0.6424   2.303   0.0971 .
D - A == 0  -1.7511     0.6535  -2.680   0.0368 *
C - B == 0   1.8709     0.6434   2.908   0.0191 *
D - B == 0  -1.3598     0.6544  -2.078   0.1604
D - C == 0  -3.2307     0.6520  -4.955   <0.001 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```

Appendix IV: Anova on the proportion of *X. compactus* infested Robusta coffee twigs before weekly treatment with *P .megacephala* baits

```
> anova(GLM2, test="Chisq")
Analysis of Deviance Table

Model: Negative Binomial(1745.351), link: log

Response: Infestedtwigs

Terms added sequentially (first to last)

          Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                       2585     1020.6
Treatment  2   1.5548      2583     1019.0   0.4596

> mean1<-effect("Treatment", GLM2, se=TRUE,confidence.level=0.95)

> summary(mean1)

 Treatment effect
Treatment
   Control       Fish      Honey
0.07344633 0.07110092 0.08685163

 Lower 95 Percent Confidence Limits
Treatment
   Control       Fish      Honey
0.05758904 0.05542678 0.06893081

 Upper 95 Percent Confidence Limits
Treatment
   Control       Fish      Honey
0.09366997 0.09120755 0.10943155

> modmult<-glht(GLM2, linfct = mcp(Treatment = "Tukey"))

> summary(modmult)
         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm.nb(formula = Infestedtwigs ~ Treatment, data = Datasetbefore,
    init.theta = 1745.351199, link = log)
```

```
Linear Hypotheses:
                    Estimate Std. Error z value Pr(>|z|)
Fish - Control == 0  -0.03245    0.17752  -0.183    0.982
Honey - Control == 0  0.16765    0.17110   0.980    0.590
Honey - Fish == 0     0.20010    0.17326   1.155    0.480
(Adjusted p values reported -- single-step method)
```

Appendix v: Anova on the proportion of *X. compactus* infested Robusta coffee twigs after weekly treatment with *P .megacephala* baits for one month compared to the untreated control, in an on station farm at NaCORI Mukono during 3rd August and 3rd September 2015.

```
> anova(GLM2, test="Chisq")
Analysis of Deviance Table

Model: Negative Binomial(1030.093), link: log

Response: Infestedtwigs

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                       2810     855.65
Treatment  2   30.873      2808     824.78 1.977e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> mean1<-effect("Treatment", GLM2, se=TRUE,confidence.level=0.95)

> summary(mean1)

 Treatment effect
Treatment
   Control       Fish      Honey
0.08629989 0.03430353 0.03362256

 Lower 95 Percent Confidence Limits
Treatment
   Control       Fish      Honey
0.06931034 0.02438357 0.02364188

 Upper 95 Percent Confidence Limits
Treatment
   Control       Fish      Honey
0.10745398 0.04825923 0.04781669

> modmult<-glht(GLM2, linfct = mcp(Treatment = "Tukey"))

> summary(modmult)
```

```
         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts


Fit: glm.nb(formula = Infestedtwigs ~ Treatment, data = Datasetafter,
    init.theta = 1030.093277, link = log)

Linear Hypotheses:
                     Estimate Std. Error z value Pr(>|z|)
Fish - Control == 0  -0.92258    0.20689  -4.459 2.08e-05 ***
Honey - Control == 0 -0.94263    0.21157  -4.455 2.03e-05 ***
Honey - Fish == 0    -0.02005    0.25013  -0.080    0.996
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```

Appendix VI: Anova on number of population across the various treatments before one month application of treatments.

```
> anova(Mod2, test="Chisq")
Analysis of Deviance Table

Model: Negative Binomial(9.8144), link: log

Response: Population

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                          29     35.126
Treatment  2   4.6802        27     30.446  0.09632 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> mean1<-effect("Treatment", Mod2, se=TRUE,confidence.level=0.95)

> summary(mean1)

 Treatment effect
Treatment
Control    fish   Honey
   23.4    30.9    33.0

 Lower 95 Percent Confidence Limits
Treatment
 Control     fish    Honey
18.28317 24.36176 26.06508

 Upper 95 Percent Confidence Limits
Treatment
 Control     fish    Honey
29.94885 39.19299 41.78004

> modmult<-glht(Mod2, linfct = mcp(Treatment = "Tukey"))

> summary(modmult)

         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts
```

```
Fit: glm.nb(formula = Population ~ Treatment, data = Datasetpopn,
    init.theta = 9.814352486, link = log)

Linear Hypotheses:
                  Estimate Std. Error z value Pr(>|z|)
fish - Control == 0   0.27802    0.16700   1.665   0.2189
Honey - Control == 0  0.34377    0.16638   2.066   0.0969 .
Honey - fish == 0     0.06575    0.16323   0.403   0.9144
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```

Appendix VII: Anova population across the various treatments after **one month application of** treatments.

```
> anova(Mod2, test="Chisq")
Analysis of Deviance Table

Model: Negative Binomial(0.9443), link: log

Response: Population

Terms added sequentially (first to last)


          Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                         29     41.619
Treatment  2   4.4996        27     37.120   0.1054

> mean1<-effect("Treatment", Mod2, se=TRUE,confidence.level=0.95)

> summary(mean1)

 Treatment effect
Treatment
Control    fish   Honey
   35.0    12.8    20.9

 Lower 95 Percent Confidence Limits
Treatment
 Control     fish    Honey
17.79105  6.40801 10.56056

 Upper 95 Percent Confidence Limits
Treatment
 Control     fish    Honey
68.85484 25.56800 41.36237

> modmult<-glht(Mod2, linfct = mcp(Treatment = "Tukey"))

> summary(modmult)

         Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts
```

```
Fit: glm.nb(formula = Population ~ Treatment, data = Dataset1, init.theta = 0.9443082754,
    link = log)

Linear Hypotheses:
                   Estimate Std. Error z value Pr(>|z|)
fish - Control == 0   -1.0059     0.4717  -2.133   0.0833 .
Honey - Control == 0  -0.5156     0.4684  -1.101   0.5136
Honey - fish == 0      0.4903     0.4737   1.035   0.5547
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```

Appendix VIII: Research Photographs

A photograph of a researcher formulating baits

A Photograph showing *Xylosandrus compactus* moving out of a dissected twig.

Researcher counting insects

Researcher marking the field for field sprays experiment

P. megacephala trapped in beef bait

BCTB larvae


Coffee field where the experiment was done

Researcher setting up *P. megacephala* baiting experiment