

**OPTIMIZATION OF AN ETHANOL-BAITED TRAP FOR
MANAGEMENT OF THE BLACK COFFEE TWIG BORER IN ROBUSTA
COFFEE-BANANA SYSTEMS OF UGANDA**

BY

NAKITENDE ALICE

BSc Science Technology - Biology (Hons), KyU.

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DECLARATION

I hereby declare that the work embodied in the research report has never been submitted to any institution in partial fulfilment of the requirements of any award.

Signed

Date.....

NAKITENDE ALICE

21/U/GMCS/14011/PE

APPROVAL

This work has been submitted with the approval of my academic supervisors.

1. Dr. Tinzaara William
Department of Agriculture Production
Faculty of Agriculture
Kyambogo University.
Email: wtinzaara@gmail.com

Signature.....

Date.....

2. Dr. Tumuhaise Venansio
Department of Agriculture Production
Faculty of Agriculture
Kyambogo University
Email: vtumuhaise@kyu.ac.ug

Signature.....

Date.....

3. Dr. Kagezi Godfrey
Principal Research Officer
National Coffee Research Institute
National Agricultural Research Organization
Email: gkagezi@gmail.com

Signature.....

Date.....

DEDICATION

I dedicate this work to the Almighty God, my mentor and supervisor; Dr. Kagezi Godfrey, my Mother; Nakiyemba Elizabeth, Uncle Mugerwa Patrick and friends.

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LIST OF ACRONYMS

NARO- National Agricultural Research Organization

BCTB- Black Coffee Twig Borer

UCDA- Uganda Coffee Development Authority

RCBD- Complete Randomized Block Design

CWD-r- Coffee Wilt Disease-resistant

ICO-International Coffee Organization

IITA-International Institute of Tropical Agriculture.

ABSTRACT

The Black Coffee Twig Borer (BCTB), *Xylosandrus compactus* poses a significant threat to Uganda's coffee industry, causing yield losses of up to 9.6% and an economic loss of about US\$42.9 million. Farmers currently manage this pest using labor-intensive cultural practices and costly chemicals, which can also harm humans and the environment. In response, the National Coffee Research Institute (NaCORI) developed an ethanol-baited NARO-BCTB trap to assist in their management. Although farmers see promising results, the trap's effectiveness can be improved through modifications. A study was therefore conducted at NaCORI to evaluate trap design (with or without flaps as well as the number and size of windows), ethanol concentration, and trap spacing on the catch of adult. Trap design was assessed through a field experiment utilizing a split-split plot design, and trap catches were assessed weekly for six months. In addition, the ability of five (5) different ethanol concentration levels to attract adult *X. compactus* beetles was evaluated in a Y-tube olfactometer laboratory assay. For each concentration, 250 adult beetles were released individually in a Y-tube and observed for 10 minutes, and response towards air, ethanol sample, or no response was recorded. Furthermore, a combined effect of ethanol concentrations and trap spacing on BCTB trap catches was tested in a Robusta coffee-banana field in a split-plot design. Traps without flaps captured 8% more beetles, than those with flaps. The flapless trap with two 6x4 cm windows were the most effective. No interaction was found among trap type, window number, and size. BCTB response rose with ethanol concentration, peaking at 70% (62.8%) and declining at 96% (23.6%). A significant interaction occurred between ethanol concentration and spacing, with the highest captures (18.0 beetles) at 70% ethanol and 30 m spacing, comparable to 50% ethanol at 20 m (15.3 beetles). Thus, flapless traps with two 6 × 4 cm windows, baited with 50 and 70% ethanol and spaced 20 and 30 m apart are recommended to reduce *X. compactus* infestation and increase yields in Robusta coffee-banana systems.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

Coffee is one of the most traded commodity in the world after oil (Gil-Gómez et al., 2024). Robusta coffee and Arabica coffee are the two coffee species that are preferred in the coffee beverage industry due to their special characteristics, like the aroma of Arabica coffee (Ogundeji *et al.*, 2019). Uganda is among the ten top coffee-growing countries in the world, and it is ranked second in Africa after Ethiopia (ICO, 2020; Ategeka, 2024). Robusta coffee accounts for 80% of all coffee grown in the country and the remaining 20% is Arabica (UCDA, 2019). In Uganda, Robusta is grown in the low-altitude regions of central, eastern, southwestern, and some northern regions. Arabica coffee is grown in the highland areas of Bugisu, southwestern and west Nile regions (UCDA, 2019).

Coffee plays a major role in generating foreign exchange for the country. For example, coffee exports for the coffee year 2023/24 totaled 6.43 million bags worth US\$ 1.14 billion (UCDA, 2024). Coffee is also an important income-generating crop, with more than nine million people deriving their livelihood from coffee-related activities along the value chain (NCP 2013). The crop is cultivated on an estimated 353,907 hectares of land by approximately 1.7 million smallholder farmers. About 90% of the farmers possess plantations that are between 0.5 and 2.5 hectares in size, with a quarter of them being female-headed (Hill 2005; NCP 2013; Mugoya 2018). The crop has potential of providing employment and reducing

poverty, thus, enhancing people's incomes and livelihoods. Coffee is therefore considered a priority commodity in Uganda (NPA 2020).

Despite the importance of coffee in Uganda, its yield per tree is far below the expected. For example, the 0.55kg of clean coffee per Robusta coffee tree is far below the 4.8 kg clean coffee/tree yield by the improved KR10 coffee wilt disease resistant (CWD-r) Robusta coffee variety (Musoli *et al.*, 2017). Both abiotic and biotic factors contribute to this challenge. Some of the abiotic factors include environmental challenges, especially drought and floods, market problems, agronomic challenges. Biotic factors are majorly pests and diseases (Wang *et al.*, 2015).

Currently, the Black Coffee Twig Borer (BCTB), *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae) is the major biotic factor causing yield losses to farmers. The pest causes losses up to 9.6% which converts to US\$42.9 million loss to the country (Kagezi *et al.*, 2016). *Xylosandrus compactus* is an ambrosia beetle because it is associated with ambrosia fungi such as *Fusarium solani* and *Ambrosiella xylebori*. The fungi which supply the diet for the larvae in the galleries (Bateman *et al.* 2016; Morales-Rodríguez *et al.*, 2020). BCTB is believed to have originated from South Asia, but it has now spread to most of the Robusta-growing regions in the world (Roca & Gallego, 2020). The pest is polyphagous in nature, attacking more than 200 species worldwide and more than 50 species in Uganda. Despite its broad range, coffee is a primary economic target. (Kagezi *et al.*, 2012; Greco & Wright, 2012). Since the pests' advent in Uganda in 1993 in areas of Bundibugyo, it has spread to other districts within the western

region, central region, and eastern regions that grow Robusta coffee (Kagezi *et al.*, 2016). The female beetle makes small pin-sized entry holes on coffee branches, making galleries where it lays eggs and it spends its entire life there (Ngoan *et al.*, 1976; Hara, 1977). These tunneling activities then lead to the drying of the infested primaries (Dixon *et al.*, 2003).

Because of the cryptic character of spending almost its entire life in the host galleries, developing control measures for BCTB remains difficult (Burbano *et al.*, 2012). Nevertheless, the National Agricultural Research Organization (NARO) of Uganda has developed a cultural-based package for managing this insect pest. This package also includes incorporation of chemicals when the infestation is very high (Kagezi *et al.*, 2016). However, cultural control approaches are time consuming, tedious, and require a collective community effort to control the pest (Jones & Jones, 2000; Egonyu *et al.*, 2009; Kagezi *et al.*, 2016). Chemical control on the other hand, is disadvantaged by the cryptic nature of this insect pest, the high expenses are involved and the chemicals being not friendly to the environment (Kagezi *et al.*, 2016).

The serious losses caused by *X. compactus* on coffee and other commercial crops have prompted research to search for alternative pest management strategies for protecting these plants, such as the use of the ethanol-baited trapping (Galko *et al.*, 2014). Plants under stress generate ethanol, which attracts ambrosia beetles like *X. compactus* (Coyle *et al.*, 2005). This knowledge has been utilized to develop a trapping technology that uses ethanol as a bait, and it is widely used in monitoring and mass trapping the *X. compactus* and thus, its management (Burbano *et al.*, 2012;

Werle *et al.*, 2017). Similarly, NARO developed an ethanol-baited trap and together with her partners, the trap is currently being promoted for management of *X. compactus* (Kucel *et al.*, 2016).

A number of trap features such as trap color, spacing, placement, type and concentration of the bait have been studied and found to affect trap captures depending on the targeted pest (Bacca *et al.*, 2006; Hanula *et al.*, 2011; Burner, 2020; Cavaletto *et al.*, 2021). For example, a study by Avinash *et al.* (2021) showed that *X. compactus* was significantly attracted to 95% ethyl alcohol spaced at 15m compared to other concentrations. The plastic bottle trap caught more *X. crassiusculus* than the Lindgren trap (Reding *et al.*, 2011). These studies have shown the importance of trap features in affecting trap efficiency. This implies that these features can be modified to increase the effectiveness of the traps (Monterrosa *et al.*, 2021).

Therefore, this study aimed at evaluating the different trap designs, ethanol concentration and spacing of the NARO ethanol-baited trap for management of *X. compactus* in Uganda.

1.2 Problem statement

Recently, the National Agricultural Research Organization (NARO) developed an ethanol-baited trap coded ‘NARO BCTB trap’ to be incorporated in the Integrated Pest Management (IPM) package. It is used for managing one of the most important insect pest of coffee in Uganda, BCTB (Kagezi *et al.*, 2016; Kucel *et al.*, 2016; NaCORI, 2018). This trap is made from a transparent water bottle (volume=500 ml) with two window slots measuring 6 x 4 cm, cut on both sides and with the flaps

left on (Kucel *et al.*, 2016). Such kinds of traps are known to be cheap, durable and easily modifiable (Susanto *et al.*, 2020; Steininger *et al.*, 2015). However, to effectively manage the population of insects by using plastic bottle traps, attention to the shape, material and other special effective modifications is required (Eliopoulos, 2007; Rizki *et al.*, 2013; INRA, 2019; Monterrosa *et al.*, 2021).

Research studies have shown that the trap catches of insects including ambrosia beetles are influenced by the number of windows or funnels on the trap. For example, simple, one-window traps have been reported to perform comparably at capturing ambrosia beetle species in traps painted or with multiple windows (Steininger *et al.*, 2015). For the NARO BCTB trap, Kucel *et al.* (2016) recommended two windows with a flap left intact. However, scientific studies to evaluate the effect of varying the size of the window and the presence/absence of the flaps on the traps are yet to be thoroughly conducted.

Kucel *et al.* (2016) also recommended the use of ethanol of about 30% concentration as the bait lure. However, several research studies have demonstrated that different concentrations of ethanol attract *scolytid* insect species including *X. compactus* (Roobakkumar *et al.*, 2022; Avinash *et al.*, 2021) and other ambrosia beetles (Cavaletto *et al.*, 2021; Santos *et al.*, 2018; Reding *et al.*, 2011; Ranger *et al.*, 2011). Nevertheless, the optimal ethanol concentration level for maximum capture of adult *X. compactus* by the NARO BCTB trap is yet to be determined. This information is critical in developing the trapping technology for mass trapping of adult *X. compactus* to reduce its population and thus, its damage (Cavaletto *et al.*, 2021).

Optimizing the spatial configuration of traps for the control of insect pest has always been a subject of interest among farmers, researchers and extension officers (Vega *et al.*, 2007; Guimapi *et al.*, 2020). This is in part due to the fact that spacing distance of traps can interact with the attractiveness of semiochemicals to affect trap catch (McMahon *et al.*, 2010). Several research studies and models have been applied to estimate the attractiveness and probability of capturing an insect using baited traps for estimating distances between the trap and the insect (Branco *et al.*, 2006), with varying results. For example, INRAE (2022) reported that traps positioned at 20 and 30 m from each other were most effective in capturing *X. compactus* and *X. germanus*, respectively. In case of the NARO BCTB trap, Kuchel *et al.* (2016) recommended a trap density of 15 traps per hectare or 7 traps per acre. However, the actual effective distance between the traps for maximum capture of *X. compactus* was not determined, though this information is vital for optimizing monitoring and/or mass trapping strategies for ambrosia beetles (McMahon *et al.*, 2010; Hanula *et al.*, 2016). This study therefore evaluated effect of trap design (trap type, window numbers and size), ethanol concentration, and the combined effect of ethanol concentration and trap spacing on trap catches of BCTB.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to optimize the efficacy of the ethanol-baited trap for sustainable management of the Black Coffee Twig Borer in coffee agro-systems in Uganda.

1.3.2 Specific Objectives

1. To identify the optimal window number, size and flap configuration of the ethanol-baited NARO BCTB trap for maximizing the capture of adult *X. compactus* in Robusta coffee agro-systems.
2. To determine the optimal ethanol bait concentration for maximizing the attraction and capture adult *X. compactus* under both laboratory and field conditions.
3. To evaluate the interactive effect of varying ethanol bait concentration and trap spacing on the field capture efficiency of adult *X. compactus*.

1.4 Hypothesis

1. Trap captures are the same when using traps with different flap configurations, window numbers, and sizes.
2. Trap captures for adult *X. compactus* beetles are the same when using different ethanol concentrations under both laboratory and field conditions.
3. The field capture efficiency of adult *X. compactus* capture is not influenced by the interaction between ethanol bait concentration and trap spacing.

1.5 Significance of the study

The Black coffee twig borer stands as the most significant pest affecting the Ugandan coffee industry. The losses it causes do not only affect farmers but also the revenue of the country due to the fact that coffee is the leading export crop. For example, the country lost 9.6% yield in the past years due to BCTB damages, and

it has increased to over 50% if no control measures are applied (Kagezi *et al.*, 2016; UCDA, 2025). More knowledge and innovations on how to manage the pest is very important in order to reduce the losses incurred. One of the management options that is incorporated in the management packages is the use of the ethanol-baited trap; a method that is very friendly to the environment. However, more studies on this trap are needed, aiming at maximizing captures of BCTB and reducing their population and damage. For example, knowing the best design, the optimal ethanol-bait concentration, the optimal trap spacing and placement height relative to the ground, suitable trap color, are some factors that can further be studied to come up with a more efficient trap BCTB management.

This study identified the most appropriate trap design for trapping BCTB, identifying the most effective trap design ensures that farmers and researchers can adopt a standardized, efficient, and environmentally friendly tool for controlling BCTB. This minimizes trial-and-error practices, reduces the costs of pest management, and increases trap efficiency in the field. A well-optimized trap design can be mass-produced and commercialized, benefiting not only farmers but also entrepreneurs and extension systems.

The study also provides information on the optimal ethanol concentration for baiting the BCTB trap, establishing the right ethanol concentration prevents under- or over-use of ethanol, ensuring cost-effectiveness and sustainability. Farmers will have reliable guidelines on the correct formulation, reducing wastage of resources while maintaining high trap efficiency. Optimized bait concentration enhances consistency in captures, providing researchers and policymakers with reliable monitoring data for BCTB management.

Furthermore, the study also provided information on the interactive effect of varying ethanol bait concentration and trap spacing on BCTB trap captures,

understanding how trap spacing interacts with ethanol concentration provides practical recommendations for field deployment, ensuring wide coverage of coffee fields without unnecessary costs. This information supports integrated pest management (IPM) strategies by improving efficiency and scalability of trapping systems at farm and landscape levels. Farmers will benefit from reduced BCTB infestation and yield losses, while Uganda's coffee industry gains in terms of improved quality and export revenue.

1.6 Conceptual framework.

The study aimed at trapping Black Coffee Twig Borer using an ethanol-baited trap. Trap captures were affected by ethanol concentration, trap design, and inter-trap distance. Difference in any of these factors affected trap captures; for instance, trap captures varied with ethanol concentration.

There are also other factors that could affect trap captures but could not be controlled, especially environmental factors, and these included rainfall, temperature, wind direction, wind speed and relative humidity. These factors which were out of the scope of this study do affect flight activity of the beetles but also diffusion of ethanol hence affecting trap captures. For the study in the laboratory, beetle response only varied with difference in ethanol concentration since extraneous factors were under control.

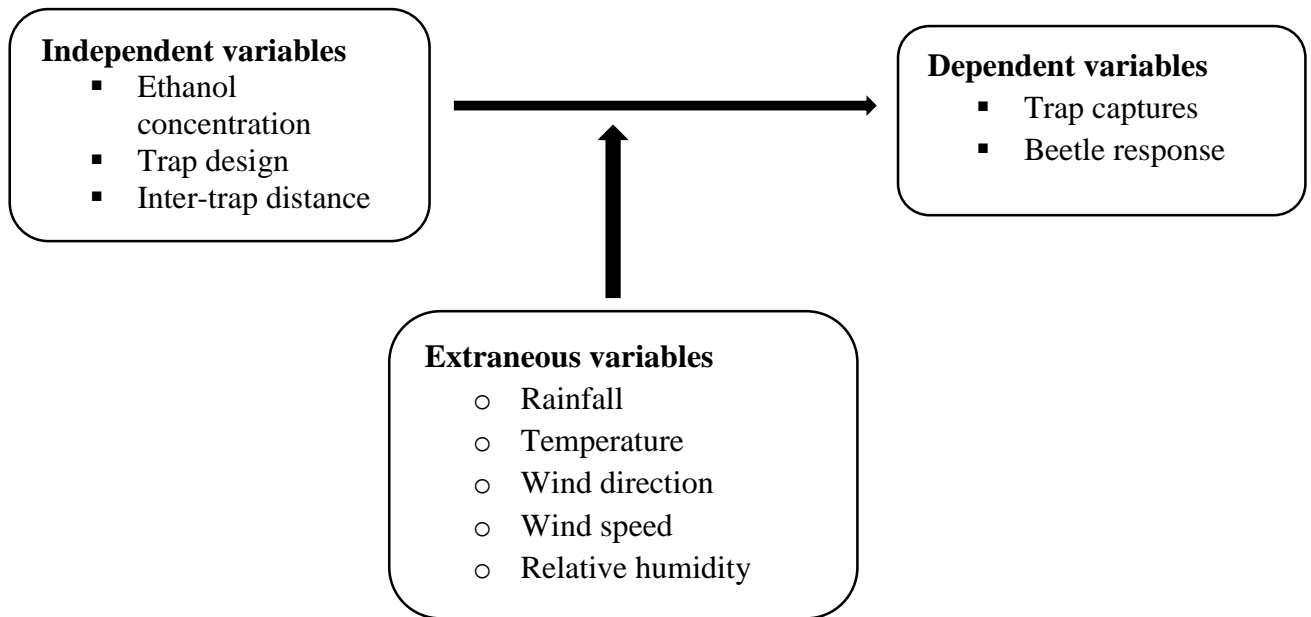


Figure 1 : Conceptual framework of the study.

CHAPTER TWO: LITERATURE REVIEW

2.1 The coffee cash crop

Coffee is the world's most widely traded agricultural commodity, with a large population consuming it as a beverage (Slavova & Georgieva, 2019). Coffee is a member of the Rubiaceae family and belongs to the genus *Coffea* L, which comprises around 105 species, primarily found in Africa (Akpertey *et al.*, 2019). The two primary species traded in the beverage industry are *Coffea canephora* Pierre, commonly known as Robusta, and *Coffea arabica*, known as Arabica. Arabica is typically preferred for its superior beverage quality and aroma (Merot *et al.*, 2019). Robusta is recognized for its unique and diverse organoleptic attributes, making it highly sought-after for coffee blends. Additionally, Robusta coffee is known for its resilience against diseases and extreme climatic conditions, such as drought, compared to Arabica (Musoli *et al.*, 2009; Li, 2022). Furthermore, Robusta coffee exhibits rich genetic diversity due to its mode of reproduction. Arabica lacks genetic diversity because it is primarily self-pollinated (Akpertey *et al.*, 2019).

Arabica coffee is cultivated in highland regions at 1400 meters above sea level elevations, while Robusta coffee thrives in lower-altitude areas (UCDA, 2019; Bongers *et al.*, 2015). Several factors influence coffee growth, including soil type, rainfall, temperature, humidity, and the area's topography (Bongers *et al.*, 2015; Vieira, 2008). Given that coffee is believed to have originated in forested

environments, it is classified as a shade-loving crop, and the shade helps mitigate the extremes of both low and high temperatures.

2.2 Challenges faced by coffee farmers in Uganda

Since the early 1990s, Uganda's coffee production and yield per hectare have faced significant challenges, which have hindered overall productivity (Heap & Bennett, 2014). Various impediments have been documented, contributing to the stagnation of production levels. Among the primary challenges challenging farmers are:

2.2.1 Climate change

Climate change represents a critical challenge for the agricultural industry around the globe (Pörtner *et al.*, 2022). Coffee is highly susceptible to the impacts of climate change, resulting in significant risks for its production (Bilen *et al.*, 2023). Factors such as drought, erratic weather patterns, and flooding from heavy rainfall have adversely affected coffee flowering, yield, and overall quality (Bilen *et al.*, 2023). Additionally, climate change has intensified issues related to soil health, nutrient availability, and the prevalence of pests and diseases. To mitigate these challenges, farmers are encouraged to adopt good agronomic practices that can adapt to the evolving climate (Ahmad *et al.*, 2024). One widely promoted approach is coffee agroforestry, which enables farmers to intercrop coffee with trees, thereby enhancing the microclimate (Gomes *et al.*, 2020).

2.2.2 Quality challenges

While Uganda is globally recognized for its coffee production, the quality of this coffee remains a significant concern (Wakulira, 2016). Quality issues often arise during the harvesting process, particularly when farmers engage in stripping, which involves the collection of both ripe and unripe berries (Nalunga, 2021). Additionally, many farmers and stakeholders lack knowledge on proper post-harvest handling practices. Problems such as drying coffee on bare ground, storing undried coffee, which encourages the growth of fungus, and inadequate storage after drying have severely impacted coffee quality (Arslan *et al.*, 2024). As a response, farmers have been trained on effective coffee harvesting techniques and encouraged to use tarpaulins instead of drying coffee on the ground (UCDA, 2017).

2.2.3 Market problems

Selecting an effective marketing channel is one of the most challenging decisions faced by farmers, as noted by Kabatoro (2023). Indigenous farmers often lack a clear market strategy and access to vital market information. Despite of the availability of coffee market information through UCDA media platforms, farmers predominantly depend on insights from fellow farmers and the local market where they sell coffee, relying heavily on middlemen, cooperatives, and private companies. (Rugerinyange & Buyinza, 2023). Unfortunately, these coffee buyers, especially middlemen, manipulate prices and take advantage of the information gaps, leading to unfair treatment of vulnerable farmers (Margiotta, 2019).

2.2.4 Agronomical challenges

One of the primary causes of low yields in farmers' fields is the failure to adopt good agronomic practices (Sseremba *et al.*, 2021; Kagezi *et al.*, 2014). Another significant contributing factor is the age of the coffee plants; many farmers hesitate to rejuvenate their coffee fields by cutting old stems, allowing them to grow. Consequently, most trees in these fields display very old stems with small canopy heads, and the primary branches on these canopies are short, exhibiting few nodes where flowering can take place (Bongers *et al.*, 2015). Due to the age of the trees, they cannot provide the necessary nutrients in adequate amounts, leading to large coffee fields with low productivity. Variations in coffee production among farmers can be attributed to management practices, soil fertility management, and investments in agro-inputs (Bongers *et al.*, 2015; Asha Nalunga, 2021). Since coffee farming is predominantly conducted by smallholder farmers, they often lack the financial resources to address these needs fully.

2.2.5 Pests and diseases

Pests and diseases are the primary factors impacting coffee production and productivity in Uganda (Wang *et al.*, 2015). Below are some of the pests and diseases affecting the coffee crop in Uganda.

2.2.5.1 Major coffee diseases in Uganda

Robusta coffee in Uganda has several diseases but some of them are not of economic importance. Some important diseases include:

Coffee wilt disease

Coffee wilt disease is a fungal infection caused by the vascular wilt pathogen *Fusarium xylarioides* (Pinard *et al.*, 2016). This soil-borne pathogen primarily targets the plant's vascular system, leading to wilting of the entire coffee plant due to its defense reactions (Flood, 2021). Symptoms of the infected tree include premature ripening of berries that remain attached to the tree, dieback and defoliation of leaves, as well as blue-black stains observed beneath the bark (Gaitán, 2015).

The pathogen was first identified in Uganda during the 1990s, and by 2002, it had infiltrated 90% of coffee fields, resulting in 45% tree mortality (Heap & Bennett, 2014). Coffee exports, which stood at 4.2 million 60-kg bags in 1996, plummeted to 2 million 60-kg bags by 2006, indicating a 50% loss in exports during that period (Heap & Bennett, 2014). Coffee wilt disease has posed a significant challenge to Robusta coffee until the development of wilt-resistant varieties by the National Agricultural Research Organization (Heap & Bennett, 2014; UCDA, 2018).

Red blister disease

Red blister disease is a fungal infection caused by the pathogen *Cercospora coffeicola* (Waller, 2007). This pathogen targets coffee berries, appearing as small red spots on green fruit. Currently, it poses a significant challenge in Uganda, as highlighted by the Uganda Coffee Development Authority (UCDA, 2019), and if left unmanaged, it can lead to yield losses exceeding 50% (Olango, 2024).

2.2.5.2 Major Coffee Pests in Uganda

Black Coffee Twig Borer

The Black Coffee Twig Borer is the most significant pest affecting the Ugandan coffee industry (Kagezi *et al.*, 2016). The pest attacks mostly Robusta coffee although some infestations have been reported in Arabica coffee (Kagezi *et al.*, 2016). It has also spread to every Robusta coffee growing region in the country (data). The pest is characterized by making pin-sized holes on the infested twigs where it lays eggs. Its tunneling activity damages the infested branches causing leaves to drop off and eventually the branches die days after and cannot produce berries (Dixon *et al.*, 2003). BCTB causes a national loss of about 10% valued at US\$ 40 million per year (Kagezi *et al.*, 2016).

Mealybugs

Mealybugs are oval-shaped, flattened insects that cluster on the leaves, flowers, berry clusters, and roots of coffee plants (Gaitán, 2015; Rutherford & Phiri, 2006). These pests are coated with a mass of white, sticky wax that makes their bodies difficult to see (Waller *et al.*, 2007). Mealybugs excrete a sugary substance that attracts ants, which feed on them and, in turn, transport the mealybugs from one plant to another (Mugo *et al.*, 2017). Additionally, a black mold can develop on infested leaves and other areas, blocking sunlight from reaching the leaves and hindering coffee plant growth (Magina *et al.*, 2012). Severe infestations can ultimately lead to the death of the plant, and this pest is particularly prevalent around Victoria Crescent (Melville, 2015).

Coffee berry moth

Coffee berry moth is a small golden pest that attacks coffee berries (UCDA, 2019). The pest lays eggs near coffee green berries, the eggs hatch into larvae and bore into coffee berries to feed on the beans (Gaitán, 2015). The larvae can damage several berries on the cluster leading to huge losses.

In Uganda, the coffee berry moth was first reported in the 1960s with low infestations, but of recent the pest is now of economic importance (Liebig, 2017; Kagezi *et al.*, 2021). A study at Kaweeri coffee plantation showed that the pest can cause 40% yield loss (Kagezi *et al.*, 2023). The pest caused to yield losses up to 80% as reported in the islands of Sao Tome (Derron, 1997).

Coffee Berry Borer

The coffee berry borer is the most devastating pest in the whole world, from the Scolytidae family that infests coffee (Alba-Alejandre *et al.*, 2018). Damage is started by the female beetle forming an entry hole at the apex of the berry into the bean (Husni *et al.*, 2022). The pest lays eggs that hatch into larvae, the feeds on the coffee bean, destroying it completely or lowers its quality (Johnson *et al.*, 2019). The damaged berries fall prematurely or remain on the tree until harvesting time, but their bean quality is very low. The coffee berry borer destroy 100% of the infested berry and 30 – 35% yield loss during the harvest period (Abewoy, 2022).

2.3 The leading pest constraint of coffee

The black coffee twig borer (BCTB) is an ambrosia beetle from the Curculionidae family, specifically in the Scolytinae subfamily and the Xyleborini tribe. This pest has a symbiotic relationship with an ambrosia fungus, and it is reflected in its name (Forestale, 2021). Lacking the necessary nutritional structures and enzymes to decompose lignin in the wood, the beetle has a symbiotic relationship with an ambrosia fungus, which it feeds on (Greco, 2010; Monterrosa, 2021). Furthermore, the fungus helps the beetle by weakening plant tissues, facilitating its boring activities. In return, the fungus benefits from being inoculated in favorable growth conditions and also offer both protection and a breeding ground (Gugliuzzo *et al.*, 2021; Ranger *et al.*, 2018).

The ambrosia beetle possesses specialized structures known as mycangia, which are used for storing fungus (Gorzlancyk, 2013). Various fungi have been found in association with these beetles, including *Fusarium* sp. and *Candida* sp., which have been isolated from the mycangia (Hayato, 2007). Additionally, several fungal species, such as *Cladosporium* spp. and *Penicillium* sp., were isolated from the beetles' body surfaces (Hayato, 2007). Among these, *Fusarium solani* stands out as the only plant pathogen. This species is known to cause root rot, cankers, and rapid wilting in affected areas of some woods (Greco & Wright, 2015a; Kendra *et al.*, 2020).

2.3.1 Lifecycle of BCTB

The adult beetle is cylindrical and is characterized by a stout, short form. After pupation, the female transitions from a brown color to a lustrous black within 3 to 4 days, while the male changes from light brown to reddish brown (Monterrosa *et al.*, 2021). The adult female beetle is larger than the male beetle. The female beetle measures approximately 1.4-1.8 mm in length and 0.7 – 0.8 mm in width, whereas the adult male measures about 0.8 – 1.3 mm long and 0.42 – 0.6 mm wide (Greco & Wright, 2015). The female-to-male population ratio is approximately 9:1, with males rarely found outside the host galleries (Roca & Gallego, 2020).

Once the female beetle enters the host galleries through the access hole, the beetle starts releasing and establishing the ambrosia fungus. The next step is laying eggs in the gallery about 4-7 days after entering. The eggs are clustered, oval with shiny white color, and several 1-23 eggs have been found in the galleries (Gaitán *et al.*, 2015). According to Greco *et al.* (2015), the incubation of eggs takes about 4 – 6 days. The overall cycle takes place at a temperature of 23°C to 27°C and 50 to 60% relative humidity (Greco *et al.*, 2015). Male beetles can develop from unfertilized eggs but females develop from only fertilized eggs (Greco & Wright, 2012). About 4 – 6 days after laying eggs, the larvae start developing. These feed on the ambrosia fungus inoculated by the mother beetle before laying eggs, the larvae undergo about 3 instars before they pupate, and the pupa takes about 4 – 6 days to mature (Ngoan, 1976). Mating within off springs takes place in the host galleries and female offspring will fly out looking for new hosts to oviposit (Ngoan, 1976). It undergoes

complete metamorphosis (Fig 5) and the whole process from eggs to adult takes about an average of 28 days (Dixon *et al.*, 2003).

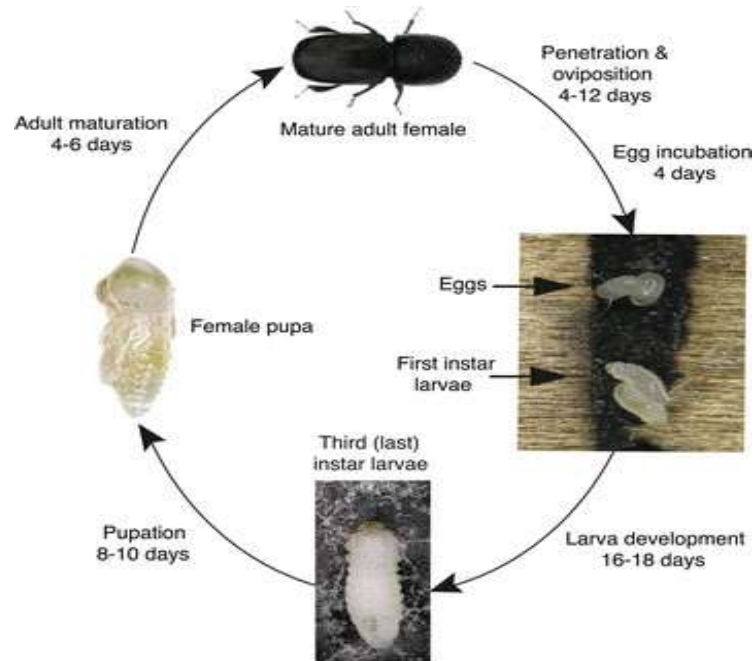


Figure 2: Life Cycle of Black Coffee Twig Borer (Sourced: O'Donnell *et al.*, 2016).

2.3.2 Damages of BCTB

Two types of damage are seen with BCTB, one where the damage is superficial and where it goes through where the brooding occurs. According to Greco & Wright (2012), *X. compactus* prefers lateral branches to vertical branches. The pest mostly attacks stressed coffee plants as a characteristic of all ambrosia beetles Greco, (2010), but research of recent shows that even healthy plants can be infested (Sun *et al.*, 2023). The adult female makes an entry hole of about 1mm in diameter (Rutherford & Phiri, 2006) and creates galleries in the host plant, the excavation activities of the female beetle damages the water and food systems of the twigs

obstructing the movement of water and nutrients (Gugliuzzo *et al.*, 2021). Early infestations may be hard to detect but with time the affected area turns light green, yellowing of leaves, and then turns black (Dixon *et al.*, 2003). Wilting becomes visible weeks after infestation and ultimately the twig is killed. A survey by Egonyu *et al.*, (2009) that revealed a 40.3% infestation rate of the field trees while the twig infestation rate stood at 8.3%.

2.3.3 Alternative Hosts for BCTB

Adult female beetles can fly up to 200 meters in search of hosts, while males are flightless and predominantly remain within the host galleries (Túler, 2019). The Black Coffee Twig Borer is polyphagous, reportedly having over 200 alternative hosts worldwide, with more than 50 identified in Uganda, including both crops and forest trees. (Greco & Wright, 2015; Kagezi *et al.*, 2012). Notably, nurseries of ornamental trees have experienced considerable damage, and reports have confirmed attacks on cocoa plants in Uganda and Hawaii. (Reding *et al.*, 2010; Waller *et al.*, 2005; Kagezi *et al.*, 2014). Additionally, BCTB affects tea, mangoes, and *Maesopsis eminii* (Kagezi *et al.*, 2013; Kagezi *et al.*, 2019). Although some trees have been intercropped within coffee-agroforestry systems to counteract climate change effects, their status as alternative hosts have discouraged farmers from planting them alongside coffee crops. In Uganda, for instance, planting *Albizia chinensis* in coffee fields is not recommended, despite its valuable characteristic of fixing nitrogen in the soil (UCDA, 2019).

The black coffee twig borer has infested some other plants, and the attacks have failed to be successful in the process, due to the release of specific chemicals in the

form of sap that have deterred the pest (Greco & Wright, 2015). For example, when the pest penetrates the avocado tree, the tree secretes sap, which, upon drying around the entry point, has been identified as calcium oxalate. This sap has been observed during successful and unsuccessful attacks (Hara, 1977). Furthermore, a reaction was noted in *Acacia koa* seedlings with stem diameters exceeding 10 mm (Hara, 1977). No brood was discovered in the entry holes of plants that produced sap. In the case of *Limonia acidissima* (L), female beetles were unable to create entry holes due to the excretion of gum, leading to the death of these females at the entry sites (Mannakkara & Alawathugoda, 2005).

2.3.4 Management of BCTB

A number of management practices have been used that include cultural, biological, mechanical, and chemical methods to manage BCTB.

2.3.4.1 Cultural methods

Cultural control methods are the most used management practices, these are majorly phytosanitary activities that include pruning, de-suckering, removing infested branches, and burning them (Ogundeji *et al.*, 2019.). Other agronomic practices that improve plant health and vigor are also encouraged (Ranger *et al.*, 2015). A survey done by Wu (2016) showed that 80% of the farmers in Uganda use phytosanitary practices as a way of managing the pest. According to Egonyu *et al.* (2009), these practices need a communal effort to get good results since the pest has no boundaries.

2.3.4.2 Biological methods

The biological control method employs natural enemies of the Black Coffee Twig Borer as a defense mechanism. These natural enemies include predators, entomopathogens, and parasitoids (Egonyu *et al.*, 2015). Some insect predators feed on different stages of the beetle. The predators *Plagiolepis* spp. and *Pheidole megacephala* are found to feed on all stages of the beetle. For instance, the *Plagiolepis* spp. was found to colonize 18% of the BCTB galleries and preyed on all the life stages of the pest (Egonyu *et al.*, 2015). The larvae of *Callimerus* spp. (Coleoptera: Cleridae) were observed in galleries found on coffee branches and according to (Husein, 2023), laboratory tests in India showed that the predator larvae fed on 18 larvae or pupa of BCTB per day, leaving the exoskeleton.

Entomopathogens have also been found to be potential biocontrol methods for BCTB, for example, the L strain of *A. flavus* was found to be highly virulent on all stages of BCTB, and it killed 70-100% of all stages of BCTB and 50% results would be achieved with 2-3 days in the laboratory (Mukasa *et al.*, 2018). The entomopathogenic fungi *B. bassiana* has also been found to infect all the stages of BCTB in India. Spraying Robusta coffee with spores of *B. bassiana* infected 21% of beetles present in branches (Uma *et al.*, 2021). Spores of *B. bassiana* are currently used in the management of Coffee berry borer in Hawaii, which may help suppress BCTB in the long run (Greco *et al.*, 2015).

2.3.4.3 Chemical methods

Chemical control is one of the intervention done to manage the BCTB. However, the biggest challenge about this method is the concealed nature of the pest, since it spends most of its entire life cycle in the host galleries (Egonyu et al., 2009), yet the entry hole is about 0.7 to 0.9 mm (Dixon *et al.*, 2003). Time of insecticide application is very important, and studies have revealed that targeting the flight time of the beetles gives better results (Reding *et al.*, 2013).

A number of insecticides have been studied and recommended in the management of black coffee twig borer. Laboratory tests conducted by Mangold *et al.* (1977) revealed that chlorpyrifos was effective in killing 72% to 92% of all life stages of BCTB. According to the Robusta coffee hand book, a farmer's guide by Uganda Coffee Development Authority, (UCDA, 2019) use of imidacloprid is encouraged and for better results, farmers are advised to mix it with Tebuconazole.

Trapping of BCTB using baited traps has been used in reducing damages, monitoring population, and distribution studies of the black Coffee Twig borer(Adams *et al.*, 2017). Different attractants to the pest have been studied and used in attracting the beetle. Ethanol, Manuka oil, ginger oil, α -pinene, and eugenol have been evaluated as single attractants or in combination, in different studies (Greco, 2010; Sweeney *et al.*, 2016). Among the baits used for attracting BCTB, ethanol has shown outstanding results, and it has been widely used to manage the pest. However, it must be emphasized that for best results, farmers need to deploy the integrated pest management strategy for BCTB (Kekeunou *et al.*, 2006).

2.4 Use of ethanol-baited traps in the management of BCTB

Ethanol is one of the attractants used in traps and according to (Sweeney et al., 2016) it is the major attractant for beetles in the Scolytidae sub-family. Ethanol is produced by plants under environmental stress like drought and floods that trigger fermentation processes within the plants producing ethanol (Miller *et al.*, 2015). Ethanol is used by ambrosia beetles to locate suitable hosts and their quality. This was evident in an experiment where trees were injected with ethanol. The beetles *X. germanus* and *X. crassiusculus* attacked most the injected trees and left out the neighboring trees that were not injected at all or injected with water (Ranger *et al.*, 2013). Scientists have used this knowledge to come up with a trapping technology using ethanol as the attractant, in search for alternative management strategy (Greco, 2010). Various types of traps that differ in designs, but using ethanol as the attractant have been developed and recommended for use against ambrosia beetles.

One of the ethanol-baited traps is the Lindgren trap used to trap scolytinae beetles, this trap consists of vertical multi-funnel through which beetles go through down the collection container. Lindgren traps have been used in forests to monitor absence or presence of ambrosia beetles (Sweeney *et al.*, 2024). Another trap design used in trapping is the Japanese trap, it consists of a yellow funnel and four vertical planes fitted in a collection container using 95% ethanol. Bsurbano *et al.*, (2012) used this trap to monitor *X. compactus* in Arabica coffee. One of the simplest models is the Baker-type, which is a 2-liter soda bottle (round, plastic) with two cuts made opposite each other. This trap is similar to one developed by the National Agricultural organization (NARO) in Uganda that is used by farmers to trap BCTB.

The trap is made of a 0.5-liter water bottle with cut windows opposite each other, using 70% ethanol tied in the small vial (Kuchel, 2016). Lures in all these traps are filled in a capped vial that steadily dispenses ethanol.

Ethanol-baited traps have been used in monitoring, mass trapping, and assessing temporal and spatial patterns of beetles (Sweeney *et al.*, 2016; Miller *et al.*, 2018). For example, these traps have been used in a study involving monitoring the ornamental seedlings damaged by ambrosia beetles in the nursery, and have also been used in the management of bark and wood-boring beetles in the forests (Ulyshen & Sheehan, 2019). In Spain, the traps have been used in monitoring *X. compactus* and *X. crassiusculus* in carob trees. The trapping technology has been integrated into the pest management packages of BCTB across nearly all coffee-growing countries worldwide.

obin2.5 Factors affecting efficiency of ethanol-baited traps

A number of factors have been reported as affecting the efficiency of traps. Such include concentration of the ethanol, trap placement, design of the trap and inter-trap distance which have been reported as significantly affecting trap catch (Kong *et al.*, 2014).

2.5.1 Trap design

A number of studies have shown how different trap designs affect trap catch. The design of the trap can affect the release rate of the bait, capture and it is also highly affected by environmental condition (Steininger *et al.*, 2015).

Trap design has affected catch of different insects when studies about it were carried out. For example, trap design affected catch of orange worms at peak abundance when different traps were used (Burks & Higbee, 2015). The single-window bottle trap was more effective at capturing *X. crassiusculus* compared to the multiple-window trap (Ruzzier et al., 2021). The Japanese baited trap was more effective at trapping *X. compactus* than the Lindgren trap (Ruzzier et al., 2021). The Lindgren trap was favorably more efficient in capturing ambrosia beetles and mountain pine beetle than the sticky and Scandian drainpipe trap. The trap showed high efficiency in mass trapping of scolytids in surveys, and pheromone-based research (Ruzzier et al., 2021). Numerous experiments were also carried out to get the best design to monitor and conduct early detection of the exotic Red bay beetle. In that study, funnel traps caught significant numbers compared to sticky and Cross-vane traps (Tanner et al., 2018).

Different studies have also shown that different insects are attracted to different trap designs (Burks & Higbee, 2015; Kendra et al., 2020; Burner, 2020). A comparison of ambrosia beetle catch was done between the multi-funnel and bottle traps, using ethanol and conophthorin as the attractant; their effectiveness depended on beetle species (Miller et al., 2015). Scolytidae trap effectiveness was compared among four trap types and effectiveness depended on the scolytidae species. Field tests have also confirmed that the variation in the effectiveness of traps generally varied with the species (Gugliuzzo et al., 2021). Exploring different trap designs to identify the best design that can effectively capture a particular species is highly important.

2.5.2 Trap placement

Trap placement is one of the key factors that should be given careful attention when deploying traps in the field, a good trap design can be failed by an inappropriate trap height (Ulyshen & Sheehan, 2019). Studies on trap placement have shown that different insects are trapped highly at a specific height and this height varies with species (Byers, 2012; Schmeelk, 2015; Sheehan *et al.*, 2019). In an experiment designed to determine the effect of trap height on bark and wood-boring beetles, Hanula *et al.*, (2016) discovered that trap height greatly affect catches in that different scolytidae species are caught at different height. Coffee Berry Borer was captured highly at 0.5 meter and best capture range was 0.5-0.8 meters (Sylvia *et al.*, 2022). In an experiment to determine the effect of trap height on catch of fruit moths, traps were placed up to 3m but moths were significantly caught at 2.5m (Kong *et al.*, 2014). Male potato tuber moths were significantly caught at 0.6m above the ground when its pheromone trap was tested (Hashemi, 2015). Trap placement is seen to be among the major factors affecting capture rates (Gugliuzzo *et al.*, 2021). Understory traps are said to capture more *X. crassiuscullus* than the canopy traps (Miller *et al.*, 2020). *Xylosandrus compactus* is highly captured when traps are placed at 1.5meters (Burbano *et al.*, 2012). Traps set at 0.5 meters above the ground captured more *X. germanus* compared to those set at 1.7 or 3.0 meters.

Trap placement is also very important in determining the vertical distribution and flight layers of flying insects. In experiments to determine effective attraction radius of a trap, traps are placed at various heights to get the mean flight height because it is believed that different scolytidae can be captured at different heights

(Byers, 2011; Byers, 2012). The trap height should therefore be carefully studied as Gugliuzzo *et al.*, (2021) recommends that optimal trap placement should be studied in other *Xylosandrus* species which includes BCTB.

2.5.3 Trap spacing

The inter-trap distance is also another factor affecting trap catches. It refers to the distance from one trap to another. Recent studies have shown that if the inter-trap distance is not observed, it may affect captures (Caiti *et al.*, 2025). Traps that are placed near to each other can bring about overlapping in the attraction radius of traps hence reducing captures (Hanula *et al.*, 2016). Trap interferences occurred when trap spacing was < 30m, and no competition was seen above 30m and captures densities stabilized. Traps baited with frontalin, and turpentine aggregation pheromones increased catch when another pheromone, (p)-endo-brevicommin, was placed between 4-6 meters with high catches at 4m (Mori, 2009). In an experiment to determine the effective trap spacing for *Ips pini*, a bark beetle, a spacing of 15 meters produced independent results. Below this distance, there was interference between traps (McMahon *et al.*, 2010). Research by Bacca *et al.* (2006) indicates that interference occurs between traps when they are spaced closer than 10 meters apart, specifically for capturing coffee leaf miners. These findings highlight the importance of trap spacing, which should be determined based on the target pest and the type of bait used.

Trap spacing is very important in determining trap density, an important variable to get population density and trap capture relationships. Burks & Higbee (2015)

says that trap density should be considered if pheromone traps are to be effective to predict damage. Dodds *et al.* (2024) confirmed that trap captures of pheromone traps are greatly affected by trap density. Inter-trap distance should be given keen attention to get effective trap results.

2.5.4 Concentration of the attractant

Beetles from the scolytidae family respond differently to different ethanol concentrations. Some pests of the Scolytidae sub family are attracted to low concentrations of ethanol while others are attracted to high concentrations (Cavaletto *et al.*, 2021). It is said that ambrosia beetles are more attracted to high ethanol concentrations (Santos *et al.*, 2018) and host selection by ambrosia beetles is determined by ethanol concentration of the host (Santos *et al.*, 2018). In an experiment where different bolts of different tree species were injected with different ethanol concentrations, entry holes for *X. germanus* decreased with increase in ethanol concentration and those of *X. saxesennii* increased with increase in concentration (Cavaletto *et al.*, 2022). Addeso *et al.* (2019) recorded high attacks with trees injected with high doses of ethanol compared with those with low doses. Ranger *et al.* (2020) showed that ambrosia beetles would attack the injected trees with ethanol and leave out the un-injected ones, or those injected with water. Studies on effect of different ethanol concentration on scolytidae beetles have been conducted using (0, 25, 70, 75 and 95%) ethanol and found that 70% and 75% ethanol concentration were effective, and observed that 95% caught very few beetles (Santos *et al.*, 2018). Recommendations according to Cavaletto *et al.* (2021) confirm that identifying the most effective ethanol concentration in attracting

scolytidae beetles would help in mass trapping of these beetles to reduce their damage. Ethanol remains the most commonly used semiochemicals for attracting Scolytidae pests, making it essential to identify the optimal concentration for the target beetle, as confirmed by Dorval & Souza (2020). Studying the effectiveness of different ethanol concentrations on BCTB is very important.

2.6 Integrating ethanol-baited traps with other semiochemicals

Ethanol has been used in combination with other semiochemicals to form a synergetic effect. Some of the attractants used in combination to form a synergetic effect include: Manuka oil, ginger oil, α -pinene, eugenol and conophthorin (Miller *et al.*, 2015; Vanderlaan & Ginzel, 2013; Burbano *et al.*, 2012).

Although studies about synergy of the compounds with ethanol have been conducted, some studies have shown that ethanol performs better when in combination while others show that ethanol works better when alone. In a study by Ranger *et al.* (2011) where ethanol traps were combined with α -pinene, results showed that ethanol alone was more effective than when it was in combination. Conophthorin was also combined with ethanol and Miller *et al.*, (2015) recommended that ethanol baited traps can be used alone since Conophthorin causes an interruption in trapping some ambrosia beetles. Results by Burbano *et al.* (2012) showed that there was no significant difference when ethanol was combined with α -pinene, ginger oil and ethanol alone, tested on different ambrosia beetles. Ethanol has also been successful when combined with other semiochemicals; an ethanol baited trap enhanced with Conophthorin increased the catches of *X.*

crassiusculus and *Xyleborinu saxesenii* when using a bottle trap (Vanderlaan & Ginzl, 2013). A combination of 95% ethanol and 100% methanol captured more coffee berry borer than 95% ethanol alone (Kurnianto *et al.*, 2024).

Ethanol has also been studied and used with ant-aggregation pheromones to come up with a push-pull effect. A combination of ethanol, Limonene and verbenone when using the Japanese trap reduced *X. compactus* more than the ethanol alone (Tobin *et al.*, 2025). Although Limonene and verbenone are anti-aggregation pheromones, verbenone is said to be more promising on BCTB since it is said to be effective on beetles that go for fresh tissues (Ranger *et al.*, 2021). Putting verbenone in the fields and applying ethanol baited traps at the edge of the field can be an area of interest, for management of beetles in the scolytinae family (Audley *et al.*, 2021). Combining ethanol with other aggregating and ant-aggregating semiochemicals can be a good management alternative.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area description

All the study experiments were conducted at the National Coffee Research Institute (NaCORI), located in Kituza village, Ntenjeru sub-county, Mukono district. Mukono lies in a tropical climate, experiencing a bimodal rainfall pattern, with annual rainfall ranging from 1400 to 1600 mm. The area experiences two rainy seasons per year, with the first season occurring from March to May and the second from September to November (Kobusinge *et al.*, 2023). NaCORI is located in the Lake Victoria Crescent agro-ecological zone, located at an elevation of 1200 m above sea level, at 0015'26.9874"N and 32047'27.69648"E in the low-to-medium altitude area (Kobusinge *et al.*, 2023). NaCORI was purposively selected because it has a high population and damage to the test insect pest, *X. compactus*.

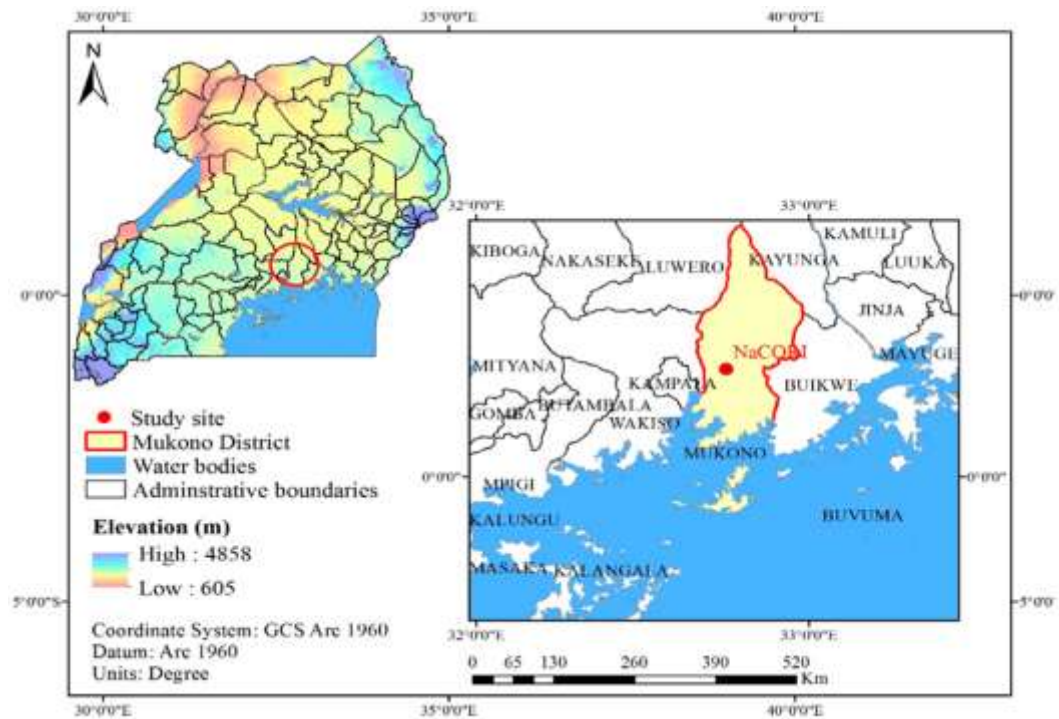


Figure 3: Location of National Coffee Research Institute (NaCORI) (source: Kobusinge et al., 2023)

3.2 Experimental field

The experiments for studies 1 identify the optimal window number, size and flap configuration of the ethanol-baited NARO BCTB trap for maximizing the capture of adult *X. compactus* in Robusta coffee agro-systems. and 3 were conducted in an already existing Robusta coffee-banana agro-system of about 5.5 acres. These coffee trees were planted in 1995 as trial experiments to assess the susceptibility of some coffee varieties to Coffee Wilt disease (CWD), which was a challenge to the Ugandan coffee by then, and it is from where the current resistant Kituuzza Robusta varieties were selected. The coffee trees have been undergoing regular stumping,

and the last one was done in 2019. The average height of the trees under study was 2.5m.

3.3 Experimental trap

This study aimed at refining the NARO BCTB trap for improved efficiency. The original trap (Fig. 4) designed by Kucel *et al.* (2016) is made out of a 500 ml transparent mineral water plastic bottle, a 25 ml plastic dispenser vial with a pin-hole on the cover for allowing vaporization of the lure, a nylon string, and a lure (ethanol). The bottle has two dispensing windows cut on two opposite sides to allow the entry of the beetles (Steininger *et al.*, 2015). In addition, soapy water is filled in the lower third of the bottle to reduce the surface tension of the water and thus, make the trapped beetle sink (Bouget *et al.*, 2009; Tarno *et al.*, 2021).

Although the ethanol concentration to be utilized is not specified by Kucel *et al.* (2016), he suggests using at least 30% ethanol concentration. Farmers are mostly using local distillates, which are readily available on the market. However, in this present study, industrial ethanol was used because it was easy to profile the different concentrations, which might be difficult in the case of the local distillate. Additionally, some detergent was added to reduce surface tension (Bouget *et al.*, 2009; Tarno *et al.*, 2021). Furthermore, a 25 ml laboratory universal bottle with a metallic cover having a tinny hole (0.5 mm in diameter) was used as the vial for allowing vaporization of ethanol (Kucel *et al.*, 2016).



Figure 4: The NARO BCTB trap under study

3.4 Data collection methods

3.4.1. Identifying the optimal window number, size and flap configuration of an ethanol-baited trap design for capturing BCTB

The experiments were conducted in the coffee-banana system because it is one of the major and commonest systems practiced by smallholder farmers in Uganda (Van Asten *et al.*, 2011). A split-split plot experiment was laid out in a randomized complete block design (RCBD) (Fig 5) with the main plot being the presence or absence of flaps, the sub-plot being the number of windows (1, 2, and 3) and the sub-sub plots being the window size (6x2, 6x4 and 6x6 cm). These were replicated three times in the coffee-banana agro-systems. Each experimental sub-sub plot measured 10 x 10 m and the distance between them was 10m, whereas the distance

between sub-plots was 10 m the distance from one block to another was 50m. An ethanol concentration of 70% was used in this study, and the trap was suspended on a string in the upper canopy (2m from the ground) of the middle coffee tree in a sub-subplot.

The traps were maintained in the field for 24 weeks, and trap captures were collected weekly. The collected samples were stored in well-labelled vials containing 70% ethanol, taken to the laboratory at NaCORI, sorted from other related beetles under a hand magnifying glass of magnification 10x (handle length = 85mm, glass diameter = 100mm, and glass thickness = 10mm), and counted. Features such as a stout, cylindrical body, shiny black color, wide convex pronotum with distinct punctures, and a rounded posterior end of the elytra were used to identify the beetle. Traps were refilled with soapy water each time beetles were collected, whereas ethanol was replaced every 14 days (Kucel et al., 2016).

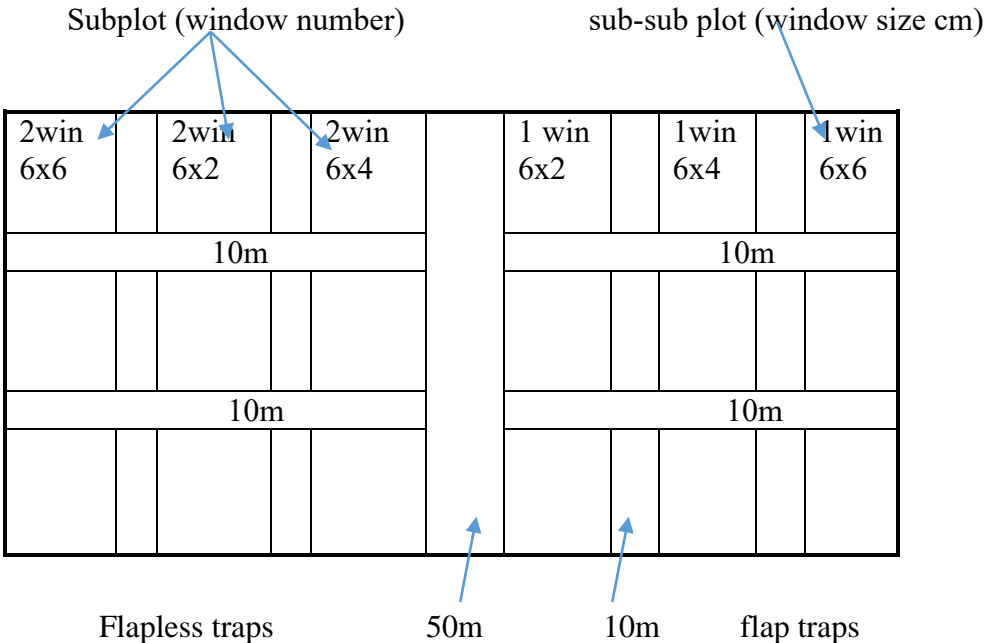


Figure 5: Experimental set up of traps in the field

3.4.1 Determining optimal ethanol bait concentration for capturing BCTB

Bioassays were conducted at the National Coffee Research Institute (NaCORI) Entomology laboratory to evaluate the ability of five ethanol concentrations (0, 25, 50, 75, and 96%) to attract adult *X. compactus* (Santos *et al.*, 2018). A Pyrex glass Y-tube olfactometer (diameter = 10 mm; stem 85 mm; arms 75 mm at 60° to the stem) (Analytical Research Systems INC, Gainesville, FL, USA) (Egonyu *et al.*, 2018; Qiu *et al.*, 2022) was used to test the attraction of adult *X. compactus* towards the different ethanol concentrations (Fig. 5). One arm of the olfactometer contained the test sample and another one had air. The bioassay studies were conducted under ambient laboratory conditions.



Figure 6: Y-tube olfactometer used to study BCTB response towards different ethanol concentrations.

Infested BCTB twigs were cut from the field, brought to the laboratory. In the laboratory, beetles were harvested from the twigs and subjected to different ethanol samples. For each of the five ethanol concentrations tested, 250 adult *X. compactus*

beetles were used, making a total of 1,250 beetles. Each concentration had 25 groups of beetles of 10 beetles each. In each group, one beetle was released at a time into the Y-tube and observed for 10 minutes for its response – whether it went toward air, ethanol sample, or no response if the beetle did not move at all, or if it went back to the entry point (Qiu *et al.*, 2022).

3.4.3 Evaluating the effect of ethanol concentration and trap spacing on BCTB trap captures

This study was conducted in a coffee – banana cropping system at the National Coffee Research Institute. From the study to identify the best trap design described in section 3.2, a flapless trap with 2 windows and size 6 x 4 cm which outperformed the rest of the trap designed was selected for use in this study. The experiment was set in a split-plot design with ethanol concentration as the main factor and trap spacing as a sub factor. All the five ethanol concentrations previously tested in the laboratory bioassay (0%, 25%, 50%, 70%, and 96%) were subsequently evaluated under field conditions (Santos *et al.*, 2018). For each concentration, four traps were deployed and suspended on coffee trees (2m from the ground) along a linear transect. The traps within each transect were positioned at intervals of 10 m, 20 m, 30 m, and 40 m, respectively (Samfix, 2019; Jactel *et al.*, 2018). To minimize interference between treatments, the distance separating one transect from another was maintained at 50 m. Due to limited number of coffee trees and a uniform coffee cropping system of interest (coffee-banana system) considered for this study, the treatments were replicated only two times (Meyners *et al.*, 2020; Yan, 2021). Although this may reduce the statistical power to detect subtle effects, it remains

sufficient for identifying significant trends, especially given the prevalence of the pests.

3.5. Statistical data analysis

For objective 1, interaction between trap type, window size, and window numbers was determined using a three-way ANOVA at a significance level of ≤ 0.05 . Differences between treatment means were separated using Tukey's HSD test for multiple comparisons. Version 4.4.0 of the R program (R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

For objective 2, the mean percentage response of adult BCTB to different ethanol concentrations was presented using a bar graph. Relationship between ethanol concentration level and the percentage of adult black coffee twig borer (BCTB) response within the Y-tube olfactometer, was determined using a nonlinear quartic polynomial regression analysis. All the analyses were done using R software (R4.4.0).

For objective 3, a Two-way ANOVA was used to determine the interaction between concentration and spacing at a significance level of ≤ 0.05 . Furthermore, a combined effect of ethanol concentration and trap spacing on BCTB trap captures was performed and means were separated using Tukey's HSD test at a significance of ≤ 0.05 . Version 4.4.0 of the R program (R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

CHAPTER FOUR: RESULTS

4.1 Optimal ethanol-baited trap design for maximum capture of BCTB

Results from the study on the effect of different trap types showed a significant difference in the average number of BCTB captured between the two trap types (Table 1). BCTB trap captures were also significantly affected by window numbers and size. Additionally, there was a significant difference in the effects of trap type and window size on BCTB captures. On the other hand, there was no significant difference in the effect of trap type and the number of windows on BCTB captures, and also no significant difference in the effect of window number and size on BCTB captures (Table 1). There was no interaction in the three factors.

Table 1: Interaction effects of type, number, and size of windows on the number of adult BCTB captured in the ethanol-baited traps

Source of variation	DF	ANOVA SS	Mean square	F value	P value
Type	1	2133.343915	2133.343915	50.06	<.0001
Number	2	2809.083333	1404.541667	32.96	<.0001
Size	2	615.189337	307.594669	7.22	0.0008
Type*Number	2	214.414021	107.207011	2.52	0.0812
Type*Size	2	372.726389	186.363194	4.37	0.0128
Number*Size	4	339.821670	84.955417	1.99	0.0931
Type*Number*Size	4	148.294811	37.073703	0.87	0.4813

4.1.2 Optimal window number for capturing BCTB

Results showed significant differences in BCTB captures in both flap and flapless traps. For flap traps, the number of captures per week increased with larger window sizes, with 3 windows capturing the highest and 1 window capturing the lowest. On the other hand, flapless traps exhibited higher overall captures, peaking at 3 windows and least at 1 window. Trap captures in 2 windows were not significantly different from those in 3 windows.

Table 2. Mean trap captures (per week) for window numbers across different trap types.

Trap type	Number of windows		
	1	2	3
	Mean trap captures per week ± SE		
flap	3.9 ± 0.2 ^c	5.4 ± 0.3 ^b	6.6 ± 0.5 ^a
Pval	8.097e-07 ***		
Fval	14.291		
Flapless	5.3 ± 0.3 ^b	8.5 ± 0.5 ^a	9.2 ± 0.6 ^a
Pval	7.365e-09 ***		
Fval	19.2		

Letters in superscript indicate significant differences at ($P \leq 0.05$) using Tukey's pairwise comparison (HSD).

4.1.2 Optimal window number for capturing BCTB

The results in table 3 show the effect of window size of both flap and flapless traps on BCTB trap captures per week. Results showed a significant difference in BCTB trap captures in both flap and flapless traps. Both flap and flapless traps recorded an increase in mean captures, as the window sizes increased from 6x2cm

to 6x6 cm. Specifically, flap traps captured the highest number of beetles in 6x6 cm and lowest in 6x2 cm. In contrast, flapless traps consistently recorded higher captures, with the highest captures in 6x4 cm although not significantly different from 6x6 cm. The lowest captures were recorded in 6x2 cm

Table 3: Mean trap captures (per week) for window sizes across different trap types

Trap type	Window sizes (cm)		
	6x2	6x4	6x6
	Mean trap captures per week ± SE		
flap	4.9 ± 0.2 ^b	4.6 ± 0.3 ^b	6.5 ± 0.5 ^a
Pval	0.0002857 ***		
Fval	8.2497		
Flapless	6.6 ± 0.5 ^b	8.4 ± 0.5 ^a	8.1 ± 0.5 ^a
Pval	0.01699 *		
Fval	4.097		

Letters in superscript indicate significant differences at ($P \leq 0.05$) using Tukey's pairwise comparison (HSD).

4.2 Optimal ethanol bait concentration for attracting adult BCTB.

Results showed that the percentage of the adult BCTB attracted to the ethanol samples in the Y tube varied significantly across the ethanol concentrations (Fig.7). The highest percentage of attracted beetles was recorded with ethanol concentration of 70%, and this was significantly different from other concentrations. On the other hand, the lowest response was with ethanol concentration of 96% but, this was not significantly different from ethanol concentration of 0%.

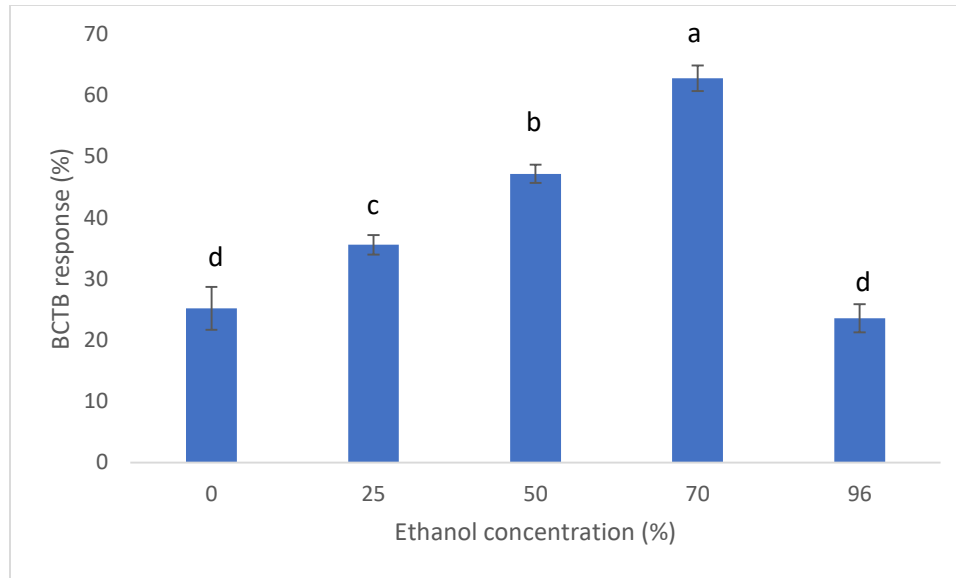


Figure 7: BCTB attraction (%) at varying ethanol concentrations (%) in the Y-tube olfactometer.

The percentage of beetles attracted to ethanol in the Y-tube, increased with increasing ethanol concentration, reached a peak at 70% ethanol concentration, and then decreased at the highest concentration of 96%. A nonlinear quartic polynomial regression analysis indicated a strong relationship between ethanol concentration and BCTB response in the Y-tube (Fig.8).

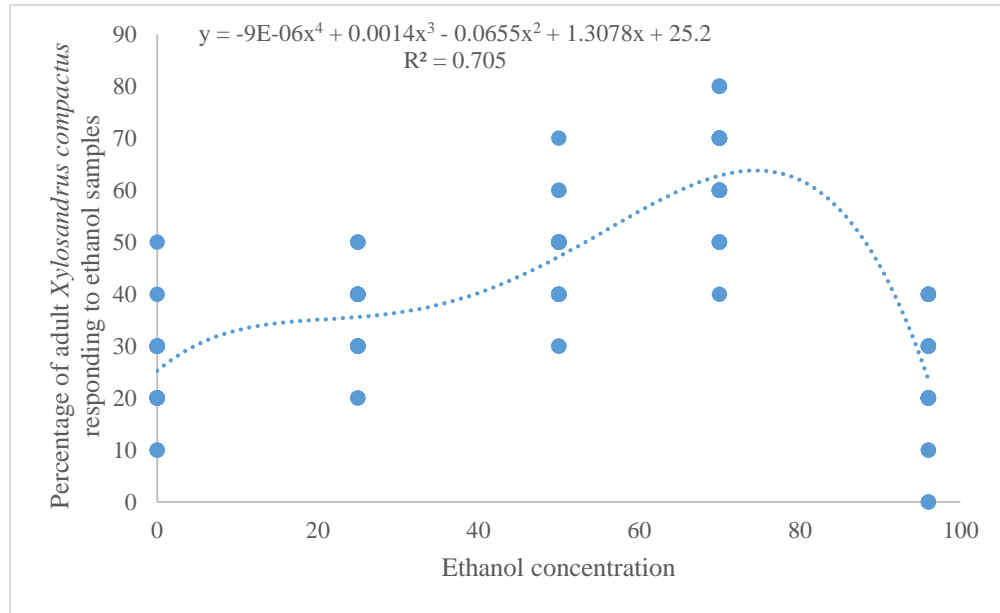


Figure 8: Relationship between ethanol concentration and BCTB response in the Y- Tube olfactometer.

4.3 Effect of the ethanol concentration and trap spacing on catches of adult BCTB under field conditions.

Results from this study showed that ethanol concentration and trap spacing significantly affected BCTB captures in the field (Table 4). There was a significant interaction between the ethanol concentration and the distance between the ethanol-baited traps placed in the Robusta coffee agro-systems (Table 4)

Table 4: Interaction effects of ethanol concentration and trap spacing on BCTB trap captures.

Source of variation	DF	ANOVA SS	Mean square	F value	P value
Concentration	4	8207.026923	2051.756731	65.22	<.0001
Trap spacing	3	613.430769	204.476923	6.50	0.0003
Concentration*trap spacing	12	1582.126923	131.843910	4.19	<.0001

The highest mean number of adult beetles was recorded in traps containing 70% ethanol and separated by 30m (18 beetles). However, this was not significantly different from traps containing 50% ethanol and spaced at 20 m (15 beetles). On the other hand, the lowest mean number of beetles was recorded in traps containing no ethanol and spaced at 10m (1 beetle) but this was not significantly different from other traps with no ethanol which also captured one or two beetles per trap.

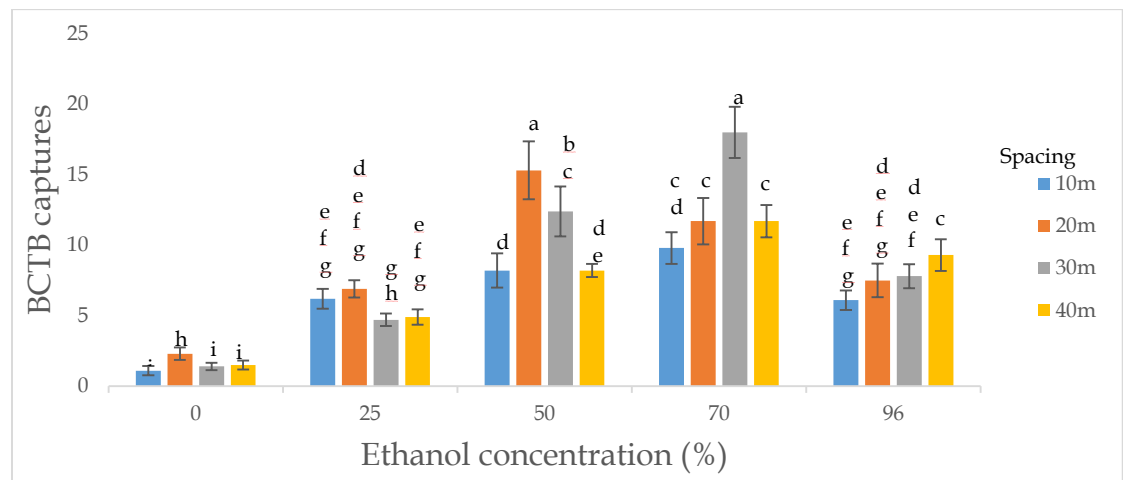


Fig9: Mean number (\pm S.E) of adult BCTB captures in traps baited with varying ethanol concentrations and spaced at varying distances apart. Means with the same letter are not significantly ($p > 0.05$) different using Tukey's HSD test.

CHAPTER FIVE: DISCUSSION

5.1 Best design of an ethanol-baited trap for capturing BCTB in Robusta coffee agro-systems

Effective trap design is critical for increasing ambrosia beetle capture rates (Tobin *et al.*, 2024), and even modest changes can have a major impact on trap performance (Burner, 2020). In this investigation, ethanol-baited traps without flaps captured significantly more beetles ($p < .0001$) than traps with flaps. These findings show that flaps may serve as physical barriers, preventing beetles from entering the trap, whereas flapless designs allow for simpler entry and improved interception opportunities. Similar patterns have been reported in previous insect investigations. For example, Soundararajan *et al.* (2020) discovered that removing the front flap of delta traps increased moth entry rates in *Ctenopseustis obliquana*. Additionally, Knight *et al.* (2002) demonstrated that removing flaps in delta traps enhanced direct moth entry, although flaps did not significantly affect captures across all trap types. These findings indicate that the influence of flaps may vary based on trap structure and insect behavior. Flapless traps are more effective than flap traps; they likely improve airflow, enhancing the dispersal of ethanol plumes that guide beetles toward the trap (Burner, 2020). Secondly, removing flaps increases the exposed surface area, allowing traps to intercept a greater number of beetles flying nearby (Hoover *et al.*, 2000; Knight *et al.*, 2002). This aligns with results from this study, where flapless traps consistently yielded higher catches. Nevertheless, the effectiveness of ethanol-baited traps is not determined by flaps alone. Several studies have documented significant differences in ambrosia beetle

captures across trap designs, highlighting the importance of design features such as entry points, color, and shape (Burks & Higbee, 2015; Miller *et al.*, 2018; Tarno *et al.*, 2021; Setiawan *et al.*, 2023; Tobin *et al.*, 2024). Thus, while flap removal appears to improve trap efficiency, comprehensive evaluations of design variables are necessary to optimize monitoring systems.

Results also showed that trap captures of *X. compactus* increased significantly ($P < .0001$) with the number of windows on the traps. Similarly, Steininger *et al.* (2015) observed that a clear trap with two windows captured more ambrosia beetles compared to a clear trap with one window. Similarly, Akist *et al.* (2024) and Basoalto *et al.* (2013), reported that increasing the number of holes in bottle traps enhanced the capture of *Drosophila suzukii*. Also, Leiva *et al.* (2019) found that traps with five openings captured the highest numbers of coffee berry borer (CBB) in Peru. Increase in numbers of *X. compactus* with increase in the number of windows on traps could in part be due to the fact that more openings increase the chances and probability of the beetles accessing and entering the traps (Ahmed *et al.*, 2023). In addition, having more windows allows the attractant (ethanol) to disperse in multiple directions, thereby improving beetle interception (Akist *et al.*, 2024). Furthermore, the number of *X. compactus* captured increased significantly ($P = 0.0008$) with increasing window sizes of the ethanol-baited traps. This could in part be attributed to not only the diffusion of attractants (ethanol) over a larger area but also, large windows reduce obstacles that could otherwise have hindered the beetles from accessing and entering the traps (Petrice *et al.*, 2004; Sweeney *et al.*, 2006). Additionally, the ConVerT trap was more effective than similar guardian

traps, attributing it to the larger openings that facilitated more air and light, making it easier for animals to locate the trap (Proulx, 1997). Whitfield *et al.* (2019) and Reardon *et al.* (2006) also reported that larger cone metal traps significantly outperformed smaller cone traps in moth captures, underscoring the role of trap diameter in capturing these insects. All in whole, the results obtained in this study highlight the importance of improving trap design and this should be tailored to the target pest (Kelsey *et al.*, 2024).

5.2 Optimal ethanol bait concentration for attracting borer BCTB.

Ethanol has been reported to aid several species of ambrosia beetles in locating suitable hosts to colonize (Ranger *et al.*, 2015, 2018). Researchers have therefore exploited this fact to develop ethanol-baited traps for controlling, monitoring, sampling, and studying ambrosia beetles (Cavaletto *et al.*, 2023). However, the efficiency of ethanol for attracting these beetles varies according to the concentration of the ethanol lures, and this can significantly impact beetle responses and therefore trap effectiveness (Rassati *et al.*, 2020; Cavaletto *et al.*, 2023; Govindaraju *et al.*, 2024). Identifying the most attractive ethanol concentration could therefore increase the efficacy of monitoring, surveillance, and management programs for ambrosia beetles (Addesso *et al.*, 2019). Studies were therefore conducted in the laboratory using the Y-tube technology to determine the best ethanol concentration for attracting adult *X. compactus* beetles.

Results showed that the percentage of the adult *X. compactus* beetles attracted to the ethanol samples varied significantly ($P < .0001$) across the ethanol concentrations. This finding supports earlier studies that showed that the response

of red flour beetle, *Tribolium castaneum* (Stevenson *et al.*, 2017; Dooley, 2020), and *T. confusum* (Wenda-Piesik *et al.*, 2017) towards several volatile organic compounds (VOCs) differed significantly across the range of concentrations tested in a Y-tube experiment. Similarly, Webster *et al.* (2010) and Roberts *et al.* (2019) also reported that the concentrations of individual VOCs influenced the behavioural responses of the adult vine weevil and black bean aphid (*Aphis fabae*), respectively, in the Y-tube.

Results showed that the percentage of adult *X. compactus* attracted to ethanol varied significantly ($p < .0001$) across concentrations, confirming that beetle responses are concentration-dependent. Attraction increased with increasing ethanol concentration, peaked at 70%, and declined thereafter, indicating a nonlinear relationship. Such concentration-specific responses are common in different insect olfactory behavior. For example, studies on *Tribolium castaneum* and *T. confusum* demonstrated that attraction to volatile organic compounds (VOCs) varied across concentration ranges in Y-tube assays (Stevenson *et al.*, 2017; Wenda-Piesik *et al.*, 2017; Dooley, 2020). Similarly, Webster *et al.* (2010) and Roberts *et al.* (2019) reported that the behavioral responses of vine weevil and black bean aphid were strongly influenced by VOC concentrations. Nonlinear responses have also been documented in other species. Kim *et al.* (2023) showed that *Drosophila melanogaster* exhibited stronger preferences for higher concentrations of ethanol and acetic acid, while *T. castaneum* larvae responded most strongly to intermediate doses of their aggregation pheromone, dimethyldecanal (DMD). These findings, together with results from this study,

suggest that *X. compactus* can discriminate among ethanol concentrations and preferentially responds to an optimal range, consistent with earlier studies on ambrosia beetles (Ranger *et al.*, 2011, 2015; Cavaletto *et al.*, 2023). The decline in attraction beyond 70% ethanol may be explained by semiochemical saturation, which can disrupt insect direction or even act as a repellent (Huetteroth & Waddell, 2011; El-Ghany, 2019). These results indicate that ethanol lures optimized around 70% concentration are likely to maximize captures of *X. compactus*, thereby improving trap efficiency for monitoring and management programs.

5.3 Effect of trap spacing and ethanol concentration on trap catches BCTB.

The study revealed a significant interaction ($p < .0001$) between ethanol concentration and trap spacing in influencing captures of adult *X. compactus* (Table 4). The highest mean catch occurred in traps baited with 70% ethanol and spaced 30 m apart (18 beetles), although this was not significantly different from traps baited with 50% ethanol at 20 m spacing (15 beetles) (Table 5). This interaction highlights that both lure strength and spatial arrangement determine trap effectiveness. Similar effects of trap spacing on dose–trap–catch relationships have been reported in other insect systems (Wall & Perry, 2011; Liu *et al.*, 2015). These results suggest that ethanol concentration and spacing act synergistically, consistent with findings by McMahon *et al.* (2010) and Heber *et al.* (2023). In practice, traps baited with high ethanol concentrations performed better when widely spaced,

whereas traps with lower ethanol concentrations were more effective at closer spacing (Govindaraju *et al.*, 2024). This pattern can be explained by odor plume dynamics: the strength of odor cues declines with distance from the source (Schlyter, 1992; Byers, 2008; Schumann *et al.*, 2021). Thus, beetles encountering a plume farther from the source have a reduced likelihood of locating the trap compared to those encountering a plume closer to its origin (McClendon *et al.*, 1976; Branco *et al.*, 2006). Furthermore, insects rely on the continuity of odor plumes for navigation (Murlis *et al.*, 1992), and inconsistent or weak signals may disrupt positioning (Jactel *et al.*, 2019).

Generally, these findings demonstrate that ethanol concentration and inter-trap spacing jointly influence *X. compactus* captures. Optimizing trap density based on lure strength is therefore essential for developing species-specific monitoring protocols (Ranger *et al.*, 2021; Miller & Crowe, 2017; Dissanayaka *et al.*, 2020). Strategically deploying stronger lures at wider intervals and weaker lures at closer distances could improve surveillance efficiency and provide more reliable population estimates for pest management.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Results revealed that the number of adult *X. compactus* beetles captured in the NARO-BCTB ethanol-baited trap was significantly influenced by the trap design and the number and size of windows on the traps. The highest number of beetles was captured in the flapless trap having two windows of size 6 x 4 cm, thus identifying this as best the trap design for trapping adult BCTB.

Furthermore, the number of adult *X. compactus* attracted to the ethanol samples in the Y-tube laboratory bioassays significantly increased with the ethanol concentration, reaching a peak at 70% ethanol concentration, and then decreased at the highest concentration of 96%. This implies that an ethanol concentration of 70% is the best among the options tested in this study, for attracting adult BCTB beetles.

Similarly, the mean number of adult beetles captured in the ethanol-baited traps under field conditions in Robusta coffee-banana cropping system significantly increased with increasing ethanol concentration, reaching a peak at 70% and then declining at the highest concentration of 96%, as was observed in the laboratory. On the other hand, trap catches varied significantly across the distances between the traps with the highest number captured in traps placed 30m from apart and baited with 70% ethanol.

6.2 Recommendations

- Farmers should use flapless traps, having two windows of dimensions 4 × 6 cm each, baited with 50-70% ethanol lures and spaced at 20-30 m apart for maximizing trap captures of *X. compactus*.

- Further studies should be conducted on:
 - ✓ Combining ethanol with other known attractants of *X. compactus* to assess potential synergetic effects in the attraction efficacy.
 - ✓ The same study to be done in other coffee systems like coffee monocrop, coffee-banana- shade to see whether same results can be obtained.
 - ✓ Assess the relationship between trap catches and beetle infestation levels.
 - ✓ The same study can be repeated using local distillates that farmers use.

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