REARING METHOD AND LIFE HISTORY OF LABORATORY BRED AFRICAN COFFEE WHITE STEM BORER, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae)

BY

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Dedication

This thesis is dedicated to the cherished memories of my late mother, Jane

Nyaguthii Gichuhi. Mum, though it has been more than a decade since you left us,

the virtues of honesty, hard work and love you instilled in me continue to inspire

and guide me. I also dedicate it to the rest of my family members: Joseph

Mbacha, Ann Nyambura, Ivy Nyaguthii, Joseph Maina, Grace Njeri, Purity

Wanjira, Ann Wambui and Samuel Mbacha.

Acknowledgement

I thank the almighty God for this far he has taken me. It has been a long journey of faith and hope; rising from a casual labourer picking coffee berries to a prospective coffee entomologist, it is, indeed, a dream come true for me./

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List of abbreviations, acronyms and symbols

a.s.l   above sea level

C.V   coefficient of variation

CABI   Centre for Agricultural Bioscience International

CWSB   coffee white stem borer

Df   degrees of freedom

FAO   Food and Agricultural Organization of United Nations

GRR   Gross reproductive rate

icipe   International center of insect physiology and ecology

ICO   International coffee organization

KALRO   Kenya Agriculture and Livestock Research Organization

L: D   Light to darkness

Lx   Proportion of surviving individuals

Mx   Age specific fertility

r   Intrinsic rate of increase

RH   Relative humidity

R_o Net reproductive rate

S.E   standard error

T°C   Temperature in degree Celsius

TaCRI   Tanzania coffee research institute

Tc   Mean generation time

Td   Population doubling time

x   Age of individuals at each stage

λ   Finite rate of increase

ABSTRACT

African coffee white stem borer, *Monochamus leuconotus* (Pascoe), Cerambycidae, is a major insect pest of coffee that causes yield losses as high as 25%. However, studying biology of Cerambycids is difficult due to the nature of their life cycle, which is mostly spent in the wood. This study aimed at developing and describing a rearing method of *M. leuconotus* using original artificial diet and its life history in the laboratory. The bioecology information generated will be useful in formulating effective management strategies. To conduct a life table analysis of *M. leuconotus*, the development and survival of 36 larvae reared on the artificial diet at 25±1°C, 85±5% RH, L:D 12: 12 and the fecundity of 25 adult females kept in pairs in cages with coffee sticks at 27°C and 42% RH were monitored daily. Using this rearing method, the duration of life cycle was reduced to 11 months from 18-24 months reported under field conditions. On average, eggs were 4.28 mm± 0.17 long with a diameter of 1.59± 0.07 mm and took 26±0.87 days to hatch. Seventh instar larvae measured 37.64 ± 1.40 mm long and weighed 0.77 ± 0.06g. Larval stage persisted for 209±12.42 days (7 months). Pupae had a mean length of 23.0 ± 0.43 mm, weighed 0.50 ± 0.02 g and took 23±0.27 days to emerge as adults. The adults on average measured 22.27± 0.27 mm long, weighed 0.49 ± 0.01g and survived for 82± 5.05 days. Survival rate was high at 78, 100 and 92% for larval, pupal and adult stages, respectively. The pre-oviposition period was 35 ±1.69 days on average and the female mean fecundity was 51.84 ± 5.92 eggs.

Life table analysis indicated the net reproductive rate (Ro) of 2.60, the intrinsic rate of increase (r) of 0.00315 with population doubling time (Td) of 220.05 day

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Coffee sector forms an important pillar of global economy (FAO, 2002). Coffee is the second most important legally traded commodity in the world after oil with value added coffee industry generating about US$ 173.4 billion worldwide in the year 2012 (ICO, 2014). Globally, Kenya is reputed in export of premium Arabica coffee. In year 2011, coffee earnings were in excess of US$ 277.7 million (Coffee Board of Kenya, 2012). In Tanzania, coffee is the largest export crop as it provides about US$115 million to the country's earning (Bafes, 2003) which contributes to about 20% of the foreign exchange earnings and has been the mainstay of the country's agro-economy since its introduction as a cash crop around 100 years ago (Tanzania Coffee Research Institute, 2005).

Coffee production is constrained by several insect pests. The African coffee White Stem Borer (CWSB) is one of the most important insect pests of coffee which is indigenous to Africa. According to Schoeman (1995), it was first recorded in South Africa in the late 1860's. It is reported to have drifted from local hosts to coffee plant with advent of coffee plantation especially in Kenya and Tanzania. It then dispersed over time from low altitude to higher altitude in

the middle of the two world wars with extension of coffee cultivation (Tapley, 1960). This pest infestation in Tanzania became more intense around 1950's due to collapse of control measures of the pests during the Second World War (Tapley, 1960). Around 1980s and 1990s, Malawi, Zimbabwe and South Africa experienced resurgence of the CWSB following the stoppage of usage of Dieldrin (Hillocks, 2000; Schoeman, 1995). Although CWSB is a major coffee insect pest, information on its biology is scarce, scattered and very old. Studies on rearing especially *Monochamus* spp are relatively few owing to the fact that the insects feed largely on inner tissues of the wood (Singh and Moore, 1985). Gathering of information from diverse sources to patch up so as to explain a phenomenon in some instances give room to deductive conclusion that Harcourt (1969) regarded as good as mere assumptions.

Life cycle of CWSB in the field takes one and half to two years (Morstatt, 1912 cited in Tapley, 1960). Larval stage lasts for 20 months. Ring barking stage of the larvae takes 3-4 months and the rest of the larval stage occurs inside the wood (Tapley, 1960). Thus the better part of CWSB life is spent inside the wood complicating the control measures. To be able to generate sufficient information on the biology and ecology of CWSB, a rearing method is necessary. Records or attempts to artificially rear *M. leuconotus* in particular seem unavailable and it can therefore be safely assumed that it has not been done or recorded. Kutywayo (2014) points out that one of the challenge in bioassays studies on this pest is getting enough number of the beetles due to lack of rearing method.

Studying life history of an insect provides an opportunity to decipher comprehensive information useful in projection of population growth and differentiation of life stages. Therefore, this study focused on developing a rearing method and carrying out life history study of CWSB in the laboratory which through analytical approach can provide updated, substantial, relevant and coherent information.

1.2 Problem statement and justification of the study

Monochamus leuconotus is a serious problem especially to smallholder coffee farmers in Africa contributing to cumulative crop losses of more than 25% as reported in South Africa in 1997 (Schoeman, 1998). Incidences of over 80% have been reported in Northern Malawi (Odour and Simons, 2003). Control measures on CWSB have been hampered by inadequate and very old information on its biology and ecology.

CWSB spends most of its life inside the wood and this makes it difficult to study its biology under field conditions. As such, a rearing method is necessary as it will make it easy to study its biology and reduce costs associated with field studies. Successful development and use of an artificial diet would minimize use of host plant material used to feed the CWSB colony.

The findings will fill some of the knowledge gaps on this pest by generating more information on its bioecology which will give insight on how to effectively manage it.

1.3 Objectives

1.3.1 General objective

The overall objective of this study was to document life history of *Monochamus leuconotus* in the laboratory, using an original rearing method with an artificial diet.

1.3.2 Specific objectives

The specific objectives were:

i.   To develop a rearing method for *Monochamus leuconotus,*

ii.   To elucidate the life cycle of *Monochamus leuconotus* under laboratory conditions,

iii. To determine life table parameters of *Monochamus leuconotus* under laboratory conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1. Coffee plant description

Coffee plant is a bushy, perennial, woody, evergreen, dicotyledonous tree. It is in the family Rubiaceae and is grown for its berries that make the coffee beverage. The main stem grows orthotropically and has plagiotropic primary, secondary and tertiary branches. The epidermal tissues of the stem (bark) are a bit soft as compared to the inner, hard cork and cambium tissues. The leaves are elliptical, shiny, dark green and waxy. The main cultivated species of coffee are *Coffea arabica* L. and *Coffea canephora* Pierre ex Frohener. African coffee White Stem Borer prefers *Coffea arabica* as compared to *Coffea canephora* as the latter has scales on the bark of the trunk where the female CWSB hides their eggs. In East Africa, *Coffea canephora* is mainly grown in Uganda while *Coffea arabica* is commonly grown in Kenya and Tanzania (Coffee Research Institute, 2012).

2.2. Coffee production in Africa

Coffee production is the economic mainstay of most of African countries (ICO, 2003). *Coffea arabica* contributes 70% of coffee production globally (Coffee Research Institute, 2012). Coffee is a known major foreign exchange earner in about 80 tropical and sub-tropical countries in Africa, Asia and Latin America (Osorio, 2002). African countries contribute about 13% of global coffee

production (Wegner, 2012) with economies of developing countries depending on coffee to a large extent. In Africa, it is a major export crop with 33 million people growing it mainly on their subsistence farms (Kotecha, 2002). Production of coffee in the year 2012/2013 was estimated at 16.7 million bags in Africa (ICO, 2014). In East Africa, about 450,000 metric tons of Arabica coffee is produced annually which is worthy more than US$ one (1) billion (Technoserve, 2013). In the period between 1990/91 and 2012/13, the average production of coffee in Kenya was one million bags while in Tanzania it was 792,000 bags during the same period (ICO, 2014). Small holding forms the largest share of Kenya's coffee production. Coffee in Kenya is grown in about 170,000 hectares with 600,000 small scale farmers owning less than two hectares. In Tanzania, it was estimated that more than 450,000 smallholders (95%) and 110 estates (5%) directly depends on coffee with about 2,000,000 additional people being employed directly or indirectly in the industry (Tanzania Coffee Research Institute, 2008).

2.3 Key insect pests of coffee in Africa

Coffee production is affected by abiotic factors such as weather factors and biotic factors like insect pests which increase cost of production besides lowering quality and quantity of coffee yield. Most major coffee pests are endemic to Africa as coffee has an African origin. The insect pests that impact on economic benefit of coffee are less than twenty (Odour and Simons, 2003). Coffee production in East Africa is heavily hampered by a number of major insect pests, namely, Coffee berry borer (*Hypothenemus hampei* Ferrari), African coffee white

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stem borer (*Monochamus leuconotus*), Antestia bugs (*Antestiopsis* spp.), Leaf miners (*Leucoptera* spp.) and Scale insects (*Coccus* spp.) (Odour and Simons, 2003; Mugo *et al.,* 2011).

Globally, economic losses attributed to Coffee berry borer have been estimated to be over USD 500 million annually (Jaramillo *et al.,* 2011). In Africa it has been reported to have caused losses as high as 96% (Waterhouse and Norris, 1989). Its attacks result in pre-mature fall of the berries or they remain on the trees but the yield quantity and quality is adversely affected.

Antestia bugs (*Antestiopsis* spp.) are serious pests, whose adults and nymphs attack flower buds, young shoots and berries. As the bug feeds on berries it transmits spores of the fungus *Nematospora* leading to rotting of beans. It is reported that owing to this transmission of fungus their economic threshold is low: two bugs per tree (Hill, 1983). In general it lowers both the quantity and quality of coffee yield (Department of Agriculture, Forestry and Fisheries report, South Africa, 2012).

Leaf miner is a coffee pest which according to Abasa (1975) becomes a serious problem when insecticides are heavily applied. Leaf miner larvae mine the leaves resulting in dark brown patches on the upper surface of the leaf. In case of severe attack, leaves are shed which affect growth of the plant by limiting surface area of photosynthesis.

Scale insects are common pests that attack coffee. At low altitude, below 1300 m *Coccus viridis* are common while *Coccus alpinus* are mostly found at altitude above 1300 m. *Coccus celatus* and *Coccus viridulus* have also been reported in Africa (Odour and Simons, 1999). They cause the damage through sucking the sap or facilitating growth of sooty mould on honeydew they deposit on the upper surface of the leaf. The plant becomes weak since it is deprived of essential nutrients while in some cases some deformities are reported due to the toxic saliva they emit. Economic losses of up to US$ 5 billion annually have been reported worldwide (Rutherford and Phiri, 2006).

African coffee white stem borer is major coffee pest in Africa. It causes crop losses mainly through ring barking of coffee stem. It is found in many countries in Africa with varying levels of infestation and damage. For instance, in Kenya, economic viability of coffee farming continues to be threatened by *M. leuconotus* according to a report by Coffee Research Foundation (1989).

2.4 African coffee white stem borer, *Monochamus leuconotus* Pascoe

The African coffee white stem borer (CWSB), *Monochamus leuconotus*, originated from Africa and it is widespread in East Africa inhabiting almost all the areas where Arabica coffee is grown (Tapley, 1960). This pest was first described by Pascoe in 1869 as reported by Tapley (1960). But it was Gooch who, in 1874, attempted to describe its habitats and lifecycle. In 1957, Duffy reviewed the biology of this pest (Tapley, 1960).

2.4.1 Biology of Monochamus leuconotus

Life cycle of Monochamus leuconotus on coffee plant

The life cycle of *M. leuconotus* in coffee tree takes 18 to 24 months (Morstatt, 1912 cited in Tapley, 1960). The egg is 4-5 mm long, 1.5-2.0 mm in diameter, light cream in colour with tapering ends (Tapley, 1960; Schoeman *et al.,* 1998). It takes about 21 days for egg hatching to occur (Schoeman *et al*., 1998). After hatching, feeding is localized around egg niche but it is later extended to other areas under the bark. Fully grown larvae are 3-5 cm long, 10 mm wide at head, 5 mm at posterior end and white or cream in colour. Larval mortality in the field was noted to be as high as 75% (Tapley, 1960). Early instar larvae tend to be positively geotactic during ring barking stage while late instar larvae at wood boring stage tend to be negatively geotactic (Schoeman *et al*., 1998).

During ring barking phase, the larvae tend to feed downward if the eggs were laid close to the ground. At this stage it is not easy to detect the presence of the larva, perhaps the only pointer could be the frass. The damage becomes clearly visible at wood boring stage as a lot of frass is exuded. Tapley (1960) established that there are seven larval instars based on measurement of width of head in application of Dyar's law. Larval stage of the stem borers takes about 20 months and is the most destructive due to ring barking which destroys phloem while its frass interrupts translocation of metabolites. In the first month the larva bores into the wood about one inch but in the preceding three weeks it digs deeper by about three more

inches. Later, it bores a quarter of an inch a day (Knight, 1939). This wood boring phase is estimated to start when the larva is around three to four months old (Tapley, 1960). Towards the end of wood boring phase, the larva makes a pupal chamber adjacent to stem surface at the top of the tunnel (Schoeman *et al.*, 1998). The larva then get into a dormant phase prior to pupation referred to as pre-pupal stage.

Pre-pupal stage is a transition stage to pupal stage which is marked by development of external appendages though they remain inactive. At this stage, no feeding occurs and is thought that physiological changes take place. It appears condensed with thick cover of subcutaneous fat that conceal visceral organs which are ordinarily visible at larval stage due to the thin integument (Schoeman *et al.*, 1998). Both Tapley (1960) and Schoeman *et al.* (1998) estimated the pupal stage to be between 21 days to 32 days depending on temperature. Schoeman *et al.* (1998) noted that the female pupa is larger (about 31 mm) than male (about 28 mm) and the last somite of female abdomen bears two genital lobes. The average weight of the female pupa is higher than male pupa (Tapley, 1960). After emerging, the adult remains in the pupal chamber for about five weeks to allow sclerotization and melanization (Tapley, 1960). On maturity, the adult makes a small circular exit hole about 1 cm in diameter mostly within one to two weeks after commencement of rain. The adult beetle can be as long as 30 mm, white-grey in colour with black head, thorax and black patches around wing apex close to both hind legs. Like other cerambycids, it has conspicuously long antennae,

twice the length of the body in male. Adult males emerge slightly earlier than female adults (Tapley, 1960). Female crawl to the crown of the coffee tree to feed on leaf petiole, green berries exocarp and green bark of shoots before flying off to mate with male.

Reproduction and longevity of *Monochamus leuconotus*

The adults mate frequently until the demise of the male. After mating the female moves down to the stem to locate oviposition site (Kutywayo *et al*., 2013). Lamiines Cerambycids nip into the bark or stem to make an egg niche which after oviposition is covered by a material that hardens (Hanks, 1999). Depending on temperature the female lays between 10 and 40 eggs, but in East Africa it is normally between 20 and 25 eggs (Moffat and Allan, 1934; Knight, 1939). Adult male Cerambycids are reported to be more active than female adults (Hanks, 1999). Eggs are laid singly mostly in darkness (Knight, 1939). On average males lifespan (112 days) is shorter than females (122 days) while the male inclined sex ratio is 1:4 (Schoeman *et al*., 1998). The early flight period of males is more prolific according to Knight (1939) and Tapley (1960).

2.4.2 Damage caused by *Monochamus leuconotus* to the coffee plant

Ring barking, yellowing of leaves, presence of exit holes and frass are the major signs of the stem borer infestation on the plant. Affected plants exhibit wilting, stunted growth, dieback and reduced yields. Tapley (1960) reported that below 1,220 meters a.s.l, infestation mostly occurs close to the base of the plant, while in

higher elevation damage is usually observed on the upper parts of the plant. Most Cerambycids prefer to attack growing plants particularly the weak ones which could be due to growing in poor soil conditions, flooded area, moderate fire damage or weakened by insect pest attack. Trees growing in urban habitat stand a higher chance of Cerambycid attack as compared to those growing in natural habitat (Hanks, 1999). Presence and level of infestation also depend on age of coffee plant, mulching and shade. For instance coffee trees which are less than 2 years old are severely affected while the older trees show some level of tolerance. Areas with high precipitation are likely to have higher infestation as rainfall determines emergence of the adult and onset of breeding, thereby determining their distribution (Kutywayo et al., 2013).

2.4.3 Economic impact of *Monochamus leuconotus*

The economic damage caused by CWSB, *M. leuconotus*, is great as its infestation eventually leads to total loss of the crop. In S. Africa, over 25% crop losses due to CWSB was reported over three years (Schoeman *et al*., 1998). Incidences of CWSB in smallholder farms were reported to be 90% in Malawi and 70% in Zimbabwe (Centre for Agriculture and Biosciences International, 2008). One stem borer in one 15-year old coffee tree was estimated to cause crop losses of 8% in Tanzania (Tapley, 1960).

2.4.4 Control of *Monochamus leuconotus*

African coffee white stem borer, like other borers, is difficult to control as most of their life time is spent inside the wood. Control measures, such as chemical, biological and cultural have been used though their efficacy is debatable.

Insecticides such as Aldrin® and Dieldrin® have been used in the past with high degree of success but they were banned due to their non-selectivity and environmental concern. Chlorpyrifos® has been used in Zimbabwe. In field trials in Malawi Fipronil®, Imidacloprid® and a solution of 10% lime were found to be effective (Rutherford and Phiri, 2006).

Cultural control measures such as proper nutrition help to reduce chances of infestation and its severity as healthy and strong trees are less susceptible to attack. Traditionally, farmers smooth the bark of the stem by using rough object like maize cob within 0.5m from the ground to make it unsuitable for female oviposition (Rutherford and Phiri, 2006).Other practices carried out to control the pest include driving a wire spoke right into the larval gallery to kill it and covering the stem with banana leaves to obstruct the female from ovipositing on it (Rutherford and Phiri, 2006).

Information on biological control of stem borers is scanty. Tapley (1960) reported predation of stem borers by woodpeckers. Woodpeckers and ants predate on larvae in the galleries. *Beauveria bassiana* and *Metarhizium anisopliae* are also used as bio-control agents as they act on contact and they are host specific

(Karanja *et al*., 2012). Braconid wasps of *Aprostocetus* species have been reported to parasitize the eggs of the stem borer in Tanzania and South Africa. This parasitoid can be effective since up to 12 individuals can develop within one egg but unfortunately they are rare hence causing little impact. Tapley (1960) reported larval mortality of 45% due to parasites and predators. Most of these parasitoids have not been identified up to species level due to difficulties in rearing with exception of *Hybogaster varipalpis* (Cameron), a braconid recorded in Kenya and Zimbabwe, which was reported to have caused larval mortality of 48% in a study in Kenya (Knight, 1939) and *Afrocoelichneumon didymatus* (Morley), an ichneumonid from Tanzania.

2.5 Use of life table

Efficient control measures are definitely required to curb the problem of pest attack. To make this a reality, more information on *Monochamus leuconotus* is necessary, more so its bioecology. Mortality factor analysis is a key element in integrated pest management. Knowledge on when and why the highest percentage of mortality occurs is vital in timing the pest control measures in a way that will be environment friendly and minimize effects on non-target organisms (Kakde *et al*., 2014). A life table provides an opportunity to compare the population growth potential of an insect under different environmental conditions. A life table is indeed regarded as a form of book keeping system used by scientists to monitor various parameters in the development of an insect. Its significance was first recognized by Leopold who used it to study natural population of insects

(Harcourt, 1969). It is a useful tool in providing information on population dynamics of insect throughout its lifecycle including and not limited to survival rate, life expectancy and mortality.

Studies on life table can be used together with studies on consumption rate of a pest to estimate the damage of an insect population on a plant (Harcourt, 1969). Similarly, studies on predation rate of predatory insect can be used with studies on its life table to estimate its potential as biological control agent (Harcourt, 1969).

2.6 Natural diet of wild *Monochamus leuconotus*

During the early larval stage (ring-barking stage), larvae mostly feeds on phloem tissue while during late larval stages they mostly feed on xylem tissue. Adults feed on leaf petiole, green berries exocarp and green bark of shoots.

2.7 Insect rearing with artificial diet

Applied and basic research mostly relies on artificially reared insects as they are largely uniform and of high quality (Aloo and Katagiri, 1994). Artificial rearing of insects in laboratory is necessary so as to derive its life table. It provides a scientist with ample time to study and scrutinize finer details of a particular organism. In India, the Asian coffee stem borer (*Xylotrechus quadripes*) is mostly reared on a natural diet but this has proved to be labour intensive and time consuming. Consequently, there are efforts geared towards developing effective method of rearing of this species (Murphy *et al*., 2008).

It is a contemporary practice globally to maintain laboratory colonies of insects with artificial diet (Alfazairy *et al*., 2012; Assemi *et al*., 2012). Generally, the quality of the bark in terms of the nutrients is low compared to subcortical tissues which have a higher concentration of nutrients (Hanks, 1999). To boost the level of nutrients in the artificial diet, sucrose or glucose, and vitamin E are necessary, in addition to host plant. Gardiner (1970) reported that inclusion of plant tissue in the diet increases chances of acceptance by the insect and act as phago-stimulant, while Aloo and Katagiri (1994) reported that use of host plant tissue in the diet have a significant impact on growth and development of larvae. Temperature is a major factor that impacts on growth and development of the insects. Warm temperature enhances completion of CWSB lifecycle (Kutywayo *et al.,* 2013). The major causes of mortality in artificial rearing of insects are climatic variables and microbial contaminations. Kutywayo *et al.* (2013) noted that, low temperatures affect egg development, survival of larvae and result in mortality of adults. *Aspergillus* spp*., Cladosporium* spp*., Fusarium* spp*., Penicillium* spp*.,* some yeast and bacteria have been pointed out as the major threats in insect rearing through artificial diet (Zha and Cohen, 2014). These microbes have an effect on feeding of the insect as their contamination changes the nutrient level and pose as health risk to insectary staff. *Aspergillus* and *Penicillium* produce a toxin known as ochratoxin making the diet harmful to insect (Zha and Cohen, 2014). An uncontaminated diet guarantees the health of insect and reduces the cost and labour involved in repeated diet preparation. Since most of the common

microbes that invade artificial diet are fungal, antifungal agents such as methyl-paraben, sorbic acid, formaldehyde, sodium benzoate, butyl paraben and potassium sorbate are used. In deciding on the particular one to use one must consider its effects on the reared insect besides considering its effectiveness to keep off fungal infection. Most insectaries prefer methyl paraben and sorbate compound (Zha and Cohen, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of *Monochamus leuconotus* from the field

Coffee stems infested with *M. leuconotus* were collected in 24 randomly sampled plots at six different elevations (1100 m to 1600 m a.s.l) along a transect delimited on the south-east slope of Mount Kilimanjaro running from Uparo (S 03.34986^{o} E 037.46235^{o}) to Nduoni (S 03.28266^{o} E 037.45391^{o}) in Moshi, Tanzania (Figure 1). Mean temperature is between 17°C and 27° while the annual rainfall ranges between 1000mm to 1300mm. The 24 plots were spread within agroforestry systems known as Chagga home gardens in Mt. Kilimanjaro. These home gardens are characterized by broadleaved, evergreen, closed to open canopy of 3-30 m tall trees like *Cordia abyssinica*, *Albizia schimperiane* and *Grevillea robusta*, shrubs mostly of *Coffea arabica* (0.3-5 m tall), bananas and herbaceous crops like maize, beans, vegetables and fodder crops (Hemp, 2004). Based on ring barking and presence of entry holes with frass, infested coffee stems were cut and transported to *icipe* for extraction of larvae. These larvae were used to start a laboratory colony of *M. leuconotus* at *icipe* in Nairobi.

3.2 Preparation of artificial diet for *Monochamus leuconotus*

An artificial diet for *M. leuconotus* was prepared based on nutritional requirement and acceptability of the diet by the stem borer with some ideas borrowed from formulations of maize stem borer's diet by Onyango and Ochieng-Odero (1994).
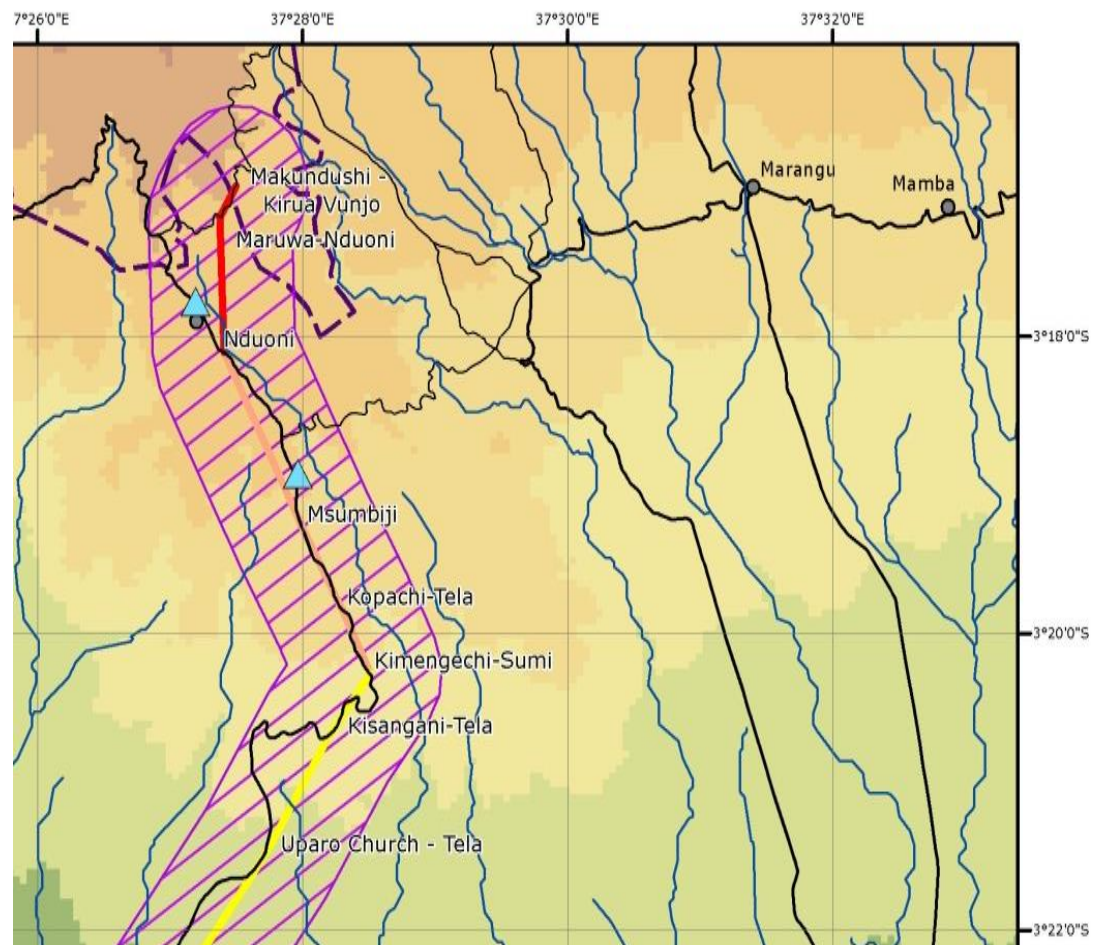
Figure 1. A map of a section of Mt. Kilimanjaro area in Tanzania with the purple shaded area showing the region where the original sample of *Monochamus leuconotus* was collected.

During formulation and preparation of the artificial diet, selected ingredients were clustered into three fractions. The first fraction comprised of coffee bark and leaves, sucrose, brewer's yeast, ascorbic acid, sorbic acid, methyl-paraben, vitamin E acetate and distilled water. Coffee bark and leaves were dried and ground into powder. The foresaid ingredients with exception of distilled water were thoroughly mixed by wooden spoon in aluminium pan. 403.3 mls of distilled water was boiled, cooled to 60° C then added to the mixture and blended for one minute. Methyl-paraben dissolved in 20 ml of absolute ethanol was put into the mixture and further blended for two minutes.

In preparation of the second fraction, appropriate amount of cold distilled water was measured depending on the intended amount of diet and put in a different pan. Agar powder weighing 12.6 grams was put into 403.3 mls of distilled water which was boiled and then cooled to 60° C. The mixture was stirred frequently to avoid sticking at the bottom of pan. This second fraction mixtures was added to first fraction mixture in the ratio of 1:1 and blended for three minutes.

In the third fraction, 2.0 g of 40% formaldehyde was added to the mixture of first and second fractions described above and further blended for three minutes. The diet was then doled out into respective containers and left to cool overnight at room temperature before being used. The amount of each ingredient required to make a litre of the diet is shown in the Table 1.

Table 1. Quantity of ingredients used to make a litre of the artificial diet for coffee stem borer *Monochamus leuconotus*

Fractions	Ingredients	Quantity
First fraction	Distilled water	403.3 ml
	Coffee barks and leaves	113.3 g
	Sucrose (table sugar)	35.3 g
	Brewer's yeast	22.6 g
	Ascorbic acid	2.5 g
	Vitamin E acetate	2.1 g
	Methyl-paraben	2.0 g
	Sorbic acid	1.3 g
Second fraction	Distilled water	403.3 ml
	Agar tech. no. three (3)	12.6 g
Third fraction	Formaldehyde 40%	2.0 g

3.3 Laboratory rearing and maintenance of *Monochamus leuconotus*

A colony of *M. leuconotus* was bred and reared in the coffee laboratory of *icipe*, Nairobi, Kenya. The infested stems collected from the field were carefully split using steel splitting wedges and sledge hammer to expose the larvae that were collected by fine forceps and camel hair brush.

A *M. leuconotus* colony was started with adults obtained from infested coffee stems collected in the field plots. These stems were kept in the laboratory and monitored daily until emergence of adult. After emergence, adults were

introduced into 12 Plexiglas cages (50 cm long, 40 cm breadth and 80 cm depth) with freshly cut coffee sticks (60 cm long) to provide food and oviposition substrate. These reproduction cages were kept at room temperature at 27 ± 0.06°C, 42 ± 0.72% RH and photoperiod L12: D12 (from 6 a.m. to 6 p.m.). Coffee sticks were slightly buried in a container with moist soil while the other end was covered with paraffin wax to avoid drying. A petri dish with a moist cotton wool was put at the base of the sticks to provide water to the adults and increase humidity. The stick was removed from the cage after one week and a fresh one introduced, until the death of the female. The sticks with the eggs that were removed from the cage were maintained in the same conditions and observed daily for egg hatching as indicated by the frass (Figure 2). After hatching, the neonate larvae were extracted from the sticks. Extraction was done by carefully peeling thin pieces of wood close to the frass outlet to expose the gallery using a sharp scalpel blade. The gallery was continuously opened until the larva was found. The larva was carefully removed by a camel brush and introduced into the artificial diet in plastic containers maintained in an incubator at a temperature of 25±1°C, a relative humidity of 85±5% and a photoperiod of L:D 12:12 (from 6 a.m. to 6 p.m.). These containers were 11.5 cm wide, 6.5 cm deep with a capacity of 0.5 litres and a lid containing a window of fine muslin (0.1 μm) for ventilation. Before using, these containers were soaked in sodium hypochlorite (jik) overnight to disinfect them and rinsed with 95% ethanol. In each container, about 3 cm thick diet weighing approximately 60 grams was

supplied. The diet was pricked with a sterilized spatula to ease entry of the larvae. Each larvae was put in the container singly (they cannibalize each other if two or more are put together) and placed in the incubator. The diet was replaced after every three weeks. The individuals that pupated were removed from the diet and put in a plastic container of the same size, lined with paper towel. After adult emergence, a small piece of moistened cotton wool and coffee twig were introduced in the container. Adults remained in the containers in the incubator until they began feeding and then they were taken to the reproduction cages and treated the same way as the adults of previous generation.

3.4 Observations on life history of *Monochamus leuconotus*

Observations were made on development and survival of immature stages and the reproduction behavior of adult females including fecundity and longevity.

3.4.1 Development and survival of immature stages

The immature stages were monitored by daily observation of each individual to check for survivorship, moulting, pupation and adult emergence. For life table analysis, a sample of 36 neonate larvae were monitored which were obtained from eggs hatched on the sticks, and reared on artificial diet in the incubator at $25\pm1°C$, $85\pm5\%$,RH and L:D 12:12 photoperiod (Figure 2). In order to get adequate information on the biology especially the life cycle of CWSB, the weight and the length of the larvae of the whole colony were recorded once a week, while those of the pupae and adults were taken once on emergence. The larvae were weighed

individually using electronic balance while the individual's body length was measured by Vernier caliper.

To determine the number of larval instars, growth curves were made from a sample of 120 larvae from the colony reared in the same conditions.

To further verify the number of larval instars, the width of head capsule of 92 individuals was measured. A photograph of the dorsal view of the larva's head capsule was captured with digital Leica camera attached to a binocular microscope. Epicranium's widest section was measured using "Measure" module of Qwin software programme (Leica imaging system, Wetzlar, Germany) (Figure 8).

3.4.2 Reproduction behavior, fecundity and longevity of adult *Monochamus leuconotus*

The adults obtained from pupae and kept on coffee sticks in the reproduction cages were observed daily and the number of eggs laid by each female were recorded. Survival and behaviour of the adults was also noted by daily visual observation. The number of eggs laid determined fecundity of each female. Pre-oviposition period was also recorded. The sex ratio obtained from 42 emerging adults was F: M 1: 0.88. Egg survival and duration of egg development were assessed from observation of 510 eggs, which were checked daily to detect hatching. A sample of twenty five females was monitored to get information on fecundity for life table analysis. The mean fecundity per day of these 25 females was plotted to portray the trend of egg laying in a week.
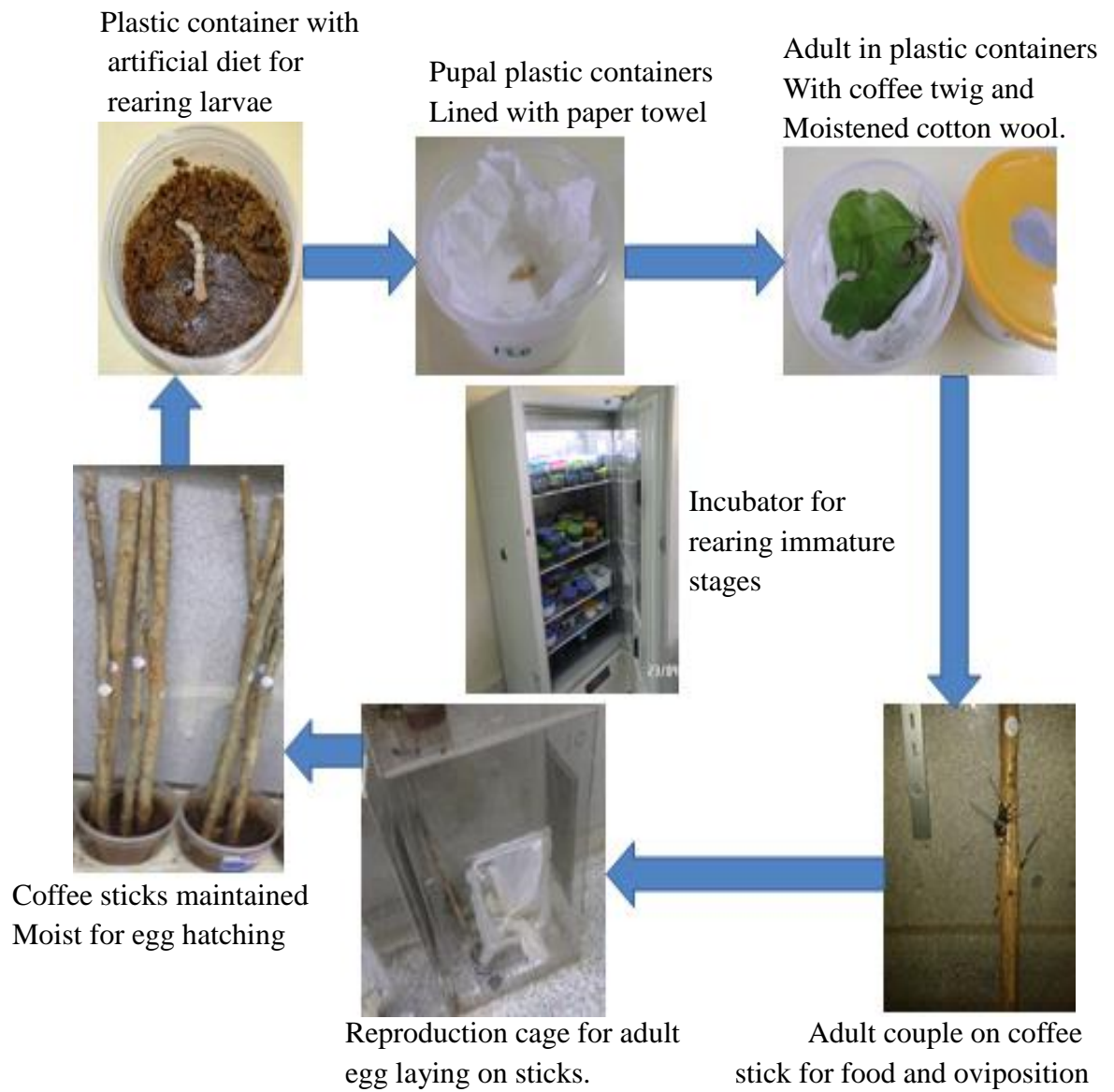
Figure 2. Stages in the laboratory rearing method for *Monochamus leuconotus*

Plastic container with
artificial diet for
rearing larvae

Pupal plastic containers
Lined with paper towel

Adult in plastic containers
With coffee twig and
Moistened cotton wool.

Incubator for
rearing immature
stages

Coffee sticks maintained
Moist for egg hatching

Reproduction cage for adult
egg laying on sticks.

Adult couple on coffee
stick for food and oviposition

3.5 Determination of life table parameters

The major components of a life table are fecundity and survivorship. The age of individuals at each stage was denoted by x while proportion of individuals surviving at age x by l_x. Age specific fertility was represented as m_x referring to the number of female progeny per female in each interval class. This was obtained by dividing the mean number of eggs at age x by sex ratio (1.88). The values of life table parameters were derived following the method of Birch (1948) adapted by Elkinton (1993). The gross reproductive rate (GRR) was calculated as Σm_x. Gross reproductive rate (GRR) refers to the mean number of female progenies born by a female throughout her oviposition lifespan. The net reproductive rate (R_o) was determined through $\Sigma l_x\, m_x$. Net reproductive rate refers to the net number of female offsprings produced per female or average net contribution of a female to the next generation. Mean generation time (T_c) is the average duration in days between the birth of individuals of a generation and that of next generation or between births of parents to that of their offsprings (Babin *et al*., 2008). It was expressed as $\Sigma(xl_x\, m_x)/R_o$. Intrinsic rate of increase (r) or innate capacity of increase was obtained through iteration of the Euler's equation $\Sigma(e^{-rx}l_xm_x) =1.0$, such that the final figure on the $e^{-rx}\, l_xm_x$ column was approximately 1. Finite rate of increase which is the factor at which population increases per unit of time was determined by $\lambda = e^r$. The population doubling time (T_d) which is the number of days required for population to double its size was estimated by ln (2)/r.

3.6 Data analysis

Data analysis was done using R statistical software version 2.15.1 (2012-06-22) and Microsoft Excel 2010. The body morphometrics, developmental duration, longevity, percentage survival, average fecundity and life table were all calculated using MS Excel. Correlation, t-test and graphical presentation of fecundity and survival rate was done through R software.

RESULTS

4.1 Life cycle of *Monochamus leuconotus* and a description of development

stages

The mean duration from egg stage to initial egg laying by the female adult emerging from those eggs was 293±5.32 days (9 months) while the whole life cycle took 340±4.66 days (11 months) on average (Figure 3).

Egg

26 ±0.87 days

**Larva
(7 instars)**

35±1.69 days

**209±12.42 days/
7 months**

Adult

Pupa

23±0.27 days

Figure 3. Life cycle of laboratory reared *M. leuconotus* with mean durations of life stages.
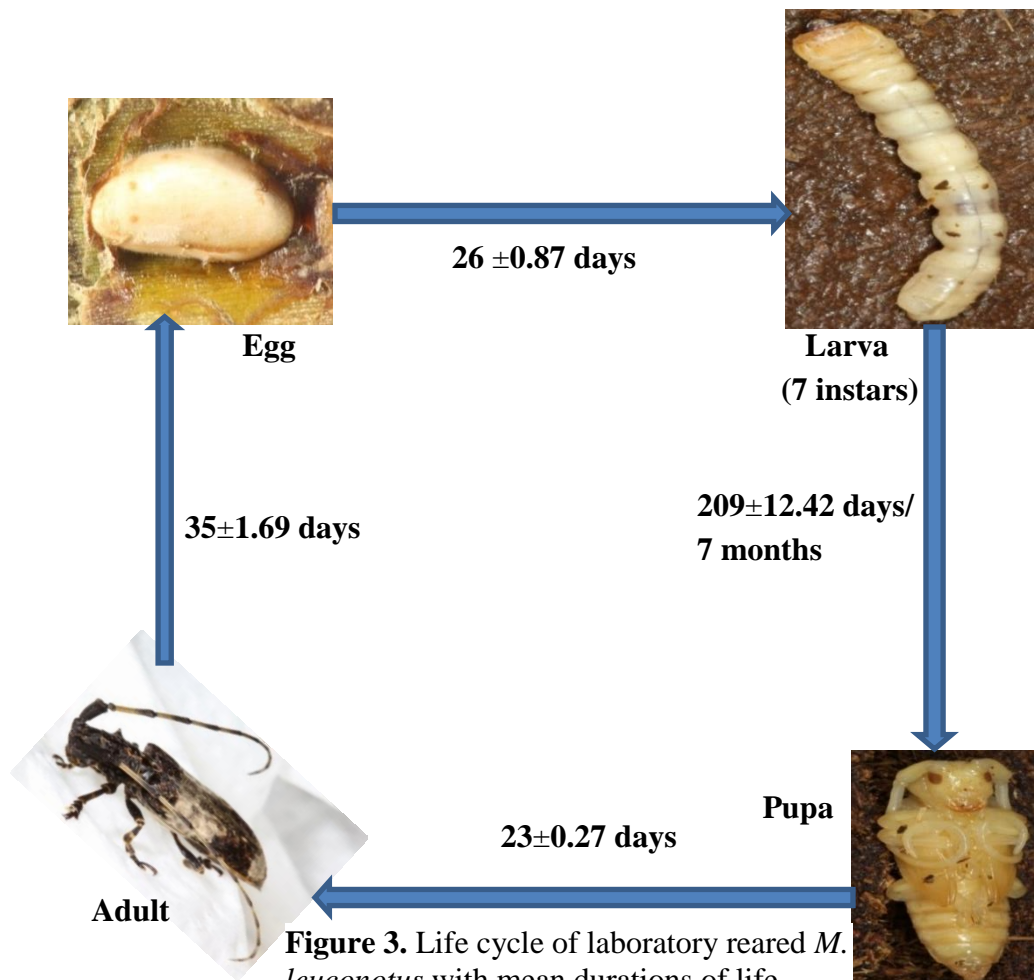
4.1.1 Egg stage

The egg is white in colour but enveloped in brown secretion that makes it appears brown (Figure 3). It is densely covered by minute white setae. It is cylindrical, fusiform in shape. The average length of an egg was 4.28 ± 0.17 mm while the diameter averaged 1.59 ± 0.07 mm. The eggs were incubated on sticks that were maintained moist and took an average of 26 ± 0.87 days to hatch. This was estimated by checking the presence of frass and is subject to negative deviation of 2-3 days. Egg mortality was high at 61% (Appendix 1).

4.1.2 Larva stage

The larvae are vermiform or apodous (Figure 4). Newly hatched neonate is creamy white to light brown with thin, delicate, depigmented, pellucid integument exposing tubular visceral organs. As it matures the colour gets whiter but prior to pupation it reverts to creamy white to brown. The body is well covered by light brown setae. The Entognathous, gouge-shaped mandibles are black, head capsule is reddish brown and is normally retracted in the prothorax unless it is provoked or moving. The body attenuates anteriorly and tapers gradually posteriorly (Figure 4). It secretes brown salivary fluid perhaps to soften the piece of wood to be ingested.

Changes in body weight and length revealed seven larval instars (Figures 5, 6 and 7).
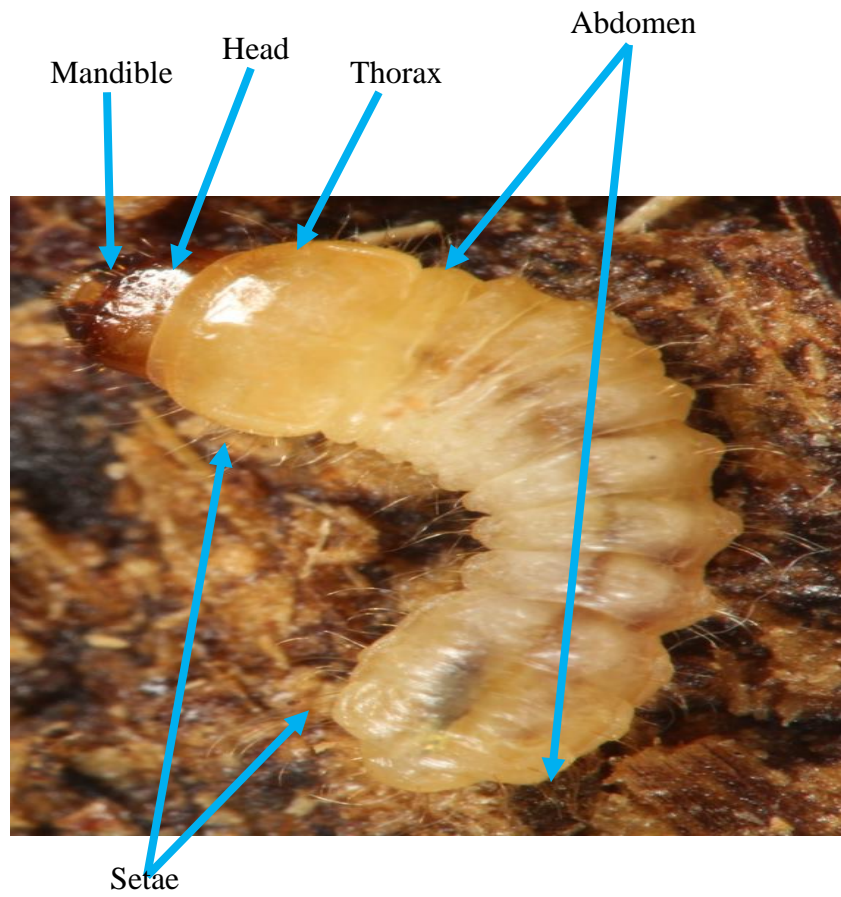
Figure 4. Major external parts of larval stage of laboratory bred *Monochamus leuconotus*

The prothorax is light brown on dorsal side. On hatching, the neonate larvae actively feed exuding frass.
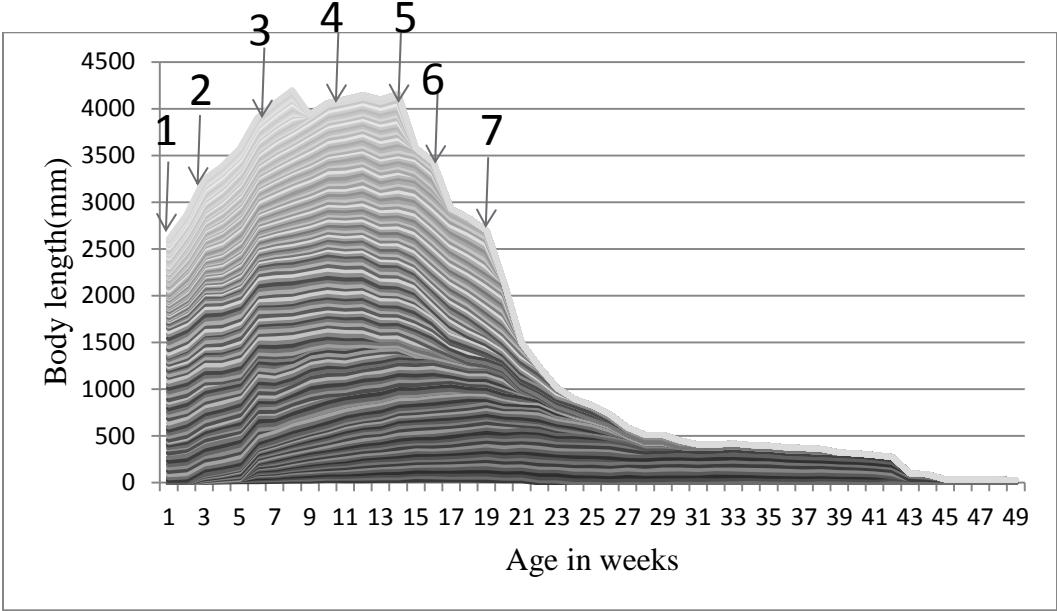
Figure 5. Body length of laboratory-bred *Monochamus leuconotus* larvae during development with arrows indicating points of ecdysis or larval instars (n=120).
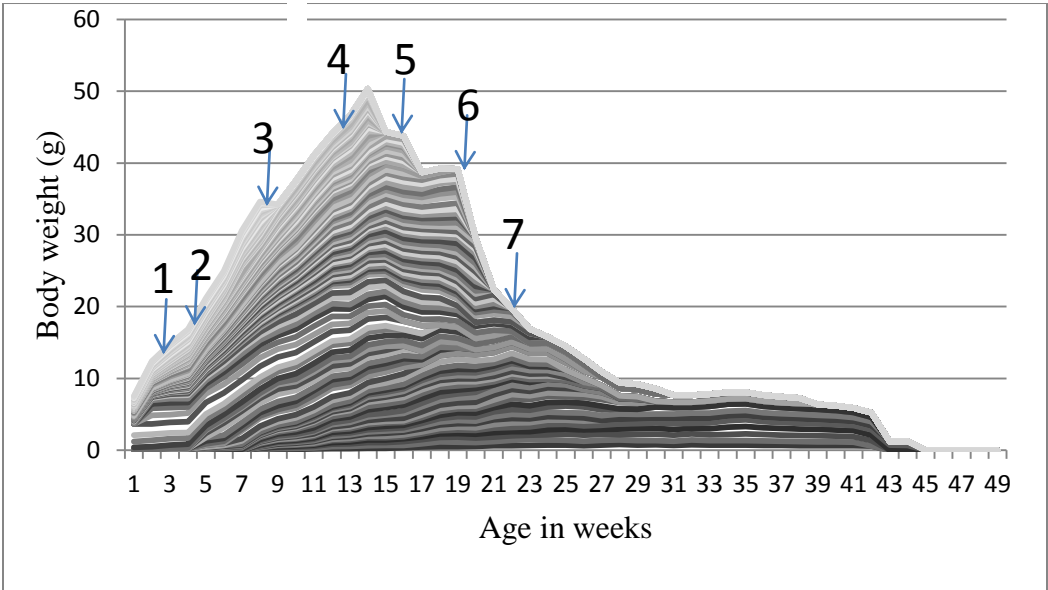
Figure 6. Body weight of laboratory-bred *Monochamus leuconotus* larvae during development with arrows indicating points of ecdysis or larval instars (n=120).
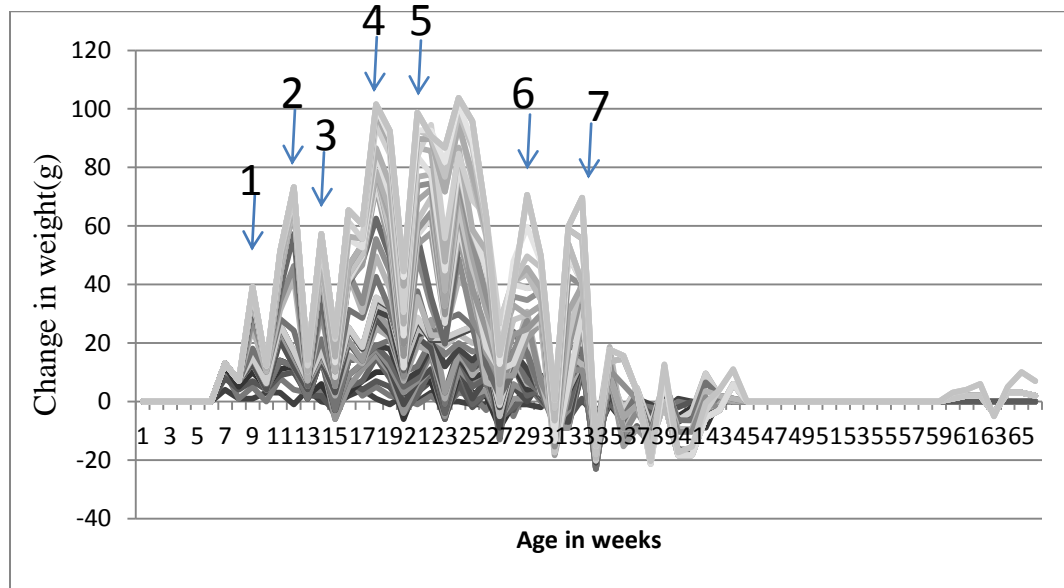
Figure 7. Change in body weight between two consecutive weeks of laboratory-bred *Monochamus leuconotus* larvae during development with arrows indicating points of ecdysis or larval instars (n=120).

A total of 92 larvae of different ages and sizes were sampled from the laboratory bred colony and the width of their head capsule was measured (Figure 8). Measurements further confirmed that there were seven larval instars (Table 2).

Figure 8. Measurement of head capsule width (widest part of epicranium) in laboratory bred *Monochamus leuconotus*.

The mean age indicated in the table 2 shows the average number of days of the sampled individuals as at the time of measurement and not the actual age of each instar. The body length increased progressively but there was a reduction in the seventh instar. The mean head capsule width of the individuals in 13 days (first instar) was 0.90 ± 0.02 mm, 38 days (second instar) 1.27 ± 0.03 mm, 57 days (third instar) 1.69 ± 0.03 mm, 81 days (fourth instar) 2.22 ± 0.05 mm, 130 days (fifth instar) 2.78 ± 0.02 mm, 145 days (sixth instar) 2.94 ± 0.02 mm and 3.09 ± 0.01 mm for 184 days (seventh instar) (Table 2). The rate of increase of the width of the head capsule from the first to second instar was 1.40; second to third instar, 1.33, third to fourth instars, 1.31; fourth to fifth instar, 1.25; fifth to sixth instar, 1.25 and sixth to seventh, 1.05.

Table 2. Mean head capsule width (mm), body length (mm), body weight (g) and mean age (days), in laboratory reared *Monochamus leuconotus* indicating different larval instars

Larvae numbers (n)	Size measurements (mean ± SEM)			Mean age (days)	Matched instar
	Head capsule width (mm)	Body length (mm)	Body weight (g)		
13	0.90 ± 0.02 [0.81-0.99]*	9.07 ± 0.45 [6-12]*	0.01 ± 0.00 [0.00-0.02]*	13	First
19	1.27 ± 0.03 [1.02-1.47]*	17.00 ± 0.49 [12-20]*	0.07 ± 0.01 [0.01-0.11]*	38	Second
24	1.69 ± 0.03 [1.50-2.07]*	20.79 ± 0.72 [14-26]*	0.14 ± 0.01 [0.06-0.23]*	57	Third
9	2.22 ± 0.05 [2.05-2.51]*	27.33 ± 0.93 [24-32]*	0.27 ± 0.02 [0.15-0.35]*	81	Fourth
7	2.78 ± 0.02 [2.67-2.86]*	35.57 ± 1.07 [31-38]*	0.59 ± 0.05 [0.41-0.71]*	130	Fifth
9	2.94 ± 0.02 [2.87-2.99]*	39.00 ± 0.83 [34-41]*	0.72 ± 0.04 [0.51-0.81]*	145	Sixth
11	3.09 ± 0.01 [3.01-3.16]*	37.64 ± 1.40 [29-47]*	0.77 ± 0.06 [0.57-1.19]*	184	Seventh

* Numbers in square brackets are minimum and maximum values (range)

There was significant correlation at $P < 0.01$, of mean age (days) to width of head

capsule, body length and body weight (Table 3).

Table 3. Pearson's correlation coefficients of mean age (days) (df = 6) to body length (mm), weight (g) and head capsule width (mm) in laboratory reared population of *Monochamus leuconotus*

	Mean age	Mean body length	Mean body weight
Mean body length	0.96**		
Mean body weight	0.98**	0.96**	
Mean head capsule width	0.98**	0.99**	0.97**

** Correlation is significant at 0.01 level (2-tailed).

The body weight and length increased considerably from first instar to the seventh

instar. The margin of change in size gradually reduced where the weight of the

fifth, sixth and seventh instars was very close (Figure 8). The body length of

seventh instar was significantly lower than that of sixth instar (t $_{8 (2)}$ = -0.12; $P >$

0.05) (Figure 9).
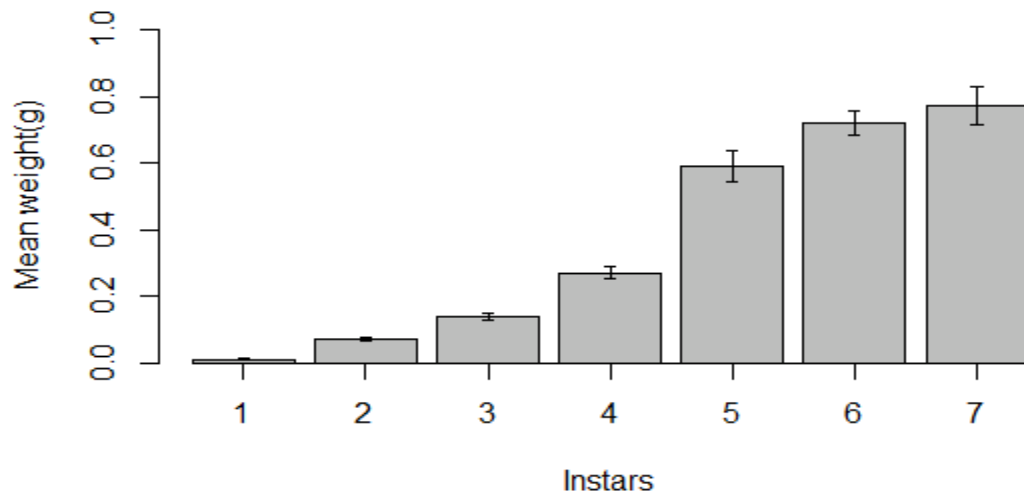
Figure 9. Mean body weight (±SE) of seven larval instars of a laboratory reared population of *Monochamus leuconotus*
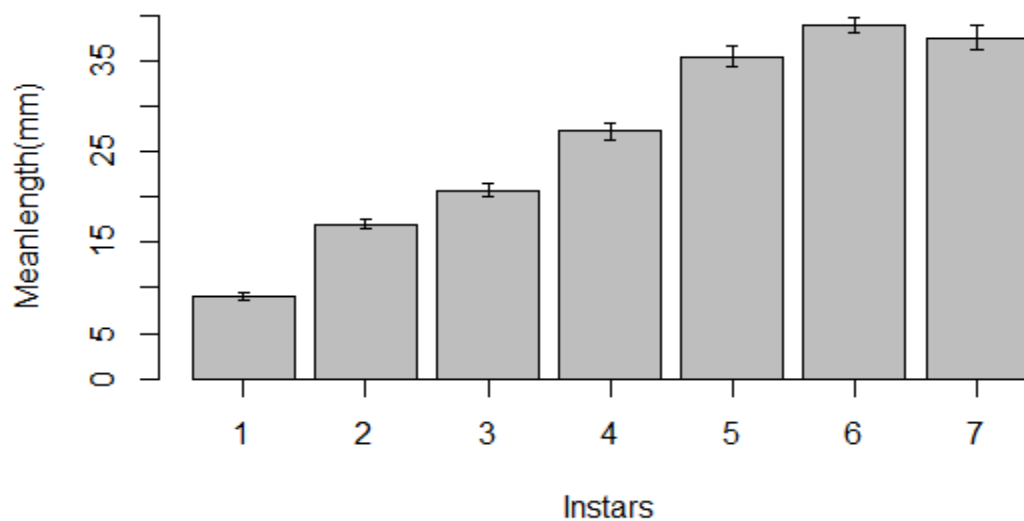
Figure 10. Mean body length (±SE) of the seven larval instars of a laboratory reared population of *Monochamus leuconotus*

The average body length in the first instar larvae reared in the laboratory was 9.07 ± 0.45 mm. The seventh instar larvae had a maximum length of 50 mm while the mean length in this stage was 37.64 ± 1.40 mm as shown in Table 4 together with body length of the other life stages. The average body weight in first instar larvae was 0.01 ± 0.00 g. Maximum weight recorded in seventh instar was 1.38 g while the average weight in this stage was 0.77 ± 0.06 g (Table 5). Prior to pupation, the last larval instar gets into a transition stage known as pre-pupal stage where there is no feeding, colour gets creamy white, the body gets constricted while the integument becomes thick and opaque (Appendix 2). The duration of this pre-pupal stage varied considerably among the individuals with some taking two months but on average it took 19±1.06 days. One to three days before pupation the larva rolled profusely. Larval stage was the most persistent stage where on average it took 209±12.42 days (seven months) under the laboratory conditions. The larva proved very hardy, despite the frequent handling, the survival rate was 78% with death mostly occurring in the first instar. From the second instar onwards, the survival rate was 100%

Table 4. Comparison of the mean body length (mm) of the different life stages of a laboratory-reared population of *Monochamus leuconotus*

Life stage	df	Range (mm)	Mean ± SEM (mm)	*p*-value
Larva (7th instar)	26	21.85 − 50.00	37.64 ± 1.40	
Pupa	26	20.00 − 27.00	23.04 ± 0.43	< 0.05
Adult	73	17.00 − 28.00	22.27±0.28	> 0.05

P-value for *t*-test, testing the difference between mean body length of the corresponding stage and that preceding it.

Table 5. Comparison of the mean body weight (g) of the different life stages of a laboratory reared population of *Monochamus leuconotus*

Life stage	df	Range (mm)	Mean ± SEM (mm)	*P*-value
Larva (7th instar)	26	0.24 − 1.38	0.77 ± 0.06	−
Pupa	26	0.31 − 0.69	0.50 ± 0.02	< 0.05
Adult	73	0.27 − 0.87	0.49 ± 0.02	> 0.05

P-value for *t*-test, testing the difference between mean body weight of the corresponding stage and that preceding it.

4.1.3 Pupal stage

The pupa of *Monochamus leuconotus* is exarate, creamy white with brown tinge but it gradually darkens as it matures to adult. The body is opaque and cylindrical. Abdomen is elongate and tapers gradually to a posterior end. The antennae are

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tightly coiled against both sides of the body. The body is covered by ferruginous setae which appear denser at the frons, intersegmental tergite of the abdomen and the last abdominal metamere. The ventral side has few scattered setae. The eyes appear as distinct, black and round (Figure 3). The average body length was 23±0.43 mm while the average weight was 0.50±0.02 g (Table 4 and 5).

Since it was difficult to differentiate male and female pupae, the body sizes of pupae were recorded and the sex was confirmed on emergence to adults. The females were on average longer (23±0.55 mm) than males (22.2±0.57 mm) ($t_{9(2)}$ = 2.66; $P < 0.05$) and heavier (0.55±0.03 g) than males (0.44± 0.02 g) ($t_{9(2)}$ = 3.90; $P < 0.05$). The pupal period took 23±0.27 days on average. Males emerged earlier on average (23±0.27 days) than females (24±0.27 days)

4.1.4 Adult

The sex ratio obtained from 42 adults was one female to 0.88 males. The body was relatively flattened dorsally, elongate and slightly curved at ventral sternite of the metathorax. The beetles measured 22.27±0.27 mm long and weighed 0.49±0.01g on average (Table 4 and 5). In a laboratory sample of adults measured, on average, females were significantly longer than males ($t_{33(2)}$ = 3.26; $P < 0.05$) at 23.09±0.36 mm and 21.51±0.35 mm, respectively. The females were also significantly heavier than males ($t_{33(2)}$ = 3.61; $P < 0.05$) at 0.54±0.02 g and 0.44±0.02 g, respectively.

The adult is an alate with strongly sclerotized integuments and appendages. It generally appears white-grey with gray-brown patches but a closer look shows that it is brown but covered by grey-white pubescence at the elytra. Dorsal view shows that the head, prothorax, mesothorax and a blotch at the elytra close to apical region have a mosaic of black and brown patterns (Appendix 3). The better part of the ventral side is dark brown with scattered white pubescence which extends to all the legs with exception of ventral metathorax that is densely covered by white pubescence making it appear white (Appendix 4). Both sexes have hypognathous mouth parts with spines on both sides of propleuron. Adult is torpid and takes some time to respond to a stimulus or acclimatize to new surroundings. The filiform antennae emanating from visible tubercles were conspicuously long (29.8±1.85 mm against a body length of 20.93±0.54 mm) as measured from 15 adults from the laboratory colony. The male antennae (34±2.76 mm) were almost twice the body length (19.43±0.81 mm) while female antennae measured 25.5±1.20 mm against body length of 22.25±0.25mm. On emergence, the lethargic beetles remained dormant lying on the paper towel for an average of 21±2.11 days before feeding. Initial feeding was on coffee leaf margin then on leaf petiole and eventually on twig stalk. This was followed by egestion of dark grey, pellet-like excreta. Females took an average of 23±2.69 days to start feeding while males took an average of 15±1.88 days, but males seemed more interested to mate than to feed at first. Extensive ring barking was observed in the cage

mostly close to the crown of the stick. Although some twigs and leaves were provided in the cage, the adult seemed to prefer barking the stem.

Once a couple was introduced to the cage, mating started almost instantly where the male advanced towards the rather reluctant female that seemed more interested on feeding at this point. Mating occurred repeatedly, copulation lasted for few minutes but in some, it prolonged even for hours. The pre-oviposition period was 35 ± 1.69 days. In most cases it appeared like laying eggs occurred at night but on rare occasion at dawn. A female would palpate trying to locate a suitable oviposition site on the stick. Once it settled on one, mostly within 3 cm from the soil level, it would nibble a small cavity, turn around, insert her ovipositor and deposit a single egg, and then seal with a dark brown, gummy, shiny secretion. Oophagy was observed in some adults though it was difficult to clearly identify whether it was due to the males or the females. The average longevity of the adults in the laboratory was 82 ± 5.05 days. Males lived for 81 ± 6.11 days while females took 84 ± 8.27 days on average.

The larval stage took the longest developmental period while pupae took the shortest (Figure 10).
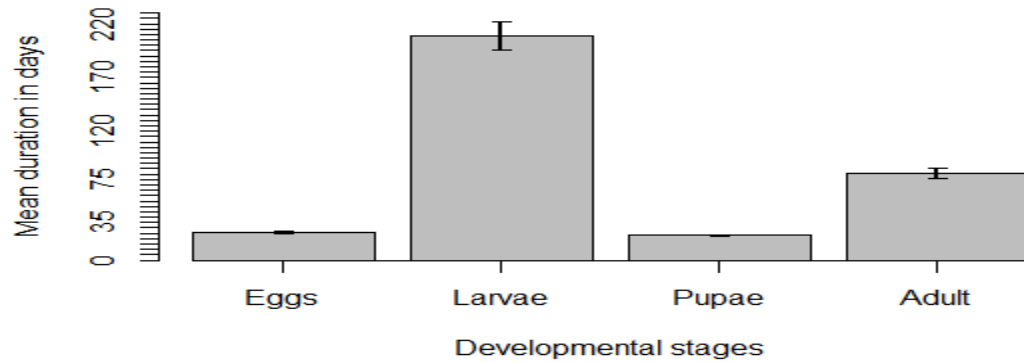
Figure 11. Developmental duration of life stages of a laboratory reared population of *Monochamus leuconotus*.

The eggs were small in size but on hatching, the length of the larvae increased sharply to the maximum, then started declining close to pupation. The difference in length between pupae and adult was marginally small (Figure 11).

Pupae recorded the highest mean body weight but there was a slight reduction as the pupae turned to adults.The mean body weight of larvae varied considearably depending on the larval instar but on overall, the average was lower than for pupae and adults (Figure 12).
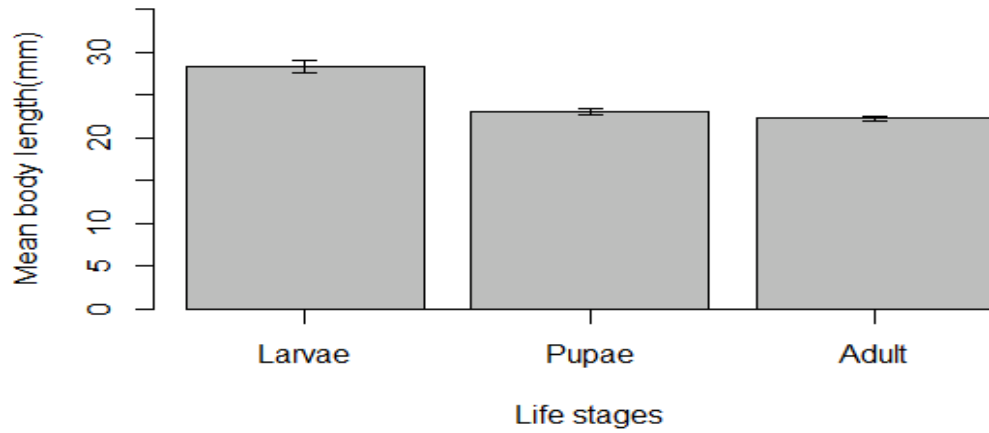
Figure 12. Relative mean body length (mm) of the different life stages of a laboratory reared population of *Monochamus leuconotus*
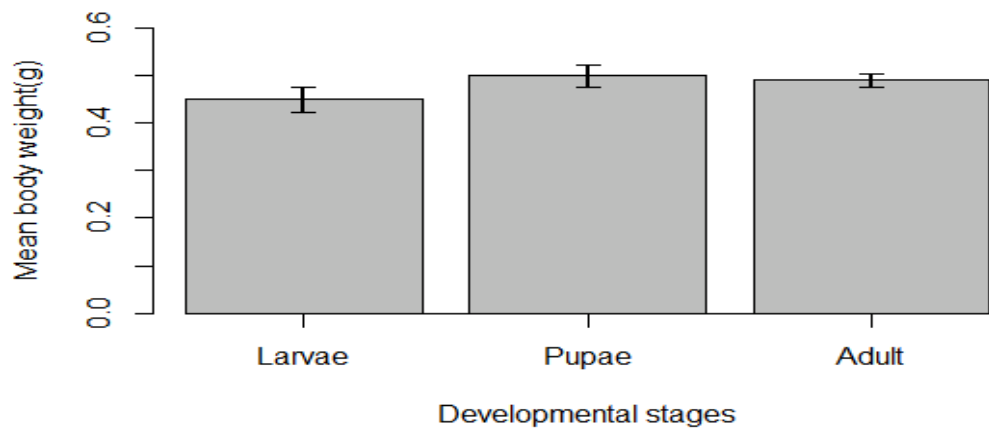
Figure 13. Relative mean body weight (mm) of the different life stages of a laboratory reared population of *Monochamus leuconotus*

4.2 Fecundity

The average number of eggs laid per female throughout the oviposition period was 51.84±5.92 eggs, with 0.73 eggs laid per day. There was a wide variation of fecundity among individuals with a female laying as many as 131 eggs in the entire oviposition period. There was a positive correlation between fecundity and body size, initial age of laying and female longevity as shown in table 6.

Table 6. Pearson's correlation coefficients (r) of fecundity to mean body length (mm), average weight (g), average initial laying age (days) and mean longevity (days) of laboratory reared population of *Monochamus leuconotus*

Variables	No. of eggs	Length	Weight	Laying age	Longevity
No. of eggs		0.25	0.46		
Length	0.25		0.71**	0.23	0.37
Weight	0.46	0.71**		0.22	0.40
Laying Age		0.23	0.22		0.44
Longevity		0.37	0.40	0.44	

** Correlation is significant at 0.01 level

The female laid eggs continuously until they die and although the survival rate gradually declined with advancement of age, fecundity seemed to take a reverse pattern (Figure 13). The highest number of eggs laid per female per day (0.89) was at the 128th day with a survival rate of close to 20%.

Females preferred laying eggs on the particular sticks they were feeding on and on specific areas of the stick such that even if another stick was introduced they would continue laying on the stick where they laid initially. The number of eggs laid per day per female in a week is shown in Figure 14. On the first day after changing the stick, fecundity was noted to be low, but gradually peaked before declining towards the end of the week. Lowest fecundity was recorded on first and last day of the week while maximum fecundity was on fifth day of the week.
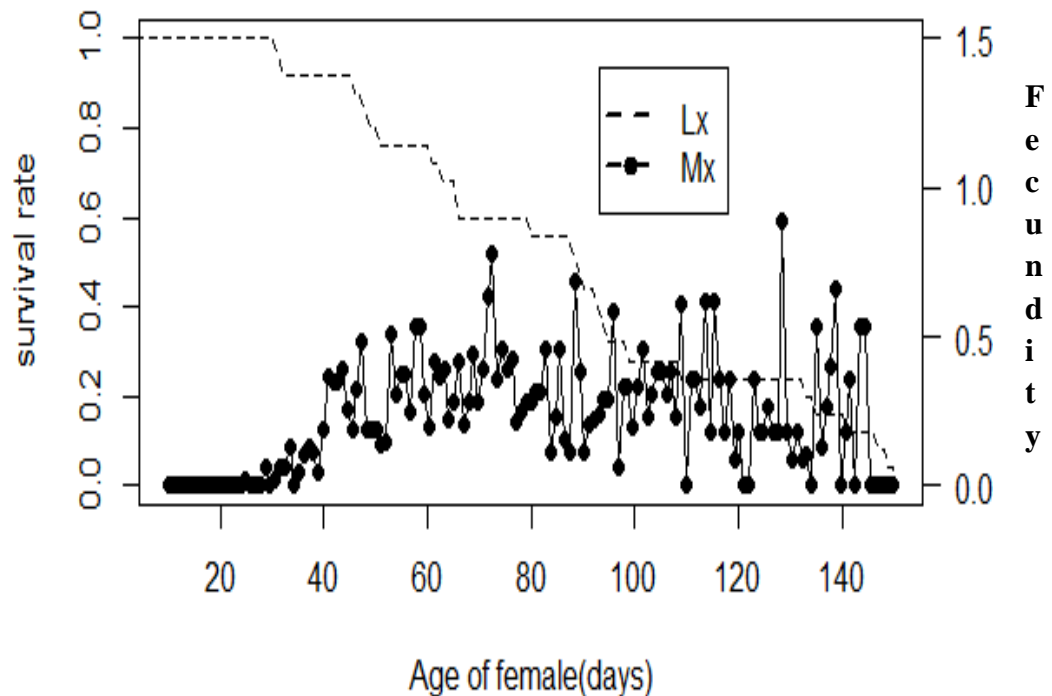
Figure 14. Age of female versus survival rate (Lx) and fecundity (Mx) of a laboratory reared population of *Monochamus leuconotus*
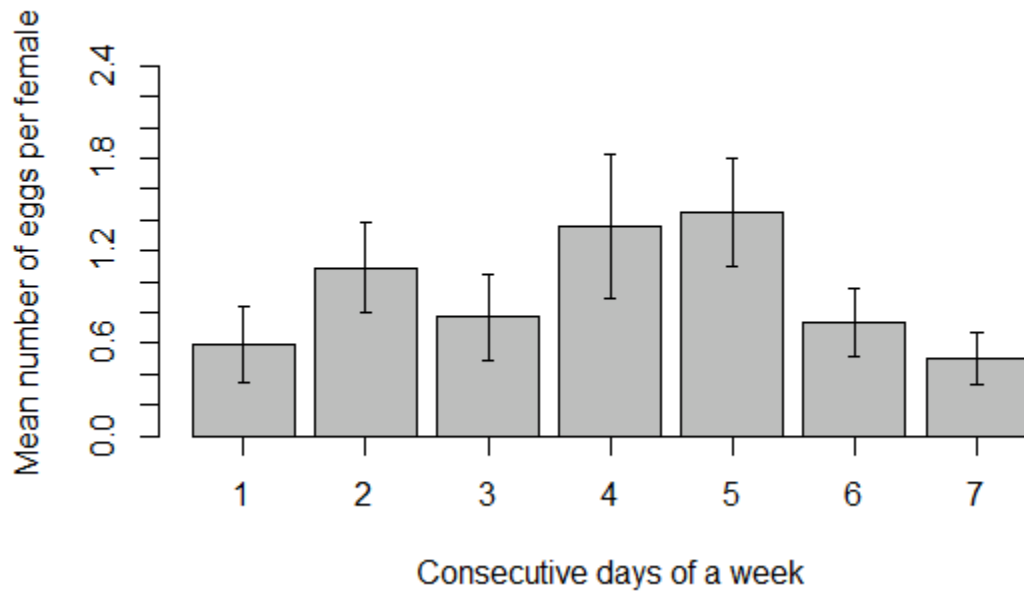
Figure 15. Daily fecundity trend within a week in laboratory reared population of *Monochamus leuconotus*

4.3 Life table analysis

Life table parameters were calculated in a spreadsheet, a summary of the numbers used, mean duration and survival of eggs, larvae and pupae are shown in Table 7.

Table 7. Number of eggs, larvae, pupae used in life table analysis

Life stage	Numbers	Mean duration (days)	Survival (lx)
Egg	510	26	1.00
Larvae	36	209	0.14
Pupae	21	23	0.14

The gross reproductive rate of *Monochamus leuconotus* reared population was 35.36 daughters per female, the net reproductive rate was 2.60 daughters per female, the mean generation time was 304.66 days, the population doubling time was 220.05 days, the intrinsic rate of increase was 0.0032, while finite rate of increase was 1.0010, as shown in Table 8.

Table 8. Life table parameters of a reared population of *Monochamus leuconotus*

Parameter	Value
Gross reproductive rate (GRR)	35.36
The net reproductive rate(R_o)	2.60
Mean generation time(T_c)	304.66
Doubling time(T_d)	220.05
Intrinsic rate of increase(r)	0.00315
Finite rate of increase (λ)	1.00099

CHAPTER FIVE

5.0 Discussion, Conclusions and Recommendations

5.1 Rearing method

The innovative rearing method developed in this study where an artificial diet was used for the first time in rearing of *Monochamus leuconotus,* proved quite successful. In insect mass rearing using an artificial diet, an ideal diet must raise at least 75% of the individuals to adult stage from viable eggs. In addition, the adult size and development rate should be close to field observations (Singh, 1983). In this study, the survival rate of larvae was 78% and 100% for pupae, as such this rearing method satisfies the requirement. Rearing of insects in the laboratory using an artificial diet leads to reduction on cost of carrying out research in addition to improving on research as the researcher can easily access the insect anytime of the year and anytime of the day unlike in the field. The rearing temperature in the incubator was $25 \pm 1^{\circ}C$, as development in most Cerambycids is optimum around this temperature (Dubois, 2002; Ricardo, 2014). The common practice in developing the diet is to modify the existing diet of an insect with similar feeding habits (Cohen, 2004). The diet that was used in this study was arrived at after some trials considering its acceptability by the insect, nutritional status, affinity to infection, ease of getting the ingredients, compatibility of ingredients and cost effectiveness as suggested by Aloo and Katagiri (1994). The ingredients used are locally available. Coffee bark and leaves

act as natural diet to provide the nutrients required by the beetle. Tapley (1960) suggested that larvae tend to grow more rapidly while feeding on soft tissues (ring barking) as compared to hard ones (wood boring). Host plant further acts as phago-stimulant in neonate and raises its acceptability (Gardiner, 1970; Aloo and Katagiri, 1994).

In a study on making artificial diet of bark borer, a Buprestidae (Coleoptera), a higher survival rate was recorded on dry brewer's yeast (used in the current study) as compared to yeast hydrolysate (Gindin *et al.,* 2009). Brewer's yeast provides major vitamins and minerals. These vitamins help in metabolism of major macronutrient such as carbohydrates, proteins and fats which supply energy. Sucrose is the major source of carbohydrate. A study on effects of sucrose levels in artificial diet on growth of *Cryptomeria* bark borer, a Cerambycid, showed that sucrose is necessary for growth and development of Cerambycids (Kitajima, 1999). Ascorbic acid provides vitamin C while vitamin E acetate supplies vitamin E necessary for reproduction. It was important to supplement the natural diet as Hanks (1999) reported that the levels of nutrients in bark are low compared to subcortical zone tissues. Microbial contamination has been reported to be a major constraint in preserving the diet, particularly the mould. This can affect nutritional status of the diet and even worse have adverse effect on the insect (Zhao *et al.,* 2014). To avoid this contamination and preserve the diet, sorbic acid, methyl-paraben and formaldehyde were successfully used. In addition to acting as

bacteriostatic agent, methyl-paraben help in modifying pH while agar powder helps to solidify and cement ingredients together (Tefera *et al.,* 2010).

In making the diet, the structure of the diet is important as it determines the ability of the neonate larvae to penetrate through. Low or high viscosity of the diet hampers its acceptability and therefore it is important to determine the right viscosity depending on the species. Gindin *et al.,* (2009), in his study, found that neonate larvae were not able to penetrate diet with more than 70% moisture content and dry crumby diet but the neonate were able to penetrate the paste-like diet with moisture content of 60-62%. In this study, the right consistency of the diet was achieved through adjustment of the amount of the distilled water and the powder of coffee bark and leaves to 806.6ml and 113.3g respectively. After making the diet, it was left open overnight to reduce moisture levels to less than 60%. Initially the diet was replaced after one month but there was slow growth rate in the last week of the month perhaps due to accumulation of the body excrement which made it less palatable. As such, replacement of the diet after three weeks proved to be better.

5.2 Life cycle of laboratory reared *Monochamus leuconotus*

The rearing method drastically reduced the duration of the life cycle of laboratory reared CWSB to 11 months unlike the 24 months reported in field conditions by Tapley (1960). Artificial rearing of Cerambycids reduces duration of life cycle as observed by Rogers *et al.* (2010).

Schoeman *et al*. (1998) reported that the whole life cycle under semi- field conditions took 18 to 24 months. Naves *et al.* (2008) also found out that artificially reared *Monochamus galloprovincialis* had univoltine life cycle. The implication is that more studies can be carried out on this pest with ease due to reduction on the costs of travelling and other logistics. A researcher can get any stage of the pest at any time including the adults which hitherto to this study were available seasonally during rainy season.

Eggs

In the laboratory reared colony, the eggs were pure white, contrary to the observation of Tapley (1960) and Schoeman *et al.* (1998) but were covered by a brown secretion that made them appear creamy. The length of egg (4.28± 0.17 mm) and diameter (1.59± 0.07 mm) were similar to the findings of both Tapley (1960) in the field and Schoeman *et al.* (1998) under laboratory conditions (26 ± 2 °C) of 4-5 mm length and 1.5-2.0 mm diameter. The incubated eggs on the sticks took on average 26 days slightly higher than 21 days reported by Schoeman *et al* (1998) but within the range of 20-30 days observed by Lewin (1936) cited by Tapley (1960). But in this particular study hatching was indicated by presence of the frass and this duration could be less by 2-3 days as the neonate larvae start by feeding on the egg shell before extruding the frass. Egg hatching was a bit low at 39%. Generally, hatchability in cerambycids is relatively low due to unfertilized eggs (Lee and Lo, 1996) and this explains why although cerambycids are highly prolific in terms of egg production this does not correspond to levels of larval

infestation in the field. For instance, mean incidence of *M. leuconotus* in Mt Elgon region in Uganda was reported to be 28.3% despite high egg production by this species (Kyamanywa, 2011). In this study moisture levels of the sticks could have also contributed to low hatchability. Hanks (1999) noted that moisture levels affect hatchability.

Larvae

The larval period took 7 months in the laboratory while Tapley (1960) estimated it at 20 months in the field. Larvae were reared singly due to cannibalism as reported by Togashi (1990). Number of instars in some species varies based on temperature or season, for instance the number of instars in *Monochamus carolinensis* varied depending on temperature (Pershing and Lint, 1988).The number of instars as determined in the laboratory were seven as earlier reported by Tapley (1960) and Schoeman *et al.* (1998). This was determined by making growth curves and measurement of head capsule width. Graphical presentation of the larval growth on diet was done so as to show the pattern of moulting and therefore number of instars. To further proof this, width of head capsule was measured. Measurements of width of head capsule is more reliable in determining number of larval instars as compared to the body length, weight and age since according to Dyar's law a change from one instar to the other is accompanied by change in size of the sclerotized body parts. The mean head capsule width of the individuals in the first instar was 0.90 mm, second instar 1.27 mm, third instar 1.69, fourth instar 2.21 mm, fifth instar 2.78 mm, sixth instar 2.93 and 3.09 mm

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for seventh instar. This was consistent with observation of Tapley (1960) who recorded 0.98 mm for first instar, second instar 1.27 mm, third instar 1.6 mm, fourth instar 2.1 mm, fifth instar 2.7 mm, sixth instar 3.5 mm and 4.5 mm for seventh instar. According to Dyar's law the ratio of increase from one instar to the other should be almost the same for the sclerotized parts of the body. In this study the ratio was significantly small, that is, ratio of increase of second to first instar was 1.40, third to second instar 1.33, fourth to third instar 1.31, fifth to fourth instar 1.25, sixth to fifth instar 1.25 and seventh to sixth was 1.05. Tapley (1960) recorded 1.29 for the second to first instar, 1.25 for third to second instar, 1.31 for fourth to third instar, 1.28 for fifth to fourth instar, 1.29 for sixth to fifth instar and 1.28 for seventh to sixth instar. The positive correlation between larval instars to head capsule width, body length, weight and age shows that there was progressive growth and development over time from one instar to the other. The seventh instar or pre-pupal stage recorded the highest weight as this was in preparation to pupation where there is no feeding, similar to observations of Schoeman *et al.* (1998). Body length of the larvae increased progressively upto sixth instar where it slightly declined in seventh instar due to body constriction and re-organization of the tissues prior to pupation. The mortality in the laboratory was low at 22% which was experienced mostly at the first instar similar to report of Kosaka and Ogura (1990) in *Monochamus alternatus*.

Pupae

The pupal period took 23±0.27 days on average similar to field observations of 21-30 days by Tapley (1960). Males took less days (23±0.27 days) than females (24±0.27 days) to emerge as adults hence protandrous, similar to observations of Tapley (1960) and other Cerambycids (Hank, 1999).This enables the male to mate with many females, establish territories in the best habitat and defend their territories (Timmerman *et al* 2007).

 Pupal weight was the highest among the life stages as observed in other cerambycids like *Apomecyna saltator* (Khan, 2012). The females were longer (23 ± 0.55 mm) than males (22.20 ±0.57 mm). Schoeman *et al*. (1998) also noted that the female pupa is larger (about 31 mm) than male (about 28 mm). Female pupae were also heavier (0.55 ± 0.03 g) than males (0.44 ±0.02 g) which concur with observation of Tapley (1960). The colour of the pupae changed gradually from creamy white to grey-black due to melanization. This was also accompanied by hardening (sclerotization) and further development of the body and appendages where the coiled antennae and legs disentangled as reported by Schoeman *et al*. (1998). Mortality was nil at this stage which corroborates with findings of Togashi (1990) and Naves *et al*. (2008) in other Cerambycids.

Adults

The laboratory adult beetles appeared shorter (22.27 ± 0.27 mm) than field beetles (30 mm) as reported by Tapley (1960).The length and weight of the adult was slightly lower than that of the pupae as observed in other cerambycids

(*Apomecyna saltator*) (Khan, 2012). Females were longer and heavier than males similar to the findings of Schoeman *et al.* (1998). The antennae were long measuring 29.80 ± 1.85 mm against body length of 20.93 ± 0.54 mm which is within the range of the length of the antennae in field adults as reported by Tapley (1960). On emergence, the callow beetle seemed weak and remained dormant for a period of 21 days perhaps to allow for morphological, physiological development and sexual maturation. This also ensures that emergence in the field coincides with favourable conditions in most cerambycids (Gupta and Tara 2013). Emergence of *M. leuconotus* in the field is normally two weeks before onset of rains. Prevalence of this pest is greatly influenced by precipitation (Kutywayo, 2013). Females rested for a longer period (23 days) than males (15 days). This could be associated with development of reproductive system (Kutywayo, 2014). The adults took 14 days feeding before laying eggs. Maturation feeding is a common behaviour in cerambycids to gain the necessary energy required for production of eggs. In *Anoplophora glabripennis*,a cerambycid, ovary took 10 days to mature after emergence (Keena, 2006). The sex ratio was one female to 0.88 males which is in contrast to a male biased ratio of 1:4 reported by Schoeman *et al.* (1998). Mating and feeding were the major activities in the cage. Copulation lasted for some minutes though in some cases it would extend beyond an hour. After mating, the male would remain mounted on the dorsum of the female for hours mate guarding similar to the observation of Kutywayo (2014). This is to increase chances of fertilization as females are polyandrous. Mate

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guarding also benefits the male by reducing risks of looking for another mate (Morewood *et al.,* 2004). In *Monochamus scutellatus* there is evidence that the latest mate is likely to fertilize most of the eggs that are laid (Morewood *et al*., 2004). Oophagy was observed in some cages especially towards the end of the week. This could be due to nutrient deficiency as the sticks desiccated or sometimes the adult landed on it accidentally as it barked. Longevity was 82 days on average, males lived for 81 days while females took 84 days. This duration could vary based on temperature, humidity and food availability. Initially the longevity was low but it gradually increased due to frequent moistening of the cotton wool and ensuring food is available throughout. This was informed by presence of soil on the frons of the dead adult an indication that it was trying to get into more humid habitat in the moist soil. Keena (2006) noted that humidity and temperature have an influence on longevity. To provide ample food, some coffee twigs were provided in the cage as the sticks became less palatable due to reduction of moisture level. Longevity of females in Lyamungu, Tanzania, was 40-60 days (Tapley,1960) while in Mpumalanga, in South Africa, females took 122 days and males 112 days (Schoeman *et al*., 1998).

Fecundity

Egg laying varied among the individuals with an individual laying as many as 137 eggs while the average was 51.84 ± 5.92 eggs translating to 0.73 eggs per day. Knight (1939) recorded a range of 10-40 eggs depending on temperature but in East Africa it is normally between 20 to 25 eggs (Moffat and Allan, 1934; Knight,

1939) while Schoeman *et al*. (1998) reported an average of 80.5 eggs (0.66 eggs per day) in South Africa. As stated earlier, sexual maturity especially in female affected fecundity. Fecundity was higher in older adults as compared to the younger ones and this could be attributed to sexual maturity by age. Longevity and survival rate were inversely proportional meaning that although fecundity increased with age, the net effect was lower due

to less number of adults laying eggs as age advanced. Longer feeding period also meant larger body size, more energy, consequently higher fecundity. These findings are similar with those of *Monochamus alternatus* (Togashi and Magira, 1981). Akbulut *et al*. (2007) noted that adult nutrition affects fecundity and longevity of the adult *Monochamus galloprovincialis*. The females laid fewer eggs on the first and last days of week. This indicates that the female takes time to gain confidence on the stick as a suitable oviposition site. Principle of natural selection suggests that an adult will only oviposit where her progenies are assured of survival to ensure continuity of generation (Morewood *et al*., 2003). The pattern of egg laying in majority of cerambycids is of two kinds where in protogynous the eggs are laid in the later stages of adult life while protandrous lay their eggs in early part of adulthood (Timmerman *et al*., 2007). But in some members of cerambycids the pattern is reversed and this seem to be the case in this study, although the males emerged earlier than females (protandrous) egg laying occurred at late stages of adulthood.

5.3 Life table

Life table is important in monitoring population dynamics of insects. Mortality is key in population regulation. Mortality of eggs stood at 61% while mortality of the first instar was 75%. From the life table calculation in this study, the cumulative mortality was 76%. Dodds and Stephen (2000) observed generational mortality of between 60.94-98.61% in *Monochamus titillator* under field conditions. The reasons for this mortality were explained earlier in this section. To gauge the performance of this rearing method, the life table parameter values obtained were compared with those of *Monochamus galloprovincialis* on pine wood by Akbulut (2007). In *M. galloprovincialis,* the gross reproductive rate was 48.34, net reproductive rate was 3.66, mean generation time was 220.05 while intrinsic rate of increase was 0.0166. In this study, the gross reproductive rate was 35.36, net reproductive rate was 2.60, mean generation time was 304.66 while intrinsic rate of increase was 0.00315.This indicates that the rate of population growth (intrinsic rate of increase) in this study was a bit lower mostly due to high mortality at early stages of development. Further to explanations given earlier on causes of early mortality, it was noted that after hatching some larvae moved down to the moist soil and died as early larval stage is positively geotactic (Schoeman *et al*., 1998). In some cases, some bored into the wood after hatching while some were accidentally injured in the process of extraction. All this contributed to low survival rate at stage two which effectively lead to low intrinsic rate of increase. Ruiz (2012) also got a low intrinsic rate of increase (0.01) on

Xylotrechus arvicola, a cerambycid and attributed it to the long duration of development from egg to adult which could have also contributed in this study

5.4 Conclusions

The rearing method developed in this study proved successful as it was able to rear 78% of the colony to adult stage. Through this rearing method, there was a great reduction on the duration of the life cycle of *Monochamus leuconotus* reared in the laboratory compared to duration of the life cycle in the field. The implication is that, future studies on this pest will take shorter period of time. Further, it will be easy to carry out more studies on larval and pupal stage as they will be easily accessible unlike in field conditions where these stages occurs inside the wood. The life history traits of laboratory bred colony were very similar to the traits of wild *Monochamus leuconotus* meaning that the findings can be easily applied in the field.

5.5 Recommendations

I would encourage other researchers to use this rearing method as it will help to gather comprehensive information on all stages in addition to reduction on travelling costs and time spent on research.

Further research ought to be carried out to ascertain the effects of temperature and moisture on fecundity and egg hatching.

This study showed that laboratory bred *Monochamus leuconotus* are protandrous but eggs are laid in the later stages of adulthood. This observation need to be

validated through field studies as it can form a basis of formulating control

strategies aimed at preventing oviposition.

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Appendix 1. Morphometrics, mortality and incubation period of eggs from

laboratory bred *Monochamus leuconotus*

Parameter	Numbers (n)	Mean ± SEM	Range
Body length (mm)	21	4.28 ± 0.17	2.95-5.61
Diameter (mm)	21	1.59 ± 0.07	1.11-2.15
Mortality	510	61%	-
Incubation period	103	26 days	17-54
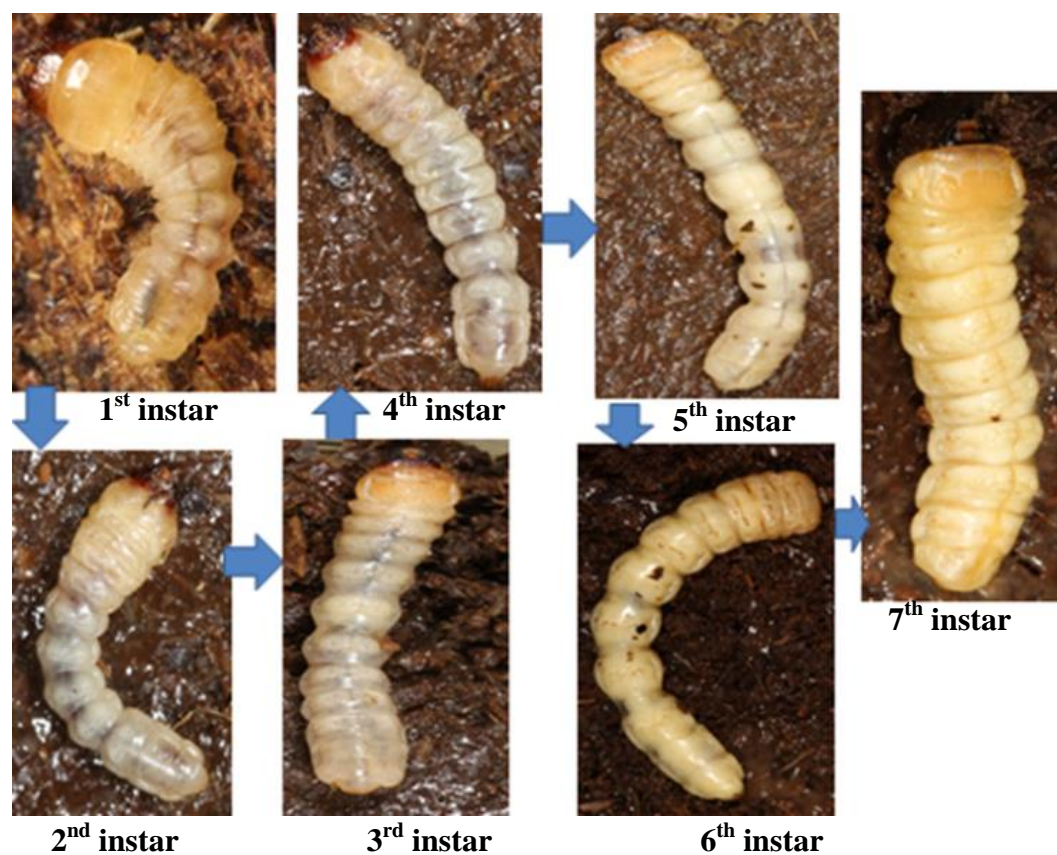
Appendix 2. Larval instars in laboratory bred *Monochamus leuconotus*

Appendix 3. Dorsal view of laboratory bred *Monochamus leuconotus* male adult

Appendix 4. Ventral view of laboratory bred Monochamus leuconotus male adult