STUDIES OF THE MECHANISMS INVOLVED IN HOST FINDING AND MATING BEHAVIOUR OF THE AFRICAN COFFEE WHITE STEM BORER, *MONOCHAMUS LEUCONOTUS* (PASCOE) (COLEOPTERA: CERAMBYCIDAE)

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A thesis submitted in partial fulfilment of the requirements of the University of Greenwich for the Degree of Doctor of Philosophy

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To

My Mother and Late Father

ABSTRACT

The African coffee white stem borer, *Monochamus leuconotus* (Coleoptera: Cerambycidae) is a serious pest of Arabica coffee in Zimbabwe and other African countries. Very was known about the chemical ecology of *M. leuconotus* prior to the initiation of the studies described in this thesis. The objectives of this work were to investigate the mating behaviour in order to look for evidence of the existence of chemical interactions between conspecific beetles and host plants. Mating behaviour and daily activity patterns of adult *M. leuconotus* were characterised under laboratory and semi-field conditions. Mating was initiated after the male encountered a searching female and touched her with antennae or tarsi. The activities of feeding, walking, mounting and copulation were mostly done during daylight hours with the exception of oviposition, which occurred at night. Laboratory bioassays conducted to determine whether contact pheromones played a role in mate recognition showed that males were able to complete the full sequence of behavioural activities involved in mating with both live and dead conspecific females. Males did not respond to dead females washed with hexane, and the responses could be partially restored by recoating the washed females with the hexane washings, indicating that cuticular hydrocarbons are important for recognition of sex and species. A laboratory bioassay was developed for evaluating the olfactory response of *M. leuconotus* to different cues. Females responded positively to coffee leaves, coffee bark scrapings and the synthetic male-specific compound of *M. leuconotus* dispensed in a sachet while males responded positively to coffee bark and to a combination of coffee leaves and the synthetic male-specific compound dispensed in a vial. Field trapping trials were conducted in Zimbabwe using live insect baits and the synthetic male-specific compound of *M. leuconotus* dispensed in polyethylene sachet and vials and different trap designs. Significant numbers of beetles were captured in traps baited with the male-specific compound, and numbers caught were further increased when certain host-plant volatiles were added, particularly (*R*)-(-)-linalool and methyl salicylate. Intercept panel traps and MK2 rat traps were effective in retaining insects caught. Floral surveys conducted around coffee fields to identify alternative host plants did not give conclusive evidence on the existence of alternative host plants although suspected coffee stem borer symptoms of attack were observed on previously reported alternative host plants. Feeding and oviposition studies suggested that female *M. leuconotus* feed mostly on Rubiaceae and preferred to lay eggs on *Coffea arabica*, *G. ternifolia, V. infausta* and *K. venosa.* The implications of the findings in relation to possible application of chemical ecology in management of *M. leuconotus* are discussed.

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ABBREVIATIONS

ARDA	Agriculture and Rural Development Authority
CABI	CAB International
CFC	Common Fund for Commodities
cm	Centimetres
CSB	Coffee Stem Borer
d.f.	Degrees of freedom
EAG	Electoantennography
g	Grams
GC	Gas Chromatography
GC-EAD	Gas chromatography coupled to electroantennographic detection
GC-MS	Gas chromatography coupled to mass spectrometry
IR	Infrared Spectroscopy
Ha	Hectares
m	Metres
masl	Metres above sea level
ml	Millilitre
NMR	Nuclear Magnetic Resonance
NRI	Natural Resources Institute
µg	Micro-grams
µl	Micro-litre
SPME	Solid Phase Microextraction
Ng/h	Nanograms per hour
TLC	Thin Layer Chromatography

GLOSSARY OF TERMS USED

Additive effect – an effect wherein two or more substances or actions used in combination produce a total effect, the same as the arithmetic sum of the individual effects.

Adectious pupae – have non-articulate mandibles.

Anemokinesis – the flight of an insect upwind or downwind in response to a stimulus but the flight is not related to the direction of the stimulus.

Anemotaxis - the directed flight of an insect towards or away from the stimulus.

Apodous larvae –without feet or having only rudimentary feet.

Arrestants – lead responders to stop or displace less frequently or slower.

Attractants - a stimulus source that directs insect movement (taxis) towards itself.

Attraction – positive response to a stimulus that leads insect movement towards the stimulus.

Baits/Lures - a chemical source intended to be attractive to a given organism.

Copulation – is when the claspers of the male were observed to be aligned with the female ovipositor and the male bent its abdomen.

Dashing – Male moves forward quickly to position itself for copulation

Detection – the response of receptors to a stimulus.

Eucephalic larvae –with well-developed head capsule.

Exarate pupae - their appendages are not closely appressed to the body.

Feeding – is when adult beetles were observed to scrape the bark of coffee stems with their mandibles.

Infrared spectroscopy -is the spectroscopy that deals with the infrared region of the electromagnetic spectrum.

Kinesis – when an insect moves in response to a stimulus but the movement is not related to the direction of the stimulus

Klinokinesis – the frequency or rate of turning is proportional to stimulus intensity.

Licking – male observed to touch the elytra/pronotum of the female with his mouth palpi. This could be related to sex contact pheromones present in the female cuticle that are recognized by the males through chemical receptors located in the maxillary and/or labial palpi.

Mate guarding – is when a male beetle was observed to continue mounting the female beetle after withdrawal of its aedegus.

Mounting – is when a beetle was observed climbing on top of another beetle.

Nuclear magnetic resonance spectroscopy (NMR) - exploits the magnetic properties of certain atomic nuclei. It determines the physical and chemical properties of atoms or the molecules in which they are contained.

Orthokinesis – the speed of movement of the individual is dependent upon the intensity of the stimulus.

Oviposition – is when a female was observed to bend its abdomen and insert ovipositor in a stem.

Perception – is the processing of sensory signals in the brain. For example, when a chemical signal is detected through olfactory sensillae, it is confirmed through perception.

Potentiation - to enhance or increase the effect of a chemical by another chemical.

Repellents – a chemical causing a responder to make movements oriented away from the stimulus source.

Resting - is when a beetle remains motionless

Synergism – the interaction of two or more substances, or other agents to produce a combined effect greater than the sum of their separate effects.

Taxis – animals respond to a stimulus by moving towards or away from the stimulus'

Touching –The male beetle was observed to extend its antennae or tarsi on the female's pronotum

Vibrating antennae - The male beetle was observed to vibrate the antennae, quickly shaking them forward and backward in the vicinity of a female

Walking – is when a beetle was observed to move in the cages.

Chapter 1
GENERAL INTRODUCTION

1.1. BACKGROUND TO CURRENT WORK

An international project "Integrated Coffee White Stem Borer Management for Smallholder Coffee Farms in India, Malawi and Zimbabwe" funded by the Common Fund for Commodities (CFC) was launched in 2002. As part of this project, work to investigate the chemical ecology of the African coffee white stem borer, *Monochamus leuconotus* Pascoe (Coleoptera: Cerambycidae) and to identify the pheromone and any olfactory cues involved in its communication system was initiated. Work on the identification of the male-specific compound and subsequent synthesis of the compound was done by chemists at the Natural Resources Institute (NRI), UK, while field and laboratory bioassays with olfactory cues and the synthetic male-specific compound of *M. leuconotus* were done in Zimbabwe. The work done at NRI was done concurrently with the field and laboratory studies described in this thesis, which were done in Zimbabwe between December 2003 and February 2013.

1.2. THE COFFEE WHITE STEM BORER, *Monochamus leuconotus* (Pascoe)

1.2.1 Classification

The outline classification for *Monochamus leuconotus* appears below. The African coffee white stem borer also known as *Anthores leuconotus* (Pascoe) and *Herpetophygas fasciatus* (Fåhraenus) belongs to the family Cerambycidae (long horned beetles) and subfamily Lamiinae (flat-faced longhorns).

Taxonomy

Class: Insecta
Family: Cerambycidae
Subfamily: Lamiinae
Tribe: Monochamini
Genus: *Monochamus*
Species: *leuconotus* (Pascoe)

1.2.2 Description of *M. leuconotus*

Tapley (1960) reviewed the general biology and control of the African coffee white stem borer in Tanzania while Schoeman *et al*. (1998) gave an account of the morphology and phenology of the pest in South Africa. Adults of both sexes are brown with a whitish median and basal sector on the elytra (Fig. 1.1a). Body length averages 25.47 mm and 27.19 mm for males and females respectively. The species exhibits sexual dimorphism with male antennae (51.34 mm) being almost twice the body length while those of females (37.31 mm) are much shorter (Schoeman *et al*., 1998).

The egg is *ca*. 5 mm in length, 2 mm diameter, elongate, spindle shaped and cream in colour. Eggs are deposited singly beneath the bark in a slit chewed by the female prior to oviposition. Eggs normally hatch in about 2 to 3 weeks.

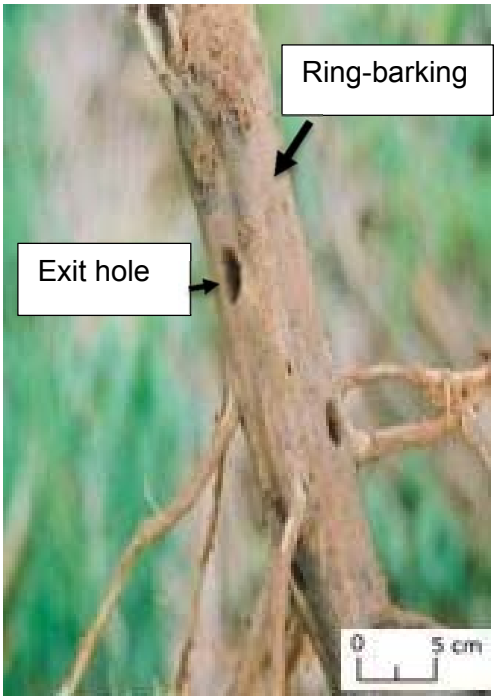
Larvae are apodous, eucephalic, cylindrical and white-to-cream in colour (Fig 1.1b). Larvae consist of the earlier ring-barking phase that feeds on phloem and cambium tissue and the later wood-boring phase that feeds on xylem tissue. The duration for the ring-barking phase is 13 -17 weeks while the wood-boring phase averages 10 weeks. There could be seven larval instars based on the head capsule width measurements (Tapley, 1960).

Pupae are adectious, exarate with females much heavier, averaging 31.41 mm, and males 28.19 mm in length (Fig. 1.1c). Pupae are normally found in pupal cells at the top end of a feeding tunnel. The duration of the pupal period is about 21 – 32 days (Tapley, 1960; Schoeman *et al.*, 1998) after which the adults emerge from the feeding tunnel (Fig. 1.1d).

(a)

(b)

(c)



(d)

Fig. 1.1 *Monochamus. leuconotus* life stages (a) adult male (b) larva (c) pupa (d) below ground damage showing complete ring-barking and exit holes (Photos by author)

1.2.3 Life history

Monochamus leuconotus is a pest of Arabica coffee at altitudes below 1,800 m above sea level (Anon. 1989) and is widely distributed in Africa from Natal, where Pascoe first described it in 1869, to latitude 5º N (Le Pelley, 1968). The geographical distribution is currently confined to Africa - Angola, Burundi, Cameroon, Congo, Congo Democratic Republic, Ethiopia, Kenya, Malawi, Mozambique, Namibia, Rwanda, South Africa, Sudan, Tanzania, Uganda, Zambia, and Zimbabwe (Fig. 1.2). Coste (1968) noted that it prefers higher and drier regions of Eastern and Southern Africa rather than the West Coast. There are no records of the pest in Southern and Central America. Its host range includes coffee (especially *Coffea arabica*) and other woody plants.

Adult beetles emerge from coffee stems about two to three weeks after the onset of the rainy season. They feed on the green bark of shoots, leaf stalks and skin of green coffee berries causing negligible damage (Tapley, 1960, Schoeman *et al*., 1998). The emergence periods of adult beetles in East Africa are coincident with the bimodal rains occurring in March to May and November to January respectively (Tapley, 1960). In Southern Africa, there is a single emergence period between November and April (Schoeman *et al*., 1998, Kutywayo, 2001). According to Schoeman *et al*. (1998), the mean longevity for female and male beetles was 122 and 112 days, respectively. Males actively search for females, which are normally sluggish. Beetles are not good fliers and copulation takes place on the trees after emergence. Beetles mated more than once with copulation duration ranging from one to several hours (Tapley, 1960). There has been no documentation on the mechanisms involved in host selection and mating behaviour and neither is there any information on long-range (> 1 m) or short-range (< 1 m) pheromone communication in *M. leuconotus*.

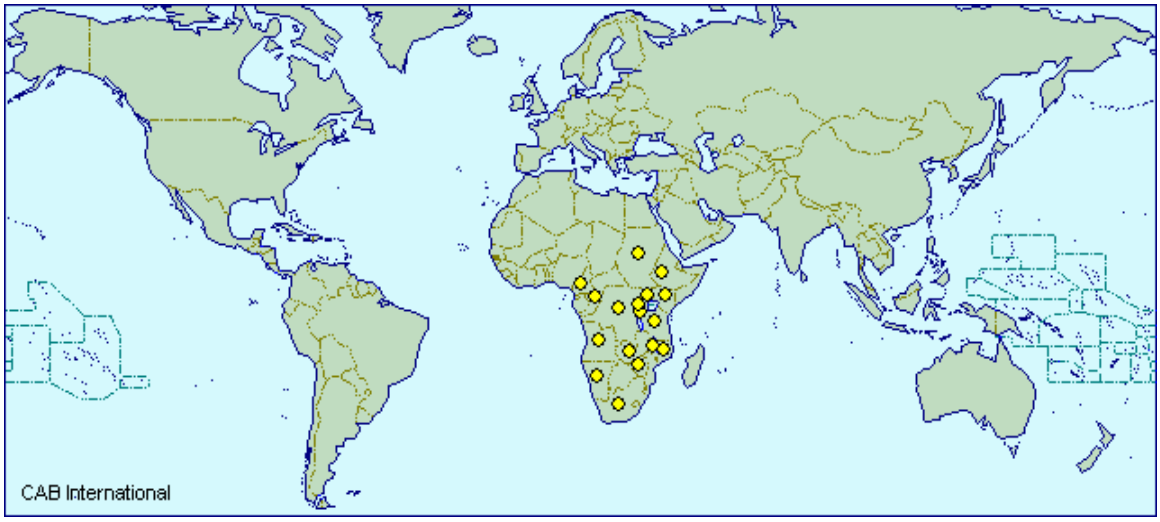


Fig 1.2 World distribution map of *Monochamus leuconotus* (CAB International, 2006). * Yellow circles denote countries where *M. leuconotus* has been reported.

1.2.4 Economic significance

Monochamus leuconotus damage is due to larval feeding activities, which lead to ring barking and wood boring (Le Pelley, 1968). Ring barking normally occurs below ground level and affected plants can be recognised by the yellowing of foliage. Damage by *M. leuconotus* larvae can lead to mortality if infested plants are less than three years old while mature trees can tolerate attack. Tapley (1960) estimated that an infestation of slightly over one borer per tree on 15-year-old coffee trees caused about 8% loss of crop in Tanzania while Schoeman *et al*. (1998) reported losses of approximately 25% over three years in South Africa. In the recent CFC project on the development of management practices of the borer in India, Malawi and Zimbabwe, the incidence of *M. leuconotus* in the smallholder sector in Malawi was 100% compared to more than 70% in Zimbabwe (CAB International, 2008).

1.2.5 Alternative host plants of *M. leuconotus*

A number of wild Rubiaceae have been reported as alternative host plants of the coffee stem borer (Table 1.1). According to Le Pelley, (1968) *M. leuconotus* breeds in all species of *Coffea*. Tapley (1960) mentioned *Coffea arabica, C. liberica, C. eugenoides* and *Lachnastoma khasiana* as the only species of coffee attacked by the borer. He added that there was resistance to the pest in robusta coffee, *Coffea canephora,* due to the splitting of the bark that does not allow successful egg development. Tea, *Camellia*

sinensis, and *Erythroxylum emarginatum* (Smee, 1936, Lee, 1971*)* are the only non-Rubiaceae reported as host plants. *E. emarginatum* is an adult food source.

Table 1.1 Plants reported as alternative host plants of *M. leuconotus*

Host	Family	Country	References
Canthium sp.	Rubiaceae	Kenya	Knight (1939)
Coffea eugenioides	Rubiaceae	Tanzania	Tapley (1960)
Coffea liberica	Rubiaceae	Tanzania	Tapley (1960)
Gardenia urcelliformis	Rubiaceae	Kenya	Knight (1939)
Gardenia sp.	Rubiaceae	South Africa	Schoeman, P.S. (pers. comm.)
Grumilea kirkii	Rubiaceae	Malawi	Lee (1971)
Kraussia floribunda	Rubiaceae	South Africa	Schoeman, P.S. (pers. comm.)
Lachnastoma khasiana	Rubiaceae	Tanzaniae	Tapley (1960)
Oxyanthus speciosus	Rubiaceae	Tanzania	Davies (1937)
Oxyanthus sp.	Rubiaceae	Malawi	Lee (1971)
Pavetta oliveriana	Rubiaceae	Tanzania	Davies (1937)
Randia sp.	Rubiaceae	Tanzania	Davies (1937)
Rytigynia schumanii	Rubiaceae	Tanzania	Davies (1937)
Vangueria linearisepala	Rubiaceae	Kenya	Knight (1939)
Vangueria sp.	Rubiaceae	Tanzania	Davies (1937)
Erythroxylum emarginatum	Erythroxylaceae	Malawi	Smee (1936)
Camellia sinensis	Theaeceae	Malawi	Smee (1936)

1.2.6 Oviposition and larval development of *M. leuconotus*

Oviposition mostly takes place at night (Knight, 1939; Tapley, 1960). Females lay their eggs singly in a slit chewed in the bark of the main stem usually within 50 cm above ground level. The number of eggs which can be laid by a single female varies from 40 to an average of 80.5 (Knight, 1939; Schoeman *et al*., 1998) during the life span. Eggs hatch within 21-23 days. Newly hatched larvae feed on the egg casing and begin to tunnel in the phloem layer, ingesting the phloem and cambium. If eggs are laid within a few centimetres of ground level, larvae burrow directly down the tree to below ground level, where they ring-bark the lateral and main roots. If eggs were laid higher up, they ring bark the tree at that level and do not attempt to reach ground level. The early instar ring-barking phase takes about 14 weeks. When larvae are 3 – 4 months old, they burrow into the wood and remain feeding and producing some frass which protrudes loosely from the entrance. Larvae then pupate in specially prepared pupal chambers at the top of the burrow adjacent to the bark. The later instar boring phase lasts about 42 weeks while the pupal period lasts 4 – 6 weeks.

1.2.7 Current management practices for *M. leuconotus*

Management of *M. leuconotus* was previously based on application of the insecticide dieldrin as a stem paint or drench just before the rains. This was effective against emerging adults and ovipositing females as well as larvae feeding on the treated bark. According to Le Pelley (1968), the wide scale adoption of the dieldrin stem treatment led to a reduction in the incidence of *M. leuconotus* in East Africa. However, ever since the withdrawal of dieldrin in the early 1980s due its environmental persistence, effective chemical control has been lacking due to the short environmental persistence of alternative insecticides. In Zimbabwe, chlorpyrifos was found to be effective and registered as a stem paint (Kutywayo, D., unpublished) while stem paints containing 2% chlordane were tried in South Africa (Schoeman and Pasques, 1993). Schoeman recommended the use of the insecticide dichlorvos as knockdown sprays against adults during the flight period but this is difficult to implement due to the absence of a reliable monitoring tool for adult emergence. In addition, blanket applications of dichlorvos can result in non-target effects on natural enemies, which can in turn lead to outbreaks of secondary pests such as the giant looper, *Ascotis selenaria reciprocaria* (Lepidoptera: Geometridae). Other insecticides have been tried as stem paints but their efficacy is still unconfirmed. For example, trials with stem paints of fipronil and imidacloprid in Malawi

(Chanika pers comm.) and fipronil and thiomethoxam in Zimbabwe (Kutywayo, D. unpublished) have not been conclusive.

Apart from insecticides, physical methods are used by farmers while biological control methods have been investigated. Physical control is based on uprooting and burning of infested plants, piercing larvae within the stems with a wire bicycle spoke, rubbing the stems with a maize cob or stick to expose ring-barking larvae and handpicking of adults. Uprooting and burning is not attractive to smallholder farmers since it results in a reduced plant population per hectare. On the other hand, the other physical control options are laborious. In terms of biological control, laboratory tests with some formulations of *Beauveria bassiana* in South Africa suggested that the fungus was effective against adult beetles and sixth instar larvae (Schoeman and Schoeman, 1997), while larvae treated with Malawian isolates of *Beauveria bassiana* were immobilised within 24 hours and died within 2 to 10 days under laboratory conditions (Kutywayo V. *et al.,* 2006).

1.3. GENERAL REVIEW OF SEMIOCHEMICALS

1.3.1 Semiochemicals

Insects communicate extensively through chemical messages called semiochemicals. Semiochemicals (Gk. semeion, a sign or mark that can be distinguished from others) are chemicals that mediate interactions between organisms (Law and Regnier, 1971). Semiochemicals are either allelochemicals or pheromones depending on whether the interactions are interspecific or intraspecific, respectively (Whittaker and Feeney, 1971). Pheromones are used in communication between members of the same species (intraspecific), while allelochemicals mediate communication between different species (interspecific).

1.3.2 Allelochemicals

Allelochemicals are detected by individuals of a species different from the source species and belong to several groups depending on whether the emitter, receiver or both benefit from the signal. A description of the different groups based on the concept of “signalling” as defined by Nordlund and Lewis (1976) is given below. However, other scholars suggest the use of the term “infochemicals” to exclude toxins and nutrients (Dicke and Sabelis, 1988).

Allomones

Allomones are semiochemicals emitted by a species to its own advantage, for example, when different species occupy the same resource. Nordlund & Lewis (1976) defined them as “chemical substances produced or acquired by an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioural or physiological response that is adaptively favourable to the emitter but not the receiver”. Allomones, such as defensive chemicals, are mostly found in the Coleoptera, Heteroptera, Hymenoptera and even the Lepidoptera. For example, ants produce formic acid to repel predators while several coleopterans also release defensive alkaloids. Some of the defensive chemicals also work as kairomones attracting predators to the insects that will be defending themselves (Blum, 1996).

Kairomones

Kairomones are semiochemicals emitted by a species to its disadvantage. For example, host plant volatiles attract pine sawyer beetle, *Monochamus alternatus* to the plants (Fan *et al*., 2007), while host odours attract tsetse flies, *Glossina* spp. to cattle (Gibson and Torr, 1999). Certain predators such as *Phytoseiulus persimilis* have been reported to use cucumber leaf volatiles in locating the prey, *Tetranychus urticae* (Takabayashi *et al*., 1994) while pheromones of bark beetles, *Ips* spp. can act as kairomones for pine sawyer beetles, *Monochamus* spp. (Allison *et al*., 2004).

Synomones

Synomones are semiochemicals whose signals are favourable to both emitter and receiver e.g. the fragrance of flowers benefits flower, bees, and fragrance of flowers or damaged leaves can attract natural enemies of the pest to the plant (Nordlund and Lewis, 1976). For example, males of the predatory mirid, *Macrolophus caliginosus* exploited host plant volatiles induced by female conspecifics previously feeding on the plants as well as to volatiles from female conspecifics (Moayeri *et al*., 2007).

Other categories of allelochemicals

Some allelochemicals derived from non-living sources mediate interactions between different species. These have been termed apneumones (Nordlund and Lewis, 1976). For example, 2-methyl-2-butanol and hexanal isolated from rabbit faeces attracted female sandfly, *Lutzomyia longipalpis*, for oviposition (Dougherty et al., 1995). These apneumones from the rabbit faeces indicate the presence of a food source for the progeny.

1.3.3 Pheromones

Pheromones (Gk. pherein, to carry; horman, to excite or stimulate) are released by one member of a species to cause a specific interaction with another member of the same species. Karlson and Lüscher, (1959), defined pheromones as "substances which are secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction such as a definite behaviour or development process".

Pheromones are volatile compounds or mixtures that are released in small quantities. For example, calling females of codling moth (*Cydia pomonella*) released pheromone at a rate of several ng/h (Witzgall *et al.,* 2010.) These compounds are detected by members of the same species and affect the behaviour often by functioning as attractants, arrestants, deterrents, stimulants or repellents. Pheromones tend to be very specific, have very notable effects on the target species and in most cases have no effect on other species. However, pheromones produced by one species can be kairomones for another species. As an example, the predator *Elatophilus hebraicus* is closely associated with its prey, the pine blast scale, *Matsucoccus josephi*, and it utilises the sex pheromone of its prey, *M. josephi* and two other *Matsucoccus* spp. as kairomones (Dunkelblum *et al*., 1996).

Pheromones and other semiochemicals are often referred to as "long-range" or "short-range". These are rather subjective terms, but Wyatt (2003) considered that the former were carried over a distance by a current of air or water while the latter were transmitted by diffusion alone, perhaps over no more than a few centimetres. High molecular weight pheromones are often detected by contact and are described as "contact pheromones". However, in that these compounds will have a finite, albeit low, volatility they may also be detected at "short range".

Pheromones fall into several categories related to their function based on the interaction mediated, such as sex, alarm or aggregation pheromones. Pheromones may also be classified as long or short range with long-range pheromones being present in Coleoptera and Lepidoptera.

Sex pheromones

Sex pheromones are chemicals or mixtures of compounds that are secreted by individuals, which produce sexual behavioural responses in members of the opposite sex of the same species. Sex pheromones are the most widespread and widely documented types of pheromones used in increasing the probability of mating success. They are produced by both sexes and have been identified in several hundred species of Lepidoptera (Arn *et al*., 1997, Witzgall *et al*., 2004, El-Sayed, 2012) and other insect orders (Hardie and Minks, 1999). The emitter releases pheromones that are transmitted through the environment to the receiver. The chemical signal then mediates a sexual behavioural response in the receiver.

Ever since Butenandt (1959) first identified sex pheromones for the silkworm moths, *Bombyx mori*, these chemicals have aroused great interest because of their potential as pest control agents.

Aggregation pheromones

These chemicals attract both sexes and generally serve to capitalise on a food source as well as bring together opposite sexes for mating. According to Shorey (1973) and Borden (1977), aggregation pheromones act as indicators of a potentially suitable food source or habitat. Aggregation pheromones are common in many Coleopterans e.g. bark beetles, *Ips* spp. and *Dendroctonus* spp, which are involved in tree attacks. Aggregation pheromones have been successfully used for management of these forest pests.

Alarm pheromones

These pheromones are common in social insects such as ants and bees. Some insect species when attacked by natural enemies, release alarm pheromones, causing avoidance or dispersal behaviour in conspecifics (Hardie *et al*., 1999, Macdonald *et al*.,

2002). For example, the alarm pheromone for many aphids causes dispersal of aphids and acts as a kairomone for natural enemies of aphids (Pickett *et al.*, 1992). In the case of bees, the sting apparatus of a honey bee left in a victim's body releases an alarm pheromone that attracts other bees and stimulates them to sting (Blum, 1969).

Trail pheromones

These are semiochemicals, which govern interactions within organised societies (e.g. Termitidae, Formicidae) (Jutsum and Gordon, 1989). They are used to recruit other insects in a colony to new food sources or to facilitate migration of a colony to a new site.

Epideictic pheromones

These semiochemicals influence interspecific spacing patterns on exhaustible food sources in phytophagous insects. They elicit dispersal away from potentially crowded food sources, thereby reducing competition. They are one of the few pheromones that serve to repel rather than attract. Bark beetles, as well as other Coleoptera, Lepidoptera, Diptera, Homoptera, Orthoptera and Hymenoptera produce epideictic pheromones. Examples are oviposition deterrents and marking pheromones (Poirier and Borden, 1991; Prokopy and Roitberg 2001).Their possible role in insect pest management could be as sprays to repel insects on habitats.

1.3.4 Insect movement in response to stimuli

Dispersal is a normal activity that occurs in insects (Hsiao, 1985) and movements may be directed (taxes) or random (kineses). After leaving a habitat, food finding or host finding behaviour of an insect starts with an orientation, then flying or walking, usually by positive anemotaxis in response to a stimulus. The new habitat will be found with the response to visual or olfactory stimuli. Kineses or taxes will be induced by chemical stimuli in this process (Hsiao, 1985, Kennedy 1986). This behaviour is very common in foraging insects such as aphids, planthoppers and grasshoppers.

Attraction and Repulsion

Attraction is the directed movement (taxis) towards a stimulus source while repulsion is directed movement away from the stimulus. Catches in field traps may result not only from an initial attraction, but also perhaps from random movements (kinesis), with insects reducing speed, turning more frequently and/or stopping on detection of a localized stimulus arrived at by chance (Hardie, 2012). In this thesis attraction means more insects in a trap and more insects in a particular arm of an olfactometer. .

Baits/Lures

A bait/lure is a stimulus that is attractive to a given organism. Thus, as Hardie (2012) observed, strictly speaking, any experimental stimulus used that produces insignificant results cannot be a bait or lure. However, Hardie (2012) did concede that the terms could be used where a commercial company sells a product as a bait or lure. In this thesis experimental stimuli that are being tested as potential attractive stimuli are regarded as baits/lures.

1.4. INSECT DETECTION OF ODOUR SOURCES

Insects generally detect odours by means of olfactory receptors located in the antennae (Visser, 1986; Bernays and Chapman, 1994; Panda and Kush, 1995). Olfactory receptors have also been found on the maxillary and labial palpi of Orthoptera and on some Lepidoptera (Visser, 1986; Bernays and Chapman, 1994) although these are used mostly for contact chemoreception. However, antennae play the dominant role as they are more exposed to airborne cues when insects move or fly upwind towards a source of odour (Gewecke, 1974).

Four types of olfactory sensillae have been described according to size and shape. These are sensilla trichodea, sensilla basiconica, sensilla placodea and sensilla coeloconica (Kaissling 1987). Generally, an olfactory sensillum includes a sensory neurone with dendritic branches that are covered with pores (Fig 1.3). These pores have hairlike cuticular structures. On an antenna, a large number of olfactory sensilla respond to either pheromone or other volatiles. Up to 100,000 receptors on the antennae of some male moths and other insects have been noted to respond to pheromone and fewer sensillae

would be needed for detection of plant volatiles since there would be greater quantities of plant volatiles defusing in the air than pheromones.

Stimulatory volatiles combine with odour-binding proteins which carry them across the lymph to stimulate the dendrite of the receptor neurone. The signal is transferred via axons to the second–order neurones in the antennal lobe via synapses. There the odour signals are conveyed to the central nervous system, which produces all behavioural responses thereafter (Mustaparta, 1984; Visser, 1986; Bernays and Chapman, 1994; Panda and Kush, 1995).

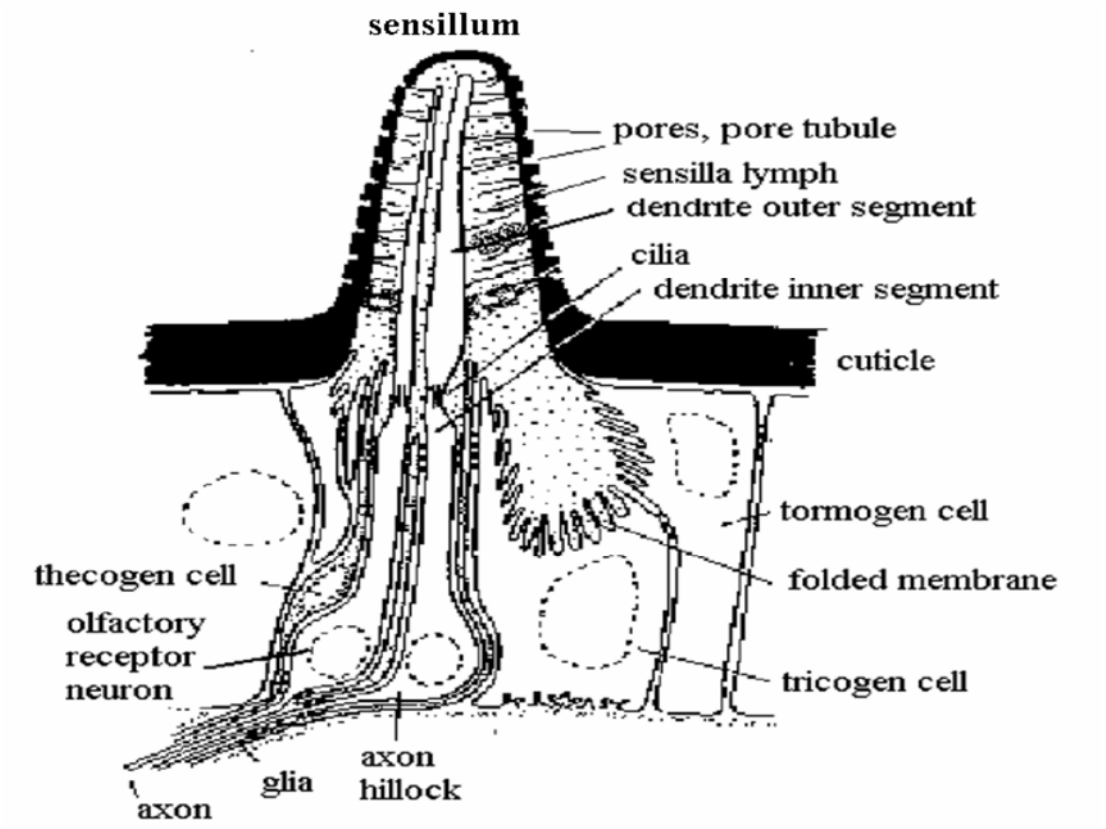


Fig. 1.3 The basic structure of an insect basiconic olfactory sensillum (Kaissling 1987)

1.5. PHEROMONE IDENTIFICATION

1.5.1 Overview of procedure

The stages in the development of a synthetic pheromone have evolved since the identification of the first pheromone from *Bombyx mori* by Butenandt *et al*. (1959). The process usually starts with live baiting in the field to discover the behaviour elicited by the pheromone and the sex that responds to the compound. Volatiles are collected by air entrainment, gland extraction, body-washings or headspace analysis and solid phase microextraction (SPME). Laboratory based bioassays such as electroantennographic detection; olfactometer or wind tunnel studies are conducted to evaluate the responses of the insects to the volatiles. Once isolation of the pheromone has been done, it is identified through techniques such as gas chromatography coupled with mass spectrometry (GC-MS), nuclear magnetic resonance spectroscopy (NMR), and Infrared spectroscopy (IR). Once the structure has been identified, the compound is synthesised and further behavioural bioassays and field tests are conducted to confirm the bioactivity of the compound (Witzgall *et al*., 2010).

1.5.2 Proof of pheromone existence

Live baiting in the field or laboratory-based behavioural bioassays are usually the first steps in the journey to establish the behaviour elicited by the pheromone and to discover which of the two sexes releases the pheromone. The field studies involve observations on the mating system of the insect under natural settings or in cages with the objective of identifying periods when pheromone is released. Host plant materials are incorporated in the field and laboratory bioassays since pheromone signalling may only occur in the presence of a suitable oviposition/food source. Field and laboratory bioassays are important at the initial stages of the studies and in the confirmation of activity of the synthetic compound (Cork and Hall, 1998, Witzgall *et al*., 2010). Laboratory bioassays rely on the establishment of insect cultures and the design of appropriate bioassay procedures to observe the insect. For example, Yun-Tai and Burkholder (1982) were able to establish that female cowpea weevils, *Callosobruchus maculatus*, emitted a pheromone, which excited males and went on to demonstrate that pheromone release began soon after emergence and continued for one week and was in synchrony with calling behaviour.

1.5.3 Collection of chemical

Jones and Oldham (1999) reviewed pheromone analysis and gas chromatographic analysis and concluded that solvent extraction and volatile trapping were the two established techniques for sampling pheromones. Other methods not involving the use of solvents include the Keele solid injection technique (Morgan and Wadhams, 1972; Bagnères and Morgan, 1990) and the Solid-phase Microextraction (SPME) (Malosse *et al*., 1995, Frérot *et al*., 2005). Solvent extraction is done when insect parts such as glands, elytra, ovipositors or whole bodies are washed in organic solvents such as pentane or dichloromethane. Solvent extraction is simple to apply and gives information on the amounts of pheromone components or precursors that are present in the insect at the time of extraction. The major disadvantage with this method is that a large fraction of the extracted chemicals may not be interesting, from an olfactory point of view, since they are non-volatiles, not functioning as olfactory cues. Volatile trapping can be done by either dynamic headspace analysis or closed-loop stripping.

Dynamic headspace analysis involves the collection of air-borne pheromones from live insects either by trapping on an adsorbent such as activated charcoal or a porous polymeric phase such as Porapak-Q, or by cryogenic trapping (Jones and Oldham 1999). In closed-loop stripping an adsorbent removes the pheromones from the air, which is constantly circulated through an entrainment chamber containing the insect. Solventless injection such as the Keele injection technique involves the placement of the sample in a sealed soft glass capillary and volatiles are then released when the material is crushed inside the hot injection port of GC injection system. This method allows samples to be collected in the field for later extraction in the laboratory. SPME consists of a fibre coated with an adsorbent that can extract organic compounds from an aqueous solution, from the atmosphere or surface of a biological material. The adsorbed compounds are desorbed upon exposure of the SPME fibre in the heated injector port of the GC system (Agelopoulos *et al.*, 2000; Frérot *et al*., 2005)

1.5.4 Pheromone Identification

Separation

The collected pheromone has to be separated from the solvent or adsorbent material before identification. Separation is normally done by distillation or through chromatography, in which compounds are separated by differential partition between a mobile and a stationary phase. Different methods such as thin layer chromatography

(TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC) have been used in the separation of pheromones. TLC has commonly been employed for preliminary separations of multi-gram amounts of material from large-scale whole body extractions while GC provides higher resolution and can be used to separate small quantities from picograms to milligrams (Attygalle and Morgan, 1988). In the case of HPLC, though it provides a resolution as good as that of GC, it is particularly useful for analysis of compounds that cannot be gas chromatographed due to their involatility, polarity and/or thermal instability. HPLC is non-destructive and useful on a preparative scale with samples up to 5 mg on analytical column and from 50 mg to 50 g on wide-bore preparative columns.

Bioassays

Bioassays are important in monitoring the separation process in order to ensure that the active compounds can be correctly identified. In order to find behaviourally-active compounds the electroantennogram (EAG) assay is a widely used method, especially in pheromone research (Roelofs, 1984). By stimulating an antenna with a volatile compound the olfactory receptor potential, as a result of receptor membrane depolarisation, can be measured. Compounds eliciting a potential larger than the spontaneous antennal activity are considered to be electrophysiologically active. The EAG method is conclusive since a compound not eliciting an electrophysiological response can be excluded as an olfactory cue, if olfaction is mediated by receptors on the antenna only. It can, however, be a poor indicator of behavioural responsiveness since depolarisation depends on the number of neurones stimulated and does not distinguish between inhibitory or synergistic effects of odours. With GC, it is possible to couple the separation and biological testing with EAG allowing for the simultaneous electrophysiological testing of each separated compound (Moorhouse *et al*., 1969; Arn *et al*., 1975; Cork *et al*., 1990; Witzgall *et al*., 2010). However, behavioural bioassays are still required since EAG cannot predict the reaction of the insect to the stimulus.

There are many different types of walking bioassay from still-air arenas, with pheromone at the centre to Y-tube olfactometers with pheromone laden air flowing down one side, clean air down the other and more elaborate four-arm olfactometers (Baker and Cardé, 1984). Bait chambers containing calling insects, pheromone extracts or other olfactory cues are connected at either ends of the Y-tube. Air is blown over the bait chambers by means of a fan or pump. The behaviour of insects released at the main arm is observed. Insects in the main arm responding to the odour orientate towards the bait chamber and

those that have reached a certain predetermined position at the end of a certain time period are counted. The olfactometer may be set up with two or more identical series of chambers, one of which acts as a control.

Wind tunnels are the other main type of instruments used in behavioural bioassays (Baker and Linn, 1984). Field conditions can be simulated in wind tunnels, which are closed systems with a constant flow of air where wind velocity, humidity, temperature and other parameters can be modified. Insects flying upwind can easily be observed and their behaviour when approaching a pheromone dispenser mounted inside the chamber can be studied. Wind tunnels are useful for pheromone screening, testing of synthetic dispensers and for verification of analytical results.

Analysis and identification

After detection of the active compounds, the chemicals have to be identified using either physical (spectroscopic) or chemical methods. The most common analytical methods are gas chromatography (GC), gas chromatography–mass spectrometry (GC-MS), infra-red spectroscopy (IR), ultraviolet spectroscopy (UV), nuclear magnetic resonance (NMR) and chemical micro-derivatisation techniques (Howse et al., 1998). GC and GC-MS are the most common analytical techniques used in insect semiochemical identification owing to their high sensitivity, which complements the amounts collected using air entrainment. GC-MS provides information on the molecular mass, elemental composition and the structure of the compound. The data is normally acquired and stored on a computer, which allows detailed analysis and computer matching of unknowns against a mass spectral library also held on a computer.

An efficient method of targeting sex-specific pheromones is through the comparison of male and female volatile collections using GC-MS. For example, Hall *et al*., (2006) identified the male–specific pheromone of *Xylotrechus quadripes* after initially comparing mass chromatograms of the volatile collections from male and female beetles. Subsequent field tests confirmed the activity of the male sex pheromone.

1.5.5 Pheromone synthesis in the laboratory

Synthesis of pheromones is done once activity has been confirmed through either laboratory or field studies. The synthetic pathway followed depends on the structure of the compound as identified through GC-MS or GC-EAD. Since pheromones of insects of the same family often have similar structures, synthetic chemists find it easier when dealing with pheromones involving the same family in the Insecta. However, in cases where the existence of a pheromone has not been documented, the process is more challenging since chemists have to develop the synthetic pathways. In such cases, microreactions followed by GC/MS-investigations of the reaction products may provide additional information on the chemical structures of target compounds (Francke and Dettner, 2005, Witzgall *et al*., 2010).

1.5.6 Pheromone dispensers

In order to release pheromones in a manner that simulates natural conditions, there is a need to store, protect and moderate release. In the simplest form, carriers can be polyethylene sachets used to release volatile compounds at very high rates (Torr *et al*., 1997) and polyethylene vials (Hall and Marrs, 1989) that have been adapted for controlled release of less volatile compounds associated with lepidopterous sex pheromones. Others are the natural rubber septum (Roelofs *et al*., 1972), sprayable and nonsprayable microcapsules, twist-ties, plastic laminates, cigarette filters (Hall and Marrs, 1989) and polyvinyl chloride (PVC) and polyvinyl acetate (PVA) based monolithic polymers (Cork *et al*., 1989).

1.5.7 Pheromone trap design

Good trap design is central to the effective utilisation of pheromone-based systems for monitoring and control of insect pests. An effective pheromone lure can attract responsive adult insects to the vicinity of a trap but entry and retention of insects is dependent on the trap design. Many trap designs have been developed over the years and each has advantages and disadvantages over others for catching members of different insect families, working in different environments and meeting the needs of the different users. Commonly used designs include water traps, pit traps, funnel traps, delta and sticky traps such as the sticky disc, cross vane and panel traps.

An important criterion of selection is to have a trap that will not affect plume structure of the lure since it influences the way in which insects approach the trap and ultimately affects capture efficiency. Trap placement also affects the manner in which insects approach the trap. For example, Mason *et al*. (1997) caught more European corn borer, *Ostrinia nubilalis*, when traps were located 0.1 m below the top of the plant canopy than when located 0.5 m above. Similarly, tree crop pests are most frequently trapped within the canopy (Bartlet *et al*., 1994) although David and Horsburgh (1989) caught the highest number of leaf roller, *Platynota flavedana,* outside the apple tree canopy while sibling species of *P. idaeusalis* were caught inside the canopy. Such findings suggest optimum trap location will vary from species to species and perhaps even between generations of a single species (David and Horsburgh, 1989).

Traps with large surface areas normally have higher catches. For example, Rothschild and Minks (1977) obtained higher catches of *Cydia molesta* in traps with larger surface areas. Other important factors in the design of the trap are its ability to retain captured insects, its cheapness and durability.

1.5.8 Field testing of pheromones

For all field trials whether they are comparing pheromone blends, dispensers or trap designs each treatment should be replicated (minimum of three times). Trap positions should be changed so that each treatment occupies each trap site an equal number of times and over as short a time as possible. This would ensure that the insect population sampled remained relatively constant for the duration of the trial. Ideally, traps should be moved daily to randomise their positions. According to Cardé (1984), the design of pheromone field experiments should be such as to “minimise variation in trap catch caused by differences in population density in different areas of the test site and as well as minimise or eliminate interactions between different treatments”. Mead and Curnow (1983) recommended the Latin square design as the best but the randomised block design is also popular (e.g. Branco *et al*., 2004; Meagher and Mitchell, 1999).

1.5.9 Mixtures of host plant volatiles and pheromones

Interactions among the various constituents of an odour blend have been reported to be responsible for host plant selection in many insects and the effects may be additive, i.e. the sum of the activities of the individual components, or synergistic, i.e. the overall effect is greater than the sum of the activities of the individual components.

Linalool and farnesene when combined with a male specific pheromone of *T. fuscum* increased catches of females (Silk *et al.,* 2010). A mixture of linalool and a green leaf volatile, (*Z*)-3-hexenol, increased the responses of pheromone olfactory receptor neurons of the male *H zea* moth to (*Z*)-11-hexadecenal, the main pheromone component of the female sex pheromone (Akhtar and Isman, 2013). Binary mixtures of (±) linalool, (*E*)-ß-farnesene or (*Z*)-3-hexenol with codlemone enhanced the attraction of male *C. pomonella* moth to codlemone in a wind tunnel. A mixture of three green leaf volatiles, (*Z*)-3-hexen-1-ol, (*E*)-2-hexenal, (*Z*)-3-hexen-1-yl-acetate with benzonitrile and benzaldehyde was significantly more attractive to female oriental fruit moth, *C. molesta* than either of the compounds or the blend of three green leaf volatiles alone. A blend of methyl-salicylate, linalool and (*Z*)-3-hexenyl acetate attracted more female *Leptinotarsa decemlineata* than males. Addition of an aggregation pheromone increased the attraction of both males and females (Li *et al.,* 2010). A study on *Atrichelaphinis tigrina* beetles showed the greatest responses from combinations of aromatic compounds (anisole, methyl benzoate, methyl salicylate, benzaldehyde). Traps containing binary mixtures of phenylacetyldehyde and the floral odorants cis -jasmone, linalool, benzyl acetate, limonene, β -myrcene, methyl salicylate, and methyl 2-methoxybenzoate increased captures of several moth species (Akhtar and Isman, 2013).

1.6. USES OF PHEROMONES IN INSECT PEST MANAGEMENT

Once identification and synthesis of pheromones and other semiochemicals has been done it is possible to use the synthetic chemicals in management of insect pests. Sex and aggregation pheromones have been investigated intensively in terms of chemistry, behavioural activity and application in manipulation of insect behaviour. Pheromones can be used in insect pest management because they are species-specific, highly biologically active and effective at very low concentrations and, in addition, are not toxic to plants and animals. No evidence of insect tolerance or resistance to the use of pheromones has been documented.

The four main uses of pheromones in insect pest management are monitoring, mass trapping, mating disruption and lure-and-kill (Witzgall *et al.,* 2010). However, the selectivity of pheromones is a disadvantage where several pest species coexist. Moreover, they may be expensive to manufacture and the optimal blend of pheromone components may be difficult to obtain or dispense. Many pheromone components are unstable and decompose in the presence of light and air. To overcome this, a variety of formulations and dispensers has been developed.

1.6.1 Monitoring

The use of sex pheromones as attractants in traps is one of the oldest practical applications of semiochemicals in pest management. Presently either sex or aggregation pheromones may be used to monitor insect activity and obtain important information on the relative density of insects. Such information can be useful in making management decisions and predictions on likely outbreaks of the pests and also timing for application of conventional pesticides.

Monitoring involves catching the pests with pheromone-baited traps and counting them at set intervals. Traps of many varieties have been developed for different pests and most involve some form of adhesive surface, water or dry funnel as the trapping medium. However, much work needs to be done to interpret catches in pheromone traps for a number of reasons such as the fact that pheromone traps generally attract adults whereas it is often the larval stage that does the damage. Furthermore in many instances, male insects are attracted when it is the female that lays the eggs producing the next generation of larvae (Witzgall *et al*., 2010).

Some of the most extensive uses of pheromones for making pest management decisions have occurred in apple orchards to monitor the codling moth, *Cydia pomonella* (Kehat *et al.*, 1994; Knight, 2000); the cotton leaf worm, *Helicoverpa* in Egypt (Downham *et al.,* 1995) and California red scale, *Aonidiella aurantii* in citrus (Samways, (1988).

Pheromone traps are particularly valuable for detection because they can detect presence when pest numbers are very low. Monitoring for simple detection and quarantine has been done for the larger grain borer, *Prostephanus truncatus* in East and Southern Africa as well as in general grain storage (Burkholder and Ma, 1985, Mullen & Dowdy, 2000; Phillips, 1997). Pheromone traps have been used to monitor insect population movements of army worm, *Spodoptera exempta* in East Africa (Dewhurst, 1993) and the pink bollworm, *Pectinophora gossypiella* (Shivanna *et al*., 2012).

1.6.2 Mass trapping

The concept of mass trapping uses species-specific synthetic chemical lures, such as sex and aggregation pheromones and food/host attractants, to attract insects to a trap where they would be confined and die. The rationale behind mass trapping is to concentrate pest insects into a restricted space where they are killed easily and cheaply and with less environmental impact than, say, widespread application of conventional insecticides. Mass trapping involves deploying pheromone traps at a much higher density than they would be for monitoring purposes. It is especially applicable where the pest population is usually widely dispersed, where control by conventional pesticides is inapplicable, where resistance has developed to conventional insecticides and no other form of control is available or, most importantly, where it can be an economic form of pest control (Jutsum and Gordon, 1989).

When mass trapping is compared with mating disruption and lure-and-kill as approaches in pest management, it is the second mostly used after mating disruption. Mass trapping has been used more against coleopteran, dipteran, and homopteran species whereas lure and kill approaches have been evaluated more often against dipteran and coleopteran species (El-Sayed *et al*., 2006). This could be related to the fact that most coleopterans and dipterans tend to aggregate making them more suitable for mass trapping. In addition, this could be due to the occurrence of economically significant insect pests in those orders.

Mass trapping has been attempted for a variety of agricultural, orchard, and forest pests on scales ranging from a few to thousands of hectares. Examples on the successful use of mass trapping as a pest management approach include the control of *Spodoptera litura* in glasshouse vegetables in Japan (Takai and Wakamura 1990) and *Conopomorpha cramerella* in cocoa in East Malaysia (Beevor *et al*., 1993). Other examples are the campaign against *Ips typographus* carried out in Scandinavian forests during 1979 -1983 (Bakke and Lie, 1989), and against the pine beetles, *Dendroctonus* sp. in the forests of the USA and Canada (Smith, 1998). According to El-Sayed *et al* (2006) mass trapping has good potential to suppress or eradicate low-density, isolated pest populations

1.6.3 Lure-and-kill

In this approach, the target insect is attracted to a source of insecticide rather than to a mechanical trap. The advantage over mass trapping is that many more attractive sources can be employed, while the advantage over mating disruption methods is that less pheromone is used (Hall, 1995). De Souza *et al*. (1991) reported some promising results against the cotton leaf worm, *Spodoptera littoralis*, in Egypt by applying the lure and kill technique although Downham *et al*. (1995) showed that it was not a viable method for the control of this species. Perhaps the most successful use of the lure and kill approach is the use of odour baited traps to control tsetse fly (Torr *et al*., 1997). Suckling and Brockerhoff (1999) also worked with a lure and kill system, targeting light brown apple moth. They showed 50% reduction in trap catches as long as lure and kill traps, baited with pheromone and insecticide, were present, but the effect disappeared when the pheromone was removed.

1.6.4 Mating disruption

Mating disruption that seeks to disorient or misdirect insects searching for mates has been the most successful direct control approach principally targeting moths (Cardé and Minks 1995; Suckling 2000). Mating disruption involves the deployment of pheromone formulations with high release rates to prevent mating by affecting the attractiveness of individual females. Using this technique successful mating disruption of pink bollworm, *Pectinophora gossypiell*a was achieved in Egypt using different types of slow release formulations impregnated with sex pheromones (Campion *et al*., 1989). This system is also widely used against many lepidopteran orchard pests including codling moth (Gut and Brunner, 1998) and oriental fruit moth (Il'ichev *et al*., 2004). The mating disruption technique is common in high value crops, where insecticide usage is problematic due to the development of resistance, or is undesirable because of the risk of human poisoning during consumption.

1.7. CHEMICAL ECOLOGY OF CERAMBYCIDS

Chemical ecology is the science that seeks to understand "the origin, function, and significance of natural chemicals that mediate interactions within and between organisms" (Adams *et al*., 1984). These natural chemicals are generally classified as semiochemicals and could be host volatiles, or pheromones. Host plant volatiles and pheromones play an important role in the chemical ecology of Coleoptera in general. For example, the pine bark beetles (Coleoptera: Scolytidae) use host odours to locate suitable habitats (Byers, 1995).

The majority of studies on the chemical ecology of Cerambycids have only been reported during the last 10 -15 years and have been reviewed (Hanks, 1999, Allison *et al.*, 2004). Cerambycids are divided into 13 subfamilies (ITIS, 2002). The most common subfamilies are Anoplodermatinae, Aseminae, Cerambycinae, Lamiinae, Lepturinae, Prioninae, Spondlylidinae and Vesperinae. Beetles belonging to the family Cerambycidae are important pests in agriculture and forestry plantations. The damage caused to trees can be due to the feeding habits of the larvae that ring bark, girdle or bore the main stems leading to tree mortality. In addition, the adults can bore the stems in search of oviposition sites or can act as vectors of nematodes that transmit plant diseases. Cerambycids of economic importance include the Asian long horned beetle, Eucalyptus borer, the sugar cane borer and the Japanese pine sawyer beetle (Table 1.2). Most of the economically important cerambycids belong to the Cerambycinae and Lamiinae subfamilies and they cause damage to commodities such as timber, forests and coffee.

Some species of *Monochamus* are woodborers in North America, Asia and Europe causing significant economic wood damage (Gardiner, 1975). In addition, they are the vectors of *Bursaphelenchus xylophilus*, the causative agent of pine wilt disease. This nematode causes severe mortality in exotic pines in North America and native pines in Southeast Asia and Japan (Dwinell, 1997). Examples of the pine nematode vectors include *Monochamus alternatus, M. carolinensis, M. clamator, M. galloprovinciallis, M. notatus, M. obtusus, M. saltuaris, M. scutellatus* and *M. titillator*. The nematode larvae emerge from the spiracles of the adult beetle, drop onto the twigs and penetrate the woody tissue through the feeding wounds (Speight and Wainhouse 1989). The nematodes then mature and feed upon parenchyma cells of trees resulting in wilt symptoms and quick death of the tree (Dwinell, 1997).

Table 1.2 Examples of major Cerambycid pests (CAB International, 2007).

Pest	**Scientific name**	**Crop(s) attacked**	**Sub-family**	**Tribe**
Sugar cane borer	*Migdolus fryanus*	Sugar cane	Anoploderminae	Anoplodermini
Cryptomeria twig borer	*Anaglyptus subfasciatus*	Cryptomeria	Cerambycinae	Anaglyptini
Old house borer	*Hylotrupes bajulus*	Timber	Cerambycinae	Callidiini
Eucalyptus borer	*Phoracantha semipunctata*	Eucalyptus	Cerambycinae	Phoracanthini
Oak bark borer	*Semanotus japonicus*	Oak	Cerambycinae	Callidiini
Asian Coffee white stem borer	*Xylotrechus quadripes*	Coffee	Cerambycinae	Clytini
Asian long-horned beetle	*Anoplophora glabripennis*	Pine	Lamiinae	Monochamini
Citrus borer	*Anoplophora malasiaca*	Citrus	Lamiinae	Monochamini
Yellow headed borer	*Dirphya nigricornis*	Coffee	Lamiinae	Phytoecini
Japanese pine sawyer beetle	*Monochamus alternatus*	Pine	Lamiiinae	Monochamini
African coffee white stem borer	*Monochamus leuconotus*	Coffee	Lamiinae	Monochamini
Yellow spotted longicorn beetle	*Psacothea hilaris*	Mulberry	Lamiinae	Monochamini

All species of *Monochamus* feeding on conifers have the same general attack sequence on dead and dying trees. Hanks, (1999) classified *M. alternatus, M. carolinensis and M. scutellatus* as insects attacking stressed hosts. Beetles are attracted to the trees by host volatiles and/ or bark beetle pheromones. For example, *M. titillator* (Miller & Asaro, 2005), *M. clamator, M. scutellatus, M*. *notatus* or *M. obtusus* (Allison *et al.* 2001; Allison *et al*., 2003; De Groot & Nott, 2004, Costello *et al*.; 2008; Macias-Samano *et al*., 2012; Hanks & Millar, 2012) and *M. galloprovincialis* (Pajares *et al*., 2004; Ibeas *et al*., 2008) have all been observed to respond to host and bark beetle semiochemicals. Both sexes can be found on logs during the day and night and mating takes place on the log surface (Rose, 1957). After mating females lay eggs singly in scars excavated in the bark surface. Larvae moult through four larval instars emerging the following spring from a pupal chamber just under the bark surface. Adults feed mostly on bark until they are mature for mating.

1.7.1 Host plant kairomones

Work on the response of cerambycids to host volatiles has concentrated on economically important pests. In general, adult cerambycids use host volatiles in host finding and mate location (Schlyter and Biggerson, 1999, Allison *et al.,* 2004). According to El-Sayed (2012), more than 55 cerambycid species use host volatiles in their communication systems. Most of these volatiles are attractants with a few oviposition deterrents, oviposition stimulants and repellents. Ethanol and monoterpenes are involved in primary attraction to the host plants as kairomones and these affect mainly the Cerambycinae and Lamiinae attacking coniferous forests. Beetles respond to ethanol alone (e.g. Montgomery and Wargo, 1983; Dunn and Porter, 1991, Hanks & Millar, 2012) or in combination with the monoterpene, α-pinene (e.g. Schröder and Weslein, 1994, McIntosh *et al.* 2001; Sweeney *et al.*, 2004; Brockerhoff *et al.,* 2006; Miller, 2006; Costello *et al*., 2008, Hanks *et al*., 2012). The synergism between ethanol and α-pinene as attractants has led to commercialisation of lures for monitoring many cerambycid species. Other attractants include the floral volatiles methyl phenylacetate (Nakashima *et al.,* 1994), linalool, benzyl acetate and phenyl propionate (e.g. Ikeda *et al.,* 1993), several monoterpenes (e.g. Ikeda *et al.,* 1980, Chenier and Philogène, 1989) and oxygenated terpenes, (+)-juniperol and (+)-pimaral (Yamasaki *et al.*, 1997). Li and Zhang (2006) implicated monoterpenes as oviposition deterrents in *Monochamus alternatus* while oviposition stimulation in the same species was attributed to proanthocyanidins such as the flavonol glucoside, (-)-2,3-trans-dihydroquercetin-3´-O-beta-D-glucopyranosole, some glycosides (Sato *et al.,* 1999a, 1999b) and D-catechin (Islam *et al*., 1997).

There have also been reports of plant volatiles repelling cerambycid beetles. Hua *et al.* (1999) reported that organic extracts of branches and leaves of *Populus opera* repelled the woodborer, *A. glabripennis,* under laboratory conditions while Yan and Tan (1998) found that essential oils from *Eucalyptus* spp. repelled three species of sawyers, *Apriona germarii, Psacothea hilaris* and *M. alternatus*. Oils from inner bark and sapwood of resistant cultivars of Japanese cedar act as repellents to the bark borer, *S. japonicus,* (Yatagai *et al*., (2002). The repellents identified were α-terpineol, nerolidol, delta-cadinene, beta-eudesmol, terpinolene and cedrol.

1.7.2 Bark beetle and boll weevil kairomones

Cerambycids have been reported to respond kairomonally to bark beetle pheromones in conifer plantations, presumably to locate trees weakened by attack by bark beetles. For example, four species (*Monochamus clamator, M. obtatus, M. notatus* and *M. scutellatus*) were attracted to combinations of host volatiles and bark beetle pheromones (Table 1.3) suggesting that the pheromones could be acting as kairomones (Allison *et al*., 2001; 2004) to the cerambycids. Other species reported to respond to bark beetle aggregation pheromones are *M. galloprovincialis* (Pajares *et al.,* 2004; Ibeas *et al.,* 2007, Pajares *et al*., 2010), *M. mutator* (De Groot and Nott, 2004), *M. tittilator* (Miller and Asaro, 2005). The response of *M. alternatus* to bark beetle pheromones has not been tested (Teale *et al.*, 2011).

In most cases the bark beetle pheromones appear to synergise host volatiles. The soya borer, *Dectes texanus texanus* was attracted to boll weevil aggregation pheromones (Patrick, 1974). This kairomonal response to bark beetle pheromones was initially reported to be limited to the Lamiinae only but has now been documented for other subfamilies of the Cerambycidae (e.g. Lepturinae, Spondylidinae, Cerambycinae, Prioninae (Costello *et al*., 2008).

Table 1.3 Responses of Cerambycid beetles to bark beetle and boll weevil pheromones

Beetle species	Host	Response	Chemical	Reference
Acanthocinus obliquus	Pine	Attraction	ipsenol, ipsdienol	Costello *et al*. (2008)
A. spectabilis				
Acmaeops proteus				
Anastrangalia sanguinea				
Arhopalus asperatus				
A. productus				
Asemum striatum				
Cosmosalia chrysocoma				
Dectes texanus texanus	Soya	Attraction	cis-2-Isopropenyl-1-methylcyclobutaneethanol (Grandlure I), (E)-(3,3-Dimethyl)-cyclohexylideneacetaldehyde (Grandlure IV), (Z)-(3,3-Dimethyl)-cyclohexylideneacetaldehyde (Grandlure III), (Z)-2-(3,3-Dimethyl)-cyclohexylideneethanol (Grandlure II)	Patrick (1974)
Monochamus clamator	Conifers	Attraction	frontalin, ipsdienol, ipsenol, seudenone	Allison *et al*. (2001)
	Pine		ipsenol, ipsdienol	Costello *et al*. (2008)
M. galloprovincialis	Conifers	Attraction	ipsenol , 2-methyl-3-buten-2-ol	Ibeas *et al.* (2007)
			ipsenol	Pajares *et al.* (2004)

Table 1.3 Responses of Cerambycid beetles to bark beetle and boll weevil pheromones (cont.)

Beetle species	Host	Response	Chemical	Reference
Monochamus mutator	Conifers	Attraction	ipsdienol, frontalin	De Groot and Nott (2004)
M. notatus	Conifers	Attraction	ipsenol	De Groot and Nott (2004)
			frontalin, ipsdienol, ipsenol, seudenone	Allison *et al*. (2001)
M. obtusus	Conifers	Attraction	frontalin, ipsdienol, ipsenol, seudenone	Allison *et al*. (2001)
M. scutellus	Conifers	Attraction	ipsenol	De Groot and Nott (2004)
			frontalin, ipsdienol, ipsenol, seudenone	Allison *et al*. (2001)
M. tittilator	Conifers	Attraction	ipsenol, ipsdienol, lanierone	Miller and Asaro (2005)
Neoclytus muricatulus	Conifers	Attraction	ipsenol, ipsdienol	Costello *et al*. (2008)
Pogonocherus pictus				
Pygoleptura nigrella				
Rhagium inquisitor				
Spondlyis upiformis				
Stictoleptura canadensis				
Tetropium cinnamopterum				
Tragosoma depsaris				
Xylotrechus longitarsus				

1.7.3 Pheromones

Cerambycid species for which pheromones have been shown to be produced and their structures identified are shown in Tables 1.4 and 1.5 together with the names of the pheromones. They can be classified as long-range pheromones and contact pheromones. Long-range pheromones are relatively volatile and carried significant distances by air currents (Wyatt, 2003). Contact pheromones are relatively involatile and detected by contact or possibly at very close range by diffusion of the chemical (Wyatt, 2003). Studies have focused on species which are important pests of agriculture and forestry (Table 1.2).

Long-range pheromones

The majority of volatile cerambycid pheromones are male-produced. Twenty-four species have been confirmed to produce male-specific pheromones, 14 of which are in the Cerambycinae with the remainder in the Lamiinae (6) and Spondylidinae (4) subfamilies respectively. Most of these attract both male and female beetles, making them confirmed aggregation pheromones (Table 1.4). Female sex pheromones have been identified for nine cerambycid species in the Anoplodermatinae (e.g. *Migdolus fryanus*) Vesperinae (e.g. *Vesperus xartati*), Lepturinae (e.g. *Ortholeptura valida*) and Prioninae (e.g. *Prionus* spp.) (Table 1.4).

In terms of chemical structure, the male-specific volatile pheromones are mostly short-chain, unbranched hydroxyketones or diols with 6 to 10 carbons and hydroxyl and carbonyl groups at C2 and C3. This structure is common in the Cerambycinae. There are some exceptions notably *Rosalia funebris* where the males produce long-chain esters (Ray *et al.,* 2009) and *Hedypathes betulinus*., which produces acetates (Fonseca *et al*., 2010) Among the Lamiinae, several alkyl ethers have been shown to be components of male-produced aggregation pheromones, as in *Monochamus galloprovincialis* (Pajares *et al*., 2010). Terpenoid alcohols and acetates have been shown to be components of the male-produced aggregation pheromones of species in the Spondylidinae and Lamiinae, as in *Tetropium fuscum* (Silk *et al*., 2007).

However, in contrast to the acetogenin diol/hydroxyketone/diketone structure and the branched esters, female-specific long-range pheromones are diverse. Females of *Vesperus xatarti* produce monoterpenes while *Migdolus fryanus* produce amides and *Desmocerus californicus* produces lactones, which are a distinct structure in cerambycid pheromones (Table 1.4). Females of *Prionus* spp. produce dimethyldodecanoic acids

while the lepturine, *Ortholeptura valida* has its own acetate structure. These volatile compounds can also be regarded as being long-range pheromones acting at distances more than a metre from the source.

Many closely related species share the same pheromone chemistry motifs and this is useful for practical purposes since multiple species can be attracted to the same lure (e.g. Hanks and Millar, 2012; Allison *et al*., 2012). However, they cannot be attracted to the wrong species due to separation in terms of time of release of pheromone under natural conditions.

Contact pheromones

Contact pheromones of the Cerambycidae are mostly female-produced long chain and branched hydrocarbons, which have been reported in 10 species (Table 1.5). They are relatively involatile and detected when an insect gets into contact with its conspecific or possibly at very short distances from the source. These are cuticular hydrocarbon components mediating intraspecific sexual communication. Cuticular hydrocarbon extracts contained olefins (e.g. (*Z*)-21-methyl-8-pentatriacontene, 9-pentacosyne, 9-heptacosyne, (Z)-9-pentacosene, (Z)-9-heptacosene, 11-methyl-heptacosene), which elicited copulatory behaviour in males (Ginzel *et al*., 2003, 2006; Rutledge *et al*., 2009; Silk *et al*., 2011).

Table 1.4 Long-range pheromones identified in the Cerambycidae

Subfamily	Beetle species	Response	Chemical	Reference
Cerambycinae	*Anaglyptus subfasciatus*	male-produced aggregation pheromone	(3*R*)-3-Hydroxyhexan-2-one , (3*R*)-3-Hydroxyoctan-2-one	Nakamuta *et al.* (1997)
Cerambycinae	*Anelaphus inflaticollis*	male-produced aggregation pheromone	(3*R*)-3-hydroxyhexan-2-one, (3*S*)-2-hydroxyhexan-3-one, 2,3-hexanedione, (2*R*,3*S*)-2,3-hexanediol, (2*R*,3*R*)- 2,3-hexanediol	Ray *et. al.* (2009).
Cerambycinae	*Curius dentatus*	male-produced aggregation pheromone	(2S,3S)-2,3-Hexanediol, (2R,3R)-2,3-Hexanediol, (2R,3S)-2,3-Hexanediol, (2S,3R)-2,3-Hexanediol	Lacey *et al.* (2004)
Cerambycinae	*Hylotrupes bajulus*	male-produced pheromone	(3*R*)-3-Hydroxyhexan-2-one, (3*R*)-3-Hydroxyhexan-2-one, 2-Hydroxyhexan-3-one, (2*R*,3*R*)-2,3-Hexanediol, (2*S*,3*R*)-2,3-Hexanediol, Hexane-2,3-dione	Reddy *et al.* (2005); Fettköther *et al.* (1995)
Cerambycinae	*Neoclytus acuminatus acuminatus*	male-produced aggregation pheromone	(2*S,*3S)-hexanediol, (2*R,*3*R)*-hexanediol	Lacey *et al.* (2004)
Cerambycinae	*Neoclytus modestus modestus*	male-produced aggregation pheromone	2-Hydroxyhexan-3-one, 3-hydroxyhexan-2-one	Hanks *et al.* (2007)
Cerambycinae	*Neoclytus mucronatus mucronatus*	male-produced aggregation pheromone	*(*3*R*)-3-hydroxyhexan-2-one	Hanks *et al.* (2007), Lacey *et al.* (2007)

Table 1.4 Long-range pheromones identified in the Cerambycidae (cont.)

Subfamily	Beetle species	Response	Chemical	Reference
Cerambycinae	*Phymatodes decussatus decussatus*	male-produced pheromone	2-hydroxyhexan-3-one , 3-hydroxyhexan-2-one	Hanks *et al.* (2007
Cerambycinae	*Phymatodes lecontei lecontei*	male-produced pheromone	2-hydroxyhexan-3-one, 3-hydroxyhexan-2-one, (*R*)-2-methylbutan-1-ol, *(*3*R*)-3-hydroxyhexan-2-one, (3*S*)-3-hydroxyhexan-2-one, 2-methylbutan-1-ol	Hanks *et al.* (2007)
Cerambycinae	*Rosalia funebris*	male-produced-pheromone	(*Z*)-3-decenyl (*E*)-2-hexenoate, (*Z*)-3-decenol, (*Z*)-3-nonenyl (*E*)-2-hexenoate, (*Z*)-3-decenyl (*E*)-3-hexenoate	Ray *et al*. (2009)
Cerambycinae	*Sarosethes fulminans*	male-produced-pheromone	(3*R*)-3-hydroxyhexan-2-one, (2*S*,3*R*)-2,3-hexanediol	Lacey *et al*. (2009)
Cerambycinae	*Xylotrechus chinensis*	male-produced-pheromone	2,3-Octandiol, 2-Hydroxyoctan-3-one, 3-Hydroxyoctan-2-one (2*S*,3*S*)-2,3-Octanediol, 2-Hydroxyoctan-3-one	Kuwara *et al.* (1987) Iwabuchi *et al.* (1987)
Cerambycinae	*Xylotrechus colonus*	male-produced-pheromone	(3*R*)-3-hydroxyhexan-2-one, (3*S*)-3-hydroxyhexan-2-one, (2*S*,3*S*)- 2,3-hexanediol, (2*R*,3*R*)-2,3-hexanediol	Lacey *et al*. (2009)
Cerambycinae	*Xylotrechus nauticus*	male-produced-pheromone	*(3R*)-3-hydroxyhexan-2-one, (3*S*)-3-hydroxyhexan-2-one	Hanks *et al.* (2007)

Table 1.4 Long-range pheromones identified in the Cerambycidae (cont.)

Subfamily	Beetle species	Response	Chemical	Reference
Cerambycinae	*Xylotrechus pyrrhoderus*	male-produced-pheromone	2-hydroxyhexan-3-one, 3-hydroxyhexan-2-one (2S,3S)-2,3-Octanediol, (2S)-2-Hydroxyoctan-3-one	Iwabuchi *et al.* (1987); Iwabuchi *et al.* (1986); Iwabuchi *et al.* (1985) Sakai *et al.* (1984)
Cerambycinae	*Xylotrechus quadripes*	male-produced aggregation pheromone	(2*S*)-2-Hydroxydecan-3-one, 3-Hydroxydecan-2-one, (2*S*,3*S*)-2,3-Octanediol, (2*S*)-2-Hydroxyoctan-3-one,2,3-Decanedione, 2-Phenylethanol, Octanoic acid	Jayarama *et al.* (1998); Hall *et al.* (2006); Rhainds *et al*. (2001)
Spondlylidinae	*Megacyllene caryae*	male-produced aggregation pheromone	(2*S*,3*R*)-2,3-hexanediol, (2*R*,3*S*)-2,3-hexanediol, (*S*)-(-)-limonene, (-)-α-terpineol, nerol, neral, geranial, 2-phenylethanol	Lacey *et al.* (2007, 2008)
Spondlylidinae	*Tetropium cinnamopterum*	male-produced pheromone	(*E)*-6,10-dimethyl-5,9,-undecadien-2-ol	Silk *et al*. (2007)
Spondlylidinae	*Tetropium fuscum*	male-produced pheromone	(*E)*-6,10-dimethyl-5,9,-undecadien-2-ol	Silk *et al*. (2007)
Vesperinae	*Vesperus xatarti*	female-produced pheromone	Vesperal	Boyer *et al.* (1997)
Anoplodermatinae	*Migdolus fryanus*	female-produced pheromone	(2*S*)-methylbutanoyl 2-methylbutylamine, formyl-isoleucine methyl ester	Leal *et al.* (1994)
Lamiinae	*Anoplophora glabripennis*	male-produced pheromone	4-heptyloxy-butanal, 4-heptyloxy-butan-1-ol	Zhang *et al.* (2002)

Table 1.4 Long-range pheromones identified in the Cerambycidae (cont.)

Subfamily	Beetle species	Response	Chemical	Reference
Lamiinae	*Hedypathes betulinus*	male-produced pheromone	(*E*)-6,10- dimethyl-5,9-undecadien-2-yl acetate, (*E*)-6,10-dimethyl-5,9-undecadien-2-one, (*E*)-6,10-dimethyl-5,9-undecadien-2-ol	Fonseca *et al*. (2010)
Lamiinae	*Monochamus galloprovincialis*	male-produced pheromone	2-undecyloxy-1-ethanol	Pajares *et. al*. (2010)
Lamiinae	*M. alternatus*	male-produced aggregation pheromone	2-undecyloxy-1-ethanol	Teale *et al*. (2011)
Lamiinae	*M. scutellatus scutellatus*	male-produced pheromone	2-(undecyloxy)-ethanol	Fierke *et al*. (2012)
Lamiinae	*M. leuconotus*	male-produced aggregation pheromone	2-(4-Heptyloxy-1-butyloxy)-1-ethanol	Hall *et al*. (2006a)
Lamiinae	*M. sutor*	male-produced aggregation pheromone	2-(undecyloxy)-1-ethanol	Pajares *et al*. (2013)
Lepturinae	*Desmocerus californicus*	female-produced pheromone	(4*R*,9*Z*)-hexadec-9-en-4-olide	Ray *et al*. (2012)
Lepturinae	*Ortholeptura valida*	female-produced pheromone	(*Z*)-11-octadecen-1-yl acetate	Ray *et al*. (2011)

Table 1.4 Long-range pheromones identified in the Cerambycidae (cont.)

Subfamily	Beetle species	Response	Chemical	Reference
Prioninae	*Prionus californicus*	female-produced pheromone	(3*R*,5*S*)-dimethyldodecanoic acid methyl 3,5-dimethyldodecanoate, 3,5-dimethyltridecanoic acid, 3,5-dimethylpentadecanoic acid	Rodstein *et al*. (2009, 2011); Maki *et al*. (2011)
Prioninae	*Prionus lecontei*	female-produced pheromone	(3*R*,5*S*)-dimethyldodecanoic acid	Rodstein *et al*. (2011)
Prioninae	*Tragosoma depsarium*	female-produced pheromone	(2*R*,3*R*)-2,3-hexanediol	Ray *et al*. (2012)
Prioninae	*T. depsarium "harrisi"*	female-produced pheromone	(2*S*,3*R*)-2,3-hexanediol	Rodstein *et al*. (2011)
Prioninae	*T. pilosicorne*	female-produced pheromone	(2*R*,3*R*)-2,3-hexanediol	Ray *et al*. (2012)

Table 1.5 Contact pheromones identified in the Cerambycidae

Beetle species	Host	Response	Chemical	Reference
Anoplophora glabripennis	Pine	Contact female pheromone	(Z)-9-Tricosene, (Z)-9-Pentacosene, (Z)-7-Pentacosene, (Z)-9-Heptacosene, (Z)-7-Heptacosene	Zhang *et al.* (2003)
Anoplophora malasiaca	Citrus	Contact female pheromone	gomadalactone B, gomadalactone C, gomadalactone A	Yasui *et al.* (2007)
		Contact female sex pheromone	Heptacosan-10-one, Heptacosan-12-one, (Z,Z,Z)-18,21,24-Heptacosatrien-10-one, (Z,Z)-18,21-Heptacosadien-10-one,(Z)-18-Heptacosen-10-one	Yasui *et al.* (2003b)
		Contact female sex pheromone,	Heptacosane, Nonacosane, 4-Methylhexacosane, 4-Methyloctacosane,9-Methylheptacosane, 9-Methylnonacosane, 15-Methylhentriacontane, 15-Methyltritriacontane	Fukaya *et al.* (2000)
Callidiellum rufipenne	Pine	Contact female sex pheromone	5,17-dimethylnonacosane	Rutledge *et al*. (2009)
		Contact pheromone, male and female	9-pentacosyne, 9-heptacosyne	Rutledge *et al*. (2009)
Mallodon dasystomus	Oak	Contact female sex pheromone	2-methylhexacosane, 2-methyloctacosane	Spikes *et al*. (2010)
Megacyllene caryae	Locust tree	Contact female sex pheromone	(Z)-9-Nonacosene	Ginzel *et al.* (2006)
		Contact female sex pheromone	(Z)-9-Pentacosene	Ginzel *et al.* (2003)

Table 1.5 Contact pheromones identified in the Cerambycidae (cont.)

Beetle species	Host	Response	Chemical	Reference
Neoclytus acuminatus acuminatus	Locust tree	Contact female sex pheromone	7-methylheptacosane	Lacey *et al*. (2008)
Psacothea hilaris	Fig	Contact sex Pheromone, Female	(Z)-21-Methyl-8-pentatriacontene	Fukaya *et al.* (1996)
Tetropium cinnamopterum	Spruce	Contact sex Pheromone, Female	(Z)-9-pentacosene, (Z)-9-heptacosene,11-methyl-heptacosene	Silk *et al*. (2011)
T. fuscum	Spruce	Contact sex Pheromone, Female	11-methyl-heptacosene, (Z)-9-pentacosene, (Z)-9-heptacosene	Silk *et al*. (2011)
Xylotrechus colonus	Rustic	Mate recognition	Unidentified female contact pheromone	Ginzel & Hanks (2003)

1.7.4 Semiochemicals of *Monochamus* species

An attempt has been made to relate host preference to features of chemical ecology by Hanks (1999), who used the following species designations in categorising cerambycid beetle species based on the condition of the larval host plant at the time of colonisation: healthy host (HH), weakened host (WH), stressed host (SH) and dead host (DH) species. According to this classification *M. leuconotus* and most Lamiinae are weakened host species and are not expected to have long-range pheromones.

In *Monochamus* spp., there are many reports of attraction to host kairomones. Several species which attack conifers have been reported to respond to monoterpenes and combinations of monoterpenes and bark beetle pheromones (Table 1.3).

Long-range, male-produced aggregation pheromones have been identified for *Monochamus galloprovincialis* (Pajares *et al*., 2010); *M. alternatus* (Teale *et al*., 2011); *M. carolinensis* and *M. tittilator* (Allison *et al*., 2012); *M. scutellatus scutellatus* (Fierke *et al*., (2012), and *M. sutor* (Pajares *et al.,* 2013). These species share the same pheromone, 2-(undecyloxy)-1-ethanol, which has also been shown as a likely pheromone of *M. obtusus* (Macias-Samano *et al*., 2012). Other species of Lamiinae share pheromone components of a similar structure, e.g. 4-heptyloxy-butanal and 4-heptyloxy-1-butanol produced by male *Anoplophora glabripennis* (Zhang *et al*., 2002). The hydroxyether structure is emerging as another example of the parsimony that seems to exist among the pheromones of many of the Cerambycidae (Hanks and Millar, 2012).

Attraction of male and female, conspecific beetles to the male-produced aggregation pheromones in *Monochamus* spp. is often strongly synergised by combination with host volatiles such as ethanol and alpha-pinene and bark beetle pheromones. For example, combinations of the pheromone with host volatiles increased trap catches of *M. galloprovincialis* (Pajares *et al.,* 2010), *M. alternatus* (Teale *et al*., 2011) and *Monochamus* spp. (Allison *et al*., 2012; Hanks *et al*., 2012).

Hall *et al*. (2006b) showed that male *M. leuconotus* produced a male-specific compound, 2-(4-heptyloxy-1-butyloxy)-1-ethanol (Figure 1.4), related in structure to the alkyl ether pheromone components produced by other Lamiinae. The potential for the use of pheromone lures for monitoring and control of *M. leuconotus* needs to be explored, given the success with pine pests.

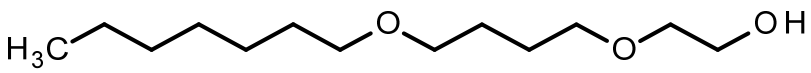


Fig. 1.4. Structure of male-specific compound (2-(4-heptyloxy-1-butyloxy)-1-ethanol) produced by *Monochamus leuconotus*

1.8. AIM AND OBJECTIVES

Aim

The aim of this study was to investigate the mechanisms involved in mating behaviour and host finding in *M. leuconotus.*

Objectives

Despite the widespread application of semiochemicals in insect pest management, very little was known on the chemical ecology of *M. leuconotus* prior to this work. Therefore, the objectives of this study were:

(i) To study the mating behaviour of *M. leuconotus* in order to look for evidence of chemical interactions between the sexes or with host plants.

(ii) To carry out field and laboratory bioassays to investigate the existence of volatile attractants to conspecific beetles or to host plants.

(iii) To carry out laboratory bioassays to determine whether contact pheromones play a role in mate recognition in *M. leuconotus*.

(iv) To carry out field and laboratory studies to investigate whether *M. leuconotus beetles* respond to the male-specific compound isolated and identified at Natural Resources Institute.

(v) To search for alternative host plants in the field as a basis for investigation of factors involved in host preference and location

(vi) To establish the oviposition preferences of *M. leuconotus* on different host plants

Chapter 2

DAILY ACTIVITY PATTERNS AND MATING BEHAVIOUR OF THE AFRICAN COFFEE WHITE STEM BORER, *Monochamus leuconotus* (PASCOE).

2.1. INTRODUCTION

The first stage in study of the chemical ecology, mating and host finding behaviour of *M. leuconotus* was to observe and describe the daily activities and mating behaviour of beetles in general and in respect of time of day. The mating behaviours of some Cerambycids have been described (e.g. Fauziah *et al.*, 1987; Kim *et al.*, 1992) but prior to this study very little was known about the mating behaviour in *M. leuconotus*.

Mating behaviour has been studied in several longicorn beetle species (Iwabuchi, 1982; Kuboki *et al*. 1985; Kim *et al*. 1992, 1993, Wang *et al*. 1991). For example, it has been shown that in the white-spotted longicorn beetle, *A. malasiaca* (Thomson), once a male touched a female with his antennae or tarsi, he dashed towards the female and held her. Then the male licked the female on her back, mounted her, adjusted his body axis to that of the female, bent his abdominal tip over that of the female and tried to copulate with her (Fukaya *et al*.1999, Fukaya 2003). This mating sequence has been found to be similar in other cerambycid beetles such as *Psacothea hilaris* or *Dectes texanus texanus* (Fukaya & Honda, 1992; Crook, *et al*. 2004). In *A. chinensis*, male antennae seemed to play a major role in sex recognition and a significant reduction of mating success rates occurred when six male antennal segments were removed (Wang, 1998). Hanks *et al*. (1996), studying the mating behaviour of the eucalyptus longhorned borer, *Phoracantha semipunctata,* observed that both sexes of this species were attracted to eucalyptus logs where males located females by antennal contact, suggesting that antennal or tarsi contact was necessary for mate recognition and contact semiochemicals were involved in the process. In the same manner, Ginzel & Hanks (2003) observed in four species of longhorned beetles that males attempted to mate with females only after contacting them with their antennae.

For the genus *Monochamus*, it has also been shown that mate recognition was initiated by antennal contact. For example, the Japanese pine sawyer, *M. alternatus,* exhibited the behavioural attributes of mounting, licking and copulation after initial antennal contact

(Fauziah *et al*. 1987; Kim *et al*. 1992) while Kobayashi *et al*. (2003) showed that in *M. saltuaris*, males recognised females after either antennal or tarsal contact. However, in other longicorns such as *Xylotrechus pyrrhoderus* and *Xylotrechus quadripes* (Ibawuchi, 1985; Rhainds *et al*. 2001; Ibawuchi, 1982) antennal contact did not seem to play a role in mate recognition. Male licking of the female's pronotum prior to copulation as is the case in the genus *Monochamus* and others was surprisingly not important in initiating copulation in *X*. *pyrrhoderus* and *X. quadripes* (Venkatesha *et al*. 1995; Rhainds *et al*. 2001; Ginzel *et al*. 2003). Once a male has been accepted by the male, the male held the female and copulation occurred. In other species, males mated more than once with the same female and there was evidence of promiscuity by both sexes (Fauziah *et al*. 1987; Wang, Q. *et al*. 1996; Rhainds *et al*. 2001; Kobayashi *et al*. (2003). However, in others such as *M alternatus* males only mated once. Males maintained a prolonged pair-bonding with females in a half-mounted position before mating, after mating and in between copulation sessions. Copulation duration in the cerambycids is very variable. For example, it was reported to be 9.80 ± 1.55 seconds in *X. quadripes* and 21.1 ± 13 seconds in *X. colonus* (Venkatesha *et al*. 1995; Ginzel *et al*. 2003) while that of *Semanotus japonicus* lasted 4.9 ± 1.072 minutes (Fauziah *et al*. 1992) and *Oemocena hirta* took 49.96 ± 19.19 minutes (Wang & Davis, (2005).

The mechanisms involved in mate recognition in *M. leuconotus* are not known. Knowledge of the mating behaviour may provide information for the future development of monitoring and control strategies for the pest, and in addition, may enhance an understanding of the biology and ecology of cerambycid beetles. This Chapter describes the daily activity patterns and mating behaviour of *M leuconotus* in laboratory and field cages. Particular attention was paid to any evidence for the possible existence of chemical interactions and either long range or contact pheromones and to find out how different sexes locate each other at close range.

Such information was useful in the design of bioassays to determine role of contact pheromones in mate location (Chapter 3) and of longer range olfactory cues (Chapter 4). Field trapping studies (Chapter 5) with live insects or the synthetic male-specific compound benefitted from knowledge of the behavioural activities involved.

2.2. MATERIALS AND METHODS

2.2.1 Insects

Infested coffee stems collected from fields at the Coffee Research Station, Chipinge, Zimbabwe during August 2003 were kept in the laboratory under 23-27°C, 13:11 h L:D regime and 60% RH conditions. Emerging beetles were collected and sexed twice daily between November 2003 and March 2004. The sex of the beetles was determined using antennal length and body size. Male beetles have longer antennae (51.34 mm) and generally smaller bodies (25.47 mm) than females (37.31 mm and 27.19 mm respectively) (Schoeman *et al*.1998). Males and females were stored individually in glass cages and fed on coffee twigs. Adult beetles were used in the study one day after emergence (< 24 h).

2.2.2 Daily activity studies

Laboratory cage observations

Cylindrical cages (20 cm diameter x 40 cm height) made from acetate sheets were used for the mating observation studies with one pair in each cage and some coffee twigs as a food source. Temperature varied between 25°C and 30°C with a 13:11 h L:D regime and RH of 60%. Insects were assumed to be unmated since they were collected from infested coffee stems that were monitored regularly for emergence of adults. Ten pairs were observed over 5 days (between 05:00 and 20:00) with sunrise around 05:30 and sunset around 18:30. The following records were taken at 30-minute intervals:

(i) Feeding was recorded when adult beetles fed by scraping the bark of coffee stems with their mandibles
(ii) Walking was recorded when a beetle was moving in the cages.
(iii) Mounting was recorded when a beetle was observed climbing on top of another beetle.
(iv) Copulation was recorded when the claspers of the male were aligned with the female ovipositor and the male bent its abdomen.
(v) Resting was recorded when a beetle was stationary and not involved in any of the above activities.
(vi) Mate guarding recorded when a male remained mounted on a female after copulation with claspers.

Data on the duration of copulation and mate guarding by mating pairs was recorded once pairs were spotted to be mating in a separate set of experiments involving 10 pairs over a five day period.

External cage observations

A cylindrical field cage (diameter 1.5 m, height 2 m) was constructed using wooden poles as supporting material (Fig. 2.1). The sides of the cage were made up of shade cloth (40% shade netting, Nets and Ropes, Martin Drive, Harare, Zimbabwe) while the top was covered with clear polythene sheet to allow sunlight to penetrate and prevent beetles from escaping.

Two potted Arabica coffee plants (about 1.2 m high) were enclosed in the cage. Three pairs of virgin adult male and female coffee white stem borer were released into the cage. Behavioural activities were recorded. Mating was counted when the genitalia of the beetles were physically connected. Feeding was recorded when a beetle was feeding on bark, walking was recorded when a beetle was walking on the plant, flight was recorded when a beetle flew in the cage while oviposition was recorded when a female chewed an oviposition chamber, withdrew its ovipositor and inserted an egg into the bark crevices. The number of beetles involved in a particular activity was recorded. For mating, when one or two pairs were involved a count of 2 or 4 was taken respectively. Monitoring was done continuously for 48 h with hourly scanning to determine the activity of each beetle. The experiment was replicated three times.

General field observations

Records on the position of beetles within the coffee canopy, height above ground level and activity were taken during random scouting for collection of insects for use in laboratory bioassays (Chapters 3 and 4) and field trapping studies in Zimbabwe (Chapter 5) during the flight period of *M. leuconotus* December 2006 to February 2007 . Insects were sexed according to the method of Schoeman *et al*., (1998) described earlier. Height above ground level was measured by a standard metre rule graduated in centimetres.

Fig. 2.1 External cage used for observations on daily activity patterns at Coffee Research Station, Chipinge, Zimbabwe.

2.2.3 Characterisation of mating behaviour

Interactions with one male and one female

A male and a female were placed in a plastic box (20 cm x 12 cm x 7.5 cm) containing coffee twigs as food. These were observed continuously under 25°C, 13:11 h L:D regime and 60% RH conditions over an eight-hour period (07:00 -15:00 h). Twenty pairs were observed. Records on the behavioural interactions involved before, during and after mating by the beetles were taken. Coffee twigs that provided food were placed in the cage. A pair of beetles was only used once for the observations.

Interactions with one male and three females

One male and three female beetles were placed together in a plastic container (20 cm x 12 cm x 7.5 cm) where observations on behavioural interactions involved in mating were done continuously from 07:00 to 15:00 h. (8 h). Coffee twigs that acted as a food source were placed in the cage. Female insects were numbered on the elytra by a magic marker. Ten such groups of insects were observed.

Interactions with one female and three males

One female and three male beetles were placed together in a plastic container (20 cm x 12 cm x 7.5 cm) and records on behavioural interactions involved in mating were taken over a ten-minute interval due to the complexity of visual observations involving a group of four insects. Twenty such groups were observed.

2.2.4 Data analysis

Laboratory observations

Data on the behavioural activities were pooled to depict the frequency for each activity of the beetle pairs at two-hourly intervals except for the last time interval, which was 1 h only over the 5-day observation period. Data were classified into eight time intervals between 05:00 and 20:00 h. Data were then analysed by single factor ANOVA to find if there were any differences in each activity due to time of day or between days of observation. Significance level was set at 5%. Differences between the means were separated using

the Least Significant Difference (LSD) test (Zar, 1994). Data on copulation duration were classified into classes based on ten minutes intervals and analysed by χ^2 analysis.

External cage observations

The hourly counts for each activity were analysed using the pivot table facility in Excel. Daily activity patterns were plotted against the time of day (*N* = 36).

General field observations

Total counts of insects in respective positions on the trees were used for data analysis. Heights were grouped into class intervals (0.0- 0.5, 0.1 -1.0, 1.1 -1.5, 1.51 -2.0, >2.0 metres above ground level) for the purpose of data analysis.

Characterisation of mating behaviour

Counts of insects involved in a specific behavioural activity (touching, holding, licking, mounting, dashing forward, abdominal bending, and copulation) were summarised and a graphical presentation to depict the sequence of events involved in mating was done.

Data on the mating frequency of females and males when a male was put in a cage with 3 females and also when 3 males were placed in the same cage with one female were analysed by ANOVA using GenStat (Release 10.1 for Windows). Differences between means were determined using the LSD test at $\alpha = 0.05$.

2.3. RESULTS

2.3.1 Daily activity patterns under laboratory conditions

Eight out of the 10 pairs successfully copulated at least once in cages during the five-day laboratory study period while two pairs did not mate. This is evidence that the beetles mated under artificial conditions.

Prevalence of beetle activities according to days

Results are summarised in Fig. 2.2. Copulation ($F_{4,35}$ = 3.49; P = 0.017) and mounting ($F_{4,35}$ = 3.34; P = 0.020) by *M. leuconotus* beetles differed during the five days of observation. Copulation was high during the first two days with the maximum on the second day. It then tailed off with the minimum recorded during day 5.

Feeding activity by the *M. leuconotus* beetles was different ($F_{4,45}$ = 5.53; P = 0.001) between days. Feeding steadily increased according to days before reaching a peak on day 5.

Resting was low on day 2 and remained almost constant comparable to walking activities between the days, which were more or less constant ($F_{4, 45}$ = 0.383, P = 0.820) during the five–day observation period.

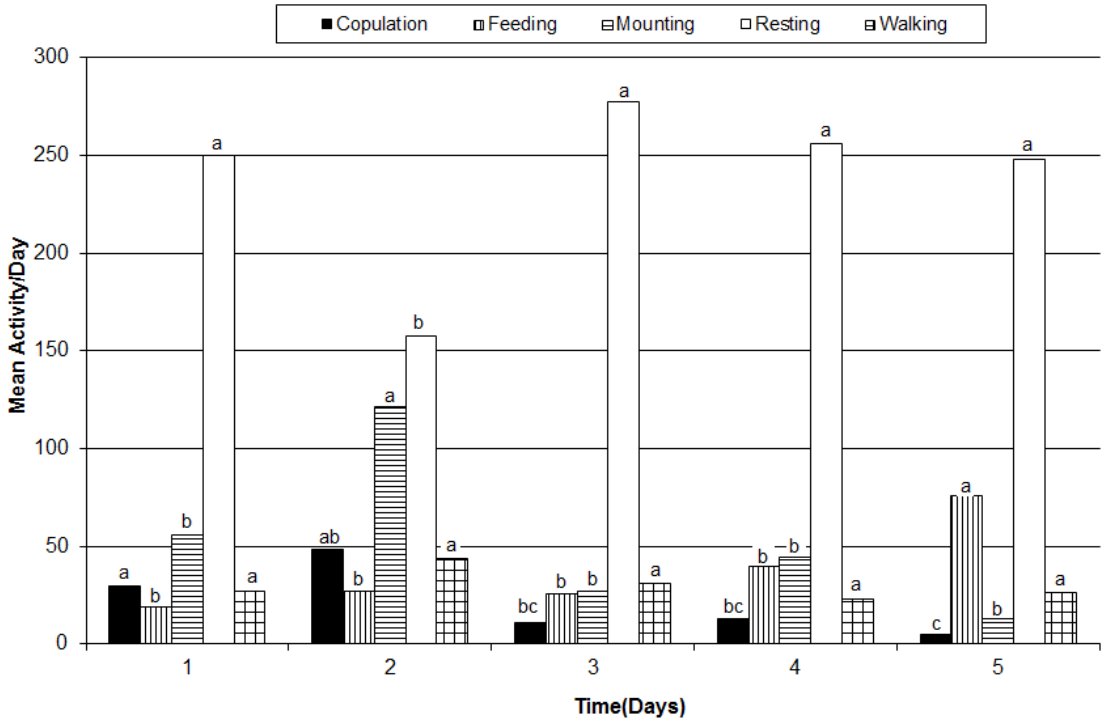


Fig. 2.2 Behavioural activities of *Monochamus leuconotus* in laboratory cages over a five-day observation period. Ten pairs observed at 30-min intervals. Data depicts average frequency per cage per day for each behavioural activity (Bars with different letters for each activity across days are significantly different by LSD test (P < 0.05)

Prevalence of activities according to time of day

Beetles mated at any time during the period of observation (Fig. 2.3) as shown by insignificant differences in copulation frequency (F = 1.24, *d.f.* 7,56, P = 0.295) and mounting (F = 1.01, *d.f.* 7,56, P = 0.436) activities.

Adult feeding activities differed during the day (F = 10.11, *d.f.* 7,56, P < 0.001) with a peak early morning which gradually declined before peaking again after mid-day.

Resting by *M. leuconotus* beetles did not differ according to time of day (F = 2.95, *d.f.* 7,72, P = 0.089).

Walking by *M. leuconotus* beetles was constant throughout the day (F = 0.959, *d.f.* 7,72, P = 0.468).

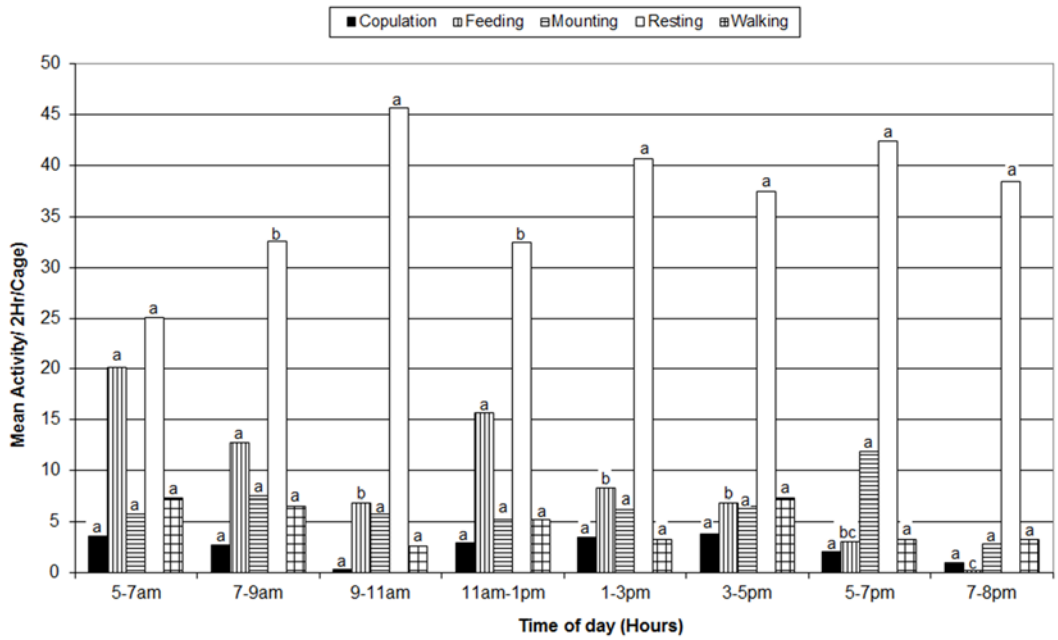


Fig. 2.3 Behavioural activities of *Monochamus leuconotus* in laboratory cages between 05:00 -20:00h over a five-day observation period. Ten pairs observed at 30 min interval. Data depicts average frequency per cage per day for each behavioural activity. (Bars with different letters across time of day for each activity are significantly different by LSD test (P <0.05).

Copulation duration varied from 6 min to 116 min (χ^2 = 14.13, *d.f* = 5, P = 0.015). Average copulation time was 32.38 min with a mean of 3.12 copulations per pair. Most of the beetles took between 20 and 30 min to copulate (Fig. 2.4). Mate guarding ranged from 0

to 148 min (χ^2 = 393.25, *d.f* = 5, *P* <0.001) with a mean duration of 47 min. Most of the beetles had mate guarding duration greater than 50 min (Fig. 2.4).

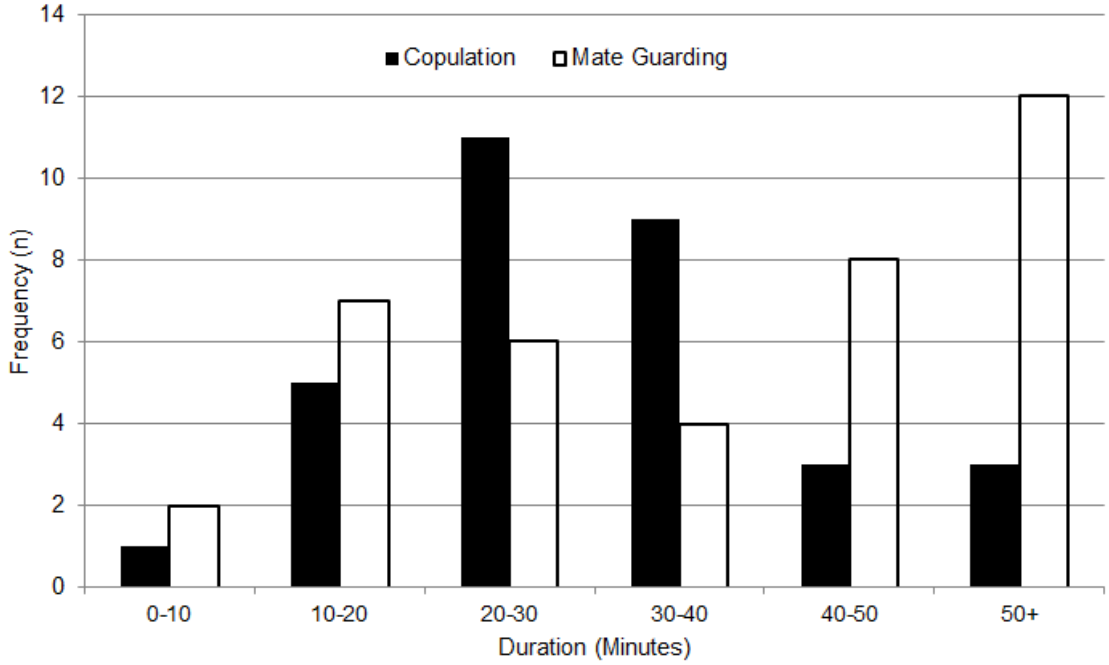


Fig 2.4 Copulation and mate guarding duration of *Monochamus leuconotus* beetles under laboratory conditions (Ten pairs observed and frequency and duration of each copulation recorded)

2.3.2 Daily activity patterns under field cage conditions

Most adult activities occurred during the day between 05:00 and 18:00 h (Fig. 2.5). Mating occurred throughout the day with three major peaks after sunrise and later after mid-day. Oviposition was observed during very early morning (02:00 h) and mid-morning (10:00 h).

The beetles flew throughout the day with peak flying at 14:00 h and 18:00 h. Mounting occurred during the day with peaks during 09:00 h – 14:00 h and 16:00 h – 1900 h. Beetles walked and fed throughout the day. There was very little feeding after dark.

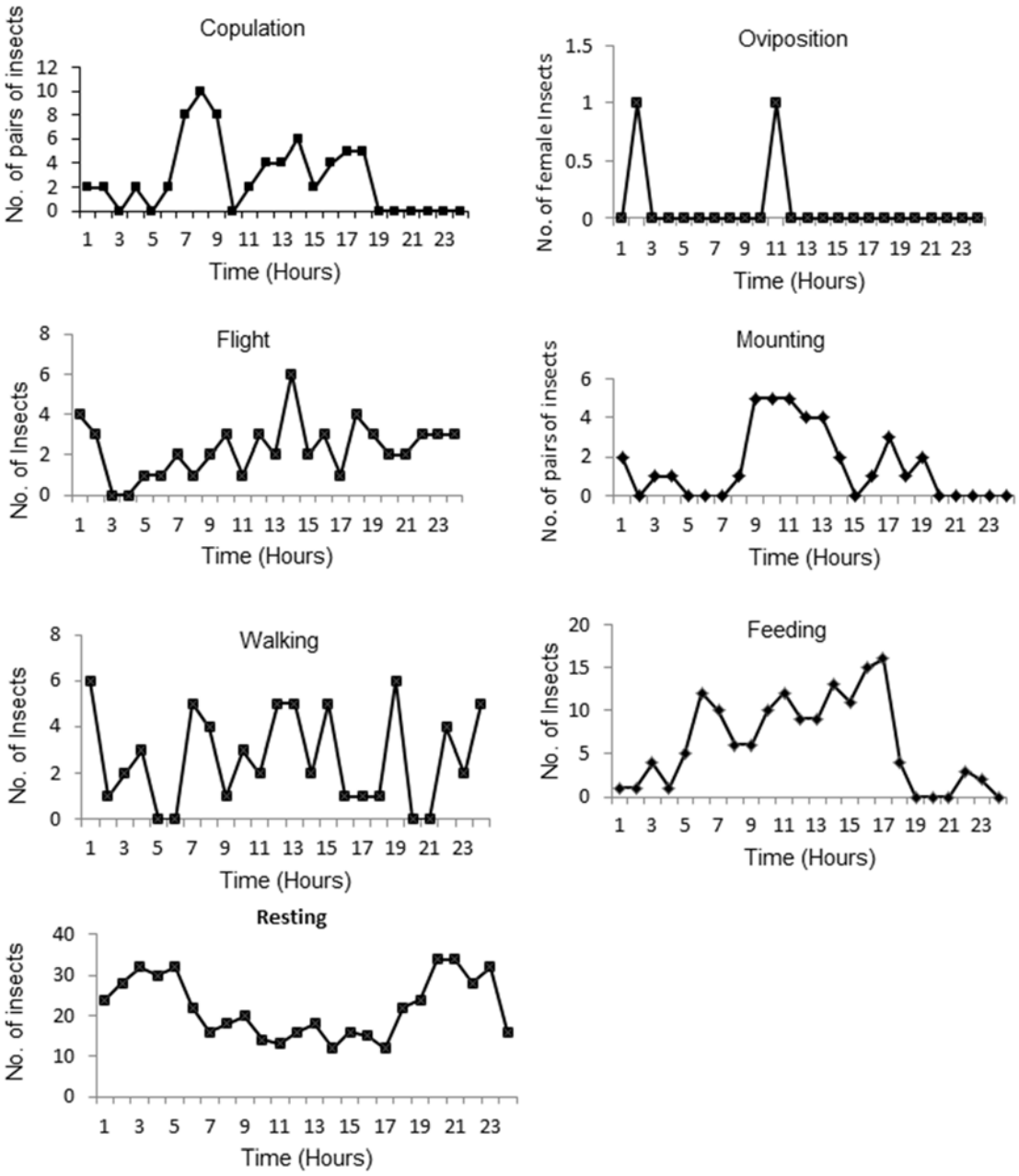


Fig 2.5 Behavioural activities of adult *Monochamus leuconotus* in a field cage over a twenty-four hour observation period (3 replicates of 3 pairs of insects observed over a 48-h period). Data depicts totals for insects involved in a given activity over the observation period. Daylight is 05:00 - 18:00 h.

2.3.3 General field observations

Position of beetles within the canopy

Most beetles were found on the main stem of the coffee trees followed by the primary branches. There were no females and mating pairs found on the leaves (Table 2.1). The occurrence of beetles on different parts of the canopy did not appear to be influenced by sex (χ^2 = 4.96, *d.f.* = 2, *P* = 0.084).

Table 2.1 Position of *Monochamus leuconotus* beetles in coffee trees during field observations at New Years' Gift, Chipinge

Position	No. males	No. females	No. mating pairs	Total beetles
Leaf	1	0	0	1
Primary branch	40	34	0	74
Main stem	171	83	32	318

Height above ground level

The height at which *M. leuconotus* adults were found varied from 0.09 to 2.2 m above ground level. Most insects were found between 1 m and 2 m above ground level with females mostly in the 1.1 to 1.5 m class while males were present in equal numbers in the 1.5 to 1.5 m and 1.51 to 2.0 m height classes. The number of mating pairs increased with height above ground level (Fig. 2.6).

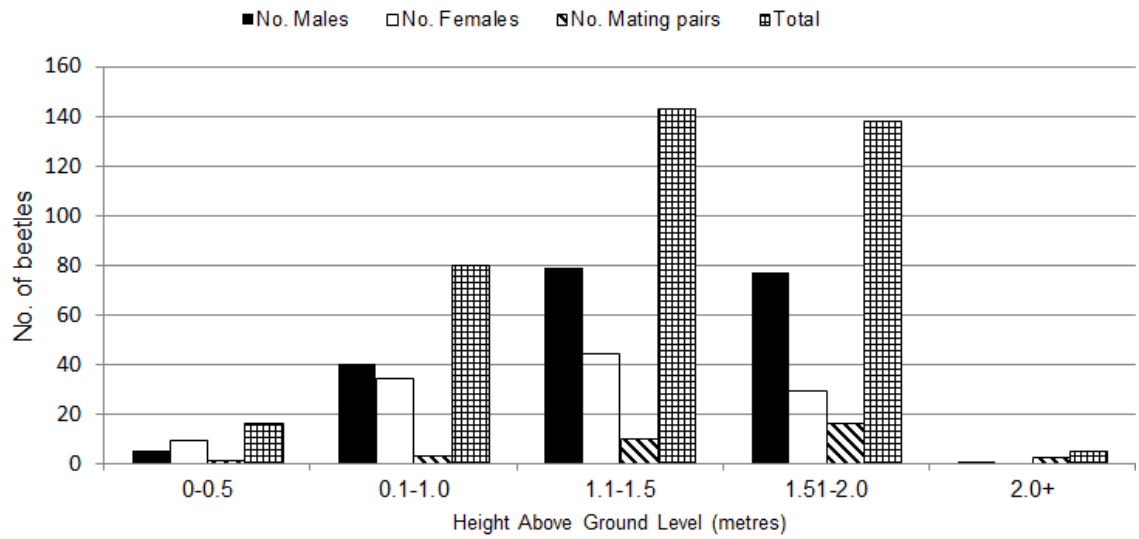


Fig 2.6 Position of *Monochamus leuconotus* beetles within the coffee canopy under field conditions at New Year's Gift, Chipinge, Dec 2006 –Feb. 2007

2.3.4 Mating behaviour

Pairs of single insects

Mating behaviour of pairs of single male and female *M. leuconotus* beetles is summarised in Fig. 2.7. Of the twenty pairs observed during the study, sixteen pairs moved or waved their antennae prior to contact with each other. Four males did not wave antennae but proceeded to approach slowly or dashed/leaped forward towards the females. Of the sixteen that moved/waved their antennae, seven touched the females with their antennae while six touched with tarsi. The remaining three did not touch with either antennae or tarsi but proceeded to approach slowly or dashed/leaped forward. Of the twenty that either approached slowly (*N* = 12) or dashed/leaped forward (*N* = 8), twelve proceeded to hold the females (holding).

Of the twelve males that held the females, nine were observed to touch the elytra/pronotum of the female with their mouth palpi before holding. This behaviour is referred to as "licking" (cf Fauziah *et al*. 1987; Kim *et al*., 1992) and could be related to sex contact pheromones present in the female cuticle that are recognized by the males through chemical receptors located in the maxillary and/or labial palpi. Three males proceeded directly to holding without licking. After holding, nine pairs licked the elytra/pronotum before mounting while three proceeded to mounting without licking. After mounting, the nine bent their abdomen and subsequently copulated. When the male approached a female, she stood motionless and either ran away or submitted by opening

her ovipositor or she continued to walk with the male mounted on her back. Abdominal bending by the male occurred before copulation finally took place. The female remained motionless during copulation with her antennae drooping forwards. After copulation the males remained on the back of the female in a half-mounted position with the female either walking around or feeding with a male on her back. Mating by the same pair occurred more than once.

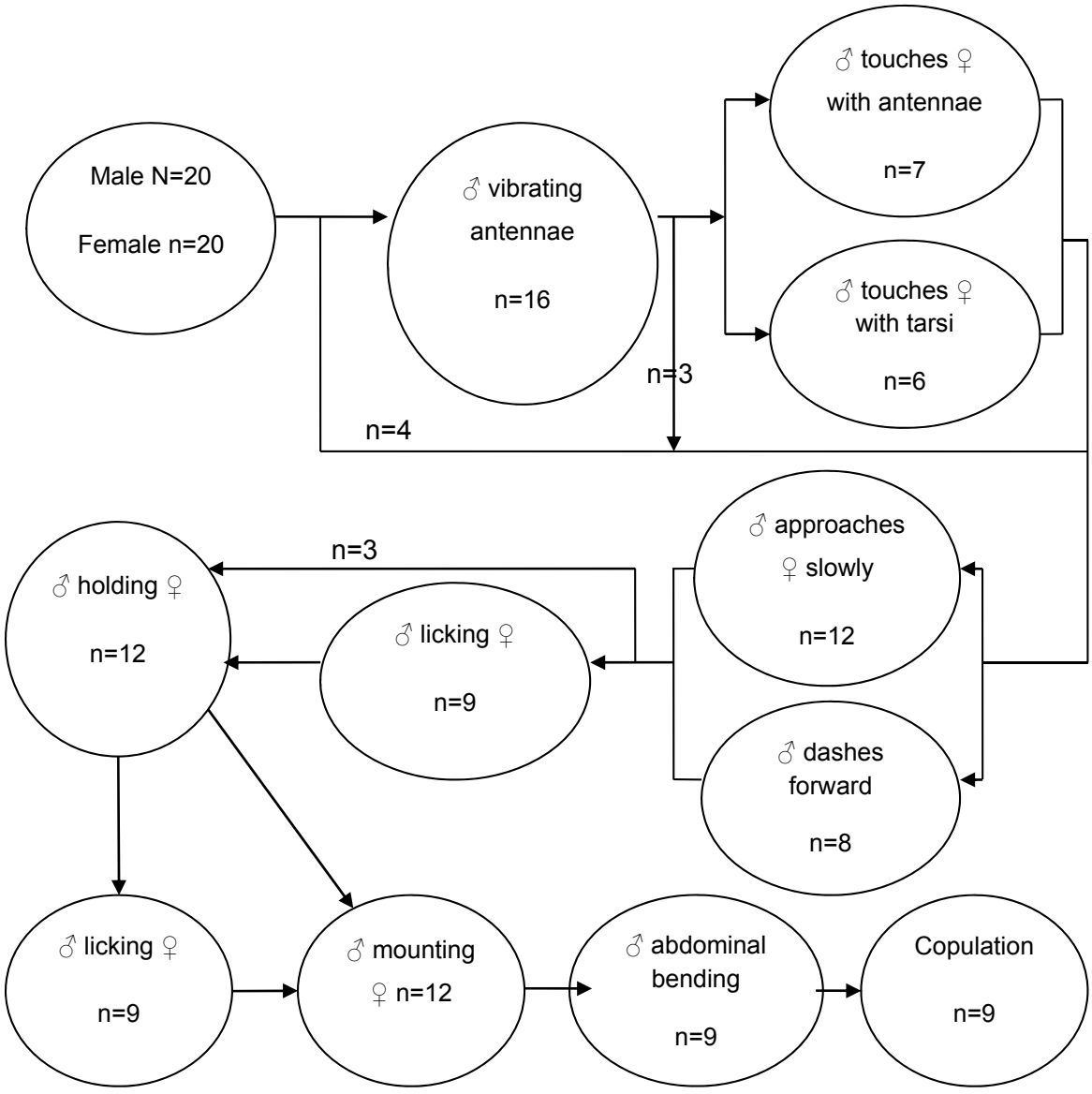


Fig. 2.7 Observed mating sequence in *Monochamus leuconotus* males under laboratory conditions after being approached by a searching female. Each numeral indicates the number of pairs exhibiting the behaviour in 20 observations of 20 pairs over 8 h.

One male and three females

When three females were placed in a single cage with one male, there was evidence of competition for the male. Female interference with mating pairs was very high (*N* = 32) and occurred in several ways: walking over mating pair, resting near mating pair, fighting (biting each other resulting in broken antennae) with the mounted female, mounting male, antennal contact with either male or female and following the couple. When a male was mounting, he could leave the female (*N* = 5) in pursuit of another female after antennal or elytral contact. In certain cases fighting with the male (*N* = 1) or other females (*N* = 7) occurred. Mounting of males by females was prevalent (*N* = 26). There were no significant differences between mating frequency per female (F = 0.4, *d.f.* 2,29, P = 0.857). Males mated more than once with different females but there were no significant differences in mating frequency (F = 0.92, *d.f.* 9,18, P = 0.465) (Table 2.2).

Table 2.2 Copulation frequencies by *Monochamus leuconotus* in a cage with three females and a male under laboratory conditions (10 replicates) over an 8 h observation period.

	Female number			**Mean number of copulations by**
Replicate	**1**	**2**	**3**	**male per female**
1	2	2	5	3
2	1	3	0	1.33
3	3	2	1	2
4	1	2	1	1.33
5	3	0	0	1
6	1	0	3	1.33
7	2	4	0	2
8	2	0	0	0.67
9	0	0	0	0
10	1	0	3	1.33
Mean	1.6	1.3	1.3	1.4

Fighting (biting each other resulting in broken antennae) between females resulted in some females having broken legs. Males also fought with females and a sound was produced when a mounting male bit a female on the antennae. In between copulation and fighting, the insects moved randomly in the cage, rested or fed on the coffee bark.

Females either walked slowly or remained stationary with some feeding during copulation.

One female and three males

Copulation occurred in 11 of the 20 groups observed while there was mounting only in another three (Table 2.3). The remainder did not exhibit any copulatory behaviour. Males fought (biting each other resulting in broken antenae) each other (*N* = 7) or the female competed for two males (*N* = 5) by pushing them away. Females were also found mounting males (*N* = 5) and males mounted copulating pairs. There were also instances where two males competed to mount the same female at the same time from different directions. The correctly aligned male would succeed in mating with the female and no further competition was experienced.

Table 2.3 Behavioural interactions of *Monochamus leuconotus* in cages containing one female and 3 males under laboratory conditions (*N* = 20)

Activity	Number of times
Males mounting other males	5
Fighting between males	7
Females touching males	5
Female fighting males	5
Mounting	14
Copulation	11

2.4. DISCUSSION

2.4.1 Daily activity patterns

The results obtained in both laboratory and outdoor cages suggest that most activities of *M. leuconotus* occur during daylight hours with less activity during the night (1800 h to 0500 h). Peaks for mating and feeding observed in the laboratory and outdoor cages appear to coincide with each other suggesting that all activities of this beetle on coffee trees are mainly associated with feeding and mating. Beetles mated readily soon after

emergence and there was evidence of multiple mating, polygynous and polyandrous behaviour under laboratory conditions (Table 2.2 and 2.3). Mating appeared to be most prevalent in the early morning and later after mid-day. The occurrence of flight at any time of the day or night suggests that the beetles can disperse at any time of the day or night and this has potential application in monitoring studies. Though no measurement of the flight distance was done during the current study, beetles have been observed to fly up to about 90 m in the field (Le Pelley, 1968).

Feeding was a major activity in terms of time spent in both laboratory and outdoor studies. Intensive feeding observed in this study could be part of maturation feeding or for the maintenance of vigour. Cerambycid beetles of the subfamily Lamiinae typically undergo a period of maturation feeding prior to sexual maturity (Linsley, 1959, Hanks, 1999). Feeding could be supplemental to maintain normal vigour of female ovaries, which are immature at the time of emergence. Li and Liu (1997) observed that *Anoplophora glabripennis* mated immediately after emergence but continued to feed. They concluded that feeding was related to the need for normal vigour as opposed to sexual maturation. Sexual responsiveness immediately after emergence has implications in the development of monitoring strategies in cerambycid beetles. Poor trap catches during the early part of the flight season has been attributed to female maturation feeding (e.g. Morewood *et al.,* 2002, De Groot and Nott, 2003). However, the results from the current studies showed that beetles mated readily after emergence and did not seem to require maturation feeding for the initiation of reproductive activities.

Mounting was also a major activity that was observed to occur either before or after copulation. The coincidence observed in copulation and mounting during laboratory and field studies is not surprising since there is a need for mounting either before or after copulation. The pre-copulatory mounting can be explained in terms of normal mate location procedure in cerambycids whereby a male mounts a female after antennal or tarsal contact (Fauziah *et al*. 1987; Kim *et al.*, 1992, Kobayashi *et al*., 2003, Fonseca and Zarbin, 2009, Rutledge *et al*., 2009). Cerambycid males are known to exhibit mate-guarding behaviour by remaining mounted on the females after copulation. Post-mating mate-guarding often occurs in cerambycids whose females are polyandrous and require many brief matings (Hanks, 1999; Morewood *et al*., 2004; Wang and Zeng, 2004).

In the current study, mating duration varied from a few minutes to more than an hour and females oviposited during the early hours of morning.. Tapley (1960) noted that copulation in *M. leuconotus* took from one to several hours which is in contrast with observations in the current study where it was observed that the majority were less than

an hour. According to Hanks (1999), post-copulation mate-guarding does not occur in species where the mating duration is long. Therefore, it would appear that in *M. leuconotus* males perform mate-guarding in order to fend off rivals and ensure reproductive success. During the current studies males showed polygynous behaviour while females showed polyandrous behaviour (Tables 2.2 and 2.3), suggesting that this could be a strategy to ensure reproductive success.

Oviposition was observed to take place at dawn and in the morning. Tapley (1960) reported that oviposition mostly took place only during the dark hours. Occurrence of oviposition at dawn agrees with Tapley (1960) although more work is required to confirm the occurrence of oviposition in the mid-morning hours observed in this study.

Resting of beetles was high and this is consistent with general cerambycid ecology whereby adults of most species are sedentary since they emerge on the same host plant where they feed and find mates. This also tallies with findings in the current study whereby most activities are to do with mating and feeding both of which are done while the insects are stationary. Flight was observed during both day and night. This shows that flying could be a strategy for dispersal or inbreeding.

2.4.2 Field observations

Beetles were mostly found on the main stem during the current studies and this is consistent with their attraction to bark scrapings during the olfactometer bioassays (see Chapter 4). Only one male beetle was found on the leaves. The fact that they do not rest on leaves could be related to the size and weight of the beetles in relation to that of leaves, although they do not feed on leaves. Appearances of beetles on primary branches and the main stem are related to their feeding habits since they feed mostly on bark (Tapley 1960). Some beetles were observed feeding on coffee cherries during the latter part of the flight season and were perched on primary branches.

Height of beetles above ground level could be an important parameter in the design of trapping trials and the results from the current study suggest that both male and female beetles can be found at any height within the coffee canopy with most preferring to be between 1 and 2 m above ground level although the coffee canopy can be up to 3.5 m high. This could imply that traps placed below 1 m above ground level might have limited chances of catching beetles. In addition, the height of beetles within the coffee canopy could be important in spraying and manual monitoring operations for the adults.

2.4.3 Possible roles of chemicals in mating behaviour

The mating behaviour of *M. leuconotus* observed during the current studies suggests that the female approaches a sedentary male who then responds to the female on antennal or tarsal contact. This is consistent with the mating behaviour of other cerambycids. For example, the Japanese pine sawyer, *M. alternatus,* exhibited mounting, licking and copulation after initial antennal contact (Fauziah *et al*. 1987; Kim *et al*., 1992) and *M. saltuaris* showed the same pattern of the male reacting to the female by touching with his antennae or tarsi, dashing towards the female and mounting her back (Kobayashi *et al*., 2003,). Copulation consisted of three stages involving first, abdominal bending and insertion of the male's aedeagus into the female's genitalia, secondly, a motionless male whose aedeagus is withdrawn by the violent post-copulation shaking of the female followed by a prolonged pair bonding (mate guarding). The sequence appears to be common in all cerambycids in the Lamiini subfamily, which according to Hanks (1999), produce short range pheromones.

Antennal contact is the major factor in mate recognition since the male appears sedentary prior to antennal contact with a female. Males probably identify females through contact pheromones present on the body surface of the insects and are sexually aroused after the detection of the contact pheromone by sense organs present on the antennae. Evidence of the existence of a contact sex pheromone on the body surface of females of *S. japonicus,* was given by Kim *et a*l., (1993), who found that female ether extracts elicited mating behaviour in males. Fukaya & Honda (1995) showed that female body extracts of *Psacothea hilaris* elicited mating behaviour in males under laboratory conditions and provided the evidence of the existence of two pheromone components.

Given the behavioural activities noted here, it is most likely that the males of *M. leuconotus* attract females through a short-range pheromone and that both sexes could have contact pheromones since females were also observed to touch males with their antennae (Table 2.3). Wickam *et al*., (2012) suggested that females of *A. glabripennis* colonised suitable host plants first and released a pheromone to attract males. The results from the current study indicate that male *M. leuconotus* attract females (since females were observed to approach the male insect) followed by antennal and tarsal contact prior to mating. Although most insects mated readily less than 24 h after emergence, the failure to mate by other pairs could be due to the insects not being sexually mature at the time of the observations or to the importance of other cues such as auditory and visual cues, which were not investigated in the current study. Cerambycid beetles normally require some maturation feeding before being sexually active.

Visual observation methods employed in the current study were able to give important insights into behavioural activity patterns and mating behaviour of *M. leuconotus.* However, there were some limitations such as the failure to distinguish activities per sex in the preliminary observation studies and difficulties in recording behavioural interactions involving more than two insects in the same cage. Insects were marked in order to improve on these limitations. Other workers overcame these limitations by using videos linked to computers. For example, mating behaviour of *Nazedelia cantori* (Ginzel & Hanks, 2005) and *M. galloprovincialis* (Pajares *et al.*, 2006) were characterised using video cameras linked to computers. Another limitation was the availability of adequate insects for experimentation since beetle emergence from most of the infested stems failed. Hanks (1999) attributed the limited documentation on cerambycid chemical ecology to the problem of insufficient insect numbers for laboratory experimentation.

Chapter 3
RESPONSES OF ADULT *Monochamus leuconotus* BEETLES TO CUTICULAR HYDROCARBONS.

3.1. INTRODUCTION

In Chapter 2, observations of mating behaviour showed that females approached males and then males touched the females with their antennae or tarsi followed by the full sequence of events involved in mating behaviour. These observations suggested the involvement of contact cues in intra-specific communication of *Monochamus leuconotus*.

Cuticular hydrocarbons located on the body surface of beetles mediate mate recognition in some cerambycids (e.g. Ginzel & Hanks, 2003; Zhang *et al.,* 2003, Ibeas *et al*., 2009, Rutledge *et al*., 2009, Silk *et al*., (2011), Table 1.5). Evidence of the role of cuticular hydrocarbons in mate recognition is usually obtained by testing for the response of males or females to dead or hexane-washed conspecifics. In addition, whole body or elytra washings can be applied to inert materials/dead insects and the response of female or male beetles is then evaluated.

Prior to this study, nothing was known about the role of cuticular hydrocarbons in mate recognition in *M. leuconotus*. In this chapter, experiments aimed at testing the possible role of cuticular hydrocarbons in mate recognition are described.

3.2. MATERIALS AND METHODS

3.2.1 Insects

Adult beetles were collected from coffee fields in the morning and brought to the laboratory during the periods December 2004 - April 2005, December 2005 - February 2006 and November 2006 - February 2007. These were separated according to sex and stored in cages where they fed on fresh coffee twigs before the bioassays. The insects were kept under a 25ºC, 13:11 h L:D regime and 60% RH conditions.

3.2.2 Experimental procedures during 2004/5 and 2005/06 seasons

A female beetle ranging from 1 day to 7 days after field collection was killed by placing the insect in a freezer for 12 h. The insect was brought back to room temperature by leaving it to stand for about 30 min. It was then placed at the centre of a plastic box arena (20 cm x 12 cm x 7.5 cm), and offered to individual males introduced into the arena. Male response to dead unwashed females was classified according to four categories of holding attempt (HA: male touched/contacted the dead female with its antennae, palpi or tarsi), holding (HD: male dashed forward and licked the dead insect), mounting/ climbing (MT) and abdominal bending (AB: male aligned its body with the dead female and attempted to mate by bending the abdomen). The behaviour of each male within 10 min of exposure to a female was recorded according to the different behavioural categories. Twenty males were tested for their response to three dead females during the 2004/2005 season. The plastic box was cleaned with ethanol in between bioassays.

In the next experiments, the above freeze-killed, female beetles were then washed by immersing in 6 ml hexane twice for 5 minutes each. The insect was left to dry for 30 min before the bioassay with air being blown over it to assist the drying process. A dead, hexane-washed female was placed at the centre of a plastic box where male beetles were released individually. The behaviour of the males towards the dead washed females was observed and records on male behaviour towards the dead female over a period of 10 min were taken. When the experiments were started the time of observation was 20 min. This was changed to 10 min, since most beetles reacted within a period of less than 10 min. Each male or female beetle was used once per day. Thirty males were tested for their response to the five dead, washed female during the 2004/2005 flight season. The plastic box was cleaned with acetone in between bioassays.

The hexane body wash extracts from each female beetle were evaporated to 1 ml at ambient temperature and used to recoat the washed females by pouring carefully over the body. Recoated females were dried for 30 min before being used in the experiments. The recoated females were offered to individual males under conditions similar to those used for the dead, unwashed and washed female experiments. Male response to the recoated females was observed for a period of 10 min and was recorded according to four behavioural categories of holding attempt, holding, mounting and abdominal bending as described above. Each male insect was used once per day per experiment. Thirty males were tested during the 2004/2005 flight season.

Because of the difficulty in collecting study specimens for carrying out experiments with *M. leuconotus* adults, further replicates were carried out in 2005/06 and data were combined.

3.2.3 Experimental procedures during 2006/07 season

Male response to live conspecific females was evaluated by pairing live male and female beetles in a plastic box arena (20 cm x 12 cm x 7.5 cm). Behavioural responses were classified according to four categories of holding attempt (HA), holding (HD), mounting (MT) and abdominal bending (AB) as above. Bioassays were run for 45 min initially and if the pair did not mate they were separated and observed again for up to five days before being discarded. If the pair mated, the female was freeze-killed while the male was kept separately for use in the next series of experiments. Eighty one pairs were tested of which 64 responded.

A female beetle that a male had successfully attempted to mate with as described above was freeze-killed by placement in a freezer for 24 h. A dead beetle was brought back to laboratory room temperature before being placed at the centre of a plastic box arena. The live male beetle that had previously mated with it was placed into the plastic box and behavioural observations noted for 45 min. Records of holding attempt, holding, mounting and abdominal bending were taken as above.

A dead female insect from above was washed by immersion in 6 ml hexane twice for 5 minutes each time. The insect was allowed to dry for 30 min before the bioassay with air being blown over it to assist the drying process. Once dry, the dead washed insect was placed at the centre of the plastic box arena and its corresponding male was brought into the box and behavioural observations done for 45 min. Records on holding attempt, holding, mounting and abdominal bending were taken as above.

The female hexane body extracts from each female washing done above were evaporated to 1 ml and used to recoat each washed female by pouring over the elytra. Recoated females were allowed to dry for 30 min before being exposed to the same male counterpart. Male response to the recoated females was observed for a period of 45 min with records on holding attempt, holding, mounting and abdominal bending being taken.

3.2.4 Data Analysis

Data on male responses to the different categories of dead insects were analysed using χ^2 Goodness of fit. Significance level was set at 5%. Differences between proportions responding were separated by multiple comparison tests (Zar, 1984). Details of statistical analyses are in Appendix 1.

3.3. RESULTS

3.3.1 Male responses to female conspecifics during 2004/05 to 2005/06 laboratory experiments

When males were presented with the unwashed, freeze-killed female beetles most of them showed all behaviours in the recorded categories involved in mating behaviour (Table 3.1).

Table 3.1 Mating responses of male *Monochamus leuconotus* to female conspecifics under laboratory conditions during 2004/05 and 2005/06 seasons

		% Male Response *			
Treatment	***N***	**HA****	**HD**	**MT**	**AB**
Dead unwashed	20	80 a	55 a	55 a	55 a
Dead washed	30	33 c	17 c	17 b	0 b
Dead recoated	30	56 b	33 b	26 b	0 b
χ^2		10.68	8.05	8.65	38.26
P		0.0048	0.017	0.013	<0.001
d.f.		2	2	2	2

* HA Holding Attempt, HD Holding, MT Mounting, AB Abdominal Bending

**Figures followed by the same letter within a column are not significantly different according to the multiple comparison tests for proportions (Zar, 1984)

After the dead female was washed in hexane, males showed significantly reduced responses in all categories (Table 3.1). Significantly fewer males initiated the mating process by attempting to hold the female and none of the males tested showed abdominal bending with the dead, washed females.

When the females were recoated with hexane extract, the response of the males was partially restored since males went through the first three stages of mating (Table 3.1). More males initiated the mating process as shown by HA and HD, but none showed abdominal bending. This indicated that solvent extraction removed the chemical cues necessary for initiation of mating behaviour.

3.3.2 Male responses female conspecifics during 2006/07 laboratory experiments

In the 2006/07 laboratory experiments, males showed all behaviours in the recorded categories involved in mating behaviour with live females (Table 3.2).

Table 3.2 Mating responses of male *Monochamus leuconotus* to dead female conspecifics under laboratory conditions during 2006/07 season.

		% Male Response *			
Treatment	***N***	**HA****	**HD**	**MT**	**AB**
Live female	81	79 a	72 a	63 a	51 a
Dead unwashed	35	54 b	46 b	29 b	11 b
Dead washed	16	50 b	19 c	6 c	0 c
Dead recoated	14	86 a	79 a	50 a	6 bc
χ^2		12.05	20.92	23.76	27.61
P		<0.001	<0.001	<0.001	<0.001
d.f.		3	3	3	3

* HA –Holding Attempt, H – Holding, MT –Mounting, AB – Abdominal Bending

**Figures followed by the same letter within a column are not significantly different according to the multiple comparison tests for proportions (Zar, 1984).

After the females were freeze-killed and presented to the same males, males were able to show all behaviours in the recorded categories involved in mating behaviour, although the frequencies were reduced in all categories.

When the females were washed in hexane, males initiated the reproductive process but the frequencies of subsequent steps were reduced and none of the tested males showed abdominal bending indicating that chemical cues had been removed by the solvent (Table 3.2).

When the females were recoated with hexane extract, the response of the males was fully restored to the levels observed with live females in the HA, HD and MT categories, although the frequency of abdominal bending was only partially restored (Table 3.2).

3.4. DISCUSSION

3.4.1 Evidence for a contact pheromone

These results suggest that a contact pheromone could be present on the body of female *M. leuconotus* beetles, which is removed by washing with hexane solvent. When the recognition cues were restored by recoating dead females, males were able to recognise the females again since the percent response to recoated females was much higher than that to solvent-washed females. During the bioassays of 2004/05 and 2005/06, males responded positively to dead unwashed females in terms of exhibiting the full behavioural sequence of events involved in the mating behaviour of *M. leuconotus* as described in Chapter 2 of this thesis. Male response to dead extract-recoated females was much higher than that to washed females signifying that recognition cues were partially restored even though males did not attempt to mate with the extract-recoated females. Similarly, when males were paired with females during the 2006/07 season and experiments done to test their response to the same dead insects, washed females and recoated females, the full sequence of mating behaviour was observed for live insects, dead unwashed females and dead extract-recoated females whereas the same did not happen with the washed females.

The presence of contact pheromones in Cerambycidae has been confirmed in a number of species in the Cerambycinae subfamily (Wang *et al*., 2002, Ginzel & Hanks, 2003, Ginzel *et al*, 2006) and the Lamiinae (Fukaya and Honda, 1992, Wang, 1998, Zhang *et al.,* 2003, Fukaya *et al*., 2004). In the Monochamini, males of *M. saltuarius* exhibited behavioural activities with dead male and females under laboratory conditions suggesting that short-range chemically-mediated communication could be present (Kobayashi *et a*l., 2003). Similarly, male *M. galloprovincialis* attempted to mate with dead unwashed and dead female extract- treated females (Ibeas *et al*., 2009) and *M. alternatus* males exhibited mating behaviour on glass rods treated with female extracts (Kim *et al*., 1992)

3.4.2 Comparison of the two approaches used to test presence of contact pheromone in females

The approach used in the experiments for 2004/05 and 2005/06 seasons was to expose different males to different dead, unwashed, washed and extract-recoated females without initially testing male response to those females prior to killing them. In contrast, during the 2006/07 season insects were paired and, if they mated, the same pairs were tested through the whole series of experiments.

Both bioassay approaches confirmed the loss of recognition of a solvent-washed female by male conspecifics and the restoration of recognition of a female treated with hexane extract. The only limitation with the first approach was that the response of the males to live insects had not been tested whereas with the second approach, the mating status of the males with the same females when alive was compared with the same females when dead. In terms of getting sufficient replication for statistical analysis and conforming to the assumptions of independent observations, the first approach could be more suitable than the second approach. Similarly, the first approach offers much more opportunities for testing different hypotheses in view of the limited insect numbers for experimenting with cerambycids. For example, when there was mortality of a male in the second approach, no further experimentation could be done resulting in a progressive decline in replication. From a statistical point of view the first approach made the data more amenable for analysis with chi square whereas the second approach did not allow for ease of statistical analysis because the data were not independent since the results of the second, third and fourth experiments were dependent on the outcome from the first experiments. However, the chi square analysis was applied to the data.

It was generally observed that males no longer responded after the last weeks of January as compared to response during December implying that females will be no longer receptive as the season progresses (Personal observation). In general, using both approaches, there was poor male response to live and dead females to chemical cues. This indicates the importance of other cues in mating behaviour of *M. leuconotus*. Poor response to the recoated females could also be explained in terms of dose-response to chemicals whereby the high dose of contact pheromone on the elytra was inhibitory. Cuticular hydrocarbons were extracted from the whole body but restored onto the elytra only, possibly resulting in a higher than normal dose.

Similar responses to washed females have been reported in *Semanotus japonicus* (Fauziah *et al*., 1992), where a few males mounted but did not exhibit copulatory behaviour. In other cerambycids, males did not respond to washed females at all (Ginzel

and Hanks 2003, Ginzel *et al*., 2003a, Kobayashi *et al*., 2003, Ginzel *et al*., 2003b, Crook *et al*., 2004, Lopes *et al.,* 2005) but gave positive responses to extract-treated females and models. However, the unexpected male mounting response to washed females could be attributed to the involvement of other cues such as visual and auditory cues in mating behaviour (Lu *et al*., 2007). Despite the mounting response to the washed females, the results from the current study appear to confirm the possible existence of compounds in the body surface of the female beetles that elicit copulatory behaviour in males, since there was no copulatory behaviour.

The existence of female contact pheromones in other cerambycids has been demonstrated by evaluating male response to extract-treated females or other extract-treated inert materials. For example, male *M. alternatus* attempted to mate with glass rods treated with a female solvent extract (Kim *et al*., 1992) while *P. hilaris* (Fukaya & Honda, 1992, 1995 and 1996), *Anoplophora* glabripennis (Li *et al*., 1999) and *A. malasiaca.* Fukaya *et al*, (2000) showed similar responses to female extracts on glass rods and cuticular hydrocarbons were apparently constituents of the contact pheromones. The existence of contact pheromones in cerambycid beetles has also been demonstrated and confirmed (Ginzel and Hanks, 2003, Ginzel *et al*., 2003a, Lacey *et al*., 2008, Rutledge *et al*., 2009, Spikes *et al*., 2010, Silk *et al*., 2011).

The results suggest the possible existence of a female contact pheromone in *M. leuconotus* since males were able to recognise dead females and dead recoated females. It appears that mate recognition is mediated by contact pheromones present on the body surface. Once the male senses the contact pheromone with its antennae, or tarsi a series of behaviours such as dashing forward to align its body with that of the female, licking, mounting and copulation are initiated as previously described in Chapter 2 of this thesis. Based on the reaction to the extract-treated female, it can be inferred that there could be a female contact pheromone in *M. leuconotus* that elicits mating behaviour in the males. The results are consistent with work at NRI, which showed female cuticular hydrocarbons were different from those of males (Fig. 3.1). Although there are numerous components in common, there is both a male-specific component (retention time 28.08 min.) and female-specific component (retention time 30.15 min.) that could serve as a sex recognition factor (Fig 3.1). Cuticular hydrocarbons profiles can be species and sex specific (Howard and Blomquist, 2005). For example, female-specific hydrocarbons have been identified (Crook *et al*., 2004; Rutledge *et al*., 2009) and their roles in mate recognition confirmed while male-specific hydrocarbons are less common but also important in mate recognition (Olaniran *et al*., 2013). The male-specific cuticular hydrocarbons for *M. leuconotus* could be involved in sex recognition whereby competing

males detect the presence of other conspecifics as evidenced by fighting for females (see Chapter 2). Alternatively, the male-specific cuticular hydrocarbons are probably used to mark the presence of males in which case they could be acting as contact cues used by the females to locate the males. In Chapter 2 of this thesis, mating behaviour was initiated when a female approached a male who would then use antennal contact to initiate the mating process. However, the practical application of the contact pheromone in management of the pest appears to be limited. In general, this information contributes to more understanding of the chemical ecology of cerambycids, which could be applied in further development of management practices.

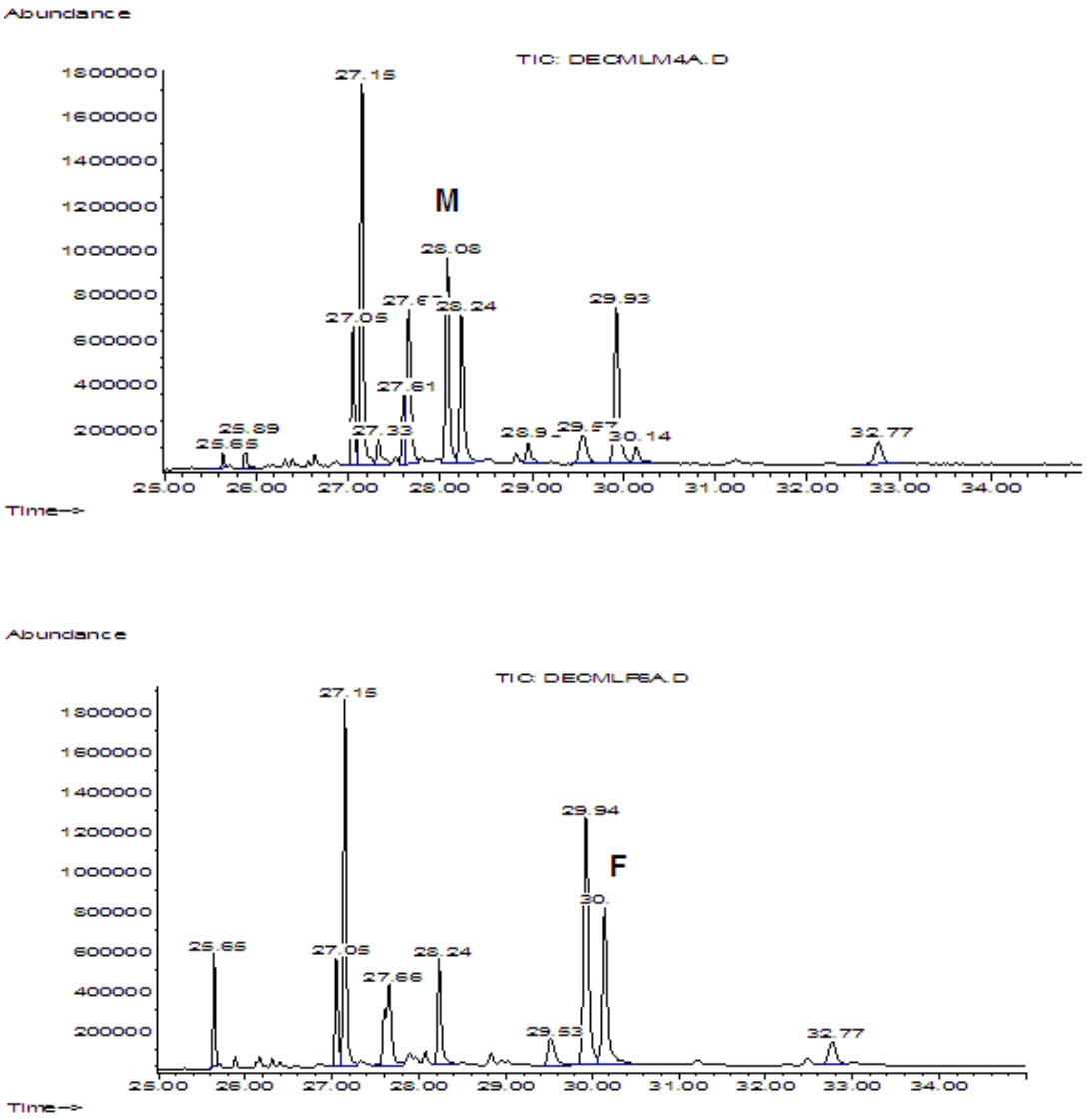


Fig. 3.1 Total ion spectra from GC-MS analyses of cuticular hydrocarbons of *Monochamus leuconotus* sampled by Solid Phase Microextraction (upper male (M), lower female (F)). (Source: D. R. Hall, Natural Resources Institute)

Chapter 4

LABORATORY BIOASSAYS OF RESPONSES OF *Monochamus leuconotus* (PASCOE) TO DIFFERENT OLFACTORY CUES

4.1. INTRODUCTION

Observations on mating behaviour and daily activities of *Monochamus leuconotus* (Chapter 2) showed that the female was attracted to the male but it did not provide sufficient evidence on possible long-range attraction between the sexes in this species. Therefore, the objective of this study was to continue with investigations for possible long-range attraction of *M. leuconotus* to live conspecific insects and the synthetic male–specific compound (MSC) under more controlled conditions. The olfactometer bioassays were also used to investigate the responses of *M. leuconotus* to different volatiles from plants, which is difficult to do under field conditions because of the presence of large quantities of host and non-host volatiles present naturally.

4.2. MATERIALS AND METHODS

4.2.1 Insects

Adult *M. leuconotus* beetles were collected from coffee fields at the Coffee Research Station, Chipinge, Zimbabwe and surrounding plantations. Insects were brought to the laboratory, separated according to sex and stored in separate cages (see Chapter 2). Insects were fed on fresh coffee twigs before the bioassays. Beetles were used within one to two days from the date of field collection. Insects were probably not virgin but field-collected insects used for previous behavioural studies mated successfully.

4.2.2 Olfactometer

A Y-tube olfactometer was constructed from Perspex tubing (diameter 6 cm, stem 60 cm long and arms 60 cm long) and placed on a table in a room in front of a window with natural light (Fig. 4.1). The olfactometer used here was constructed in Zimbabwe from clear Perspex tubing sourced in the UK. The tubing was designed to be wide enough (6

cm diameter) to allow easy movement of *M. leuconotus* beetles without risking damage to their antennae.

Plastic boxes (20 cm x 12 cm x 7.5 cm) containing the odour sources were attached to both ends of the Y tube. A pump attached to the stem of the Y tube drew in air (2 litre/min). Because of the large diameter, the flow rate was relatively low at approximately 70 cm/min in the main stem. Air was drawn in through charcoal filters (8-10 mesh activated charcoal, 35.5 cm length, 4 cm diameter) on each arm, through the source boxes, into the olfactometer, through the pump and out through a charcoal filter (Fig. 4.2). The olfactometer was placed in front of two identical windows with natural light and the Y-tube was positioned to avoid positional bias due to lighting or any other factors.

Fig. 4.1 Y-tube Olfactometer used to evaluate response of *Monochamus leuconotus* to different odours under laboratory conditions at Coffee Research Station, Chipinge, Zimbabwe.

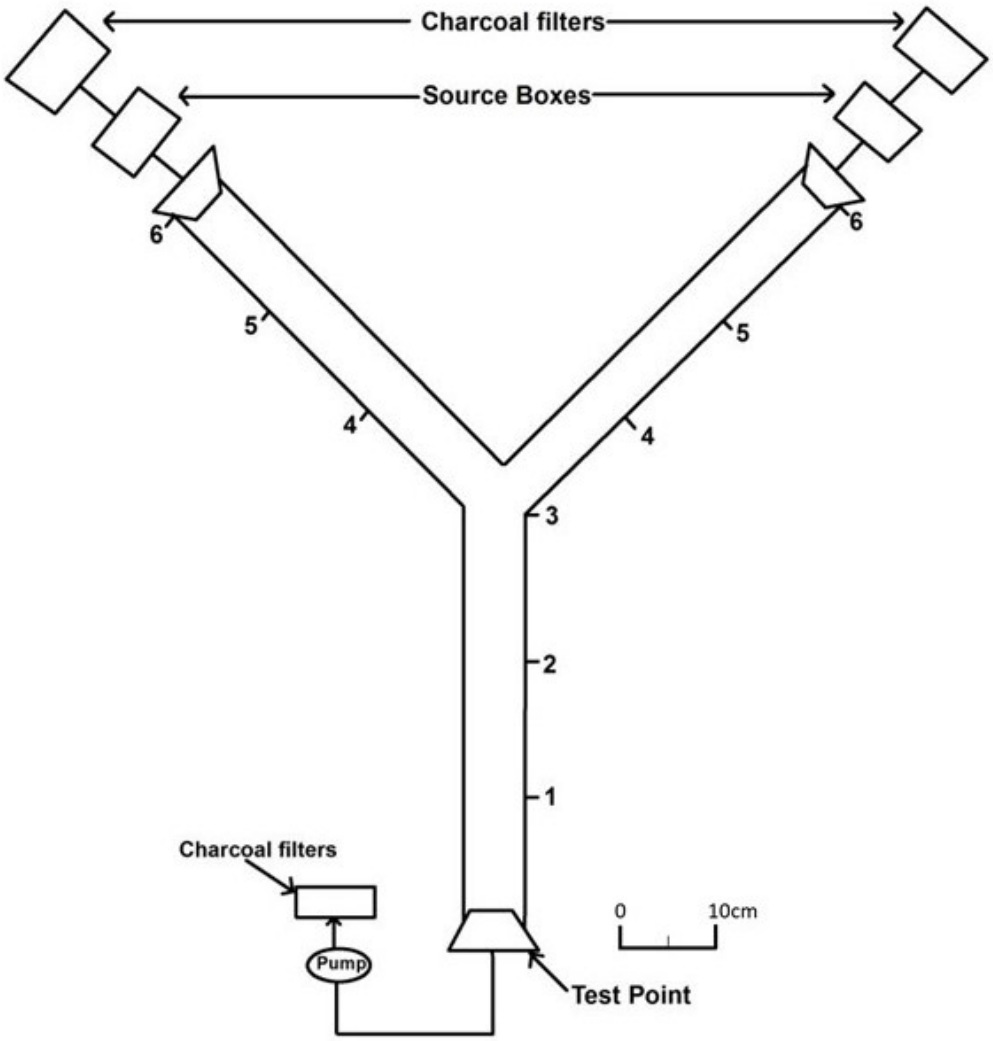


Fig. 4.2 Y-tube Olfactometer used to evaluate response of *Monochamus leuconotus* to different odours under laboratory conditions at Coffee Research Station, Chipinge, Zimbabwe.

4.2.3 Bioassay procedures

Odour sources were placed in the plastic boxes and individual test male or female insects introduced at the downwind end of the stem of the Y–tube. The olfactometer was run for 1 min before introduction of new odour sources. Preliminary studies indicated that if an insect had not made a choice after 10 min it did not generally do so after a longer interval, and so the bioassay was run for 10 min and the position of the insect noted. The arms of the Y–tube were marked at 20 cm intervals from the start position such that score 1 - 3 indicated no choice as the insect remained in the stem of the Y-tube and score 4 - 6 indicated a choice according to the arm chosen with score 6 corresponding to the insect

reaching the source box (Fig. 4.2). Test source boxes for the synthetic male-specific compound were separated from the other boxes in order to avoid contamination. Individual test beetles were released at the downwind end of the stem and observations started immediately after release. Once walking started, observations continued until the insect reached either end of the arms of the Y- tube or after 10 minutes. In certain cases, an insect that had made a choice would quickly move back and choose the other arm of the olfactometer. However, the insect's first choice became the final score.

Odour sources were swapped after every five runs to compensate for any positional bias. In between, the olfactometer was washed with ethanol and left to run for a minute without an insect to allow the volatiles to clear from the Y- tube. All bioassays were run between 10.00 h and 15.00 h under 25ºC, LD 13:11 h regime and 60% RH conditions. Treatments were alternated on any given day.

Observations were carried out during the flight season of *M. leuconotus* (November to April) in 2004/5, 2005/6 and 2006/7. Some observations were carried out during the night in 2006/7 in order to compare with daylight responses. A torch with a red plastic cover over the lens was used to observe the insects under dark conditions since insects are not sensitive to red light.

4.2.4 Odour sources

Blank

In order to check for any possible bias to the two arms of the Y-tube, the insects were tested in the olfactometer without olfactory cues in the source boxes. Blank runs without any odour sources were run daily.

Host plant sources

Arabica coffee leaves (100 g) were collected from plantations at the Coffee Research Station, Chipinge, Zimbabwe. Leaf renewal was once per day.

Freshly harvested leaves of tea, *Camellia sinensis,* (100 g) were collected from an old tea garden at the Coffee Research Station, Chipinge, Zimbabwe. Tea was used because it was previously reported as a host plant for *M. leuconotus* in Malawi (see Table 1.1)

Keetia gueinzii leaves (100 g) were collected from the bush surrounding coffee plantations at the Coffee Research Station, Chipinge. *Keetia guenzii* was selected because it is closely related to coffee and is the most abundant species belonging to the family Rubiaceae in the coffee growing areas (see Chapter 6).

Arabica coffee bark (100 g) was collected by scraping from 3 to 5 year old Catimor F6 trees at the Coffee Research Station just prior to testing. Coffee bark was renewed once per day.

Insects

Female and male beetles used as odour sources for the experiments were collected from laboratory cages containing separate sexes of field-collected, adult beetles. A male or female was then combined with either coffee bark (100 g) or coffee leaves (100 g) depending on the experiment as detailed below. In each case, the plant materials were placed in the source box first before placing the insects on top of the host materials.

Synthetic male-specific compound

The synthetic male-specific compound from *M. leuconotus*, 2-(4-heptyloxy-1-butyloxy)-1-ethanol, was provided from NRI and tested in slow-release dispensers and as neat material. Two types of pheromone dispensers were evaluated: the white polyethylene sachet (2.5 cm x 2.5 cm x 120 μm thick) and the heat-sealed polyethylene vial (22 mm x 8 mm x 1.5 mm thick) each containing 50 μl of compound. The release rate of synthetic compound from the sachet determined in a laboratory wind tunnel at 27°C and 8 km/h wind speed was approximately 0.38 mg/d while that for the vial was approximately 0.052 mg/d. The vial or sachet was hung at the centre of the lid of the source box.

Alternatively, the compound was diluted into three concentrations from an initial solution of 50 mg in 5 ml hexane. The compound (0.05 mg, 0.1 mg or 10 mg) was impregnated onto filter paper strips (25 mm x 25 mm, Whatman No. 1), which were then placed at the centre of the source box during the bioassays.

4.2.5 Experimental Design and Statistical Analysis

Data were analysed according to whether insects scored 4 -6 (i.e. made a choice) or scored 6 (i.e. made source contact). Insects that scored 4 or more, (i.e. made a choice)

were classed as responders, otherwise they were considered to be non-responders. These data were used to calculate the response factor for each pair of treatments.

Data for 2004/5 and 2005/6 bioassays were combined. Data on insect responses during daytime and during the night for 2006/7 were compared. Daytime data for all the three years were combined.

The choices of the responding insects were subjected to a binomial test for deviation from a 50:50 response with SPSS 12.0.1. Statistical significance was set at 5%.

The percent response data for daytime and night time responses to insects during the 2006/07 season were normalised by arcsine transformation followed by ANOVA unbalanced design using Gen Stat Release 10.1 for Windows, Lawes Agricultural Trust. Differences between means were determined using the LSD test.

4.3. RESULTS

4.3.1 General insect response in the bioassays

Movements of *M. leuconotus* beetles consisted of either a general walking movement upwind towards the odour source (N = 875) or, at times, a direct flight towards the odour source (N = 30) (Appendix 2). Insects would move from the point of release either in straight line or side-to-side zigzag movements. Beetles that did not respond remained on the bottom, side or top of the stem of the olfactometer.

4.3.2 Response in absence of odour

In the absence of odour the percent response for males was high (76.9% for a score of 4-6 and 69.2% for a score of 6). There were no significant differences in choice by both males and females ($P > 0.05$) when both arms of the olfactometer did not contain any odours (Tables 4.1 –3). Both sexes chose either arm freely showing that there was no positional bias due to placement of the olfactometer in the laboratory or any other factors.

4.3.3 Responses to plant volatiles

During 2004/5 and 2005/6 both male and female *M. leuconotus* showed some preference for coffee leaves over clean air and coffee bark over clean air (Table 4.1). Using a score

of 4 -6, the responses of females to coffee leaves and males to coffee bark were significant ($P < 0.05$). With a score of 6, the responses of both males and females to coffee bark were significant ($P < 0.05$) although responses to coffee leaves were not significant. The percent of insects responding to host volatiles varied between 46.2% and 90% when a score of 4-6 was used against 33.3% to 72% when a score of 6 was used.

Table 4.1: Response of *Monochamus leuconotus* to plant volatiles in a laboratory olfactometer during 2004/5 and 2005/6 bioassays

Test Insect	Stimulus A	Stimulus B	*N*	No. Insects Responding		Percent Response[a]	*P*[b]
				A	B		
Female (score 4-6)	Coffee leaves	Blank	49	22	11	67.4	**0.04***
	Coffee leaves	Tea leaves	25	10	11	84	0.499
	Coffee bark	Blank	15	7	2	60	0.089
	Blank	Blank	13	3	3	46.2	0.656
Male (score 4-6)	Coffee leaves	Blank	42	15	13	66.7	0.425
	Coffee leaves	Tea leaves	25	4	8	48	0.194
	Keetia leaves	Coffee leaves	10	3	6	90	0.254
	Coffee bark	Blank	15	10	1	73.3	**0.006***
	Blank	Blank	13	4	6	76.9	0.377
Female (score 6)	Coffee leaves	Blank	49	14	9	46.9	0.202
	Coffee leaves	Tea leaves	25	9	9	72	0.592
	Coffee bark	Blank	15	6	0	40	**0.016***
	Blank	Blank	13	4	3	53.8	0.499
Male (score 6)	Coffee leaves	Blank	42	12	8	47.6	0.252
	Coffee leaves	Tea leaves	25	3	7	40	0.172
	Keetia leaves	Coffee leaves	10	3	4	70	0.5
	Coffee bark	Blank	15	5	0	33.3	**0.031***
	Blank	Blank	13	3	6	69.2	0.254

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.

[b] Binomial test for differences from 50:50 response

* Significant ($P < 0.05$)

Preferences were apparent but less marked in experiments carried out during daylight in 2006/7 (Table 4.2). Percent response varied from zero to more than 90% with uniformly higher responses in the presence of coffee bark compared with coffee leaves.

Table 4.2: Response of *Monochamus leuconotus* to plant volatiles in a laboratory olfactometer during 2006/7 bioassays

Test Insect	Stimulus A	Stimulus B	*N*	No. Insects Responding		Percent Response[a]	*P*[b]
				A	B		
Female (score 4-6)	Coffee leaves	Blank	9	1	2	33.3	0.500
	Coffee bark	Blank	26	12	8	76.9	0.252
	Blank	Blank	20	12	7	95.0	0.180
Male (score 4-6)	Coffee leaves	Blank	9	3	0	33.3	0.125
	Coffee bark	Blank	26	12	8	76.9	0.252
	Blank	Blank	20	11	8	95.0	0.324
Female (score 6)	Coffee leaves	Blank	9	1	2	33.3	0.500
	Coffee bark	Blank	26	9	8	65.4	0.500
	Blank	Blank	20	11	7	90.0	0.240
Male (score 6)	Coffee leaves	Blank	9	0	0	0.00	
	Coffee bark	Blank	26	7	7	53.8	0.604
	Blank	Blank	20	9	7	80.0	0.402

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.[b] Binomial test for differences from 50:50 response

* Significant ($P < 0.05$)

When all data were combined, (Table 4.3) the response of males to coffee bark was significant ($P < 0.05$) using a score of 4 -6 but not with a score of 6. Female beetles showed preferences for coffee leaves and bark using a score of 4-6, but these were not significant ($P = 0.07$).

No preference was shown between coffee leaves and tea leaves by either sex of the beetles. Both sexes did not show preference between *Keetia* and tea leaves (Table 4.1). However, the general response to the plant volatiles was high (40 – 90%).

Table 4.3: Response of *Monochamus leuconotus* to plant volatiles in a laboratory olfactometer over three seasons (2004/5, 2005/6, 2006/7).

Test Insect	Stimulus A	Stimulus B	*N*	No. Insects Responding		Percent Response[a]	*P*[b]
				A	B		
Female (score 4-6)	Coffee leaves	Blank	58	23	13	62.1	0.070
	Coffee leaves	Tea leaves	25	10	11	70.73	0.499
	Coffee bark	Blank	41	19	10	84.0	0.070
	Blank	Blank	33	15	10	75.8	0.212
Male (score 4-6)	Coffee leaves	Blank	58	18	13	53.5	0.237
	Coffee leaves	Tea	25	4	8	48.0	0.193
	Keetia leaves	Coffee leaves	10	3	6	90.0	0.254
	Coffee bark	Blank	41	22	9	75.6	**0.015***
	Blank	Blank	33	15	14	87.9	0.500
Female (score 6)	Coffee leaves	Blank	58	15	11	68.9	0.279
	Coffee leaves	Tea leaves	25	9	9	72.0	0.593
	Coffee bark	Blank	41	15	8	56.1	0.105
	Blank	Blank	33	15	9	72.7	0.154
Male (score 6)	Coffee leaves	Blank	58	12	8	34.5	0.252
	Coffee leaves	Tea	25	3	7	40.0	0.172
	Keetia leaves	Coffee leaves	10	3	4	70.0	0.500
	Coffee bark	Blank	41	12	7	46.3	0.180
	Blank	Blank	33	12	13	75.6	0.500

[a]Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.[a] Binomial test for differences from 50:50 response

* Significant (p<0.05)

4.3.4 Response to live insects in the presence of plant materials

Responses of *M. leuconotus* to live conspecific insects plus plant material are shown in Tables 4.4-4.6.

General insect response varied from 74.7 to 90% when a score of 4-6 was used against 66.7 to 80% for a score of 6. No preferences to live conspecific male or female beetles in the presence of plant material over coffee bark and leaves were shown by either sex during the 2004/5 seasons ($P > 0.05$) (Tables 4.4).

Similarly, neither sex of *M. leuconotus* showed any preference for either male nor female conspecifics over coffee leaves and coffee bark during the 2006/7 season (Table 4.5). The response of both sexes to the treatments varied between 60 and 95% when a score of 4-6 was used.

When data were combined, there were no significant preferences ($P > 0.05$) for either male or female insects over coffee leaves and bark (Table 4.6) indicating the absence of any long-range chemical cues between the insects under these conditions.

Table 4.4: Response of *Monochamus leuconotus* to live conspecific insects and plant material in a laboratory olfactometer during 2004/5 and 2005/6 seasons

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response[a]	*P*[b]
			N	A	B		
Female (score 4-6)	Male + Coffee bark	Coffee bark	15	7	5	80.0	0.387
	Female + Coffee bark	Coffee bark	15	7	7	93.0	0.605
	Male + Coffee leaves	Coffee leaves	10	3	6	90.0	0.254
Male (score 4-6)	Male + Coffee bark	Coffee bark	15	8	5	86.7	0.290
	Female + Coffee bark	Coffee bark	15	7	7	93.3	0.605
	Male + Coffee leaves	Coffee leaves	10	5	4	90.0	0.50
Female (score 6)	Male + Coffee bark	Coffee bark	15	6	4	66.7	0.377
	Female + Coffee bark	Coffee bark	15	7	4	73.3	0.274
	Male + Coffee leaves	Coffee leaves	10	2	5	70.0	0.225
Male (score 6)	Male + Coffee bark	Coffee bark	15	6	2	53.3	0.144
	Female + Coffee bark	Coffee bark	15	6	6	80.0	0.613
	Male + Coffee leaves	Coffee leaves	10	4	3	70.0	0.499

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.

[b] Binomial test for differences from 50:50 response

Table 4.5: Response of *Monochamus leuconotus* to live conspecific insects and plant material in a laboratory olfactometer during 2006/7 season

Test Insect	Stimulus A	Stimulus B	*N*	No. Insects Responding		Percent Response[a]	*P*[b]
				A	B		
Female (score 4-6)	Male + Coffee bark	Coffee bark	20	12	7	95.0	0.180
	Female + Coffee bark	Coffee bark	20	10	9	95.0	0.500
	Male + Coffee leaves	Coffee leaves	20	8	9	85.0	0.500
	Female + Coffee leaves	Coffee leaves	30	13	13	86.7	0.577
Male (score 4-6)	Male + Coffee bark	Coffee bark	20	9	8	85.0	0.500
	Female + Coffee bark	Coffee bark	20	9	6	75.0	0.304
	Male + Coffee leaves	Coffee leaves	20	10	8	90.0	0.407
	Female + Coffee leaves	Coffee leaves	30	9	9	60.0	0.593
Female (score 6)	Male + Coffee bark	Coffee bark	20	12	6	90.0	0.119
	Female + Coffee bark	Coffee bark	20	10	8	90.0	0.407
	Male + Coffee leaves	Coffee leaves	20	9	8	85.0	0.500
	Female + Coffee leaves	Coffee leaves	30	13	11	80.0	0.419
Male (score 6)	Male + Coffee bark	Coffee bark	20	5	8	65.0	0.290
	Female + Coffee bark	Coffee bark	20	9	4	65.0	0.133
	Male + Coffee leaves	Coffee leaves	20	8	7	75.0	0.499
	Female + Coffee leaves	Coffee leaves	30	9	13	73.3	0.262

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*n)*.

[b] Binomial test for differences from 50:50 response

Table 4.6 Response of *Monochamus leuconotus* to live conspecific insects and plant material in a laboratory olfactometer during three seasons (2004/5, 2005/6, 2006/7).

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response [a]	P [b]
			N	A	B		
Female (score 4-6)	Male + Coffee bark	Coffee bark	35	19	12	88.6	0.141
	Female + Coffee bark	Coffee bark	35	17	16	94.3	0.499
	Male + Coffee leaves	Coffee leaves	45	11	19	66.7	0.100
	Female + Coffee leaves	Coffee leaves	30	13	13	85.0	0.577
Male (score 4-6)	Male + Coffee bark	Coffee bark	35	17	13	85.7	0.292
	Female + Coffee bark	Coffee bark	35	16	13	82.8	0.356
	Male + Coffee leaves	Coffee leaves	45	15	12	60.0	0.351
	Female + Coffee leaves	Coffee leaves	30	9	9	60.0	0.593
Female (score 6)	Male + Coffee bark	Coffee bark	35	18	10	80.0	0.092
	Female + Coffee bark	Coffee bark	35	17	12	82.9	0.229
	Male + Coffee leaves	Coffee leaves	45	11	13	53.3	0.419
	Female + Coffee leaves	Coffee leaves	30	13	11	80.0	0.419
Male (score 6)	Male + Coffee bark	Coffee bark	35	11	10	60.0	0.500
	Female + Coffee bark	Coffee bark	35	15	10	71.4	0.212
	Male + Coffee leaves	Coffee leaves	45	15	10	55.5	0.212
	Female + Coffee leaves	Coffee leaves	30	9	13	73.3	0.262

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.

[b] Binomial test for differences from 50:50 response

4.3.5 Response to synthetic male-specific chemical

Male-specific chemical dispensed from vials or sachets

Responses of male and female *M. leuconotus* to the synthetic, male-specific compound dispensed from polythene vials or sachets are shown in Tables 4.7, 4.8 and 4.9.

During the 20004/5 and the 2005/6 seasons there was significant attraction of female beetles to the synthetic male-specific compound dispensed from a sachet relative to clean air (Table 4.7). For males there were indications that they were repelled by the dispensers in the presence of coffee leaves (Table 4.7).

No preferences were shown for the sachet and vial dispensers over either clean air or coffee leaves ($P > 0.05$) during the 2006/07 season (Table 4.8).

When data were combined, females showed preferences ($P < 0.05$) for the sachet over clean air. There were no preferences shown between the vial, sachet, coffee leaves, and clean air by males (Table 4.9).

Table 4.7: Response of *Monochamus leuconotus* to synthetic male-specific compound (MSC) in chemical dispensers and plant materials in a laboratory olfactometer during 2004/5 season and 2005/6 season

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response[a]	*P*[b]
			N	A	B		
Female (score 4-6)	MSC in sachet	Blank	25	16	5	84.0	**0.01***
	MSC in vial	Blank	25	8	9	72.0	0.5
	MSC in vial + coffee leaves	Coffee leaves	10	6	4	100	0.377
	MSC in sachet + coffee leaves	Coffee leaves	10	5	5	100	0.623
Male (score 4-6)	MSC in sachet	Blank	25	6	9	60.0	0.304
	MSC in vial	Blank	25	10	8	72.0	0.407
	MSC in vial + coffee leaves	Coffee leaves	10	2	8	100	**0.05***
	MSC in sachet + coffee leaves	Coffee leaves	10	3	7	100	0.172
Female (score 6)	MSC in sachet	Blank	25	9	4	52.0	0.133
	MSC in vial	Blank	25	7	8	60.0	0.5
	MSC in vial + coffee leaves	Coffee leaves	10	3	4	70.0	0.5
	MSC in sachet + coffee leaves	Coffee leaves	10	3	5	80.0	0.363
Male (score 6)	MSC in sachet	Blank	25	4	8	48.0	0.194
	MSC in vial	Blank	25	8	6	56.0	0.395
	MSC in vial + coffee leaves	Coffee leaves	10	2	7	90.0	0.089
	MSC in sachet + coffee leaves	Coffee leaves	10	3	7	100	0.172

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.

[b] Binomial test for differences from 50:50 response

* Significant ($P < 0.05$)

Table 4.8 Response of *Monochamus leuconotus* to synthetic male-specific compound (MSC) in chemical dispensers and plant materials in a laboratory olfactometer during 2006/7 season

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response[a]	*P*[b]
			N	A	B		
Female (score 4-6)	MSC in sachet	Blank	15	7	6	86.7	0.499
	MSC in vial	Blank	5	4	1	100	0.188
	MSC in vial + coffee leaves	Coffee leaves	20	12	8	100	0.252
	MSC in sachet + coffee leaves	Coffee leaves	20	4	3	35.0	0.499
Male (score 4-6)	MSC in sachet	Blank	15	4	6	66.7	0.377
	MSC in vial	Blank	5	1	0	20.0	0.188
	MSC in vial + coffee leaves	Coffee leaves	20	10	8	90.0	0.407
	MSC in sachet + coffee leaves	Coffee leaves	20	4	4	40.0	0.635
Female (score 6)	MSC in sachet	Blank	15	5	4	60.0	0.500
	MSC in vial	Blank	5	4	1	100	0.188
	MSC in vial + coffee leaves	Coffee leaves	20	12	7	95.0	0.180
	MSC in sachet + coffee leaves	Coffee leaves	20	2	5	35.0	0.227
Male (score 6)	MSC in sachet	Blank	15	5	10	100	0.151
	MSC in vial	Blank	5	2	3	100	0.500
	MSC in vial + coffee leaves	Coffee leaves	20	7	10	85.0	0.315
	MSC in sachet + coffee leaves	Coffee leaves	20	4	4	40.0	0.634

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.

[b] Binomial test for differences from 50:50 response

Table 4.9 Response of *Monochamus leuconotus* to synthetic male- specific compound (MSC) in chemical dispensers and plant materials in a laboratory olfactometer over three seasons (2004/5, 2005/6, 2006/7).

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response[a]	*P*[b]
			N	A	B		
Female (score 4-6)	MSC in sachet	Blank	40	23	11	86.7	**0.029***
	MSC in vial	Blank	30	12	10	85	0.416
	MSC in vial + coffee leaves	Coffee leaves	30	18	12	73.3	0.181
	MSC in sachet + coffee leaves	Coffee leaves	30	9	8	56.7	0.500
Male (score 4-6)	MSC in sachet	Blank	40	10	15	62.5	0.212
	MSC in vial	Blank	30	11	8	63.3	0.324
	MSC in vial + coffee leaves	Coffee leaves	30	12	16	93.3	0.286
	MSC in sachet + coffee leaves	Coffee leaves	30	7	11	60	0.240
Female (score 6)	MSC in sachet	Blank	40	9	8	42.5	0.500
	MSC in vial	Blank	30	11	9	66.7	0.412
	MSC in vial + coffee leaves	Coffee leaves	30	15	11	86.7	0.278
	MSC in sachet + coffee leaves	Coffee leaves	30	5	10	50	0.151
Male (score 6)	MSC in sachet	Blank	40	10	15	62.5	0.212
	MSC in vial	Blank	30	11	8	63.3	0.324
	MSC in vial + coffee leaves	Coffee leaves	30	12	16	93.3	0.286
	MSC in sachet + coffee leaves	Coffee leaves	30	7	11	60	0.240

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.[b] Binomial test for differences from 50:50 response

* Significant ($P < 0.05$)

Male-specific chemical dispensed from filter paper

No preferences were shown to different amounts of the synthetic male–specific compound of *M. leuconotus* dispensed from filter paper over clean air by either sex of the beetles (Table 4.10). Beetles showed equal preference to all concentrations of the solution over clean air ($P > 0.05$).

Table 4.10: Response of *M. leuconotus* to different concentrations of the synthetic male-specific compound (MSC) dispensed in a filter paper in a laboratory olfactometer during the 2004/05 season.

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response[a]	P^b
			N	A	B		
Female (score 4-6)	0.05 mg MSC	Blank	10	5	3	80.0	0.363
	1 mg MSC	Blank	10	3	3	60.0	0.656
	10 mg MSC	Blank	40	20	18	70.9	0.436
Male (score 4-6)	1 mg MSC	Blank	10	5	4	90.0	0.500
	10 mg MSC	Blank	40	20	16	90.0	0.300
Female (score 6)	0.05 mg MSC	Blank	10	4	3	70.0	0.499
	1 mg MSC	Blank	10	3	2	50.0	0.500
	10 mg MSC	Blank	40	18	12	75.0	0.181
Male (score 6)	1 mg MSC	Blank	10	5	4	90.0	0.500
	10 mg MSC	Blank	40	18	16	85.0	0.432

[a] Percent response: percentage of beetles responding to Stimulus A and Stimulus B over total number of beetles tested (*N)*.

[b] Binomial test for differences from 50:50 response

4.3.6 Response to live conspecific insects during the night

Night bioassays were conducted during the 2006/07 season. Beetles were tested for their response to live insects in the presence of coffee bark and leaves. Neither sex of *M. leuconotus* showed any preference ($P > 0.05$) for live conspecific beetles of either sex in the presence of coffee leaves and bark (Table 4.11).

Table 4.11 Response of *Monochamus leuconotus* to live conspecific insects and plant material at night in a laboratory olfactometer during the 2006/07 season.

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response[a]	*P*[b]
			N	A	B		
Female (score 4-6)	Males + Coffee leaves	Coffee leaves	15	2	0	13.3	0.250
	Male + Coffee bark	Coffee bark	10	1	0	10.0	0.500
	Female + Coffee bark	Coffee bark	10	0	0	0.0	-
Male (score 4-6)	Males + Coffee leaves	Coffee leaves	15	1	0	6.7	0.500
	Male + Coffee bark	Coffee bark	10	0	0	0.0	-
	Female + Coffee bark	Coffee bark	10	2	1	30.0	0.499
Female (score 6)	Males + Coffee leaves	Coffee leaves	15	0	0	0.0	-
	Male + Coffee bark	Coffee bark	10	0	0	0.0	-
	Female + Coffee bark	Coffee bark	10	1	1	20.0	0.750
Male (score 6)	Males + Coffee leaves	Coffee leaves	15	0	0	0.0	-
	Male + Coffee bark	Coffee bark	10	0	0	0.0	-
	Female + Coffee bark	Coffee bark	10	1	2	30.0	0.500

[a] Percent response: percentage of beetles responding to treatment and control over total number of beetles tested (*N*)

[b] Binomial test for differences from 50:50 response

Response to live conspecific insects during daytime and at night

Responses to live insects during daytime bioassays for 2006/07 were significantly higher ($P < 0.001$) than for night bioassays (Fig. 4.3). This indicates that the insects are more active during the day than at night.

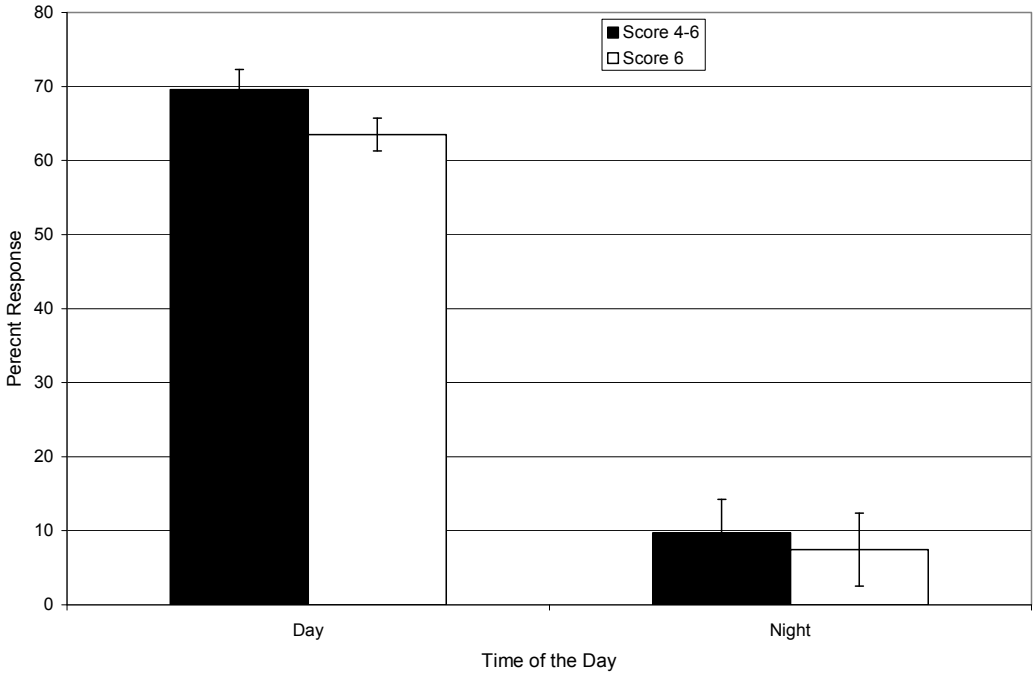


Fig. 4.3 Response of *Monochamus leuconotus* beetles to live conspecifics during daytime and at night in olfactometer bioassays. Score 4-6 and Score 6 indicate choices according to the arm of the olfactometer chosen according to Fig 4.2.

4.3.7 Response to male-specific compound during the night

The percent response of both sexes to chemical dispensers during the night was generally low when compared to daytime observations (Table 4.1-12). Using a score of 4-6, female beetles did not show preference to the sachet and vial over clean air ($P > 0.05$) while males also did not show preference to the sachet over clean air. When a score of 6 was used, both sexes did not prefer the sachet over clean air and the female did not prefer the vial over clean air.

Table 4.12 Response of *Monochamus leuconotus* to chemical dispensers during the night in a laboratory olfactometer

Test Insect	Stimulus A	Stimulus B	No. Insects Responding			Percent Response[a]	P^b
			N	A	B		
Female (score 4-6)	Sachet	Blank	60	11	10	35.0	0.500
	Vial	Blank	30	5	2	23.3	0.250
	Blank	Blank	10	0	0	0.00	1.000
Male (score 4-6)	Sachet	Blank	45	5	3	17.8	0.368
	Blank	Blank	10	0	0	0.00	1.000
Female (score 6)	Sachet	Blank	60	9	7	26.7	0.402
	Vial	Blank	30	2	0	6.70	0.250
	Blank	Blank	10	0	0	0.00	0.000
Male(score 6)	Sachet	Blank	45	1	1	4.40	0.750
	Blank	Blank	10	0	0	0.00	1.000

[a] Percent response: percentage of beetles responding to treatment (A) and control (B) over total number of beetles tested (*N*)

[b]Binomial test for differences from a 50:50 response

4.4. DISCUSSION

4.4.1 General observations

In initial studies in the absence of odour the percentage response for males was up to 77%, confirming that they could move unrestricted in the olfactometer. There were no significant differences in choice by both males and females and both sexes chose either arm freely showing that there was no positional bias due to placement of the olfactometer in the laboratory or any other factors. This could have been a response to move upwind and/or to the light from the windows but it was considered a useful system to assess the ability of the beetles to choose between the volatiles emitted by odour sources in the two arms.

Most experiments were carried out during daylight hours, as the observations described in Chapter 2 indicated activity during the night was greatly reduced. As few consistently strong responses were observed to the odour sources tested here during the day, some

of the experiments were repeated during the night. No significant responses to odours were observed and the general level of responsiveness was very low, confirming the results of Chapter 2 and that the behaviour in the olfactometer was at least consistent with that in more natural surroundings.

The two scoring methods developed in this study gave similar results with respect to male response to coffee bark and to the combination of the male-specific compound dispensed in a vial and coffee leaves, which were both significant. Despite the slight differences noted in terms of female response to coffee leaves and the male-specific compound dispensed in a sachet (4-6 scale significant) and coffee bark (6 only scale significant), it appears that use of either scale in evaluating olfactory response is fairly accurate since the bioassays were terminated after 10 minutes. Other workers used a single scoring system whereby the insect arrived at the final destination within a specified time. For example, Liendo *et al., (*2005) evaluated the response over 15 minutes, Cervantes *et al.,* (2006) after 5 minutes, Hall *et al*., (2006) after 10 minutes and Ibeas *et al., (*2006) did not specify the length of the bioassays. The fact that the two scoring methods tallied shows that there was no bias since the experiments were conducted all day (morning and afternoon).

4.4.2 Attraction to host-plant odours

The olfactory bioassays reported here suggest that female *M. leuconotus* exhibit attraction to three of the stimuli tested: coffee leaves, coffee bark and the sachet of the synthetic male-specific compound. Male coffee stem borers were attracted to coffee bark.

Studies with other cerambycids have shown that beetles were attracted to host volatiles. For example, *M. alternatus* beetles showed attraction to pine volatiles (Sakai and Yamasaki, 1989; Sakai *et al*., 1992; Yamasaki *et al*., 1997), which elicited some flight and walking responses in females. Eucalyptus borer, *P. semipunctata*, flew and walked towards host odours under laboratory conditions (Barata and Araujo, 2001; Barata *et al*., 2000). Pajares *et al*., (2004) showed that the pine sawyer, *Monochamus galloprovincialis* was attracted to pine volatiles while Reagel *et al*. (2002) reported that the red milkweed borer, *Tetraopes tetrophthalmus* was attracted to host volatiles. Four species of Cerambycidae (*Monochamus clamator, M. obtatus, M. notatus* and *M. scutellatus*) showed attraction to combinations of host volatiles and bark beetle pheromones suggesting that the pheromones could be acting as kairomones (Allison *et al*., 2001; 2003). Most of the examples on response to host volatiles involve pine and conifers which

produce large amounts of volatile compounds, but attraction of *Steirastoma breve* by cocoa is an exception (Liendo *et al.,* 2005, Liendo–Barandiaran *et al*., 2010).

There is a need to identify the active compounds in the coffee volatiles that elicited response in both sexes of *M. leuconotus* and their mode of action. Sakai *et al*. (1992), identified the active compound in pine bark volatiles as the monoterpene, *α*-pinene while Yamasaki *et al*., (1997) found the oxygenated terpenes, (+)-jupinerol and (+)-pimaral as being responsible for the flight response in female *M*. *alternatus.* Inner bark aqueous acetone extracts of *Pinus densiflora* stimulated oviposition of *M*. *alternatus* and were found to contain flavonol glucoside, proanthocynadins and some five glycosides (Sato *et al*. (1999a, 1999b) while D-catechin was identified as another oviposition stimulant for *M*. *alternatus* (Islam *et al*., 1997).

During parallel work at NRI on the pheromone of the white coffee stem borer, *Xylotrechus quadripes*, from India, some compounds were identified in volatiles from cut coffee stems and leaves. These included (*Z*)-3-hexenol, linalool, ethyl benzoate and methyl salicylate. These results were obtained after the laboratory bioassay work described here and so the compounds were not bioassayed. However, they were tested in subsequent field trapping tests (Chapter 5).

4.4.3 Responses to conspecific beetles and the male-specific compound

No responses were observed in the olfactometer for either sex of *M. leuconotus* to odours from live, conspecific beetles in the presence of coffee leaves or bark. The latter were provided as food for the beetles as production of aggregation pheromone in many species requires the beetles to feed (e.g. Ibeas *et al.,* 2008). However, they also emitted volatiles that were attractive and the response to the plant material in both arms of the olfactometer may have overridden any response to compounds from the beetles. Indeed, the overall level of responsiveness was uniformly high in these experiments.

With the male-specific compound it was possible to test this in the absence of plant material. Females were attracted to the male-specific compound dispensed from the polyethylene sachet. Despite poor responses to the neat chemical itself during the course of the current bioassays, it is probable that the sachet was releasing high enough amounts of compound for detection by the insects. The poor response to the male-specific compound dispensed in a vial by females is not surprising since the vial has a lower release rate than the sachet. Attraction of females by male cerambycid beetles or male extracts under both field and laboratory conditions has been demonstrated

(Ibawuchi, 1985, 1988; Fetthöker *et al*., 1995; Nakamura, *et al*., 1994; Noldt *et al*., 1995; Venkateshu *et al*., 1986). However, Rhainds *et al*. (2001) expressed reservations on the use of pheromones to attract females because of the complex mating behaviour of *Xylotrechus quadripes*. The male-specific synthetic pheromones for 24 cerambycid species have been identified and successfully synthesised (Table 1.4). Most of these are aggregation pheromones and are useful lures in pest monitoring programmes.

4.4.4 Conclusions

Despite the relatively few statistically significant results obtained with the olfactometer used here, the levels of overall responsiveness were generally high and the observations on behaviour of the beetles in the olfactometer were consistent with those observed under more natural conditions. Carrying out the experiments in the African laboratory had the major advantage that the beetles did not have to be transported long distances from their natural habitat and were in as good condition as possible. Similarly, conditions of light, temperature and humidity were those naturally experienced by the beetle. However, the African laboratory did not have sophisticated control of the latter conditions and particularly the uniformity of lighting, and these should probably be better controlled in future experiments. The air flow rate used in these experiments was low (70 cm/min in the main stem), although possibly not that different from the wind speed experienced within a coffee crop. However, in future experiments it should probably be increased, although not to a level that disturbed the behaviour of the beetles.

The olfactometer bioassay provided a system in which to study the effects of volatiles from individual chemicals or sources of chemicals at “long-range”, i.e. carried in an air flow (Wyatt, 2003) in the absence of other chemical or visual stimuli, and probably even auditory. The results obtained here showed some evidence for “long-range” attraction of *M. leuconotus* beetles by volatiles from host plants and the male-specific compound. There were followed up by experiments to investigate the ability of these compounds to attract beetles to traps in the field where many other chemical and visual stimuli are also present.

Chapter 5
FIELD TRAPPING STUDIES ON THE AFRICAN COFFEE WHITE STEM BORER, *Monochamus leuconotus* (PASCOE)

5.1 INTRODUCTION

In Chapter 2, the behaviour of *M. leuconotus* in cages was observed. This suggested that females were attracted to males and then males approach females, but it was not possible to determine whether long-range attractants exist. In Chapter 4, laboratory olfactometer studies provided some evidence of attraction of the beetles to volatiles from host plants and the male-specific compound produced by *M. leuconotus* at a distance of 1 m or more.

In this Chapter, trapping experiments are described which were carried out in the field in Zimbabwe to investigate whether long-range attractants are produced by *M. leuconotus*. Live insects and the synthetic male-specific compound of *M. leuconotus* in different dispensing devices were tested as lures. The synthetic male-specific compound was identified at NRI when GC-MS analysis of volatiles from virgin males contained a single major component that was not present in volatiles from females. This was then synthesized at NRI (CABI, 2008) and was tested alone or in combination with host volatiles. Four different designs of traps were tested.

In all these trials the number of beetles and mating pairs observed within a radius of 1 m of the trap were recorded as well as the number actually caught on the trap in case the beetles were attracted and not actually retained by the trap.

5.2. MATERIALS AND METHODS

5.2.1 Traps

Cross vane traps

Eight cross vane traps were made from white correx sheets. The dimensions for each vane were (50 cm x 30 cm x 4 mm thick) and each was covered with polybutene adhesive (Trappit, Agralan, Ashton Keynes, Wiltshire, UK). Traps were fitted with a glued base made from expanded polystyrene (Fig. 5.1) to catch beetles that fell from the cross vanes. These were similar to those used for the Asian coffee white stem borer, *Xylotrechus quadripes*

(Coleoptera: Cerambycidae), by Hall *et al*. (2006) except that these did not have a base at the bottom.

Fig. 5.1 Cross vane trap with base used for field trials in a coffee plantation at ARDA Katiyo, Chipinge Zimbabwe.

Rat Traps Mk 1

Sticky rat traps mounted on a paper base (12 cm x 25 cm, model no. 60RBGL, Catchmaster; Pest Control Direct, Hailsham, Sussex, UK) were used as recommended by Lacey *et al.* (2004) (Fig. 5.2).

Fig. 5.2 Rat traps MK 1 used in 2004/05 field trials at Crocodile Creek and ARDA Katiyo Estates, February 2005.

Rat traps Mk 2

During the period December 2005 to January 2006, experiments were carried out using a more weatherproof type of sticky rodent trap with a plastic backing (12 cm x 25 cm, Trapper, Bell Laboratories, Madison, WI, USA) (Fig. 5.3).

Fig. 5.3 Rat traps MK 2 used for trapping studies at New Year's Gift, Chipinge, Zimbabwe. Trap baited with live *Monochamus leuconotus* beetle placed in a 150ml plastic bottle.

Intercept traps

During the 2006/07 and subsequent flight seasons, commercially-available intercept traps (APT Inc., Marylhurst, Oregon, USA) were tested at New Year's Gift Estate, Chipinge. These were large, black cross-vane traps (panels 30.5 cm x 80.5 cm high) made of correx sheet, without adhesive and with a funnel and container of water (13.5 cm x 13.5 cm x 14.5 cm deep) at the base to collect insects colliding with the cross-vanes (Fig. 5.4). Traps were hung between poles placed within coffee rows at a height of 1 m above ground level.

Fig. 5.4 Intercept trap used for trapping studies at New Year's Gift Estate, December 2006. Lure is white sachet at centre of trap.

5.2.2 Live Insect Lures

For the first trials in 2003/04, infested stems collected from the field were maintained in the laboratory at the Coffee Research Station, Chipinge. Emerging adult *M. leuconotus* were collected each day, sexed on the basis of length of the antennae and maintained separately in glass cages under laboratory ambient light and temperature conditions. Beetles emerging from stems were regarded as virgins since monitoring stems for beetle emergence was twice per day. Beetles were provided with coffee twigs as feeding material. Insects used as baits and fresh coffee twigs were renewed once a week.

Insects used during the February 2005 and December 2005/January2006 experiments were collected as adults from coffee fields adjacent to the trials at Crocodile Creek, New Year's Gift, Chipinge and ARDA Katiyo Estates respectively. These were placed in separate boxes for males and females and later used to initiate the experiments or to

replace live insect baits each week. The virginity status of the insects used for the rat traps and intercept trials was not confirmed. It was assumed that they had mated.

During December 2006, insects were collected from adjacent coffee fields at New Year's Gift Estate. Beetles were used immediately as baits in the traps. The virginity status of the insects used for the rat traps and intercept trials was not confirmed. It was assumed that they had mated.

Male or female insects used as lures were placed in a clear plastic screw-cap bottle (150 ml) obtained from supermarkets in Zimbabwe with the wall perforated with approx 20 holes with a dissecting needle (Fig. 5.3).

5.2.3 Synthetic Lures

Male-specific compound

The male-specific compound, 2-(4-heptyloxy-1-butyloxy)-1-ethanol, was synthesised at NRI and provided in four different slow-release formulations.

Sealed polyethylene vials (22 mm x 8 mm x 1.5 mm thick; Just Plastics, London, UK) contained the synthetic male-specific compound (50 μL) and gave an approximate release rate of 0.052 mg/d determined in a laboratory wind tunnel at 27°C and 8 km/h wind speed in a constant room temperature at NRI (CABI, 2008).

The male-specific compound (100 μL) was also dispensed from a cellulose dental roll (9 mm x 36 mm, Kent Express Limited, Gillingham, Kent, UK).

For experiments from 2003-2007, white polyethylene sachets (2.5 cm x 2.5 cm x 120 μ thick; International Pheromone systems Ltd., Wirral, UK) were used containing the synthetic male–specific compound (50 μL) and giving an approximate release rate of 0.38 mg/d determined in a laboratory wind tunnel at 27°C and 8 km/h wind speed.

For experiments from 2010-2013, clear polyethylene sachets (5 cm x 5 cm x 120 μ thick; Transatlantic Plastics, Southampton, UK) were used containing the male-specific compound (100 μL) impregnated on a cigarette filter (14 mm x 6 mm; Swan, High Wycombe, Bucks., UK) with a release rate of 0.4 mg/d at 27°C and 8 km/h wind speed. A clear polyethylene sachet was used since the manufacturer has phased out white sachets. The cigarette filter was placed inside the sachet to make sealing the sachet easier and did not affect the release rate.

Host-plant volatiles

The host volatiles, (*R*)-(-)-linalool, ethyl benzoate, methyl salicylate and (*Z*)-3-hexenol were evaluated on the basis that these were found to be major volatiles found in coffee plants after air entrainment studies in India (Hall personal communication).

Synthetic compounds were obtained from SigmaAldrich (Gillingham, Dorset, UK) and used as supplied.

(*R*)-(-)-Linalool (≥ 95%; 100µL) was dispensed from sealed, clear polyethylene sachets as above, giving a release rate of 3.5 mg/d at 22°C.

Ethyl benzoate (≥ 99%; 100µL) was also dispensed from a sealed, clear polyethylene sachet as above, giving a release rate of 45.9 mg/d.

Methyl salicylate (≥ 98%; 100µL) was dispensed from a polyethylene vial (15 mm x 30 mm x 1.5 mm thick; Fisher Scientific, Loughborough, UK) with approximate release rate 1.3 mg/d at 22°C.

(*Z*)-3-Hexenol (98%; 100µL) was also dispensed from a polyethylene vial as above with approximate release rate 0.3 mg/d at 22°C.

5.2.4 Experiments

Experiment 1 (December 2003 to February 2004)

This was carried out using sticky cross vane traps with a perforated plastic bottle placed at the centre of the trap containing a live male or female *M. leuconotus* adult beetle. Traps were mounted in a coffee plantation at ARDA Rusitu Valley Estate, Chipinge, Zimbabwe (Fig. 5.5), for 8 weeks between 15 December 2003 and 6 February 2004 at a spacing of 10 m apart and 1.2 m above ground level in a randomised design with three replicates for each lure and two for the control, which had empty bottles only. Monitoring was done every two days to record the number of *M. leuconotus* beetles and other insects on the trap and within a one-metre radius of each trap. Beetles were sexed on the basis of their antennae and recorded as male, female or mating pairs. Trap positions were changed after every seven days so that each treatment occupied all positions at least once during the study period. Total insects within the vicinity of each treatment were determined.

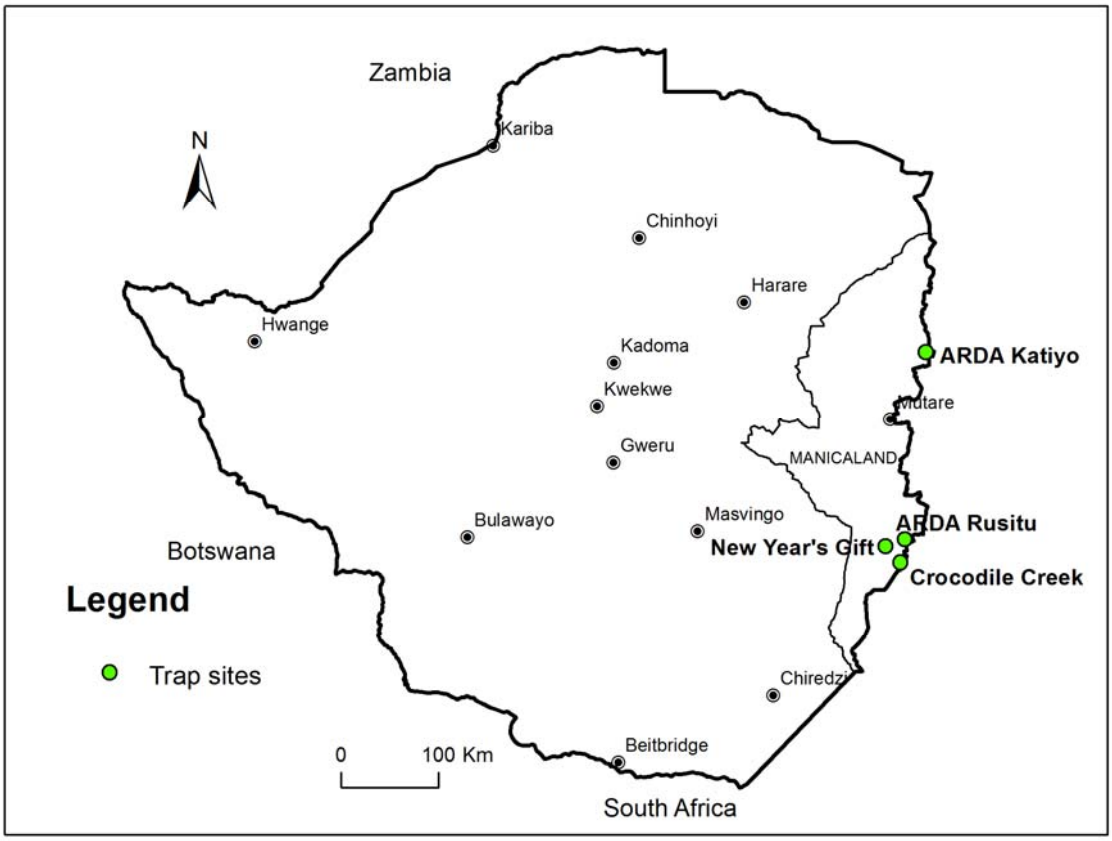


Fig. 5.5: Field sites for trapping trials in Manicaland Province, Zimbabwe during 2003 to 2012

Experiment 2 (February 2005)

Work was carried out at Crocodile Creek, Chipinge between the 2nd February and 23rd February 2005 and ARDA Katiyo, Mutasa (Fig. 5.5) between 14th February and 9th March 2005. MK1 rat traps were tied to coffee stems at a height of 30 cm above ground level and placed 4 m apart. The trials were set up as a completely randomised design with three treatments replicated three times. Treatments were the male-specific compound of *M. leuconotus* in polyethylene vials, the compound in white polyethylene sachets and an empty bottle as control. Monitoring of traps was done on a weekly basis. Adult white stem borers within a radius of 1 m from the trap were recorded.

Experiment 3 (13 December 2005 to 17 January 2006)

MK2 rat traps were used for these trials at New Year's Gift Estate, Chipinge, and ARDA Katiyo (Fig. 5.5). There were two sites at New Year's Gift located about 100 m apart at

opposite sides of a 10 ha plantation of coffee (Fig. 5.6). The design was a randomised block design with the four treatments and four replications at each site. Treatments were live male or female *M. leuconotus*, the male-specific compound in a white polyethylene sachet and an unbaited control.

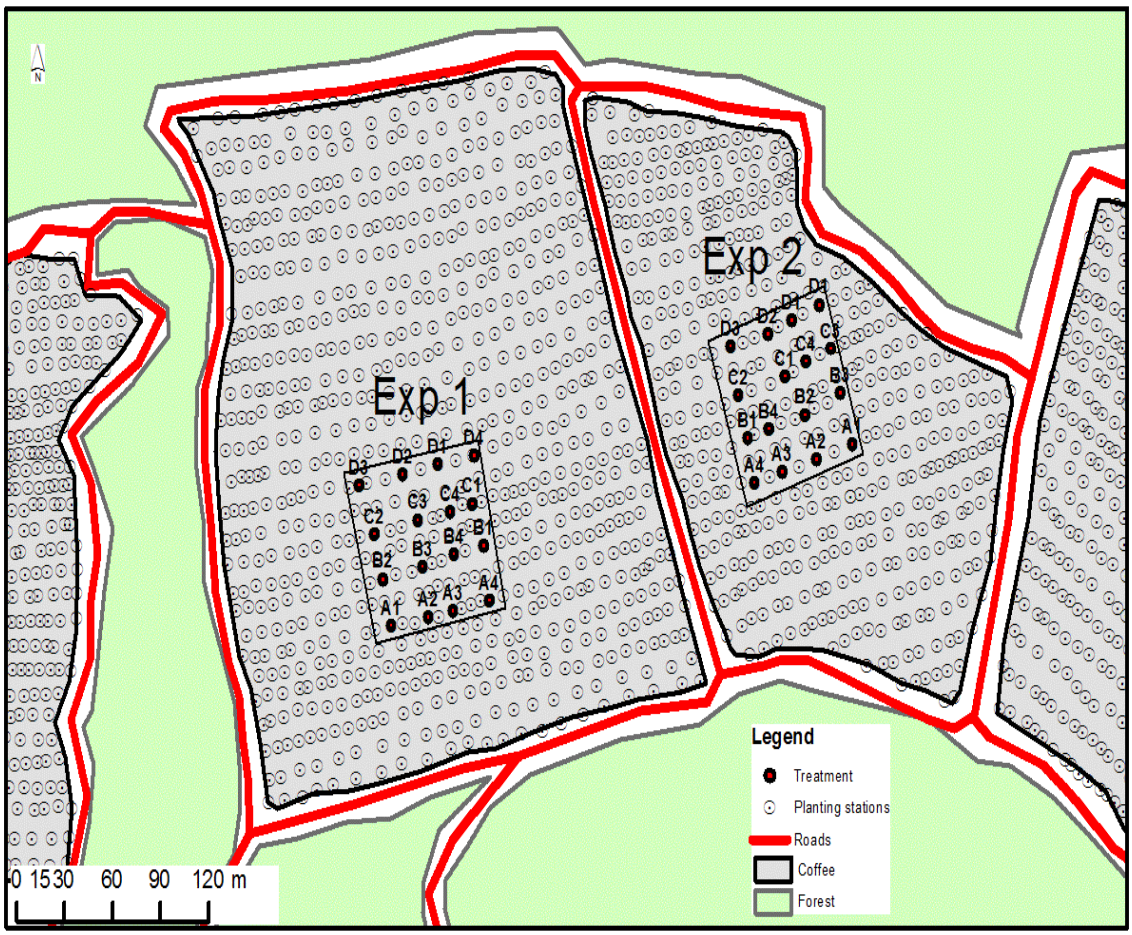


Fig 5.6: Site map for trapping trials at New Year's Gift Estate, Chipinge, Zimbabwe, 2006/2007

Traps were mounted in the field at a height of 1 m above ground level. Trap placement was done by hanging onto coffee stems using pieces of wire. Odour sources were hung in front of the trap and secured using pieces of wire. The distance between traps within a row was 12 planting stations (20 m) while that between the blocks was 200 m. Monitoring and renewal of live insect baits was done once a week. The sachets were not renewed during the course of the experiments. Records of the total number of males, females and mating pairs on the traps and within a radius of 1 m round the trap were taken.

Experiment 4 (December 2006 to January 2007, New Year's Gift)

This was carried out using intercept traps at New Year's Gift Estate (Fig. 5.5). Lures were the male-specific compound of *M. leuconotus* in white sachet and cigarette filter dispensers as well as live male or female beetles and the untreated control. Each treatment was replicated 10 times (two replicates each over the 5-day observation period) in a randomised block design with five treatments as described above. Traps were positioned 20 m apart. Replication was according to time with each day as a replicate. Treatments were randomised every day around 09:00 in the morning. The sachet and cigarette filters were hung by pieces of wire while the plastic bottles containing either live male or female baits were placed at the centre of the traps. Monitoring was done every day. Live insect baits and the cigarette filter dispensers were renewed daily. The sachet was not renewed. Records of insects on and surrounding a 1-metre radius from each trap were taken at 09:00 every day.

Experiment 5 (December 21 2010 to February 10 2011)

This was carried out using intercept traps at ARDA Katiyo (Fig. 5.5). Lures were the male-specific compound in a clear sachet with dispensers of (*R*)-(-)-linalool, ethyl benzoate, methyl salicylate or (*Z*)-3-hexenol and an unbaited control.

The design of the experiment was a randomised block design with unequal replication. Treatments 1 (male compound + linalool) and 4 (male compound + ethyl benzoate) had 2 replications while treatment 3 (male compound + methyl salicylate), treatment 4 (male compound + (*Z*)-3-hexenol) and 5 (control) had 3 replications each. Differences in replicate numbers were due to insufficient traps. Distance between traps was 20 m while the distance between blocks was 200 m. Traps were monitored on a daily basis except when it was raining when access to the site was difficult.

Experiment 6 (February 12 to April 13 2011)

During February to April 2011, more work was done with combinations of the male-specific compound with host-plant volatiles at ARDA Katiyo (Fig. 5.5). Intercept traps were used and the four treatments were the male-specific compound in a clear sachet with (*R*)-(-)-linalool, the male-specific compound alone, the male-specific compound with methyl salicylate and an unbaited control. The design of the experiment was a randomised block design with unequal replication. Treatment 1 (male specific compound + linalool) had 2 replications; treatment 2 (male-specific compound) had 5 replications while treatments 3 (male-specific compound + methyl salicylate) and 4 (control) had 3 replications each. This was necessitated by insufficient intercept traps and also the need to bulk up for the male-specific compound alone in order to clearly understand its role. Distance between traps was 20 m while the distance between blocks was 200 m. Traps were monitored on a daily basis except when it was raining. Volatiles were not changed during the trial.

Experiment 7 (January 1 to April 14 2012)

This experiment was carried out with intercept traps at ARDA Katiyo (Fig. 5.5). Treatments were the male-specific compound in a clear sachet with (*R*)-(-)-linalool, the male-specific compound alone, (*R*)-(-)-linalool alone and an unbaited control. Treatments were replicated three times in a randomised complete block design. Records were taken on a daily basis except when it was raining. Intercept trap positions were arranged at random and they were changed weekly to avoid positional bias. Distance between traps was 20 m while the distance between blocks was 200 m. Volatiles were not changed during the duration of the trial.

5.2.5 Data analysis

Data on the number of insects within the vicinity of each trap for the cross vane traps study (Experiment 1) were analysed by the Chi-square (χ^2) test for goodness of fit using SPSS Version 12.0 for Windows. The accepted level of significance was 5%.

Since no beetles were found on the traps and in the vicinity of MKI traps, no analysis was done (Experiment 2). For the second set of rat traps (MKII), data on the total males, females and mating pairs for each site were analysed separately using χ^2 goodness of fit test. Further data analysis was with the sites as replicates and finally the aggregate totals for the three

sites were analysed with the χ^2 goodness of fit test. Totals for each sex took into account individuals from the mating pairs per treatment.

The data for the December 2006 intercept trapping trials were analysed using χ^2 goodness of fit as described for the 2005/06 rat trap trials.

Data for the 2010-11 and 2012 trials (Experiments 5, 6 and 7) were transformed ($\log_{10}$ (n+1)) and subjected to one way ANOVA using GenStat Release 14.1 (VSN International, 2011). Where significant, means were separated using Tukeys' test at $P < 0.05$.

Details of data analyses are provided in Appendix 3.

5.3. RESULTS

5.3.1 Experiment 1 (December 2003 to February 2004 trials at ARDA Rusitu)

No *M. leuconotus* beetles were caught in the cross-vane traps baited with conspecific beetles. In addition, there were no significant differences in number of male beetles found within the vicinity of the traps at ARDA Rusitu Estate ($\chi^2 = 3.785$, *d.f* = 2, $P = 0.144$) (Table 5.1). In terms of females around traps, there were also no significant differences due to treatments ($\chi^2 = 0.500$, *d.f* = 2, $P = 0.779$).

Table 5.1 *Monochamus leuconotus* adults within 1m radius of cross vane traps baited with live beetles in Experiment 1 at ARDA Rusitu Estate, Chipinge, Zimbabwe (17 Dec 2003 – 6 Feb 2004).

		Mean total insects / trap	
Treatment	**Number of traps**	**Males**	**Females**
Male beetle	3	3.00	1.66
Female beetle	3	1.33	1.33
Unbaited	2	1.50	1.50

The highest number of males was observed during weeks 1 and 6 and no males were caught during weeks 2 and 4 (Fig. 5.7). There was no female observed round the traps during the first 4 weeks of the study. Female sightings started during week 5 with the highest numbers of females recorded in the fifth week.

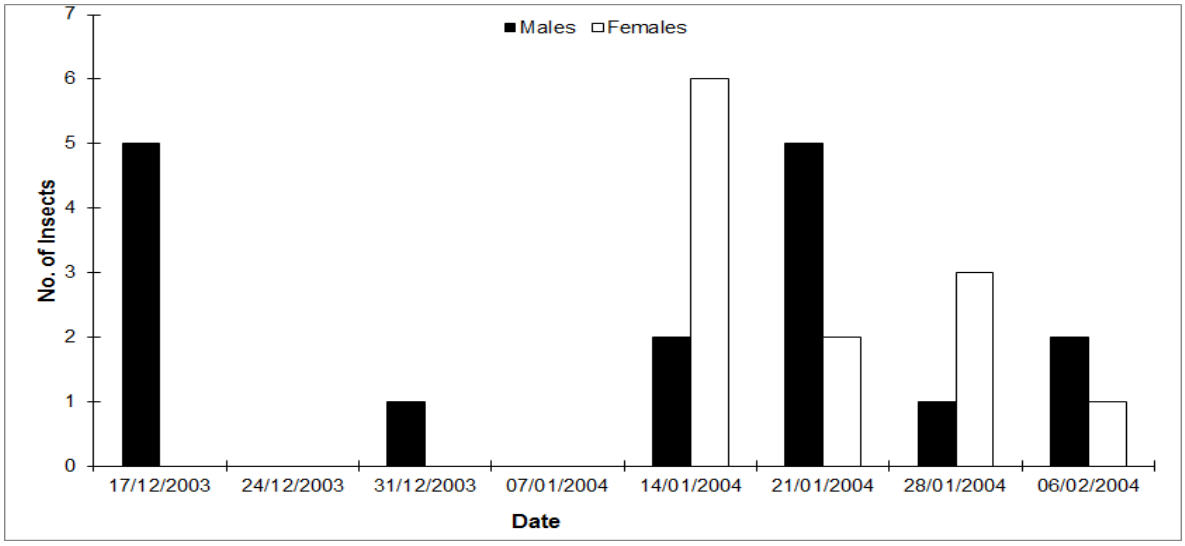


Fig. 5.7 Total numbers of *Monochamus leuconotus* around a 1 m radius of baited and unbaited traps in Experiment 1 at ARDA Rusitu Valley Estates, Chipinge, Zimbabwe (17 Dec 2003 – 6 Feb 2004).

5.3.2 Experiment 2 (February 2005 trials at Crocodile Creek, Chipinge and ARDA Katiyo)

There were no *M. leuconotus* adults caught or found within the vicinity of the traps baited with vial and sachet dispensers of the synthetic male-specific compound of *M. leuconotus* during the trials of February 2005.

5.3.3 Experiment 3 (December 2005 to January 2006, New Year's Gift, Chipinge, and ARDA Katiyo)

New Year's Gift Site 1

The numbers of individual males found within the vicinity of the traps at the first trial site at New Year's Gift were not different for the different treatments ($\chi^2 = 5.054$, $d.f = 3$, $P = 0.168$; Table 5.2)..

There were no significant differences in number of female beetles found within the vicinity of the traps ($\chi^2 = 1.174$, $d.f = 3$, $P = 0.759$).

In terms of mating pairs, no differences were found in the numbers recorded during field observations ($\chi^2 = 3.182$, $d.f = 3$, $P = 0.364$).

Table 5.2 Total numbers of *Monochamus leuconotus* beetles around a 1 m radius of sticky rodent traps in Experiment 3 at New Year's Gift Site 1 (13 Dec 2005 -17 Jan 2006; 4 replicates, 6 readings)

Treatment	No. Males		No. Females		No. mating pairs
	Alone	Total*	Alone	Total*	
Control	9	14	2	7	5
MSC in sachet	9	11	2	4	2
Live male	5	6	6	7	1
Live female	3	6	2	5	3

*Denotes totals after adding in the numbers from the mating pairs

When individual insects from the mating pairs were included into the totals for males and females per treatment (Table 5.2), the unbaited treatment had the highest number of insects within a radius of 1 m of the traps followed by the sachet and live male respectively. However, the numbers of insects found near the sachet were not significantly different from those in the unbaited treatment.

Only one adult female beetle was caught on rat trap MK2 baited with a female conspecific at New Year's Gift (Fig 5.8).

Fig 5.8 A female *Monochamus leuconotus* beetle caught on the rat trap MK2 at New Year's Gift Estate, Chipinge, Zimbabwe on a trap with live female conspecific as bait

More beetles were observed around the traps during the first week with no males being caught during weeks 3, 4 and 6 (Fig. 5.9). The highest number of males was in week 1. Females were recorded throughout the study period except week 6 when there was nothing. The highest number of females was in week 1 followed by week 3.

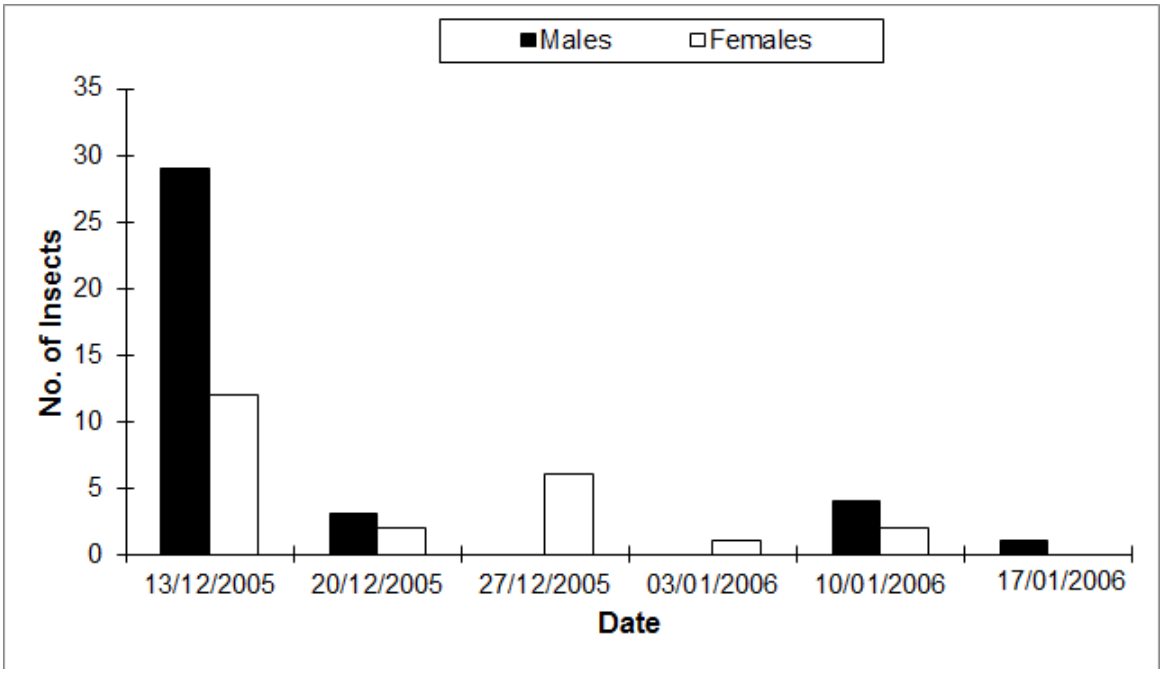


Fig 5.9 Total numbers of *Monochamus leuconotus* beetles observed around all treatments per week in Experiment 3 at New Year's Gift Site 1(Dec. 2005/Jan 2006).

New Year's Gift Site 2

Again no beetles were recorded in the traps. There were no significant differences in numbers of male beetles around the traps due to treatment at the second site at New Year's Gift (χ^2 = 1.200, *d.f* = 3, *P* = 0.753) (Table 5.3).

The number of females per treatment was not significantly different (χ^2 = 1.300, *d.f* = 2, *P* = 0.522).

The numbers of mating pairs at this site were too low such that they could not be analysed statistically (Table 5.3).

Table 5.3 Total numbers of *Monochamus leuconotus* beetles around a 1 m radius of sticky rodent traps in Experiment 3 at New Year's Gift Site 2 (13 Dec 2005-17 Jan 2006; 4 replicates, 6 readings)

Treatment	No. Males		No. Females		No. mating
	Alone	Total*	Alone	Total*	pairs
Control	1	2	5	5	0
MSC in sachet	0	4	5	9	4
Live male	2	2	6	6	0
Live female	2	2	0	0	0

*Denotes totals after adding in the totals from mating pairs

The number of insects observed within the vicinity of traps differed according to time. Male numbers were highest during the first week and thereafter the number was constant up to the completion of the study (Fig. 5.10). Female numbers were highest during the first week followed by weeks 5 and 2 respectively. No females were recorded during week 6.

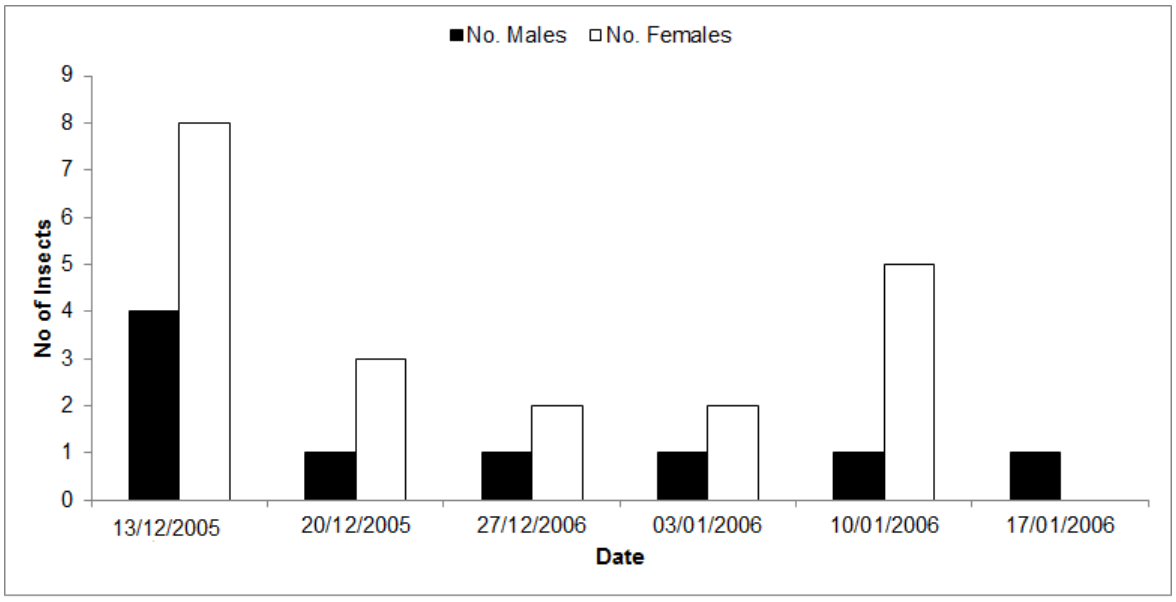


Fig. 5.10 Total number of *Monochamus leuconotus* adults observed around all treatments per week at in Experiment 3 at New Year's Gift Site 2 (13 Dec 2005-17 Jan 2006).

The number of females was generally higher than that for males throughout the period December 2005 to January 2006.

ARDA Katiyo

At ARDA Katiyo, there were no significant differences in the number of males around each trap (χ^2 = 7.293, *d.f* = 3, *P* = 0.063). In the case of females, there were no significant differences between the numbers found within the vicinity of the traps (χ^2 = 2.381, *d.f* = 3, *P* = 0.497) (Table 5.4).

Mating pairs did not respond to the traps differently at ARDA Katiyo (χ^2 = 1.118, *p* = 0.773) (Table 5.4).

Table 5.4 Total numbers of *Monochamus leuconotus* beetles around a 1 m radius of sticky rodent traps in Experiment 3 at ARDA Katiyo (29 Dec 2005-9 Feb 2006; 4 replicates, 7 readings)

	No. Males		No. Females		No. mating
Treatment	**Alone**	**Total***	**Alone**	**Total***	**pairs**
Control	9	15	8	14	6
MSC in sachet	10	14	7	11	4
Live male	1	5	6	10	4
Live female	4	7	4	7	3

*Denotes totals after adding in the numbers from the mating pairs

The number of insects within the vicinity of traps at ARDA Katiyo varied according to time. Male numbers were highest during the first two weeks and thereafter there was a decline (Fig. 5.11). There were no males observed during week 6. Female numbers were highest during weeks 1 and 2 followed by a progressive decline that started in week 3. There were no females observed during week 6.

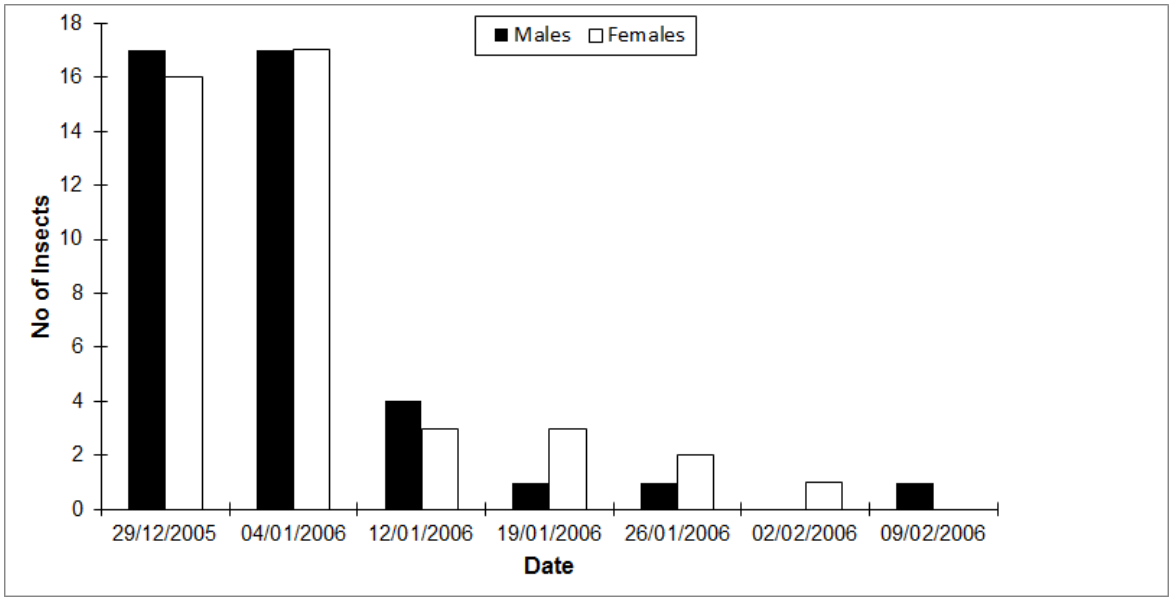


Fig. 5.11 Total numbers of *M. leuconotus* adults observed within 1 m of all treatments per week in Experiment 3 at ARDA Katiyo (29 Dec.2005- Feb 2006; 4 replicates, 7 readings)

When the totals for each sex per treatment across all sites were considered, there were significant differences in the number of males per treatment (χ^2 = 11.818, *d.f* = 3, *P* = 0.008) with the unbaited control traps having the highest number followed by traps baited with the male-specific compound dispensed in a sachet. Both the live baits had lower numbers of males (Table 5.5).

Table 5.5 Total numbers of *Monochamus leuconotus* beetles recorded within the vicinity of traps at three sites, Dec 2005/Feb 2006 (4 replicates, 19 readings)

Treatment	No. Males		No. Females		No. mating pairs
	Alone	Total*	Alone	Total*	
Control	20	31	15	26	11
MSC in Sachet	19	29	14	24	10
Live male	8	13	18	23	5
Live female	9	15	6	12	6
χ^2		11.818		5.588	3.250
p		0.008		0.133	0.355

*Denotes totals after adding in the numbers from the mating pairs

In terms of females, there were no significant differences in numbers found within the vicinity of the traps. The numbers of mating pairs per treatment were not significantly different from each other. Lower numbers of mating pairs around the live insects were observed at New Year's Gift than at Katiyo (Table 5.5 and 5.6).

5.3.4 Experiment 4 (December 2006 to January 2007, New Year's Gift)

During December 2006, there were no significant differences in the number of males around traps with the different baits (χ^2 = 0.400, *d.f* = 2, *P* = 0.819). In the case of females, there were no significant differences between the numbers found within the vicinity of the traps (χ^2 = 0.600, *d.f* = 3, *P* = 0.896) There were no significant differences in number of mating pairs found within the vicinity of traps (χ^2 = 0.600*, d.f.* = 3, *P* = 0.896) (Table 5.6).

Table 5.6 Total numbers of *Monochamus leuconotus* beetles around a 1 m radius of intercept traps at New Year's Gift, 15 -19 Dec 2006 (2 traps per treatment, 5 readings)

Treatment	No. Males		No. Females		No. mating
	Alone	Total*	Alone	Total*	pairs
Control	4	5	3	4	1
MSC in cigarette filter	1	4	4	7	3
Live female	8	8	5	5	0
Live male	6	8	4	6	2
Sachet	5	5	6	6	0

*Denotes totals after adding in the numbers from the mating pairs

The number of insects found around the traps increased with time (Fig. 5.12). Number of females was initially less than that for males.

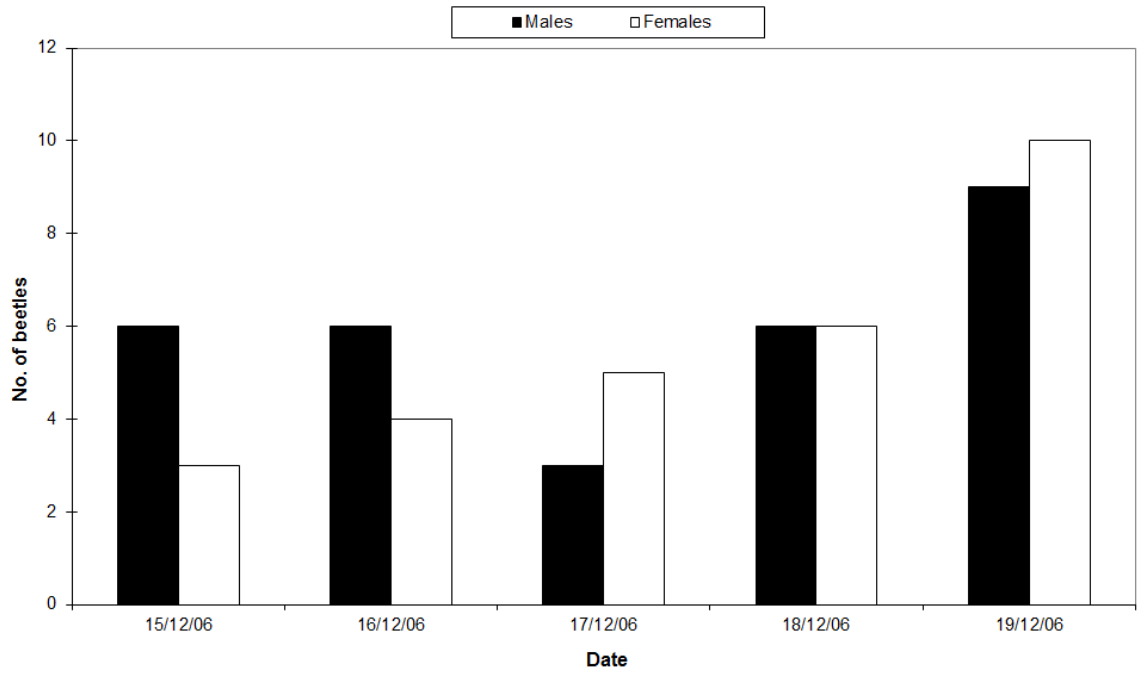


Fig. 5.12 Total numbers of *Monochamus leuconotus* beetles observed around all treatments per day in Experiment 4 at New Year's Gift (15 -19 Dec. 2006; 2 intercept traps per treatment, 5 readings)

5.3.5 Experiment 5 (December 2010 to February 2011, ARDA Katiyo)

The intercept traps caught *M. leuconotus* beetles. There were significant differences in the number of males with all combinations of the male-specific compound with host volatiles. All treatment combinations caught significantly more males than the unbaited control but were not significantly different from each other (F = 4.51, $d.f$ = 4,8, P = 0.034) (Fig. 5.13).

The combination of the male-specific compound with (*R*)-(-)-linalool caught significantly more female beetles than the combinations with ethyl benzoate and (*Z*)-3-hexenol but was not significantly different from the male-specific compound + methyl salicylate. All treatments caught more female insects than the unbaited control (F = 5.20, $d.f$ = 4,8, P = 0.023) (Fig. 5.13).

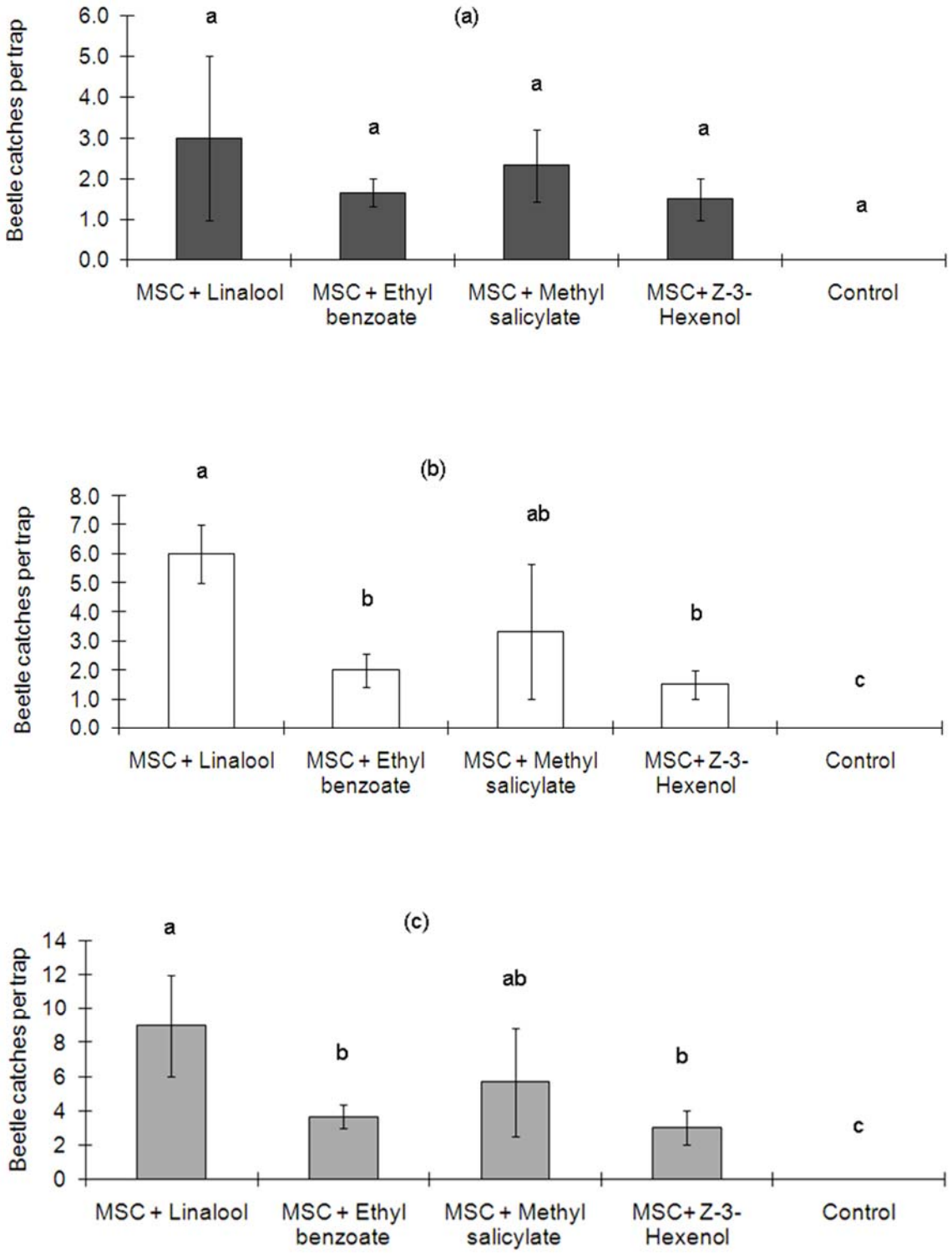


Fig. 5.13 Mean catches/trap of *Monochamus leuconotus* (±SE) in intercept traps in Experiment 5 at ARDA Katiyo (20/12/2010 -10/02/2011) in traps baited with male-specific compound (MSC). For MSC + linalool and MSC + (Z)-3-Hexenol, $N = 2$; For MSC + methyl salicylate, MSC + ethyl benzoate and Control $N = 3$. (a) Males, (b) Females, (c) Total. For each sex, bars followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

There were significant differences in the total number of beetles caught by intercept traps, when combinations of the synthetic male-specific compound with different host volatiles were compared (F = 9.14, $d.f$ =4,8, P < 0.004) (Fig. 5.13). All treatments caught significantly more insects than the unbaited control. The combination of the male-specific compound with (*R*)-(-)-linalool caught significantly more insects than the MSC + ethyl benzoate and MSC + (*Z*)-3-hexenol but it was not different from the combination with methyl salicylate.

Numbers of male beetles (F = 0.55, $d.f$ = 4,8, P =0.706) (Fig. 5.14) surrounding the traps were not significantly affected by the lures.

All treatments had significantly different numbers of female beetles around the traps than the unbaited control. More female beetles were recorded around traps baited with the combination of male-specific compound with (*R*)-(-)-linalool than round male-specific compound + (*Z*)-3-Hexenol and the unbaited control (F=3.18, $d.f$ = 4,8, P = 0.013) (Fig. 5.14). Catches in traps baited with male-specific compound + methyl salicylate, male-specific compound + ethyl benzoate and male-specific compound + (*R*)-(-)-linalool were not significantly different from each other.

The male-specific compound + (*R*)-(-)-linalool had significantly more mating beetle pairs than the unbaited control. All other treatments were not significantly different from each other (F = 0.45, $d.f$ = 4,8, P = 0.771) (Fig. 5.14).

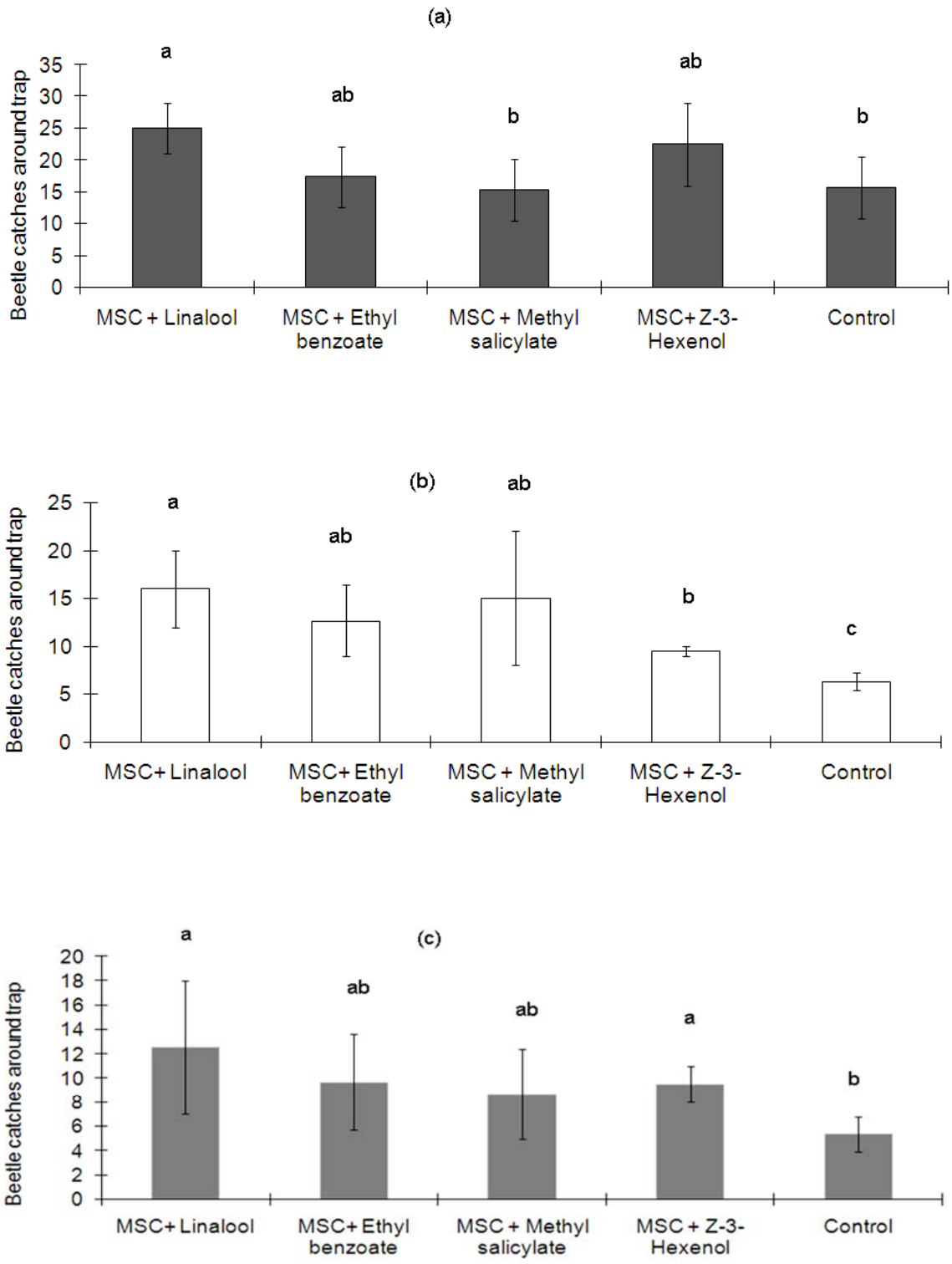


Fig. 5.14 Mean numbers of *Monochamus leuconotus* (±SE) within 1 m radius of intercept traps (ARDA Katiyo, 20/12/2010 -10/02/2011 baited with male-specific compound (MSC). For MSC + Linalool and MSC + Z-3-Hexenol, $N = 2$; For MSC + Methyl salicylate, MSC + Ethyl benzoate and Control, $N = 3$. (a) Males, (b) Females, (c) Mating pairs. For each sex, bars followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

5.3.6 Experiment 6 (February to April 2011 trials, ARDA Katiyo)

Adult beetles of *M. leuconotus* were caught on the traps during field bioassays at ARDA Katiyo. Catches with all treatments differed significantly from those in the unbaited control. The combination of the male-specific compound with (*R*)-(-)-linalool caught significantly more males than the unbaited control and the male-specific compound alone (F=7.10, *d.f* = 3,9, P = 0.010). Number of males caught by the male-specific compound with methyl salicylate was not significantly different from the male-specific compound with (*R*)-(-)-linalool.

Number of females caught was not significantly affected by the treatments (F=3.63, *d.f.* = 3,9, P = 0.058).

The combination of the male-specific compound with (*R*)-(-)-linalool caught more insects than the male-specific compound alone, male-specific with methyl salicylate and the unbaited control (F=15.81, *d.f* = 3,9, P < 0.001) (Fig. 5.15). The total number of insects caught by combining the male-specific compound with methyl salicylate was significantly different from with the unbaited control but not different from the male-specific compound + (*R*)-(-)-linalool.

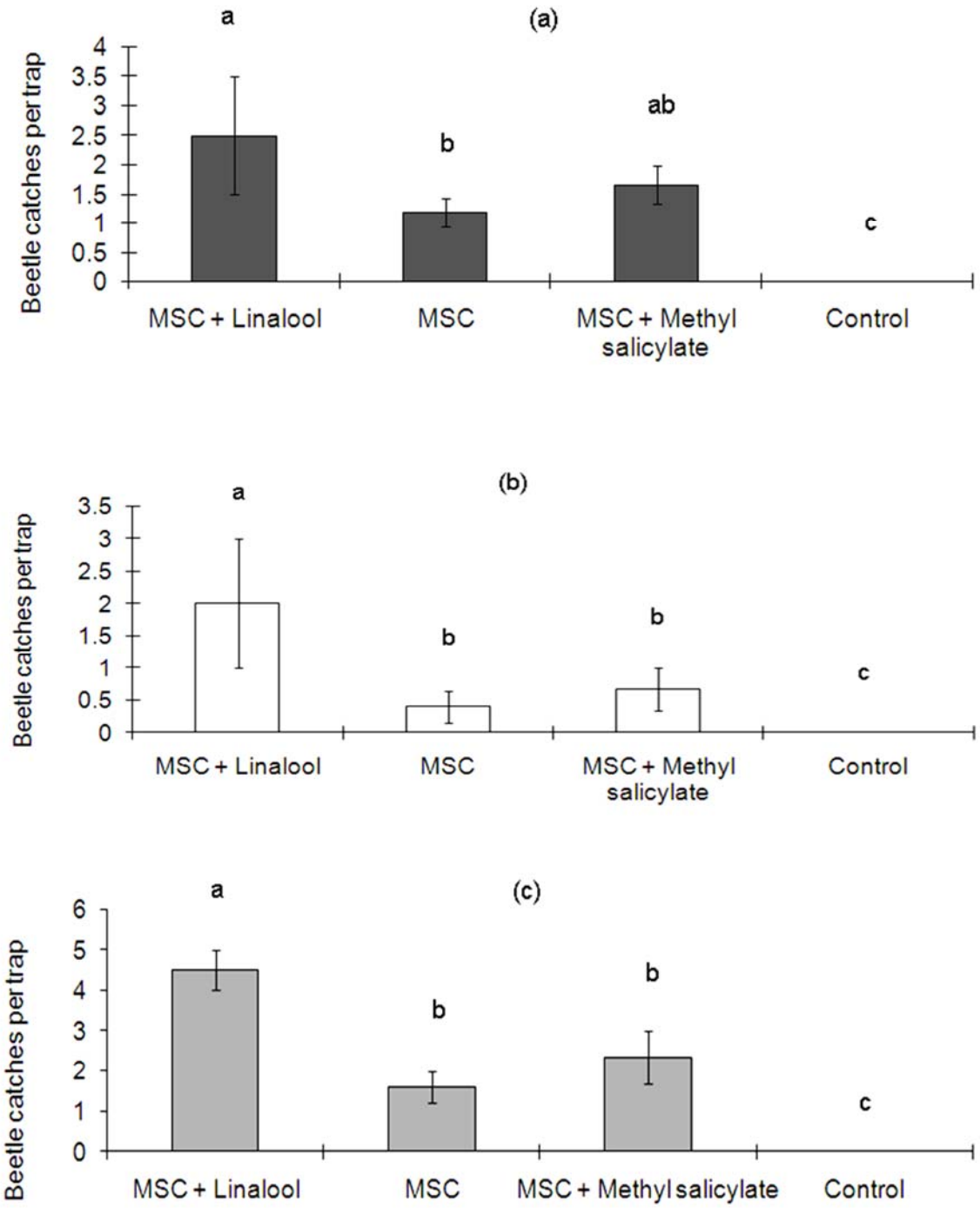


Fig. 5.15 Mean catches/trap of *Monochamus leuconotus* (±SE) in intercept traps baited with male-specific compound (MSC) (ARDA Katiyo, 20/12/2010 -10/02/2011). For MSC + Linalool, $N = 2$; For MSC + Methyl salicylate and unbaited Control, $N = 3$, For MSC, $N = 5$. (a) Males, (b) Females, (c) Total. For each sex, bars followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

Numbers of males recorded within the vicinity of traps baited with a combination of the male-specific compound with (*R*)-(-)-linalool and the male-specific compound alone were significantly different from male-specific compound with methyl salicylate, which was not significantly different from the unbaited control (F=4.64, $d.f$ = 3,9, P = 0.003, Fig. 5.16).

Numbers of females around the traps (F=1.10, $d.f$ = 3,9, P = 0.350) and numbers of mating pairs (F =0.38, $d.f$ = 3,9, P = 0.169) were not significantly affected by the treatments.

Traps baited with the male-specific compound with methyl salicylate did not result in more beetles round the traps than the unbaited control.

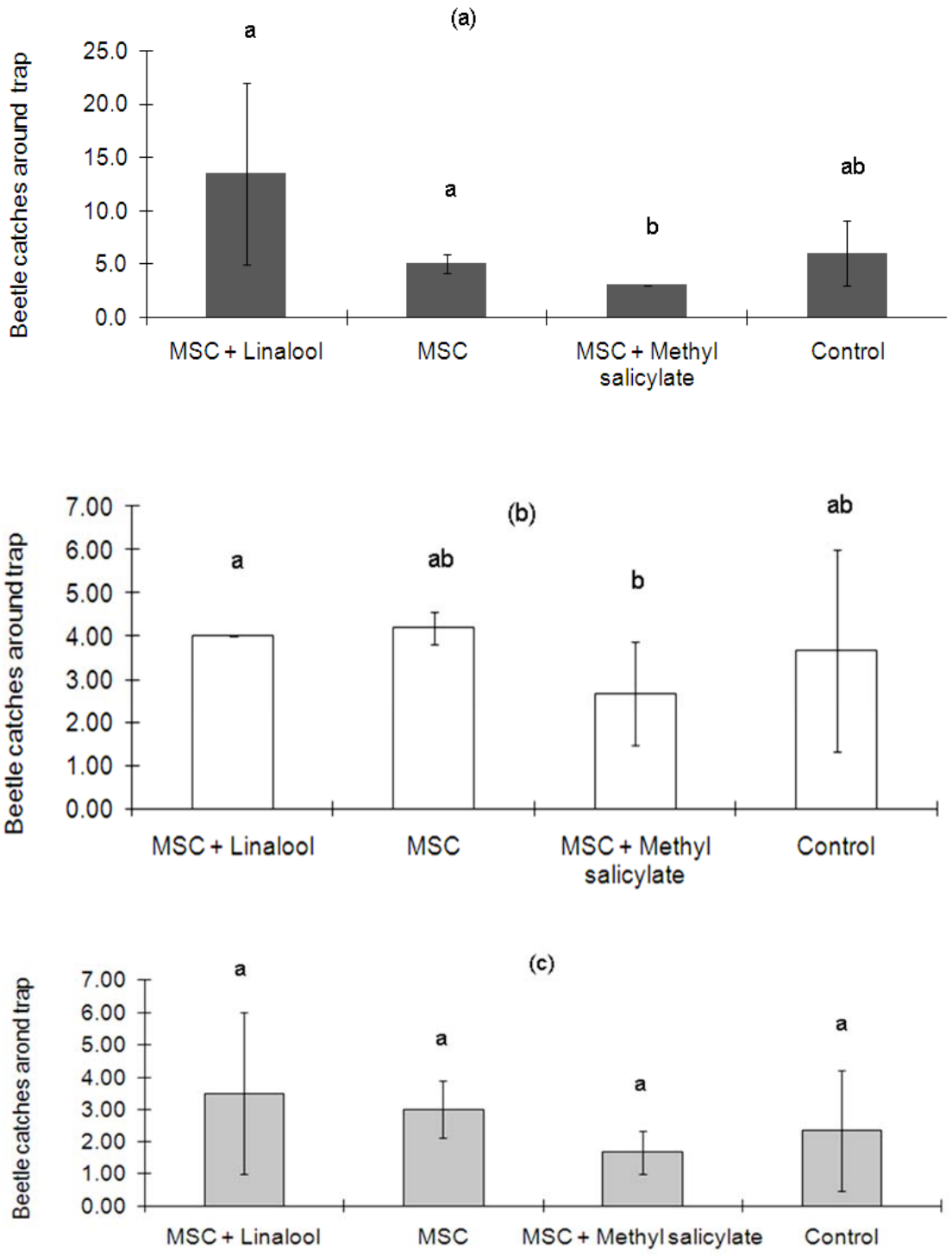


Fig. 5.16 Mean numbers of *Monochamus leuconotus* (±SE) within 1 m radius of intercept traps baited with male-specific compound (MSC) (ARDA Katiyo, 20/12/2010 -10/02/2011). For MSC + Linalool, *N* = 2; For MSC + Methyl salicylate and unbaited Control, *N* = 3, For MSC, *N* = 5. (a) Males, (b) Females, (c) Mating pairs. For each sex, bars followed by the same letter are not significantly different (Tukey's HSD test, *P* <0.05).

5.3.7 Experiment 7 (January to April 2012)

Both sexes of *M. leuconotus* were caught on traps during early 2012. There were significant differences in the number of beetles caught by traps. For males, all treatments differed significantly from the unbaited control. The male-specific compound + (*R*)-(-)-linalool caught more beetles than the male-specific compound alone, (*R*)-(-)-linalool and the unbaited control respectively ($F = 15.62$, $d.f = 3,8$, $P = 0.001$) (Fig. 5.17). Combining the male-specific compound with (*R*)-(-)-linalool caught significantly more beetles than the male-specific compound alone and (*R*)-(-)-linalool and the unbaited control ($F = 4.37$, $d.f = 3,8$, $P = 0.005$) (Fig. 5.17).

Numbers of female beetles caught were not significantly affected by the treatments ($F = 2.24$, $d.f = 3,8$, $P = 0.161$).

Total number of insects caught on traps baited with (*R*)-(-)-linalool alone was not significantly different from the unbaited control and the male-specific compound alone (Fig. 5.17).

Numbers of male *M. leuconotus* round traps baited with the combination of the male-specific compound with (*R*)-(-)-linalool were significantly different from the male-specific compound alone and the unbaited control but they were not significantly different from (*R*)-(-)-linalool alone ($F=5.59$, $d.f = 3,8$, $P < 0.001$) (Fig. 5.18). Numbers of female beetles round traps ($F=1.08$, $d.f = 3,8$, $P = 0.355$) and mating pairs ($F=0.61$, $d.f = 3,8$, $P = 0.628$) were not significantly affected by the treatments.

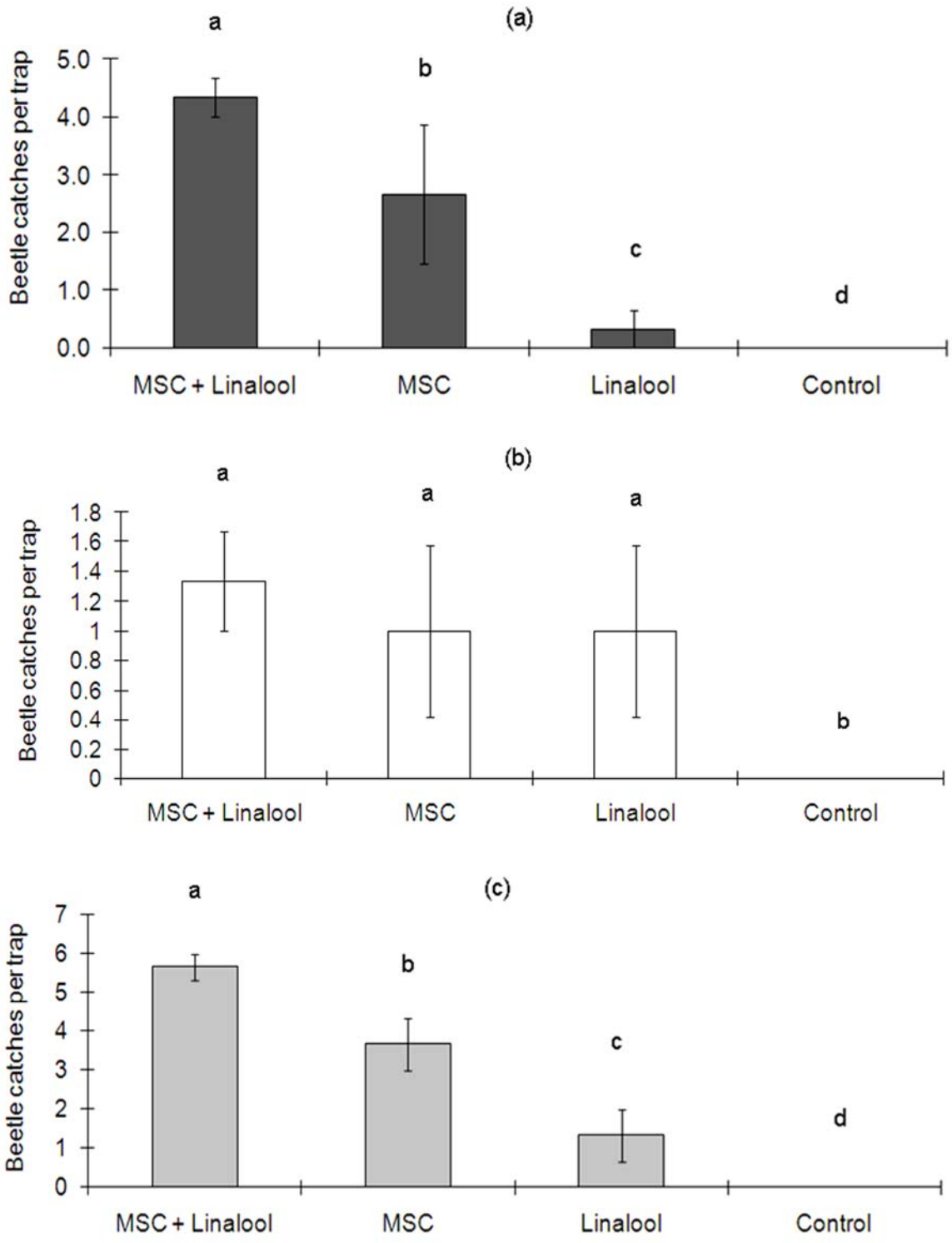


Fig. 5.17 Mean catches/trap of *Monochamus leuconotus* (±SE) in intercept traps baited with male-specific compound (MSC) (ARDA Katiyo, 02/01/2012 -31/03/2012). For all treatments, $N = 3$. (a) Males, (b) Females, (c) Total. For each sex, bars followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

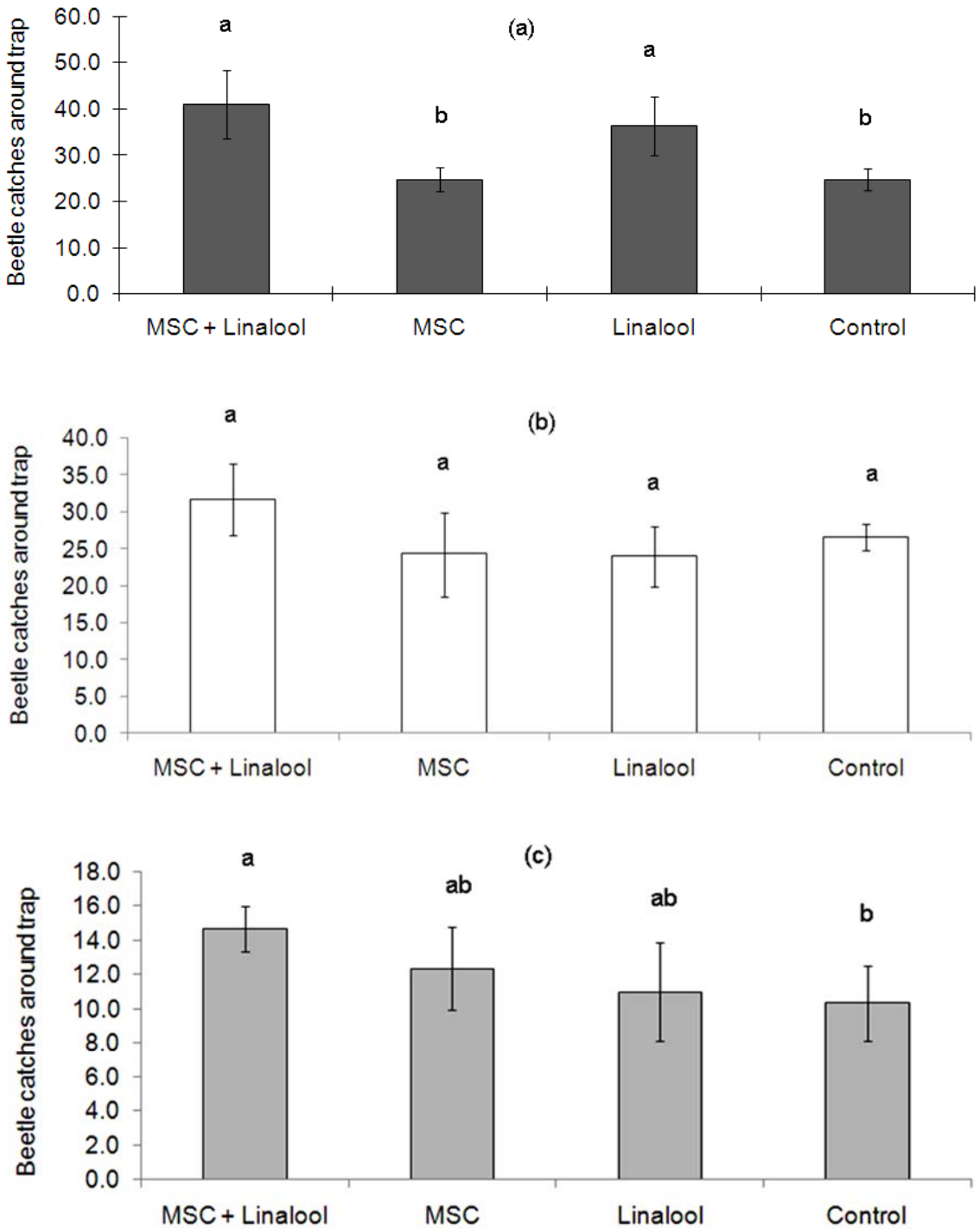


Fig. 5.18 Mean numbers of *Monochamus leuconotus* (±SE) within 1 m radius intercept traps baited with male-specific compound (MSC) (ARDA Katiyo, 02/01/2012 -31/03/2012). For all treatments, $N = 3$. (a) Males, (b) Females, (c) Mating pairs. For each sex, bars followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$). .

5.4. DISCUSSION

5.4.1 Trap catches

Live insects of both sexes and the synthetic male–specific compound of *M. leuconotus* formulated in polyethylene sachet and cigarette filter dispensers were the main lures tested for evidence of long-range attraction during the earlier parts of the study period. In addition, combinations of the male-specific compound with the host volatiles, (*R*)-(-)-linalool, methyl salicylate, ethyl benzoate and (*Z*)-3-hexenol were also tested during subsequent work. These compounds were found to be the major components in collections of volatiles from Arabica coffee plants in India (CAB International, 2008; David Hall, *pers comm*.).

There was no consistent data to indicate attraction or repellency of any of the lures in the initial work where the only significant differences are in Table 3.5 for males where fewer beetles were observed round the live baits than the male-specific compound dispensed in a white polyethylene sachet or unbaited, but this is not really borne out in the other experiments. Only one beetle was actually ever caught in a trap in the earlier trials and so aggregation around the lure was assessed. The early trapping trials followed a similar pattern of generally low numbers of *M. leuconotus* around the lures and higher numbers around unbaited traps. Despite the fact that the results were statistically insignificant, there appeared to be some specific trends in terms of the sachet and live male baits attracting more female beetles than live female baits at all the sites tested during the 2005/06 season, although the unbaited traps had the most beetles. This suggests that the males could be releasing a male-specific compound that attracts females to trees within its vicinity. Another noteworthy observation was the generally high numbers of males attracted towards the male-specific compound dispensed in a white polyethylene sachet. Attraction of males by the male-specific compound could suggest that it could be an aggregation compound. When the synthetic male-specific compound was dispensed at a high rate (10 µg) in the cigarette filter there seemed to be no suggestion of its role as a long-range male-specific compound despite the fact that more females than males were found around the cigarette filter.

However, the later trials with intercept traps yielded positive results and significantly more beetles were trapped in baited traps than unbaited. There were some significant differences in the total numbers of *M. leuconotus* actually trapped when the male-specific compound was combined with (*R*)-(-)-linalool, methyl salicylate, ethyl benzoate and (*Z*)-3-hexenol (Fig. 5.13 - 5.18). The male-specific compound in combination with (*R*)-(-)-linalool caught more beetles than the combinations with ethyl benzoate and (*Z*)-3-hexenol suggesting that (*R*)-(-)-linalool (Fig. 5.13) could have an additive effect. When the

compound alone was compared with combinations with (*R*)-(-)-linalool and methyl salicylate, more beetles were trapped by the combinations than for the male-specific compound alone (Fig. 5.15) suggesting that combinations of the male-specific compound with the host volatiles increased trap catches. The combination of the male-specific compound with (*R*)-(-)-linalool caught more beetles than the combination with methyl salicylate and the male-specific compound alone suggesting that (*R*)-(-)-linalool is important in increasing attractiveness of the male-specific compound.

Subsequent work to confirm the effect of (*R*)-(-)-linalool with the male-specific compound (Fig. 5.17, Fig. 5.18) produced consistent results, which showed that more beetles were attracted to combinations of the male-specific compound with (*R*)-(-)-linalool followed by the male-specific compound alone and (*R*)-(-)-linalool alone.

In terms of attractiveness of the lures to the different sexes of *M. leuconotus*, the combination of the male-specific compound with (*R*)-(-)-linalool was more effective in luring females to the traps when compared with the combinations with ethyl benzoate and (*Z*)-3-hexenol. When the male-specific compound was compared with the combinations with (*R*)-(-)-linalool and methyl salicylate, marginally more females were caught with the combinations than for the male-specific compound alone ($P = 0.058$). However, when the male-specific compound was compared with (*R*)-(-)-linalool, there was no significant effect on the number of females caught. Combinations of the male-specific compound with (*R*)-(-)-linalool, methyl salicylate, ethyl benzoate and (*Z*)-3-hexenol did not have a significant effect on the numbers of males caught by the traps (Fig. 5.13). When the combination of the male-specific compound with (*R*)-(-)-linalool was compared with the male-specific compound alone, the combination caught more males than the male-specific compound alone, which also attracted more males than (*R*)-(-)-linalool alone. There was evidence of attraction of *M. leuconotus* to the host volatiles, (*R*)-(-)-linalool, methyl salicylate, ethyl benzoate and (*Z*)-3-hexenol in combination with the male specific male-specific compound. (*R*)-(-)-Linalool alone was attractive to both sexes of the African coffee white stem borer. Attraction to combinations of these host volatiles with the male-specific compound suggests that the volatiles could be involved in host location whereby both sexes are attracted to suitable hosts by host volatiles and the male-specific compound, which enables females to find mates (see Chapter 2). Males then find and recognise their mates through chemical contact (see Chapter 3).

When male beetle counts within a 1 m radius were considered, there was no evidence of increased numbers of males due to combinations (*R*)-(-)-Linalool, ethyl benzoate and (*Z*)-3-hexenol (Fig. 5.14). However, the combination of the male-specific compound with (*R*)-(-

)-Linalool was not different to the male-specific compound alone but better than the combination with methyl salicylate (Fig. 5.16). Results from male beetle counts round 1 m radius indicate the potential of (*R*)-(-)-Linalool in trapping coffee stem borer as combinations of the male-specific compound with (*R*)-(-)-Linalool and (*R*)-(-)-Linalool alone was better than the male-specific compound alone (Fig. 5.8).

Combinations of (*R*)-(-)-Linalool with the MSC resulted in more female beetles within 1 m radius of the traps than the combination with (*Z*)-3-hexenol and the untreated control (Fig. 5.14) while combinations with methyl salicylate and with (*R*)-(-)-Linalool were not better than the MSC alone (Fig. 5.16) while the combination of MSC with (*R*)-(-)-Linalool was not better than MSC alone and (*R*)-(-)-Linalool alone (Fig 5.18) suggesting that female beetles are not affected much by the volatiles.

Trap catches could be a more reliable evaluation of beetle attraction to the lures than beetle counts within a 1m radius since these are based on actual catches on the traps whereas beetles around traps could be due to random or kinetic movements.

Low numbers of trapped beetles obtained during the studies are generally consistent with previous work with other Cerambycids. For example, Nakamuta *et al*., (1997) reported low numbers of *Anaglyptus subfasciatus* while similarly low catches were recorded in trapping studies with *Xylotrechus quadripes* (Hall *et al*., 2006; Rhainds *et al*., 2001). The appearance of more males than females at the beginning of the study period rather than later could be of adaptive significance since males have been reported to require maturation feeding before mating. However, in the studies reported in this thesis there was no evidence of the requirement for maturation feeding (see Chapter 2). According to Schoeman (1998), the sex ratio of *M. leuconotus* was male-biased at the beginning of the flight season. Observations in the current study also confirm this assertion.

There is evidence for long-range attraction within the subfamily Lamiinae where male-produced aggregation pheromones have been confirmed from 7 species after field tests (Table 1.4). These include the Monochaminae, *Monochamus galloprovincialis* (Pajares *et al*., 2010); *M. alternatus* (Teale *et al*., 2011); *M. carolinensis* and *M. tittilator* (Allison *et al*., 2012); *M. scutellatus scutellatus* (Fierke *et al*., (2012), and *M. sutor* (Pajares *et al.,* 2013), whose pheromones have been identified.

Attraction of male and female, conspecific beetles to the male-produced aggregation pheromones in *Monochamus* spp. is often strongly synergised by combination with host volatiles such as ethanol and α-pinene and bark beetle pheromones. For example, combinations of the pheromone with host volatiles increased trap catches of *M*.

galloprovincialis (Pajares *et al.,* 2010), *M. alternatus* (Teale *et al*., 2011) and *Monochamus* spp. (Allison *et al*., 2012; Hanks *et al*., 2012). In the present study evidence of the increase of attractiveness of the male-specific compound of *M. leuconotus* has been overwhelming (Fig. 5.13, 5.15, 5.17) suggesting that combining the male-specific compound with (*R*)-(-)-linalool could be of practical benefit in monitoring the pest.

There is evidence of the attraction of a number of Monochaminae species to host volatiles in coniferous plantations, e.g. *M. alternatus* (Ikeda *et al*., 1980, Sakai and Yamasaki, 1989, Sakai *et al*., 1992, Yamasaki *et al*., 1997)*, M. carolinensis* (Erbilgin and Raffa, 2000), *M. notatus*, *M. scutellatus* and *M. mutator* (Groot and Nott, 2004) and *M. galloprovincialis* (Pajares *et al*., 2004, 2008), which are all attracted by *α*-pinene and ethanol. In addition, Nehme *et al*., (2009) demonstrated that (*R*)-(-)-linalool was an attractant for the Asian longhorn borer, *Anoplophora glabripennis,* at both close and long range. In both cases the effect was greatest when combined with the sex pheromone. In the current study, results on the long range attraction being synergised by (*R*)-(-)-linalool are in agreement with those for *A. glabripennis*. The results from the study reported in this thesis suggest that (*R*)-(-)-linalool is an attractant for *M. leuconotus* and its combination with the male specific compound has potential for practical implementation in monitoring and detection of the pest under field conditions.

There has been no documentation on the role of host volatiles in host selection by *M. leuconotus*. According to Hanks (1999), *M. leuconotus* attacks weakened hosts and is thus unlikely to use long-range pheromones in mate recognition. This probably explains why no beetles were caught with conspecific lures. However, since weakened or stressed hosts release ethanol, there might be a need to compare the response of *M. leuconotus* adults to healthy and weakened hosts and, in addition, determine the chemical composition of the volatiles from weakened and healthy coffee plants.

5.4.2 Comparison of the different trap designs used in the experiments

Trap designs used in the current studies were the cross vane, sticky types (MK1, MK2) and intercept panel traps. Cross vane and intercept traps were used since they have been shown to be more effective than other methods of trapping large wood-boring insects (McIntosh *et al.,* 2001; Morewood *et al.,* 2002; De Groot and Nott 2003; Sweeney *et al.,* 2004; Hall *et al.,* 2006). However, when no *M. leuconotus* were caught, aggregation round the trap was taken into consideration. Sticky traps were tried because they were recommended for trapping cerambycid beetles (Lacey *et al*., 2004, Jocelyn Miller, pers.

comm.). They could also be placed easily within the coffee canopy, closer to the beetles' habitat. *M. leuconotus* female beetles are normally sluggish and remain on the tree that they emerge from where they meet their mates.

Many beetles were caught on the intercept traps and one beetle was caught on the sticky traps MK2 while none were caught on the cross vane and sticky traps MK1. The catch on the MK2 suggests that the type of glue used could be ideal for trapping of *M. leuconotus* since it managed to retain the beetle on the trap. However, the glue was not durable for a long time especially in high temperatures, which might need to be addressed in future trap designs. The MK1 trap could not withstand rain because of the paper base. The intercept trap was robust many beetles were caught. These results suggest that the intercept traps are ideal and effective in capturing the adults of *M. leuconotus*. Intercept traps also have the advantage of durability at the farmer level since they can be reused over many flight seasons. Graham *et al.* (2010) reported that the effectiveness of the intercept traps can be further increased by coating the panels with Fluon. However, given the sedentary nature of the adults, it is suggested that the MK2 sticky traps could be used as another option because they managed to capture and retain a beetle of *M. leuconotus* during the course of the current studies. However, an appropriate glue type that withstands the high temperatures in the tropics needs to be identified. More work still needs to be done on trap placement within the canopy such as height above ground level and topographic placement of the traps within coffee plantations.

Chapter 6
INVESTIGATION OF ALTERNATIVE HOST PLANTS FOR *Monochamus leuconotus* (PASCOE)

6.1. INTRODUCTION

In previous chapters of this thesis, the work described focussed mainly on determining whether volatile or involatile pheromones play a role in the chemical ecology of *M. leuconotus*. It is also possible that chemical signals play a part in host plant selection by this pest, and many cerambycid species are reported to show a kairomonal response to volatiles from the host plant and even to pheromones of other insects feeding on the host plant (See Chapter 1). Most of the reports concern species of cerambycids attacking conifers, which are rich in volatile secondary chemicals, unlike coffee (Pajares *et al*., 2004, Teale *et al*., 2011).

In Chapter 4, some evidence for attraction of both male and female *M. leuconotus* to scrapings of coffee bark was obtained. An alternative approach was thought to be to find alternative wild host plants attacked by *M. leuconotus* and then to investigate host features, chemical or otherwise, which determine whether a plant is attacked by *M. leuconotus*. As well as providing important information on the biology of *M. leuconotus*, this information could be used in management of this pest, for example, by avoiding planting coffee where alternative wild host plants are abundant and may provide a reservoir of infestation. Alternatively, it might be possible to use alternative wild hosts as trap crops. For example the trap crop could be inter-planted with the coffee or planted around the coffee plantation to attract the pest, thereby reducing infestation on the coffee plants.

Schoeman (pers. comm.) claimed that it was relatively easy to find alternative wild host plants attacked by *M. leuconotus.* Thus, initial work focused on carrying out transect surveys of the vegetation round coffee fields to describe and quantify the wild host species attacked by *M. leuconotus*. As very few, if any infestations were found, an alternative approach was adopted of cataloguing species of plants round coffee fields and then looking for any correlations between the degree of *M. leuconotus* infestation in the coffee field and the presence of particular plant species.

The objectives of the study were to search for possible alternative host plants of *M. leuconotus* in Zimbabwe and to determine their relationship with the incidence of the pest in coffee under field conditions as a basis for investigations into host preference and selection.

6.2. MATERIALS AND METHODS

6.2.1 Study locations

Selected areas were in the coffee growing districts of Chipinge, Chimanimani, Mutare, Mutasa, Goromonzi, Makonde, Hurungwe and Guruve districts of Zimbabwe (Appendix 4). Coffee fields were selected from the smallholder, intermediate and large-scale farmers. Smallholders were identified as farmers without adequate financial and human resources to manage the white borer problem and their fields were less than 1 ha each. Intermediate farmers had fields ranging from 10 to 20 ha and their management practices did not meet standard recommendations. For the large scale sector, each farmer had at least 20 ha although management practices varied according to the farmers involved. For example, the ARDA Estates at Katiyo and Rusitu Valley were state-owned and management tended to be lax due to inadequate resources while their corporate counterparts such as Tanganda, who own New Year's Gift, had adequate resources. Other individual large scale farmers either had poor or good management practices. For example, at Lonely Park in Acturus the practice of handpicking of beetles as opposed to preventative chemical sprays was a poor management practice. Altitudes and geographic coordinates at each site were determined using a global positioning system (Garmin eTrex Vista). The geographic locations, altitudes and varieties grown at the different sites for the line transects surveys are given in Table 6.1.

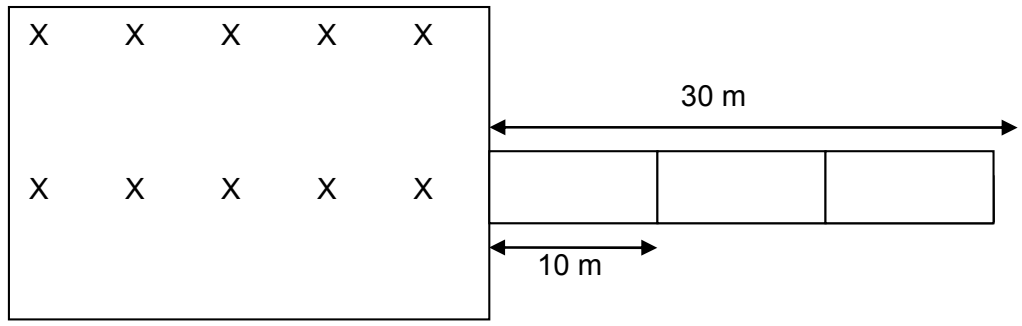
Study sites for the transect belt surveys which involved scouting for coffee stem borer symptoms on all wild trees were selected according to incidence level of *M. leuconotus* in the field with a bias towards heavily infested fields since earlier attempts to find alternative host plants had yielded negative results. Most of the plots from the first survey were included in later surveys with the major difference being the sampling area and the inclusion of one study site from a natural forest with no coffee production within its vicinity where no correlation with coffee field infestations were done.

6.2.2 Transect surveys during 2003/04 Season

Line transects surveys of trees (Buckland *et al*., 2007) surrounding coffee plantations were carried out from December 2003 to September 2004 (flight season of the stem borer in Zimbabwe is December to April) to determine species biodiversity and *M. leuconotus* incidence in wild Rubiaceae trees round coffee fields . The species distributions along a line from the edge of a coffee field were recorded. Each transect was 10 m long. For example, the first transect was from 0 to 10 m, the second from 10 m to 20 m while the third was from 20 to 30 m from the edge of the coffee plantation.(Fig. 6.1). At each site the number of transects depended on the distribution of natural vegetation surrounding the coffee field. In certain cases, there was no vegetation within 30 m from the edge of the coffee plantation. Where vegetation was present in all the four cardinal directions (North, East, South and West), four transects were taken and where it was only available in one direction, only one transect was taken. A total of 78 transects were surveyed across all the 30 sites. Trees were identified in the field with the help of a specialist from the National Herbarium and Botanic Gardens, Harare. All plants belonging to the family Rubiaceae were examined for the presence of symptoms of *M. leuconotus* such as ring barking, fresh shavings and exit holes. Stems of all Rubiaceae plants showing suspected symptoms were taken to the laboratory for further examination of symptoms such as the presence of ring barking, exit holes, frass and adult feeding damage.

The incidence of stem borers in a coffee plantation at each site was obtained by examining 30 trees selected at random in a stepped traverse style across each field whose surroundings were being surveyed for alternative host plants. Presence or absence of the stem borer (as confirmed by the presence of the symptoms described above) was recorded and expressed as proportion of trees affected (all 30 trees infested gives incidence of 1).

a



b

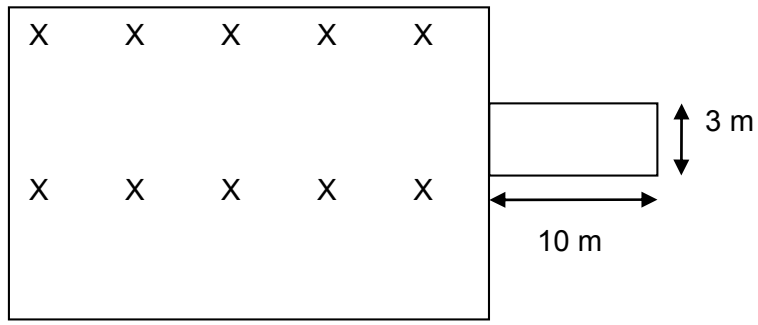


Fig. 6.1 Approaches used during the transect surveys for alternative host plants of *Monochamus leuconotus*: (a) Sampling was done along a line transect of 10 m long by 1 m wide in 2003/4, the first transect was from 0 to 10 m, the second was 10 to 20 m, and the third was 20 to 30 m from the edge of the coffee field, (b) Sampling was done for all trees in a transect belt 10 x 3 m on the edge of the field in 2005/6.

6.2.3 Transect Surveys during 2005/06 Season

Having found very few infested wild Rubiaceae plants in the earlier surveys, more surveys were carried out in Chipinge, Chimanimani, Mutare, Mutasa and Goromonzi districts to determine if *M. leuconotus* could be found on plants other than those belonging to the family Rubiaceae. Tree species diversity and symptoms of *M. leuconotus* attack on wild trees in transect belts (3 m x 10 m) along the edges of coffee fields were recorded. The

same sites used during earlier surveys were examined but transects were now 10 metres from the edge of the field and 3 metres wide compared to 30 metres and 1 metre wide used in earlier studies (Fig 6.1). Since all trees irrespective of family were being examined for stem borer symptoms, the transect belt was deemed ideal for the detailed study. The number of transects per site varied according to the distribution of natural vegetation at those sites. The survey focused on severely infested plantations and some patches of natural vegetation where no coffee was grown to see if there could be stem borer infestation under natural conditions. In total, 18 coffee fields were investigated for the presence of alternative host plants in vegetation surrounding them. In addition, the incidence of *M. leuconotus* in coffee fields was re-examined.

Table 6.1 Characteristics of the sites where line transect surveys for alternative host plants of *M. leuconotus* were carried out in Zimbabwe.

Site	Coordinates	Altitude (m)	Farming system	Variety
Mutamangira	18º 34.612 S, 32º 42.359 E	1320	Smallholder	Catimor F6
Gwiriri 315	18º 33.957 S, 32º 43.016 E	1400	Smallholder	K7
Amajuba	16º 36.984 S, 30º 44.481 E	1200	Large scale	Catimor F6/SL28
Muranda	18º 32.115 S, 32º 44.383 E	1224	Smallholder	K7
Muranganwa	18º 32.371 S, 32º 45.043 E	1162	Smallholder	Catimor F6
Charity Dumba	18º 32.090 S, 32º 45.000 E	1219	Smallholder	Catimor F6
ARDA Katiyo1	18º 22.042 S, 33º 02.862 E	629	Large scale	Catimor 129
ARDA Katiyo	18º 21.929 S, 33º 02.782 E	674	Large scale	Catimor 129
Chikomba 2/En Highlands	18º 19.206 S, 32º 59.210 E	688	Large scale	Caturra
Chikomba 1/En Highlands	18º 19.547 S, 32º 58.780 E	750	Large scale	Catimor 129
En Highlands	18º 20.264 S, 32º 56.786 E	880	Large scale	Catimor 129
Area 1-Lonely Park Farm*	17º 47.707 S, 31º 25.339 E	1313	Large scale	SL28
Area 2-Lonely Park Farm*	17º 47.707 S, 31º 25.339 E	1313	Large scale	Catimor F6
Area 3-Lonely Park Farm*	17º 47.736 S, 31º 25.314 E	1312	Large scale	SL28
Area 4-Lonely Park Farm*	17º 47.775 S, 31º 25.301 E	1313	Large scale	SL28
Petrusville Plot 9B	16º 45.321 S, 30º 10.609 E	1241	Large scale	Catimor F6

Table 6.1 (cont) Characteristics of the sites where line transect surveys for alternative host plants of *M. leuconotus* were carried out in Zimbabwe.

Site	Coordinates	Altitude (m)	Farming system	Variety
Wildene Farm	16º 45.950 S, 30º 10.045 E	1217	Large scale	SL28
Nyamuseve Farm	16º 31.963 S, 30º 42.390 E	1122	Large scale	Catimor F6
New Year's Gift	20º 06.073 S, 32º 33.081 E	743	Large scale	Catimor F6
New Year's Gift Gombati	20º 05.691 S, 32º 36.436 E	889	Large scale	Catimor F6
Gwenzi area	20º 32.731 S, 32º 38.672 E	817	Smallholder	Catimor F6
Tamandayi	20º 18.270 S, 32º 50.322 E	766	Smallholder	Catimor F6
ARDA Rusitu Valley Section 3	20º 02.366 S, 32º 40.668 E	1076	Large scale	Catimor F6
Steyn Farm	19º 42.773 S, 32º 54.400 E	1167	Intermediate	Catimor F6
Bvumba Agric Cooperative	19º 06.450 S, 32º 47.972 E	1196	Intermediate	Catimor F6
Crake Valley	19º 06.187 S, 32º 48.769 E	1102	Large scale	Catimor F6
Isis Farm	20º 18.498 S, 32º 31.348 E	1040	Large scale	Catimor F6
Stillfontein Estates	20º 20.604 S, 32º 42.150 E	1021	Large scale	Catimor F6
ARDA Rusitu Valley	20º 02.335 S, 32º 48.645 E	1100	Large scale	SL28
Farfell	20º 25.014 S, 32º 41.167 E	1009	Large scale	Mundo nova

*Adjacent fields on the same farm

6.2.4 Data Analysis

Data on the distribution of plants across the sites, biodiversity, and distribution along line transects and according to the farming sectors were collated using the pivot table facility in Excel (Appendix 5). These were analysed by ANOVA using Genstat (Appendix 6). The relationship between the incidence of *M. leuconotus* across sites and the occurrence of each plant family was determined by regression analysis for the line transect and detailed surveys. Further correlations between the incidence of *M. leuconotus* and individual species belonging to the Rubiaceae (*Vangueria infausta, Keetia venosa, Keetia guenzii, Vangueria esculenta* and *Pavetta gardenifolia*) family were done. The influence of altitude on the incidence of *M. leuconotus* was determined through simple correlations.

6.3. RESULTS

6.3.1 Distribution of species across all sites during line transect surveys in 2003/4

Sites surveyed during 2003/4 are listed in Table 6.1 by district. In all, 847 trees were recorded from natural vegetation surrounding 30 coffee fields (Table 6.2). In terms of frequency, the most common families were Fabaceae, Euphorbiaceae and Rubiaceae respectively. Numbers of species per family were highest in Fabaceae, Rubiaceae and Eurphobiaceae in descending order respectively. Other families such as Boraginaceae, Polygalaceae, Ochnacaceae, Olacaceae and Vitaceae were rare and only contained a single tree species at the sites that they were found. For example, Polygalaceae was only found only at Amajuba in North Western Zimbabwe while Vitaceae was only found at Muranganwa in the Eastern Highlands of Zimbabwe.

Table 6.2 Numbers of records by family and species identified during the line transects surveys.

Family	No. of records	No. of Species	Family	No. of Records	No. of Species
Anacardiaceae	26	5	Loganiaceae	8	3
Annonaceae	9	1	Malvaceae	2	2
Apiaceae	17	2	Melastomataceae	1	1
Apocynaceae	21	5	Meliaceae	8	2
Araliaceae	8	4	Melianthaceae	3	1
Asteraceae	21	3	Mimosaceae	2	1
Bignoniaceae	10	3	Moraceae	27	3
Boraginaceae	1	1	Myrsinaceae	13	1
Caesalpiniceae	5	3	Myrtaceae	40	6
Capparaceae	1	1	Ochnaceae	2	1
Celasteraceae	9	5	Olacaceae	1	1
Chrysobalanaceae	26	1	Pittosporaceae	10	1
Clusiaceae	7	1	Polygalaceae	1	1
Combretaceae	32	7	Proteaceae	4	3
Dipterocarpaceae	1	1	Rhamnaceae	9	1
Ebenaceae	10	2	Rosaceae	1	1
Euphorbiaceae	113	11	Rubiaceae	100	19
Fabaceae	189	36	Rutaceae	7	2
Flacourticeae	5	1	Sapindaceae	12	3
Gentianaceae	3	1	Steruliaceae	3	2
Guttiferae	4	2	Ulmaceae	27	2
Labiatae	1	1	Verbenaceae	40	2
Lamiaceae	7	2	Vitaceae	1	1

Nineteen species belonging to the Rubiaceae family were recorded across all the sites of which *Vangueria infausta, Keetia venosa, Keetia guenzii* and *Vangueria apiculata* were the most abundant in descending order respectively. Suspected symptoms of infestation by *M. leuconotus* were found on some Rubiaceae such as *Vangueria infausta, Pavetta gardenifolia* and *Keetia venosa* but no beetles emerged from the stems collected (Table 6.3).

Table 6.3 Rubiaceae species identified during the line transect surveys in 2003/4 and prevalence of *M. leuconotus* symptoms of attack.

Species	No. of trees	No. with symptoms of stem borer attack
Aidia macrantha	2	0
Canthium ngoni	1	0
Catunaregam obovata	2	0
Fadogia urncilatum	1	0
Gardenia cornuta	2	0
Gardenia resiniflua	5	0
Gardenia ternifolia	3	0
Keetia guenzii	11	0
Keetia venosa	13	5
Oxyanthus latifolius	3	0
Pavetta comostyla	1	0
Pavetta gardenifolia	7	3
Psychotria capensis	1	0
Psychotria mahonii	5	0
Rothmania manganjae	1	0
Vangueria apiculata	10	0
Vangueria esculenta	5	0
Vangueria infausta	24	7
Vangueriopsis lanciflora	3	0

There were more tree species in the estate sector when compared to the intermediate and smallholder sectors (F = 38.94, *d.f*. 2,4, P = 0.002). In the intermediate sector, there was only one tree in the first transect whereas for the smallholder sector, most of the trees were within the first transect (Table 6.4). In terms of distribution of species according to distance from the edges of coffee plantations there were no significant differences (F = 0.26, *d.f*. 2,4, P = 0.781). Most species were uniformly distributed with the exception of the family Fabaceae where most trees were found at a distance of 30 m from the edge of the field. The number of Rubiaceae in the estate sector was significantly greater (F = 10.44, *d.f.* 2,4, P = 0.026) than in the intermediate and smallholder sectors. There were no significant differences in distribution of Rubiaceae (F = 0.02, *d.f.* 2,4, P = 0.978) according to distance from the edge of the field. However, in the intermediate sector,

there were no plants belonging to the Rubiaceae in the 10 m and 30 m transects. The Apocynaceae were not found in the smallholder sector while the Arialiaceae was absent from the intermediate sector (Table 6.4).

Table 6.4 Distribution of plant species along line transects during the 2003/04 season. Transect 1 was 0 to 10 m from the field edge, transect 2 was 10 to 20 m from field edge and transect 3 was 20 to 30 m from field edge. The transect size was 10 m x 1 m.

Species abundance	Distance from field edge (m)	Farming system			Mean
		Estate	Intermediate	Smallholder	
Total	10	175	10	76	87.0
	20	227	46	36	103.0
	30	223	13	41	92.3
Rubiaceae	10	18	0	14	10.7
	20	25	6	2	11.0
	30	27	0	8	11.7
Araliaceae	10	0	0	3	1.00
	20	2	0	0	0.67
	30	3	0	0	1.00
Apocynaceae	10	5	0	0	1.70
	20	5	1	0	2.00
	30	10	0	0	3.30
Fabaceae	10	50	1	6	19.0
	20	48	3	5	18.7
	30	67	3	8	26.0

6.3.2 Distribution of species across all sites during transect belt surveys in 2005/6 season

A total of 360 trees were recorded across all sites during the surveys done on transect belts during 2005/6. Fabaceae, Rubiaceae and Euphorbiaceae were the most common families in descending order of frequency respectively (Table 6.5). Species diversity followed a similar pattern to that of the families with 21 species recorded from Fabaceae while Rubiaceae and Euphorbiaceae had 19 and 10 species respectively.

Table 6.5 Numbers of records per family and species identified during transect belt surveys in Zimbabwe in 2005/06 season

Family	No. of records	No. of species	Family	No. of records	No. of species
Anacardiaceae	14	6	Meliaceae	3	3
Annonaceae	8	3	Melianthaceae	3	1
Apiaceae	3	2	Mimosaceae	15	6
Apocynaceae	4	2	Moraceae	1	1
Araliaceae	6	3	Myrsinaceae	4	1
Asteraceae	5	2	Myrtaceae	11	4
Bignoniaceae	4	2	Ochnaceae	3	2
Boraginaceae	2	1	Olacaceae	2	2
Celasteraceae	4	2	Papilionaceae	3	2
Chrysobalanaceae	8	1	Passifloraceae	1	1
Clusiaceae	6	1	Pittosporaceae	2	1
Combretaceae	18	6	Proteaceae	1	1
Dracaenaceae	1	1	Rhamnaceae	1	1
Ebenaceae	8	2	Rosaceae	1	1
Euphorbiaceae	41	10	Rubiaceae	57	19
Fabaceae	58	21	Rutaceae	6	1
Flacourtiaceae	4	1	Sapindaceae	6	1
Guttiferae	2	1	Sterculiaceae	1	1
Lamiaceae	8	4	Thymelaeaceae	2	1
Lauraceae	2	1	Ulmaceae	5	2
Loganiaceae	7	4	Verbenaceae	16	2
Malvaceae	2	1	Vitaceae	1	1

Some families that were not recorded during the previous line transect surveys were identified notably; Dracaenaceae, Lauraceae, Papilionaceae and Thymeleaceae. In addition, some families that were reported from the line transect surveys were not found during the detailed surveys. These included Caesalpiniceae, Capparaceae, Gentianaceae, Labiatae and Polygalaceae. A total of 57 plants belonging to 19 species in the Rubiaceae were recorded with *Vangueria infausta* being the most prevalent followed by *Keetia venosa* as in the previous survey. Some species of Rubiaceae not previously found during the line transect surveys were identified during the detailed

surveys. They included *Canthium huillense*, *C. spinosa, Coddia rudis, Hyperacanthus microphyllus Psydrax* spp. and *Tarenna* spp. (Table 6.6)

A number of trees were found with symptoms of coffee stem borer such as frass, exit holes and ring barking larvae (Table 6.6). In terms of frequency of occurrence of symptoms, the most common suspected wild hosts from the Rubiaceae were *Vangueria infausta, Keetia venosa* and *Pavetta gardenifolia* respectively. The non-rubiaceous trees found with symptoms resembling *M. leuconotus* attack were *Tabernaemontana elegans*, *Cussonia natalensis* and *Senna pendula*.

Table 6.6 Prevalence of suspected *Monochamus leuconotus* symptoms on Rubiaceae and some Non-rubiaceae hosts (*non-Rubiaceae)

Species	**No. of trees**	**No. with symptoms of stem borer attack**
Canthium huillense	1	0
Canthium spinosa	1	0
Coddia rudis	1	0
Hyperacanthus microphyllus	1	0
Keetia gueinzii	2	0
Keetia venosa	9	2
Mussaenda arcuata	3	0
Oxyanthus speciosa	2	0
Pavetta gardenifolia	4	1
Pavetta schumanniana	2	0
Psychotria muhonni	3	0
Psydrax parviflora	3	0
Psydrax sp.	1	0
Rothmania manganje	1	0
Tarenna gardenifolia	2	0
Tarenna pavettoides	1	0
Vangueria apiculata	2	0
Vangueria esculenta	4	0
Vangueria infausta	14	5
*Tabernaemontana elegans**	3	2
*Cussonia natalensis**	5	1
*Senna pendula**	1	1

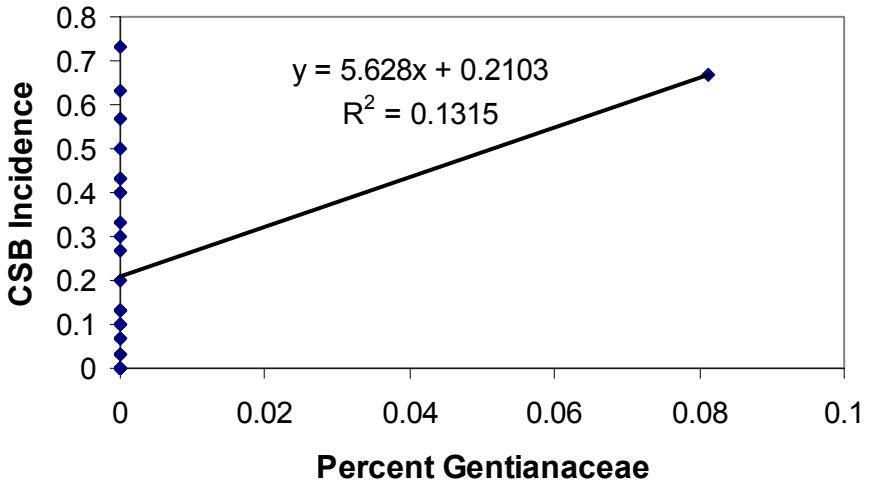
6.3.3 Relationship between the incidence of *M. leuconotus* in coffee fields, plant family abundance and individual Rubiaceae species across sites during line transect surveys

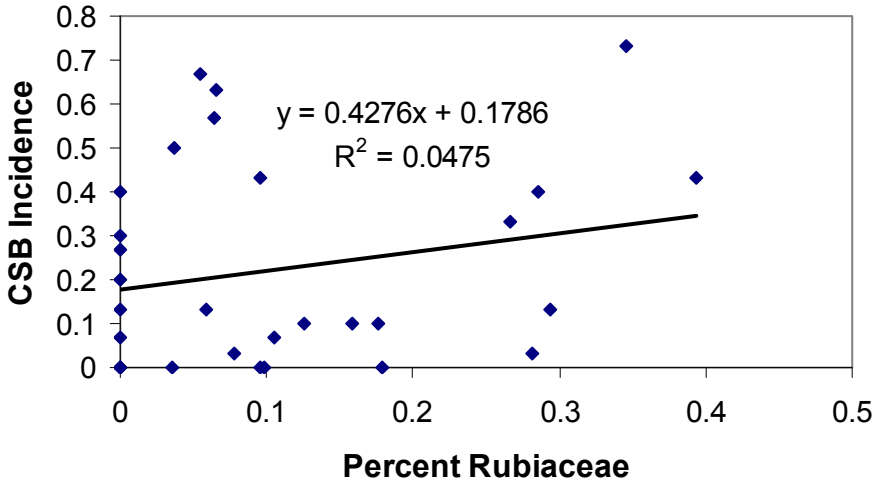
There was no evidence of the existence of any linear relationships between the percentage of plant families and the infestation of coffee white stem borer at each site ($P > 0.05$) (Table 6.7), except for one plant family (Gentianaceae, $P = 0.049$). The occurrence of the plant families was not a reliable predictor of white stem borer infestation in coffee plantations across all the study sites. For example, for the family Rubiaceae, which is the same family as coffee and was expected to be a reliable predictor of coffee stem borer infestation in coffee plantations, the relationship was not significant ($P = 0.247$) (Fig. 6.2). Though the regression coefficients were not significant, some relationships were positive with respect to the incidence of *M. leuconotus* e.g. Rubiaceae, Gentianaceae while some were negative e.g. Anarcadiaceae, Dipterocarpaceae (Table 6.7, Fig. 6.2).

For most of the families such as Anacardiaceae and Loganiaceae the incidence of *M. leuconotus* decreased with increase in the number of trees within those families (Table 6.7) while most of the relationships were very weak due to the rather low coefficients of determination (R^2) and shallow slopes.

There was no evidence of any relationships between the incidence of *M. leuconotus* in coffee fields and the presence of *Vangueria infausta, Pavetta gardenifolia,*and *Keetia venosa,* ($P > 0.05$) in adjacent wild forests (Fig 6.3).

However, the relationship between the incidence of *M. leuconotus* in coffee fields and the presence of *Keetia guenziii* was significant ($P = 0.037$). All the relationships between the incidences of *M.leuconotus* with individual Rubiaceae species were characterised by shallow slopes. The relationship with *Pavetta gardenifolia* was negative and weak while those for *Keetia venosa* and *Vangueria infausta* were positive (Fig. 6.3).





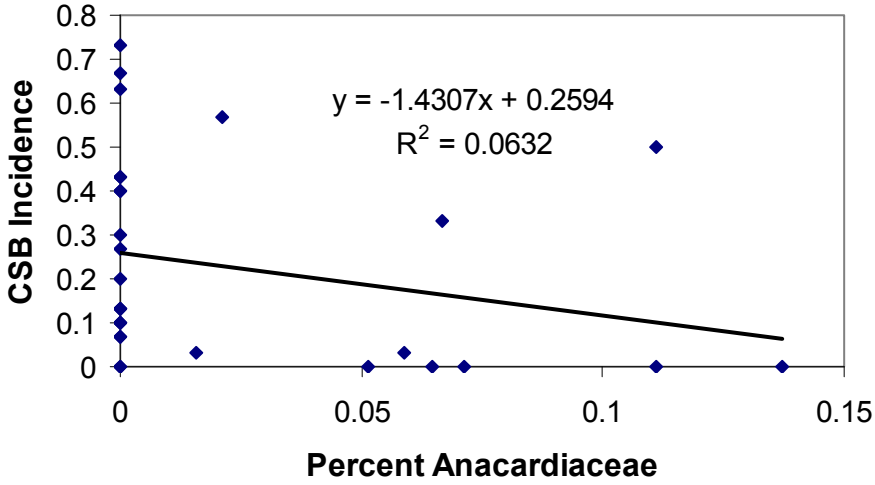


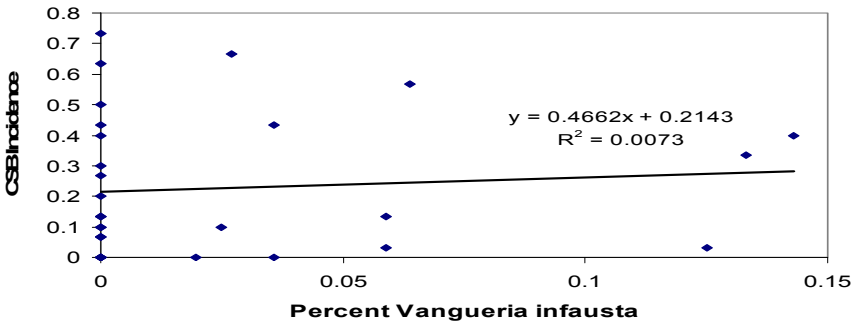
Fig. 6.2 Relationship between occurrences of selected plant families in the wild with the incidence of *Monochamus leuconotus* (CSB) in coffee fields across all study locations during line transects surveys, 2003/4 (*N* = 29).

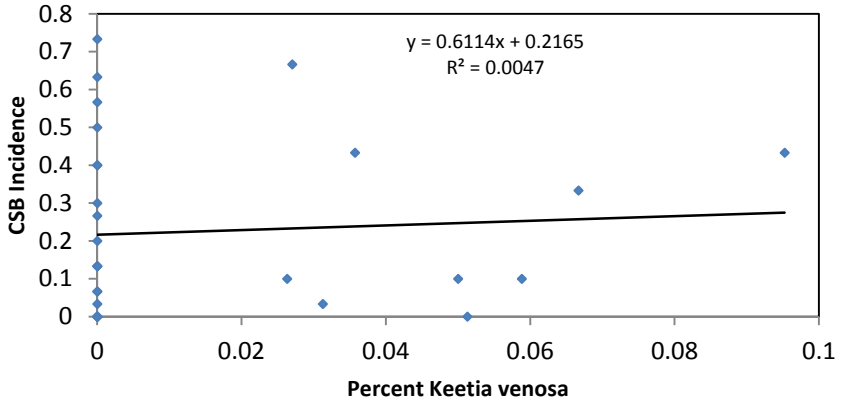
Table 6.7 Relationship between the incidence of *M. leuconotus* in the field and plant family abundance at each site during line transect surveys

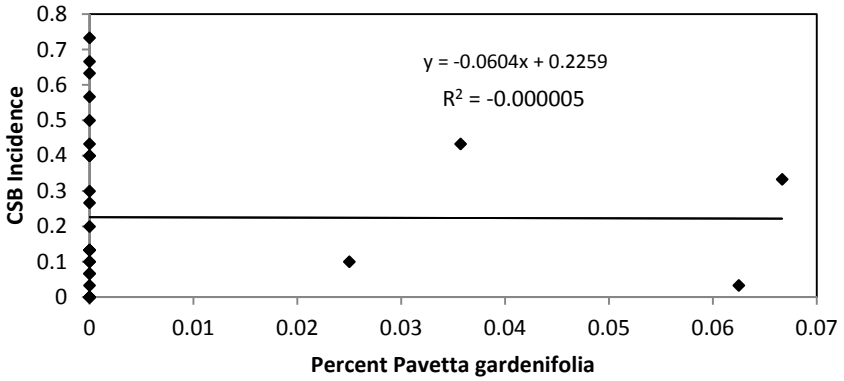
Family	Slope (b)	R^2	*P*	Family	Slope (b)	R^2	*P*
Anacardiaceae	-1.43	0.063	0.180	Loganiaceae	-0.24	0.001	0.893
Annonaceae	-0.95	0.019	0.468	Malvaceae	7.12	0.073	0.150
Apiaceae	-1.26	0.047	0.251	Melastomataceae	-7.23	0.034	0.327
Apocynaceae	1.06	0.022	0.436	Meliaceae	0.92	0.019	0.470
Araliaceae	-2.99	0.044	0.267	Melianthaceae	-4.24	0.025	0.470
Asteraceae	-0.16	0.001	0.857	Mimosaceae	-0.81	0.006	0.690
Bignoniaceae	1.58	0.037	0.310	Moraceae	-0.22	0.001	0.873
Boraginaceae	16.56	0.079	0.133	Myrsinaceae	0.22	0.001	0.849
Caesalpiniceae	1.21	0.022	0.436	Myrtaceae	-0.44	0.011	0.576
Capparaceae	-6.53	0.034	0.327	Ochnaceae	-5.95	0.034	0.327
Celasteraceae	-3.90	0.120	0.062	Olacaceae	2.53	0.021	0.450
Chrysobalanaceae	0.26	0.002	0.792	Pittosporaceae	-1.78	0.024	0.417
Clusiaceae	1.08	0.010	0.610	Polygalaceae	7.67	0.051	0.231
Combretaceae	0.31	0.004	0.730	Proteaceae	-4.32	0.044	0.264
Dipterocarpaceae	-11.9	0.034	0.327	Rhamnaceae	0.32	0.002	0.819
Ebenaceae	1.52	0.015	0.515	Rosaceae	-4.94	0.011	0.587
Euphorbiaceae	-0.08	0.001	0.848	Rubiaceae	0.43	0.047	0.247

Table 6.7 (cont) Relationship between the incidence of *M. leuconotus* in the field and plant family abundance at each site during line transect surveys

Family	Slope (b)	R^2	*P*	Family	Slope (b)	R^2	*P*
Fabaceae	-0.24	0.042	0.276	Rutaceae	-1.61	0.018	0.478
Flacourticeae	1.73	0.013	0.550	Sapindaceae	-2.14	0.046	0.257
Gentianaceae	5.63	0.131	0.049	Steruliaceae	-1.75	0.005	0.705
Guttiferae	3.34	0.038	0.304	Ulmaceae	1.28	0.073	0.149
Labiatae	6.02	0.029	0.367	Verbenaceae	0.46	0.025	0.407
Lamiaceae	-0.39	0.001	0.893	Vitaceae	2.53	0.022	0.450







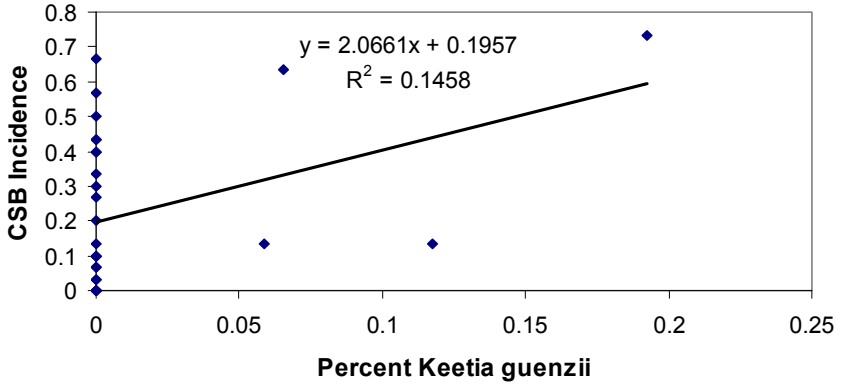


Fig. 6.3 Relationship between occurrences of selected plants belonging to the family Rubiaceae in the wild with the incidence of *Monochamus leuconotus* (CSB) in coffee fields across all study locations during line transects surveys, 2003/4 (*n* = 29).

6.3.4 Relationship between the incidence of *M. leuconotus* in coffee fields, plant family abundance and individual rubiaceae species across sites during transect belt surveys, 2005/6.

There was evidence of the existence of a linear relationship between the incidence of *M. leuconotus* in coffee fields and the presence of 3 plant families (Anacardiaceae Araliaceae and Ulmaceae) in the wild ($P < 0.05$), (Table 6.8). However, there was no evidence of the existence of any relationships with the presence of the rest of the plant families identified ($P > 0.05$). According to the predicted relationships, the incidence of *M. leuconotus* appeared to decrease with increase in numbers of species in some families (i.e. Araliaceae and Ulmaceae) while there was an increase in *M. leuconotus* incidence with increase in number of species (i.e. Anacardiaceae) (Table 6.8). Despite the insignificant regressions between *M. leuconotus* and the presence of many plant families in the wild, there appeared to be an equal distribution of families with positive and negative effects with respect to the incidence of the borer suggesting that the occurrence of plant families in the wild cannot be used as an indicator of stem borer incidence.

The incidence of *M. leuconotus* increased with increase in numbers of species belonging to the family Anacardiaceae and decreased with increase in numbers in the case of Araliaceae and Ulmaceae families (Fig. 6.4).

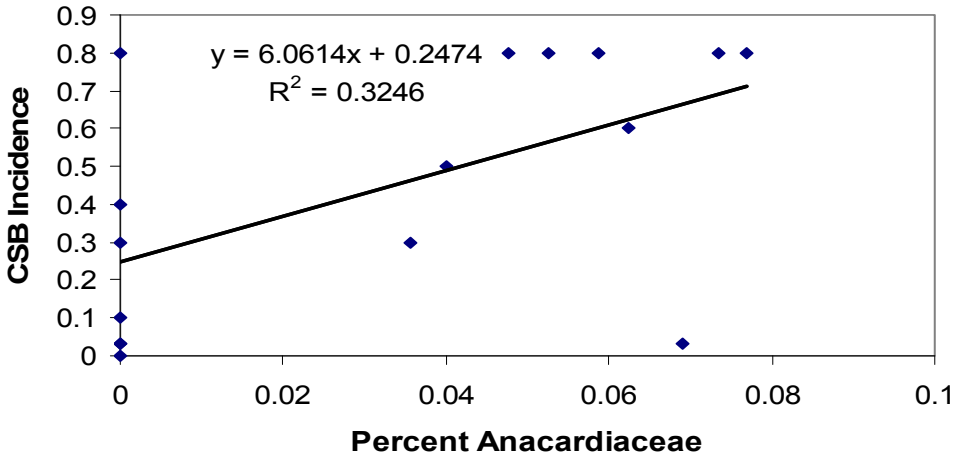
The relationships between the incidence of *M. leuconotus* and the presence of *Keetia venosa, Vangueria infausta* and *Pavetta gardenifolia* were not significant ($P > 0.05$). However, all species had positive regression coefficients with the incidence of *M. leuconotus* (Fig 6.5).

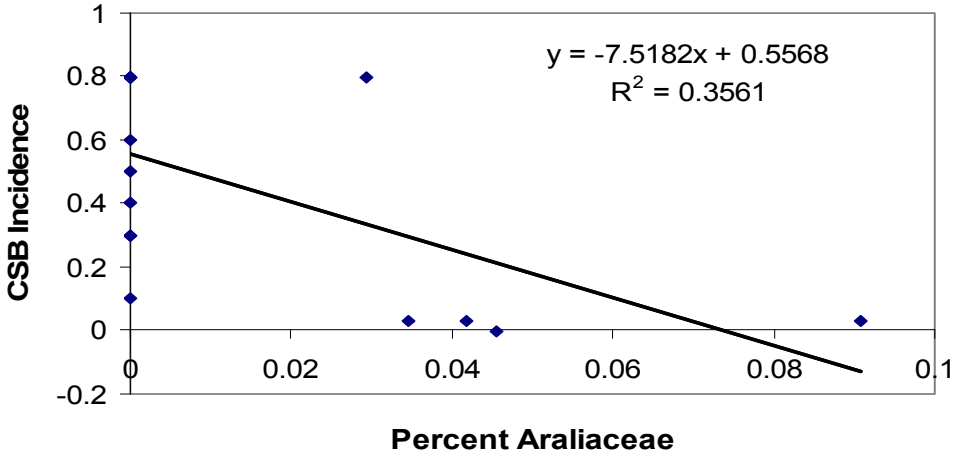
Table 6.8 Relationships between incidence of *Monochamus leuconotus* in adjacent coffee fields and occurrence of plant families in the wild across all sites during detailed surveys

Family	Slope (b)	R^2	*P*	Family	Slope (b)	R^2	*P*
Anacardiaceae	6.06	0.325	0.021	Meliaceae	-6.25	0.148	0.141
Annonaceae	1.77	0.021	0.590	Melianthaceae	-2.68	0.011	0.701
Apiaceae	7.04	0.161	0.123	Mimosaceae	-1.75	0.051	0.401
Apocynaceae	1.80	0.015	0.654	Moraceae	1.52	0.002	0.867
Araliaceae	-7.52	0.356	0.015	Myrsinaceae	-1.26	0.015	0.656
Asteraceae	-4.90	0.189	0.092	Myrtaceae	1.68	0.026	0.553
Bignoniaceae	-1.19	0.005	0.790	Ochnaceae	5.68	0.090	0.258
Boraginaceae	4.89	0.084	0.276	Olacaceae	1.03	0.003	0.843
Celasteraceae	-0.49	0.0004	0.939	Papilionaceae	2.34	0.054	0.386
Chrysobalanaceae	4.51	0.181	0.100	Passifloraceae	25.88	0.082	0.283
Clusiaceae	-0.37	0.002	0.876	Pittosporaceae	-2.63	0.022	0.582
Combretaceae	4.49	0.191	0.090	Proteaceae	25.88	0.082	0.283
Dracaenaceae	-10.40	0.126	0.177	Rhamnaceae	-5.12	0.076	0.303
Ebenaceae	-2.07	0.035	0.490	Rosaceae	7.99	0.082	0.283
Euphorbiaceae	1.31	0.058	0.368	Rubiaceae	-0.83	0.087	0.269
Fabaceae	0.36	0.026	0.553	Rutaceae	-3.51	0.041	0.453
Flacourticeae	-1.99	0.018	0.624	Sapindaceae	3.83	0.103	0.226

Table 6.8 (cont) Relationships between incidence of *Monochamus leuconotus* in adjacent coffee fields and occurrence of plant families in the wild across all sites during detailed surveys

Family	Slope (b)	R^2	*P*	Family	Slope (b)	R^2	*P*
Guttiferae	5.89	0.172	0.110	Sterculiaceae	-12.78	0.110	0.210
Lamiaceae	3.97	0.073	0.310	Thymeleaceae	-5.19	0.126	0.177
Lauraceae	-0.06	0.000	0.992	Ulmaceae	-5.72	0.281	0.035
Loganiaceae	-0.58	0.001	0.906	Verbenaceae	-2.60	0.130	0.170
Malvaceae	3.99	0.082	0.283	Vitaceae	-5.12	0.076	0.303





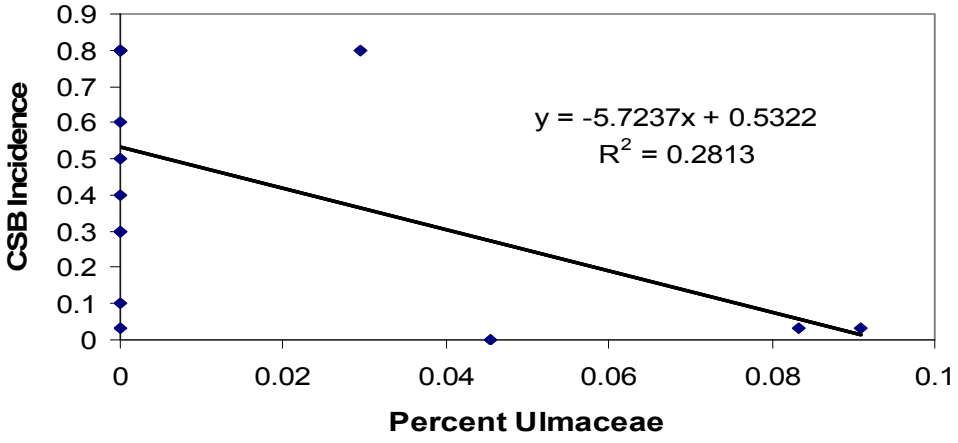
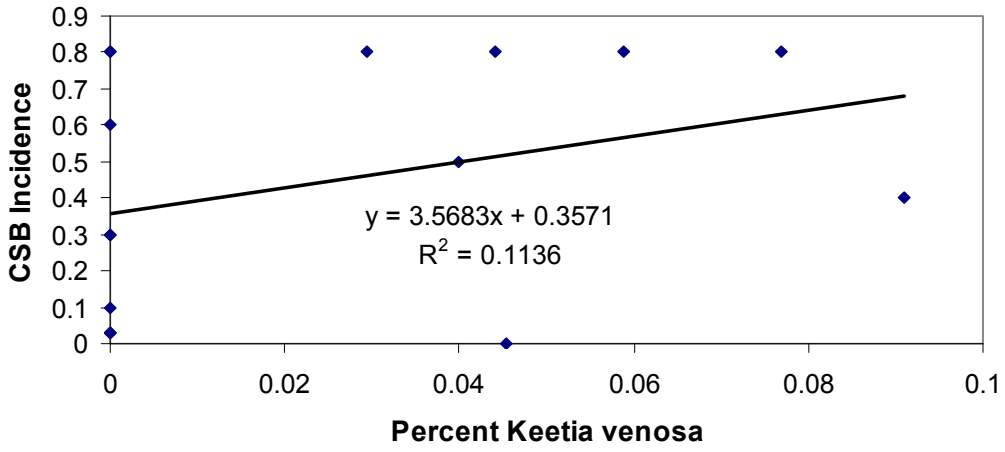
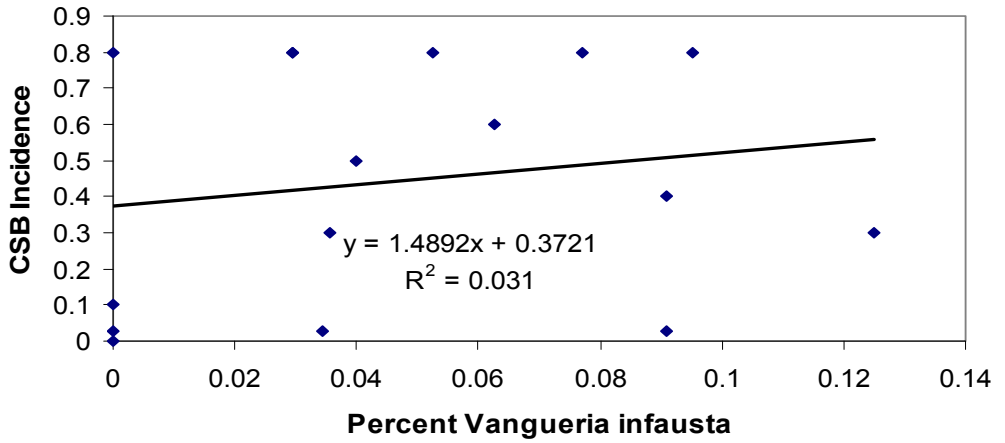


Fig. 6.4 Relationship between occurrences of some plant families in the wild with the incidence of *Monochamus leuconotus* (CSB) in coffee fields across all study locations during transect belt surveys (*N* = 16)





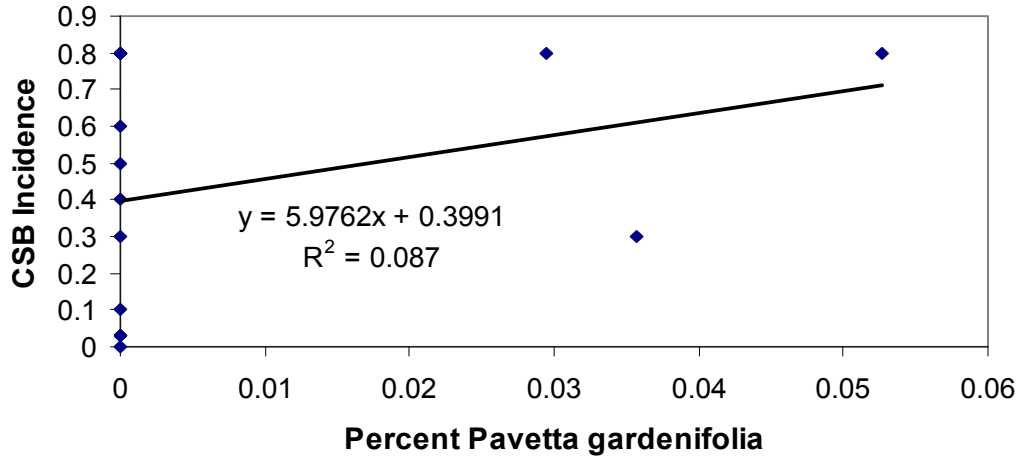


Fig. 6.5 Relationship between occurrences of selected plants belonging to the family Rubiaceae in the wild with the incidence of *Monochamus leuconotus* (CSB) in coffee fields across all study locations during transect belt surveys (*N* = 16)

6.3.5 Relationship between incidences of *M. leuconotus* with altitude

The relationship between altitude and *M. leuconotus* was significant ($P < 0.05$). Incidence appeared to decrease with increase in altitude across all sites (Fig. 6.6)

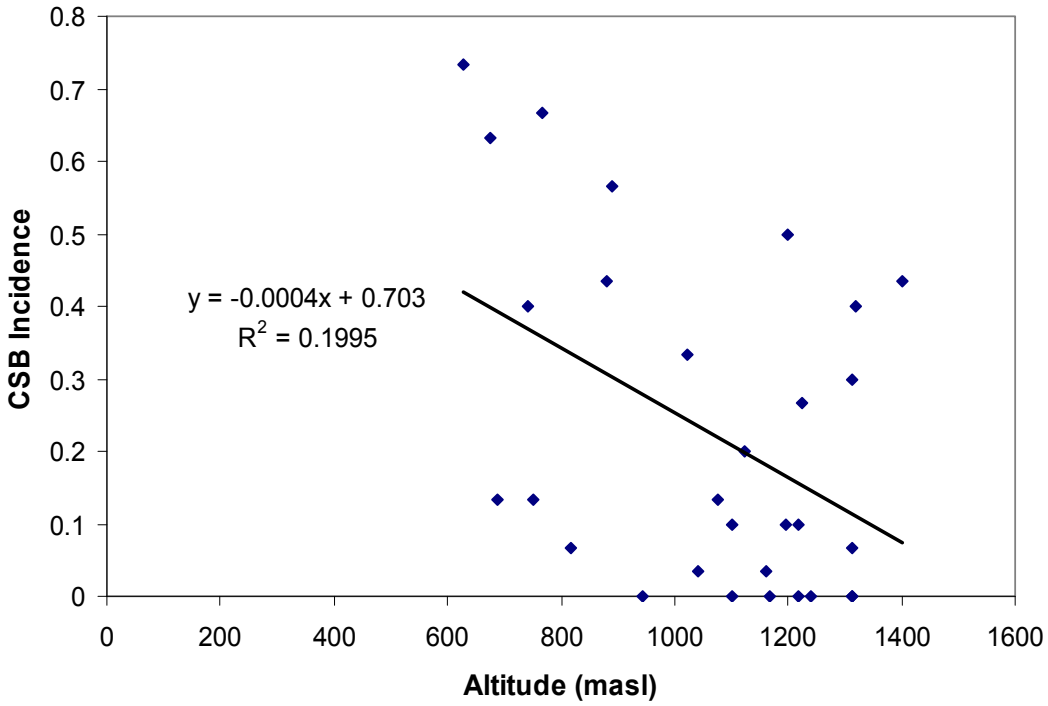


Fig. 6.6 Relationship between altitude and the incidence of *Monochamus leuconotus* (CSB) in coffee fields (*N* = 29)

6.4. DISCUSSION

Two surveys were conducted to search for alternative host plants of *M. leuconotus* in wild vegetation surrounding coffee plantations in the coffee growing areas of Zimbabwe. During the first survey, tree species along a 30 m transect from the edge of a coffee field were counted and classified with symptoms of *M. leuconotus* attack scored on Rubiaceae plants. For the second survey, plant species in a transect belt were recorded and symptoms of *M. leuconotus* attack scored on every plant species i.e. including non-Rubiaceous plants. Both surveys failed to confirm conclusively the presence of any alternative host plants of *M. leuconotus* since no beetle was found on the plants and none

emerged from stems collected for adult rearing under laboratory conditions, even though these had symptoms of stem borer infestation.

However, the study showed that biodiversity around coffee plantations was rich particularly with respect to Rubiaceae and Fabaceae. A number of trees were specific to the Eastern Highlands while others could be found in the Northern districts only. This could be related to environmental factors especially rainfall and temperature. The Eastern Highlands of Zimbabwe normally receive more rainfall than the Northern districts and temperatures tend to be much lower.

When no *M. leuconotus* beetles were found during the study, correlations between the incidence of the pest in coffee fields and the occurrence of the plant families across the sites were examined but only gave a few significant relationships with non-Rubiaceae families. This suggests that there could be some more important factors that influence the incidence of the pest in coffee fields. One such factor that was examined was altitude. The relationship between altitude and the incidence of *M. leuconotus* was determined and found to be significant with incidence decreasing with an increase in altitude. This is consistent with previous work. For example, Tapley (1960) suggested that the upper limit for coffee borer infestation was about 1,320 m above sea level. Most sites surveyed during the present study were located at less than 1,320 m above sea level. Therefore, the high incidence of the pest could be related to altitude, which could be a useful predictor of incidence. Other factors that could be useful predictors of the incidence of the pest could be temperature, rainfall and management practices. It was not possible to obtain temperature and rainfall data for the surveyed sites but based on the negative temperature correlation with altitude, it can be assumed that incidence increases with temperature. For example, some of the worst affected sites were at lower altitudes with hot weather conditions (see Table 1).

It was not possible to analyse statistically the influence of management practices on stem borer incidence in coffee fields because of unequal replication of sites with different management practices. However, the incidence of the pest was generally higher where management practices were low. In the current study, the ARDA Estates and most of the smallholder farmers were perceived to be poorly managed coffee lands as well as some research plots at the Coffee Research Station where certain treatments were favourable to the proliferation of *M. leuconotus*. Despite the generally high management practices at some sites, incidence of *M. leuconotus* was high and the relationship with wild plants could not predict the presence of the pest. For example at New Year's Gift, where incidence was high, altitude was low and management practices high, the presence of

wild hosts could not be a predictor. This suggests that altitude is an overriding factor in terms of predicting the incidence of *M. leucotonus*.

During the current study it was expected that possible alternative host plants of the borer could be found within the Rubiaceae. However, apart from the presence of symptoms on *Vangueria infausta*, *Pavetta gardenifolia* and *Keetia venosa,* their alternative host status could not be confirmed. Some wild hosts belonging to the Rubiaceae have been recorded before. For example, *Pavetta oliveriana* and *Vangueria* sp. (Davies, 1937) and *Vangueria linearispela* (Knight, 1939) have been reported from Tanzania and Kenya respectively while Schoeman (personal communication) found *Gardenia* sp. and *Kraussia floribunda* as alternative host plants. The identification of alternative host plants can only be confirmed by the emergence of beetles after successful completion of a generation within the plants. From the current work, it is very difficult to confirm the alternative hosts.

It can be inferred from the current study that finding alternative hosts of *M. leuconotus* in the wild is not easy. The study design assumed that *M. leuconotus* could be easily found especially in plants belonging to the family Rubiaceae and that, its incidence in coffee plantations adjacent to suspected wild hosts would be related to the presence of the hosts in the wild. According to the current findings, wild populations of *M. leuconotus* are probably very low and patchy as opposed to those in cultivated coffee where there is a high concentration of habitats and food. Therefore, failure to locate wild hosts could be related to the patchiness of the distribution of wild hosts, which in turn affects the distribution of the pest. For example, the Rubiaceae, which were the primary targets for the location of wild hosts, were found in very low numbers in the current studies. This explains why there is a dearth of information in the literature on how alternative host plants were identified. It is most probable that they found them purely by chance as is the case with *Kraussia floribunda* and *Gardenia* sp. in South Africa (Schoeman, P. S, personal communication).

The approach used in identifying alternative hosts focused on selecting plants with visual symptoms of attack, which were then collected for laboratory rearing of adults. This could be misleading in that symptoms could be those of other Cerambycidae. For example, *M. leuconotus* is reported to attack Rubiaceae only (CABI, 2006) but according to the results of the current study some non rubiaceous plants such as *Tabernaemontana elegans* and *Senna pendula* had some symptoms of *M. leuconotus*. On the other hand, it could be possible that they are genuine alternative host plants since some non-rubiaceous hosts of *M. leuconotus* have been reported from Malawi (Smee, 1936). One possible limitation of the design could be the failure to remove the soil below the collar of the tree to a

reasonable depth in order to expose underground ring-barking damage or frass. While this could have been helpful in increasing the proportion of infested trees identified, this is very laborious and consequently would have made the survey impractical because of the amount of time spent on each tree. Le Pelley (1968) reported that the removal of soil to a depth of 30 cm or more in order to find a high proportion of infested trees in Kenya allowed for the destruction of larvae exposed during the excavations while Tapley (1960) reported that an extensive campaign to remove alternative host plants led to only a very small proportion of the hosts being found. Therefore, finding alternative hosts is both laborious and difficult because of the long life cycle of the pest and its cryptic nature whereby the larvae and pupae are found within the stems making it very difficult to detect especially in wild hosts where the damage symptoms could be different from those in plantations.

Chapter 7
INVESTIGATION OF FEEDING AND OVIPOSITION PREFERENCES OF *Monochamus leuconotus* (PASCOE)

7.1. INTRODUCTION

In previous chapters of this thesis, the work described focused mainly on determining whether volatile or involatile pheromones play a role in the chemical ecology of *M. leuconotus.* It is also possible that chemical signals play a part in host plant selection by this pest. Many cerambycid species are reported to show a kairomonal response to volatiles from the host plant and even to pheromones of other insects feeding on the host plant (See Chapter 1). Most of the reports concern species of cerambycids attacking conifers, which are rich in volatile secondary chemicals, unlike coffee (Pajares *et al*., 2004, Teale *et al*., 2011). In Chapter 4, work on the response of *M. leuconotus* to plant volatiles and combinations with insects and chemical cues suggested that the beetles were attracted to coffee bark scrapings and leaves.

In Chapter 6, attempts to find alternative host plants of the coffee white stem borer were made through transect surveys. Some suspected symptoms of coffee white stem borer were found on three rubiaceae (i.e. *Keetia venosa*, *Pavetta gardenifolia*, *Vangueria infausta*) and the non-rubiaceae (*Tabernaemontana elegans, Cussonia natalensis* and *Senna pendula*). Further work to find relationships between plant families and the incidence of coffee white stem borer on coffee fields during line transect surveys showed that the relationship with Gentianaceae was significant ($N = 29$, $P < 0.05$). During transect belt surveys, three families (Anacardiaceae, Araliaceae, Ulmaceae) showed significant relationships with stem borer incidence ($N = 16$, $P < 0.05$). In terms of relationships with Rubiaceae, the occurrence of *Keetia guenzii* was significantly correlated to stem borer incidence ($P < 0.05$). It was concluded that it was difficult to find alternative host plants in the field through transect surveys.

The objectives of this study were to investigate further possible alternative host plants through determination of feeding and oviposition preferences on some of the suspected hosts identified during the transect surveys, *Keetia venosa, Vangueria* sp*., T. elegans and Gardenia ternifolia. Bauhinia galpinii* was also included in this study as it has been reported as a possible host by farmers.

7.2. MATERIALS AND METHODS

7.2.1 Study location

Work was carried out at Coffee Research Station, Chipinge, Zimbabwe under laboratory conditions (25°C 13:12 h L:D, 60% RH).

7.2.2 Test materials

Stems of K*eetia venosa, Vangueria* sp*., Bauhinia galpinii, Gardenia ternifolia* and *Coffea arabica* were collected from the field and the wild and stored in cages and used within a day of collection. Cut stems were sealed with paraffin wax and offered to insects for oviposition. The sizes of stems used are shown in Table 7.1.

Table 7.1. Sizes of test materials used in oviposition preference studies of *Monochamus leuconotus*

Test material	Family	Dimensions (mean ± S.D)	
		Stem diameter (cm)	Bark thickness (mm)
Coffea arabica	Rubiaceae	3.95 ± 0.64	1.5 ± 0.71
Gardenia ternifolia	Rubiaceae	6.30 ± 2.40	4 ± 1.41
Vangueria infausta	Rubiaceae	5.30 ± 0.14	3.0 ± 0
Keetia venosa	Rubiaceae	4.30 ± 0.14	3.0 ± 0
Bauhinia galpinii	Fabaceae	3.84 ± 1.40	4.0 ± 0
Tabernaemontana elegans	Apocynaceae	4.28 ± 1.06	4.1 ± 1.5

7.2.3 Insects

Insects of different ages were hand collected from fields at the Coffee Research Station, Chipinge, Zimbabwe during December 2010 and December 2011 and kept in individual cages (45.2 cm long x 45.2 cm wide x 42.1 cm high) in the laboratory under 25°C, LD 13:11 h regime and 60% RH conditions. Insects were provided with 15% sugar solution on cotton wick as food for 2 d prior to testing. Insects were assumed to have mated in the field.

7.2.4 Bioassay procedures

Two stems were selected and paired (e.g. coffee vs coffee, coffee vs *G. ternifolia*, Table 7.2 and 7.3). For each selection test, one test plant and one control (coffee) was placed vertically, one on each side of a net cage (45.2 cm length, 42.1 cm height and 45.2 cm width, Fig 7.1). A female *M. leuconotus* was introduced into the cage for 24 h. Records were taken on feeding marks, oviposition scars and number of eggs laid on the each of the stems. Feeding marks were distinguished from oviposition scars by the total removal of fed bark whereas oviposition scars were noted by plugs on the bark. A single female was used only once. The stems were marked and kept separately before being assessed for hatching of eggs after 2 weeks. Each test was repeated 20 times except for Experiment 6, which was repeated 9 times.

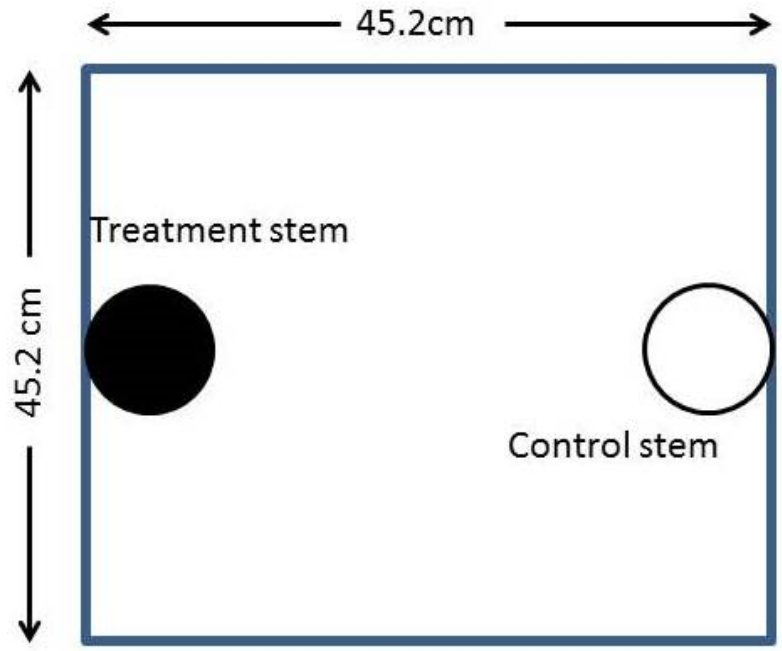


Fig. 7.1. Upper view of a cage used to investigate the oviposition preference of *Monochamus leuconotus*

7.2.5 Data analysis

Data were subjected to Wilcoxon signed rank test (Zar, 1999) and correlations between feeding marks, oviposition scars and number of eggs laid, were done using IBM SPSS Statistics 20. Significance was set at $P \leq 0.05$.

7.3. RESULTS

7.3.1 Feeding preferences and oviposition scars of female *M. leuconotus*

There were no significant differences in feeding marks and oviposition scars on coffee stems over coffee stems ($P > 0.05$, Table 7.2). Female coffee stem borers fed more on coffee than on *G. ternifolia* ($P < 0.05$) but there were no significant differences in terms of oviposition scars between coffee and *G. ternifolia* stems ($P > 0.05$). There were more feeding marks ($P < 0.05$) and oviposition scars ($P < 0.05$) on coffee stems than on *B. galpinii* stems. There were no significant differences in number of feeding marks ($P > 0.05$) and oviposition scars ($P > 0.05$) between coffee and *K. venosa*, and coffee and *V. infausta*. Significant differences in feeding scars ($P < 0.05$) and oviposition scars ($P < 0.05$) were observed between coffee and *T. elegans* stems.

Table 7.2. Feeding response and oviposition scars of female *Monochamus leuconotus* under laboratory conditions

Expt	Treatment (*n*)	No. feeding marks (mean ± SE)*	No. oviposition scars (mean ± SE)*
1	Coffee (20)	1.5 ± 0.3a	2.2 ± 0.9a
	Coffee (20)	1.4 ± 0.3a	2.0 ± 0.8a
2	Coffee (20)	1.0 ± 0.3a	0.9 ± 0.4a
	G. ternifolia (20)	0.7 ± 0.5b	0.6 ± 0.4a
3	Coffee (20)	0.8 ± 0.2a	1.2 ± 0.4a
	B. galpinii (20)	0.2 ± 0.2b	0.8 ± 0.1b
4	Coffee (20)	1.0 ± 0.3a	0.8 ± 0.3a
	K. venosa (20)	0.9±0.4a	0.8 ± 0.4a
5	Coffee (20)	0.9 ± 0.3a	0.7 ± 0.3a
	V. infausta (20)	1.3 ± 0.5a	1.8 ± 1.7a
6	Coffee (9)	1.4 ± 0.4a	1.4±0.4
	T. elegans (9)	0.1 ± 0.1b	0

* Means followed by the same letter within a column and experiments are not significantly different at $P \leq 0.05$ (Wilcoxon signed rank test for paired differences)

7.3.2 Number of eggs laid and numbers hatched

There were no significant differences in number of eggs laid between coffee stems and coffee stems (Experiment 1), coffee stems and *G. ternifolia* (Experiment 2) and coffee stems and *V. infausta* (Experiment 5) and number of eggs hatched ($P > 0.05$, Table 7.3). For coffee stems and *K. venosa*, there were no significant differences in number of eggs laid ($P > 0.05$). However, there were significant differences in number of eggs hatched with more eggs hatching on coffee stems than on *K. venosa*. There were highly significant differences in number of eggs laid ($P < 0.001$) and number of eggs hatched ($P < 0.001$) between coffee stems and *B. galpinii* where more eggs were laid and hatched on coffee stems than on *B. galpinii* (Table 7.3). Female *M. leuconotus* laid more eggs on coffee stems than on *T. elegans* stems ($P < 0.05$) while more eggs hatched on coffee stems than on *T. elegans* stems ($P < 0.05$).

Table 7.3. Oviposition of female *M. leuconotus* under laboratory conditions

Expt	Treatment (n)	No. eggs laid (mean ± SE)*	No. eggs hatched (mean ± SE)*	% Hatch
1	Coffee (20)	2.1 ± 0.3*a**	1.4 ± 0.3a	67
	Coffee (20)	1.9 ± 0.4*a*	1.1 ± 0.6a	58
2	Coffee (20)	1.1 ± 0.3a	0.5 ± 0.2a	45
	G. ternifolia (20)	0.4 ± 0.1b	0.2 ± 0.1a	50
3	Coffee (20)	2.0 ± 0.3a	1.6 ± 0.3	80
	B. galpinii (20)	0.1 ± 0.1b	0	0
4	Coffee (20)	0.9 ± 0.3a	0.8 ± 0.3a	89
	K. venosa (20)	0.3 ± 0.1a	0.1 ± 0.1b	33
5	Coffee (20)	0.6 ± 0.2a	0.5 ± 0.2a	83
	V. infausta (20)	0.3 ± 0.1a	0.2 ± 0.1b	67
6	Coffee (9)	2.2 ± 0.4	1.4 ± 0.3	63
	T. elegans (9)	0	0	

*Means followed by the same letter within a column and experiments are not significantly different at $P \leq 0.05$ (Wilcoxon signed rank test for paired differences)

7.3.3 Correlation between feeding marks, oviposition scars, eggs laid and number of eggs hatched

There were significant correlations between numbers of feeding marks and oviposition scars, oviposition scars and number of eggs laid and number of eggs laid and number of eggs hatched (Table 7.4) while the relationship between feeding marks and eggs laid, feeding marks and number of eggs hatched, and oviposition scars and number of eggs laid were not significant ($P > 0.05$) (Table 7.4). In all cases the relationship was positive (Fig 7.2).

Table 7.4. Correlation between feeding marks, oviposition scars, eggs laid and number of eggs hatched

Parameters		**r**	**R^2**	***P***
Feeding marks	oviposition scars	0.785	0.593	0.007
Feeding marks	number eggs laid	0.574	0.326	0.083
Feeding marks	number eggs hatched	0.497	0.247	0.144
Oviposition scars	number eggs laid	0.632	0.364	0.050
Oviposition scars	number eggs hatched	0.546	0.276	0.102
Number of eggs laid	number eggs hatched	0.962	0.927	<0.001

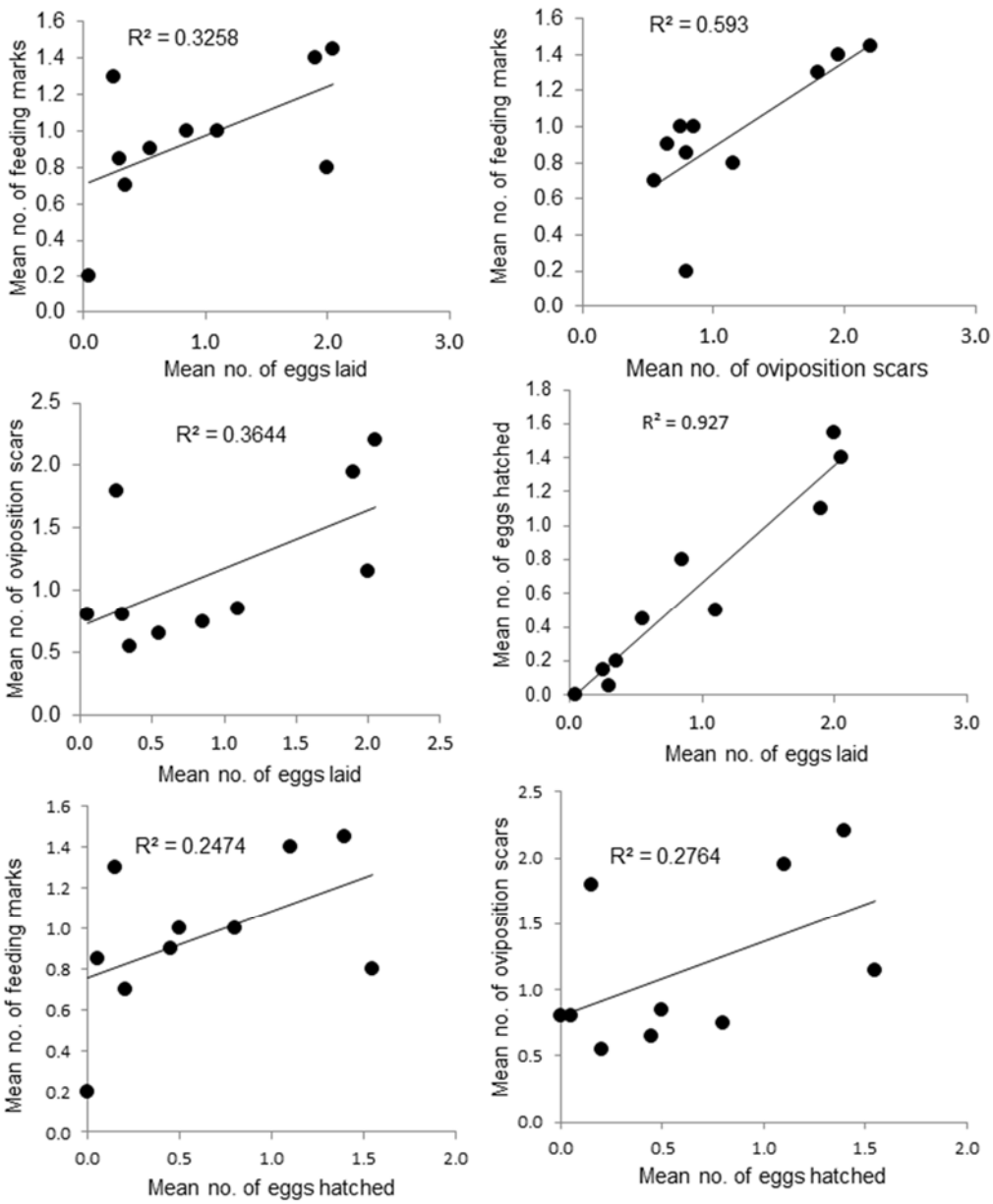


Fig. 7.2. Correlation between *Monochamus leuconotus* feeding marks, oviposition scars, number of eggs laid and number of eggs hatched under laboratory experimental conditions in Chipinge, Zimbabwe

7.4. DISCUSSION

In terms of feeding there was evidence that the female *M. leuconotus* preferred coffee stems over stems of *V. infausta, B. galpinii* and *T. elegans* as evidenced by more feeding marks on coffee stems than on the former. However, there were no significant feeding preferences between coffee stems over stems of *G. ternifolia, K. venosa* and *V. infausta.* This suggests that *K. venosa*, and *V. infausta* stems could be alternative food sources of *M. leuconotus* whereas *B. galpinii* and *T. elegans* are not preferred. According to available information, no food sources of *M. leuconotus* in the Rubiaceae other than coffee have been reported. It was expected that species within the Rubiaceae family will be preferred by *M. leuconotus* as these are in the same family as coffee. In this study, the two alternative feeding preferences identified (*K. venosa* and *V. infausta)* are in the Rubiaceae family. However, *G. ternifolia*, which is also in the Rubiaceae family could not be confirmed as an alternative food source of *M. leuconotus,* indicating that choice of feeding preference by *M. leuconotus* is at plant species level and not at family level. Prior to this study, the only reported non-Rubiaceae food source for *M. leuconotus* was *Erythroxylum emarginatum* (Smee, 1936).However, to confirm these species as food sources, more work on survival, toxicity and life-cycle completion should be carried out.

The results from this study suggest that *M. leuconotus* prefers to cut oviposition scars and lay eggs on coffee and other species in the Rubiaceae family (*G.ternifolia*, *V. infausta* and *K. venosa*) over the non-Rubiaceae species (*B. galpinii* and *T. elegans*). This suggests that the non-Rubiaceae species cannot be alternative host plants of *M. leuconotus* although results from field studies reported in this thesis (see Chapter 6) showed suspected symptoms of attack by *M. leuconotus* on these species. These findings suggest that symptoms obtained during the field could be of other cerambycid species. In terms of egg hatching, there appeared to be good oviposition success on coffee, *G. ternifolia* and *V. infausta* as evidenced by the non-significant differences between the number of eggs laid on the former species and coffee. Although *K. venosa* was comparable to coffee in terms of preference for egg laying, egg hatching was better on coffee than on *K. venosa.* This suggests that there could be other factors reducing hatchability of *M. leuconotus* eggs on *K. venosa*.

Previous studies reported *G. ternifolia* and *V. infausta* as alternative hosts of *M. leuconotus,* (see Chapter 1), which were confirmed in this study. This study showed that *K. venosa* is an alternative host on account of feeding and oviposition preference. In general, the female *M. leuconotus* preferred to feed on coffee stems over other hosts tested in this study. There were more oviposition scars on coffee stems than on *B. galpinii*

whereas no oviposition scars and eggs were laid on *T. elegans*. This may indicate that *T. elegans* has anti-feedant and anti-ovipositional properties, which deterred *M. leuconotus* beetles from feeding and laying eggs on the plant and cannot be an alternative host plant of the coffee white stem borer.

Given the significant correlations between feeding marks and oviposition scars, oviposition scars and number of eggs laid and between number eggs laid and number of eggs hatched, it is highly likely that sources of food for *M. leuconotus* are preferred as oviposition sites.

During field studies (see Chapter 6) it was expected that possible alternative host plants of the borer could be found within the Rubiaceae. However, apart from the presence of symptoms on *Vangueria infausta*, *Pavetta gardenifolia* and *Keetia venosa,* their alternative host status could not be confirmed. Some wild hosts belonging to the Rubiaceae have been recorded before. For example, *Pavetta oliveriana* and *Vangueria* sp. (Davies, 1937) and *Vangueria linearispela* (Knight, 1939) have been reported from Tanzania and Kenya respectively while Schoeman (personal communication), found *Gardenia* sp. and *Kraussia floribunda* as alternative host plants. The oviposition preferences exhibited by female *M. leuconotus* in this study could suggest that the preferred shrubs are alternative host plants. More work needs to be done to with more suspected host plants and to identify possible causes of low hatchability on alternative hosts identified in this study such as *K. venosa*.

Chapter 8
GENERAL DISCUSSION

8.1. SUMMARY OF RESULTS

The mating behaviour of *M. leuconotus* characterised under laboratory conditions during the course of the current studies suggested that it was the female beetle that approached a normally sedentary male, which would then recognise the female upon antennal or tarsal contact. Thereafter, the male would go through a series of events involved in mating behaviour culminating in copulation. Males dashed forward and touched the female with antennae or tarsi, and, if the female was receptive, the male would mount the female and proceeded to align its body with that of the female, and then bend its abdomen before extruding its reproductive organs for mating. Mating was considered successful when the claspers of the male were joined with the female's ovipositor. The duration of copulation varied from 6 minutes to 116 minutes. After copulation, the male withdrew its aedegus and continued to mount while the female walked around or was feeding. There was evidence suggesting that most activities of *M. leuconotus* occurred during daylight hours with much less activity during the night. Peaks for mating and feeding observed in the laboratory and outdoor cages appeared to coincide with each other suggesting that all activities of this beetle on coffee trees are mainly associated with feeding and mating. Beetles mated readily one day after emergence and there was evidence of multiple mating and polygynous and polyandrous behaviour under laboratory conditions. Mating appeared to be most prevalent early in the morning (5 – 7 am) and later after mid-day (1 – 5 pm).

In terms of evidence for short-range communication, during laboratory bioassays carried out during 2004/05 and 2005/06, males responded positively to dead females in terms of showing all behaviours in the recorded categories involved in mating behaviour. Responses to hexane-washed dead females were very low and males did not attempt to mate with the washed females. When males were offered dead females treated with the hexane extract, response was much higher than to hexane-washed females.

During the 2006/07 bioassays, males were tested with dead females that they had responded to while still alive. Males attempted to mate with live females and dead unwashed females but did not attempt to mate with hexane–washed females. When the females were recoated with hexane extracts the response was much higher than that to

hexane-washed females and males attempted to mate with the hexane extract recoated females. These results confirmed that hexane-soluble chemicals in the cuticle of female beetles are important for recognition by the male, presumably of species and sex. These results were further supported by analyses of the cuticular hydrocarbons done at NRI, which showed distinctive differences between the sexes.

Olfactory bioassays conducted using procedures developed during the current studies suggest that female *M. leuconotus* exhibit attraction to coffee leaves, coffee bark and the synthetic male-specific compound dispensed in a polyethylene sachet while males were attracted to coffee bark. The lack of response to either male or female beetles suggested that there are no long-range attractants present in *M. leuconotus* or that these were not produced or produced in low amounts under the conditions used. The positive response to the male-specific compound in the olfactometer bioassays could suggest that the male–specific compound acts as a short range pheromone. The attraction of both sexes of *M. leuconotus* implies that the male specific compound could be an aggregation pheromone or territorial semiochemical, which serves to mark suitable habitats.

Sticky traps baited with either live male or female *M. leuconotus* did not attract any more beetles than the controls. Similarly, traps baited with the synthetic male–specific compound of *M. leuconotus* formulated in polyethylene sachet and cigarette filter dispensers failed to attract or catch beetles during the study period. After no insects were caught on the traps in initial studies, insects within a metre radius of the traps were counted. Despite the inclusion of these insects there was no evidence to suggest the presence of long range attractants in either sex of *M. leuconotus*. However, in later work, intercept traps baited with combinations of the male-specific compound and host volatiles caught significantly more beetles than the unbaited control. In particular, combinations of (*R*)-(-)-linalool with the male-specific compound resulted in significantly more females caught than with ethyl benzoate and (*Z*)-3-hexenol. All combinations of the male-specific compound with (*R*)-(-)-linalool, ethyl benzoate, methyl salicylate and (*Z*)-3-hexenol caught more *M. leuconotus* females than the untreated control under field conditions. In terms of total number of insects, combinations of (*R*)-(-)-linalool with the male-specific compound caught more insects than with ethyl benzoate and (*Z*)-3-hexenol. Combining the male-specific compound with either (*R*)-(-)-linalool or methyl salicylate resulted in more male beetle catches than the male-specific compound alone. Combining the male-specific compound with (R)-(-)-linalool significantly caught more beetles than the male-specific compound alone and (R)-(-)-linalool and the unbaited control. Trap designs evaluated during the current studies were the cross vane, sticky types (MK1, MK2) and intercept panel traps. The intercept trap offered advantages in terms of increasing trap

catches since beetles were only caught in the intercept traps. One beetle was caught on the MK2 sticky rodent traps while the MK1 and the cross vane traps did not catch any.

Two surveys were conducted to search for alternative host plants of *M. leuconotus* in wild vegetation surrounding coffee plantations in the coffee growing areas of Zimbabwe. During the first survey tree species along a 30-metre line transect from the edge of a coffee field were counted and classified with symptoms of *M. leuconotus* attack scored on Rubiaceae plants. For the second survey, all plant species in a transect belt were recorded and symptoms of *M. leuconotus* attack scored on every plant species. Both surveys failed to confirm conclusively the presence of any alternative host plants of *M. leuconotus* since no beetle was found on the plants and none emerged from stems collected for adult rearing. However, potential symptoms of attack were found on *Vangueria infausta* and *Pavetta gardenifolia,* which have been previously reported as host plants. In the absence of unambiguous evidence of attack on alternative host plants by *M. leuconotus* attempts were made here to correlate presence of particular species around coffee fields with the degree of infestation in the field but no clear correlations were found. This may have been due to the fact that no correlations exist or due to other factors such as management practices and local environmental factors overriding any correlations. In addition, *M. leuconotus* damage to wild hosts might be different to damage on coffee under plantation conditions.

Feeding and oviposition studies conducted during the course of these studies under laboratory conditions suggest that female *M. leuconotus* feed mostly on Rubiaceae. Female beetles preferred to lay eggs on confirmed food sources such as *Coffea arabica*, *G. ternifolia, V. infausta* and *K. venosa.* However, egg hatching was low on *K. venosa*.

8.2. DISCUSSION

8.2.1 General

The African coffee stemborer, *Monochamus leuconotus*, is the most important insect pest of coffee in Africa, but nothing was known about the chemical ecology of this species before the start of the studies described in this thesis in 2004. Indeed, study of the chemical ecology of Cerambycidae in general was only just beginning (e.g. Allinson *et al*., 2004) although much research in this area has been carried out since (e.g. Hanks *et al.*, 2013).

Monochamus leuconotus is a particularly difficult species to work with since there is a maximum of one generation per year and adults are only available for a few months at the start of the rainy season from December to February. Thus the studies described here were carried out over several seasons in order to obtain sufficient data.

While these studies were being carried out, parallel field work was being carried out in Malawi and laboratory work at NRI in the UK. While the trapping studies in Malawi gave similar negative results to those obtained in the early experiments in Zimbabwe, important new results were obtained at NRI. Trapping and analyses of volatiles from cut coffee stems and leaves were carried out after completion of the laboratory bioassay studies in Zimbabwe, but provided material for testing in later field trials. More recently pentadecanal was identified in volatiles from coffee stems and it is hoped to test this in future bioassay and/or trapping experiments.

Despite these difficulties, a number of aspects of the chemical ecology of *M. leuconotus* were investigated and several important, novel results were obtained.

8.2.2 Mating behaviour

The mating behaviour of *M. leuconotus* had not been described in detail prior to the studies reported in this thesis. However, the observed sequence of mating behaviour is consistent with other that of other cerambycid beetles (Fauziah *et al*. 1987; Kim *et al.*, 1992, Kobayashi *et al*., 2003, Ibeas *et al*., 2008, Fonseca & Vidal 2009, Wickham *et al*., 2012). Information on mating behaviour of adults, particularly the rôle of semiochemicals in reproduction is important in the development of effective detection and management strategies for many Cerambycidae (Allison *et al*., 2004) The dashing response by males after antennal contact is probably due to the presence of a contact pheromone on the body surface of the female (see Chapter 2). This also tallies with the response to dead washed and dead unwashed insects whereby the restoration of recognition cues on the body surface of the dead females led to higher male response (See Chapter 3). Male response to the extract-treated females was poor possibly due to a dose-response effect. Ibeas *et al*. (2008) observed higher male response with increase in concentration of cuticular hydrocarbons in *Monochamus galloprovinciallis* while higher concentration increased mountings in *Glenea cantor* and inhibited abdominal bending (Lu *et al.,* 2007). In *Xylotrechus colonus* there was higher response with increase in concentration (Ginzel and Hanks, 2003). Current findings on poor male response in terms of holding attempt, holding, mounting and abdominal bending when extracts were reapplied on to body in *M.*

leuconotus are in agreement with Ibeas *et. al*., (2008) and Lu *et al.,* (2007) and suggest that higher concentrations of the extracts in the Lamiinae could be inhibitory since extracts were obtained from the whole body and carefully reapplied onto a small surface on the elytra.

The results from the studies described here confirm for the first time that the presence of contact pheromones on the body surface of the female is important for mate recognition in *M. leuconotus.* The reduced response to the cuticular hydrocarbon extract suggests that other cues such as visual or auditory could be involved in mate recognition as well. Hydrocarbons in the epicuticular wax layer of insects are important in interspecific and intraspecific recognition (Howard and Blomquist, 1992).

8.2.3 Trapping studies

Initial field trapping studies on *M. leuconotus* were carried out with cross-vane traps and sticky "rat traps" used successfully by other researchers to trap cerambycid beetles. However, no *M. leuconotus* beetles were caught over several seasons in traps baited with live conspecific beetles or either sex or with the male-specific compound identified from *M. leuconotus* dispensed at various rates (Hall *et al.,* 2006b). However, in subsequent trials using intercept panel traps significant numbers of beetles were trapped in traps baited with the male-specific compound and/or host-plant volatiles, particularly linalool. This is the first time this has been done for this species, and it is only recently that capture of other *Monochamus* species in traps baited with male-specific compounds has been reported (e.g. Pajares *et al*., 2010).

As pointed out in Chapter 1, catches in field traps may result not only from an initial attraction and directed movement (taxis) towards a stimulus source, but also from random movements (kinesis), with insects reducing speed, turning more frequently and/or stopping on detection of a localized stimulus arrived at by chance (Hardie, 2012). As also mentioned, the materials used to increase catches in the traps can be classified as long-range or short-range attractants. Long-range attractants are relatively volatile and carried significant distances by air currents (Wyatt, 2003). Short-range or contact attractants are relatively involatile and perceived by contact or possibly at very close range by diffusion of the chemical (Wyatt, 2003).

There is evidence for long-range attraction within the subfamily Lamiinae where male-produced aggregation pheromones have been confirmed from 7 species after field tests

(Table 1.4). These include the Monochaminae, *Monochamus galloprovincialis* (Pajares *et al*., 2010); *M. alternatus* (Teale *et al*., 2011); *M. carolinensis* and *M. tittilator* (Allison *et al*., 2012); *M. scutellatus scutellatus* (Fierke *et al*., (2012), and *M. sutor* (Pajares *et al.,* 2013). These species all apparently produce the same male-specific compound "Monochamol" which is chemically related to that of *M. leuconotus* and also to the two compounds produced by males of another member of the Lamiinae, *Anoplophora glabripennis* (see Pajares *et al.,* 2010). It is probable that the volatile male-specific compound of *M. leuconotus* could be acting over short distances since insects probably could not pick it up in earlier trapping trials (see Chapter 5) and it was picked up by females in olfactometer bioassays (see Chapter 4, Table 4.7). These findings are consistent with current knowledge on pheromones in the Cerambycidae. Volatile male-specific sex or aggregation pheromones have been identified in 24 Cerambycidae of which 14 belong to the subfamily Cerambycinae 6 to the Lamiinae and 4 to the Spondylidinae (Ray *et al.,* 2006, Hanks and Millar, 2012). Female –produced sex pheromones have been identified for seven species, *Migdolus fryanus*, *Verperus xartati* (Leal *et al.,* 1994, Boyer *et al.,* 1997), *Prionus californicus* (Cervantes *et al.,* 2006), *Desmocerus californicus* (Ray *et al*., 2012), *Ortholeptura valida* (Ray et al., 2011), *Tragosoma depsarium* (Ray *et al.*, 2012) and *Prionus lecontei* (Rodstein *et al*., 2011). Hanks (1999) related the reproductive strategies of Cerambycid beetles to the condition of the larval host plant at colonization - stressed, healthy, dying plants or dead wood. *M. leuconotus* was regarded as a weakened host species, which was unlikely to have any long range pheromone since beetles emerge from the proximity of adult feeding, mating and oviposition sites. According to this hypothesis, males encounter females by chance or by antennal contact after receiving a signal from the female. This theory appears to be correct in as far as beetles' emergence and encounter on the larval host plant but differs in that current studies indicate that the male is sedentary while the female searches, which implies the male releases the volatile compound, which probably acts at a relatively short distance. On the other hand, the theory is questionable in as far as attack on weakened hosts is concerned since observations during the current studies and general field observations (unpublished data) showed that healthy hosts were attacked by *M. leuconotus*. In light of the successful trapping of beetles in field bioassays which confirmed the existence of long range chemical communication in *M. leuconotus*, some of the specific classifications of Hanks (1999) may need revisiting, for example the implication that there are no long-range pheromones in *M. leuconotus*.

Further work is required to optimise trap lures for *M. leuconotus,* but the results here provide a potential new method of monitoring this pest and even possibly of control by mass trapping.

8.2.4 Alternative host plants for *M. leuconotus*

No alternative host plants for *M. leuconotus* were conclusively found under field conditions during the current study although some evidence that other species of Rubiaceae can act as hosts has been reported. *M. leuconotus* is probably a very low density pest in the wild and only assumes pest status under monoculture conditions due to a high concentration of habitats and food. Failure to find alternative hosts could also be related to the patchy distribution of Rubiaceae around coffee plantations. Host preference can only be confirmed by the emergence of beetles after successful completion of a generation within the plants. From the current work it is very difficult to confirm the alternative hosts of *M. leuconotus*. It is most likely that previous workers found them purely by chance. For example, *Kraussia floribunda* and *Gardenia* sp. were found purely by chance in South Africa (Schoeman pers. comm.). Laboratory oviposition preferences studies confirmed the alternative host status of *G. ternifolia* and *V. infausta* which had been previously reported. A new finding of *K. venosa* as a potentially suitable host of *M. leuconotus* was reported. Alternative host plants can pave the way in studying host-plant relations.

8.2.5 Management of *M. leuconotus*

In terms of management of this pest there seems to be potential for further developing traps for monitoring or control in the light of the attraction to the male-specific compound. Enhancement of trap catches by host volatiles shows that host volatiles play a critical role in mating systems. This could be exploited in order to come up with more effective monitoring.

It is difficult to envisage how short-range and contact chemical cues could be used in pest management unless they are also employed as trail pheromones as has been found for two other cerambycid beetle species, *Xylotrechus quadripes* (Hall, *pers comm*) and *Anaplophora glabripennis* (Hoover *et al.,* 2014).

If non-host plants were found to produce repellent chemicals and these could be identified and synthesised then they might be used to deter *M. leuconotus* from attacking coffee.

8.3. SUGGESTIONS FOR FUTURE WORK

In order to build on the work done on the chemical ecology of *M. leuconotus* so far, the following work should be considered.

- In order to obtain a more complete understanding of the role of short-range cues in mating behaviour, experiments could be carried out on the effects of removing the antennae of either sex and of blinding the insects. The sex-specific cuticular hydrocarbons should be identified and their roles determined. The role of the male specific compound at short-range should also be investigated.

- Field bioassays to find ways of enhancing trap catches and improving trap retention of insects should be considered. The intercept traps proved more effective than the other traps tested here and it has been reported that their effectiveness can be increased further by coating the panels with Fluon (Graham *et al.,* 2010). Multifunnel traps have been widely used for forest pests, including cerambycid beetles (e.g. Pajares *et al.,* 2010) and could be tested for *M. leuconotus*. The use of mixtures of host plant odours and pheromone attractants need to be pursued further in view of the positive results obtained with combinations of linalool with the male-specific compound.

- The indications that *M. leuconotus* beetles are attracted to host plant volatiles should be followed up with further olfactometer studies. Work on trapping and analyses of host plant volatiles was carried at NRI after completion of the laboratory bioassays described here, although some of the compounds were subsequently evaluated in field trapping studies. Recently pentadecanal has been identified in volatiles from coffee stems at NRI. The functions of these compounds could be investigated in more detail in the laboratory bioassay in order that their use in the field can be optimised.

- The issue of alternative host plants needs to be further investigated. Possibilities would be to take candidate host plants identified in this work and by others and compare attractiveness for oviposition in field cage studies. The life cycle of the pest would then be followed after natural or, if necessary, initiation of infestation and subsequent development of the pest. It would then be possible to compare host and non-host plants in order to elucidate the factors necessary in host plants for the pest.

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APPENDICES

APPENDIX 1. STATISTICAL ANALYSES OF CUTICULAR HYDROCARBON STUDIES (CHAPTER 3)

Chi square calculations

Experiments for 2004/2006

Holding Attempt

Treatment	Responding	Non-responding	Total
Dead unwashed female	16	4	20
Dead washed	17	3	30
Recoated	10	20	30
Total	43	27	80

χ^2 =10,67672, p = 0.0048, df =2

Holding

Treatment	Responding	Non-responding	Total
Dead unwashed female	11	9	20
Dead washed	10	20	30
Recoated	5	25	30
Total	26	54	80

χ^2 = 8.121432, p = 0.0172367, df = 2

Mounting

Treatment	Responding	Non-responding	Total
Dead unwashed female	11	9	20
Dead washed	8	22	30
Recoated	5	25	30
Total	23	53	80

χ^2 = 8.650792, p = 0.0132283, df = 2

Abdominal Bending

Treatment	Responding	Non-responding	Total
Dead unwashed female	11	9	20
Dead washed	0	30	30
Recoated	0	30	30
Total	11	69	80

$\chi^2 = 38.26087$, $p < 0.00001$, $df = 2$

Experiments for 2006/07

Holding attempt

Treatment	Responding	Non-responding	Total
Live female	64	17	81
Dead unwashed	19	16	35
Washed	8	18	16
Recoated	12	2	14
Total	103	43	146

$\chi^2 = 12.04899$, $p = 0.007217$, $df = 3$,

Holding

Treatment	Responding	Non-responding	Total
Live female	58	23	81
Dead unwashed	16	19	35
Washed	3	13	16
Recoated	11	3	14
Total	88	58	146

$\chi^2 = 10.67672$, $p = 0.000010988$, $df = 3$

Mounting

Treatment	Responding	Non-responding	Total
Live female	51	30	81
Dead unwashed	10	25	35
Washed	1	15	16
Recoated	7	7	14
Total	69	77	146

χ^2= 23.75599, p< 0.00001, df = 3

Abdominal bending

Treatment	Responding	Non-responding	Total
Live female	41	40	81
Dead unwashed	4	31	35
Washed	0	16	16
Recoated	1	13	14
Total	46	100	146

χ^2 = 27.60636, p<0.000001, df = 3

Multiple comparison tests for proportions after χ^2 analysis

2004/2006 Experiments

a) Ranking proportions in ascending order

Treatment	n	HA	Proportion	Rank	HD	Proportion	Rank
Dead unwashed	20	16	0.803	3	11	0.555	3
Washed	30	10	0.333	1	5	0.167	1
Recoated	30	17	0.566	2	10	0.333	2

Treatment	n	MT	Proportion	Rank	AB	Proportion	Rank
Dead unwashed	20	11	0.555	3	11	0.555	2
Washed	30	5	0.167	1	0	0	1
Recoated	30	8	0.266	2	0	0	1

(b) Transforming proportions to arc sine and calculation of standard error

Treatment	n	HA	Transformed Proportion	HD	Transformed Proportion	MT	Transformed Proportion	AB	Transformed Proportion	SE
Dead unwashed	20	16	1.091	11	0.833	11	0.833	11	0.833	3.163
Washed	30	10	0.621	5	0.434	5	0.434	0	0.0902	2.594
Recoated	30	17	0.850	10	0.621	8	0.551	0	0.0902	2.594

$$p' = \frac{1}{2}\left[\arcsin\sqrt{\frac{x}{n+1}} + \arcsin\sqrt{\frac{x+1}{n+1}}\right]$$

x is the number responding on a treatment and n is the common number of subjects per treatment and

$$\frac{45}{\pi\sqrt{(n+0.5)}} = SE$$

(c) Calculation of Q value and comparison of proportions

HA	PAIR	Difference	Q_{cal}	$Q_{0.05,\infty,3}$	Conclusion
	3-1	0.241	5.299	3.315	Reject H_0
	3-2	0.47	5.057	3.315	Reject H_0
	2-1	0.229	10.335	3.315	Reject H_0
HD	3-1	0.212	4.683	3.315	Reject H_0
	3-2	0.187	4.12	3.315	Reject H_0
	2-1	0.399	8.805	3.315	Reject H_0
MT	3-1	0.282	6.231	3.315	Reject H_0
	3-2	0.399	2.573	3.315	Accept H_0
	2-1	0.117	8.805	3.315	Reject H_0
AB	3-1	0.069	16.366	3.315	Reject H_0
	3-2	0.000	0.000	3.315	Accept H_0
	2-1	0.069	10.3903	3.315	Reject H_0

q = difference between two transformed proportions in degrees/SE

For pair 3-1 on AB above q = 180(0.83144-0.090293)/ (2.594*π) = 16.366

2006/2007 Experiments

(a) Ranking proportions in ascending order

Treatment	n	HA	P	R	HD	P	R	MT	P	R	AB	P	R
Live	81	64	0.790	3	58	0.716	3	51	0.667	4	41	0.506	4
Dead unwashed	41	19	0.543	2	16	0.457	2	10	0.286	2	4	0.114	3
Washed	16	8	0.5	1	3	0.188	1	1	0.063	1	0	0	1
Recoated	14	12	0.857	4	11	0.785	4	7	0.503	3	1	0.007	2

(b) Transforming proportions to arc sine and calculation of standard error

Treatment	n	HA	Transformed Proportion	HD	Transformed Proportion	MT	Transformed Proportion	AB	Transformed Proportion	SE
Live	81	64	1.090	58	1.001	51	0.915	41	0.791	1.587
Dead unwashed	41	19	0.827	16	0.744	10	0.570	4	0.361	2.371
Washed	16	8	0.785	3	0.469	1	0.298	0	0.122	3.526
Recoated	14	12	1.152	11	1.067	7	0.785	1	0.317	3.761

(c) Calculation of Q value and comparison of proportions

Response category	Pair	Difference	Q_{cal}	$Q_{0.05,\infty,3}$	Decision
HA	4-1	0.062	1.327	3.633	Accept
	4-2	0.325	6.069	3.633	Reject
	4-3	0.367	5.763	3.633	Reject
	3-1	0.305	6.739	3.633	Reject
	3-2	0.042	0.811	3.633	Accept
	2-1	0.263	7.626	3.633	Reject
HD	4-1	0.066	1.334	3.633	Accept
	4-2	0.497	6.014	3.633	Reject
	4-3	0.598	9.394	3.633	Reject
	3-1	0.532	11.834	3.633	Reject
	3-2	0.275	5.316	3.633	Reject
	2-1	0.257	7.589	3.633	Accept
MT	4-1	0.13	2.797	3.633	Reject
	4-2	0.215	4.016	3.633	Reject
	4-3	0.487	7.668	3.633	Reject
	3-1	0.617	13.632	3.633	Reject
	3-2	0.272	5.632	3.633	Reject
	2-1	0.345	5.306	3.633	Reject
AB	4-1	0.474	10.239	3.633	Reject
	4-2	0.044	0.810	3.633	Accept
	4-3	0.195	3.065	3.633	Accept
	3-1	0.669	14.772	3.633	Reject
	3-2	0.239	4.630	3.633	Reject
	2-1	0.430	12.463	3.633	Reject

APPENDIX 2. GENERAL INSECT RESPONSE IN THE OLFACTOMETER BIOASSAYS (CHAPTER 4)

Year	n	Flight	Walking
2004/2005	250	11	239
2006/2007	655	19	636
Total	905	30	875

APPENDIX 3. STATISTICAL ANALYSES OF TRAPPING RESULTS (CHAPTER 5)

Experiment 5 Analysis of Variance Results

1. On trap

Variate: Male

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.51718	0.12929	4.51	0.034
Residual	8	0.22953	0.02869		
Total	12	0.74670			

Variate: Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.91872	0.22968	5.20	0.023
Residual	8	0.35351	0.04419		
Total	12	1.27223			

Variate: Total

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	1.41252	0.35313	9.14	0.004
Residual	8	0.30914	0.03864		
Total	12	1.72166			

2. 1 metre radius (Insects round the traps)

Variate: Male

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.10991	0.02748	0.55	0.706
Residual	8	0.40108	0.05013		
Total	12	0.51099			

Variate: Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.19446	0.04862	1.32	0.341
Residual	8	0.29457	0.03682		
Total	12	0.48904			

Variate: Male_Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.13603	0.03401	0.45	0.771
Residual	8	0.60522	0.07565		
Total	12	0.74125			

Experiment 6 Analysis of Variance Results

1. On trap (Actual insects caught)

Variate: Male

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.42751	0.14250	7.10	0.010
Residual	9	0.18059	0.02007		
Total	12	0.60810			

Variate: Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.25954	0.08651	3.63	0.058
Residual	9	0.21447	0.02383		
Total	12	0.47401			

Variate: Total

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.73608	0.24536	15.81	<.001
Residual	9	0.13965	0.01552		
Total	12	0.87573			

2. 1 metre radius (Insects round the traps)

Variate: Male

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.26514	0.08838	1.74	0.228
Residual	9	0.45663	0.05074		
Total	12	0.72177			

Variate: Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.11560	0.03853	0.57	0.646
Residual	9	0.60330	0.06703		
Total	12	0.71889			

Variate: Male_Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.09631	0.03210	0.38	0.769
Residual	9	0.75675	0.08408		
Total	12	0.85307			

Experiment 7 Analysis of Variance Results

1. On trap (Actual insects caught)

Variate: Male

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1.06031	0.35344	15.62	0.001
Residual	8	0.18102	0.02263		
Total	11	1.24133			

Variate: Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.21308	0.07103	2.24	0.161
Residual	8	0.25352	0.03169		
Total	11	0.46660			

Variate: Total

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1.20986	0.40329	18.39	<.001
Residual	8	0.17542	0.02193		
Total	11	1.38529			

2. 1 metre radius (Insects round the traps)

Variate: Male

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.10010	0.03337	2.79	0.109
Residual	8	0.09568	0.01196		
Total	11	0.19579			

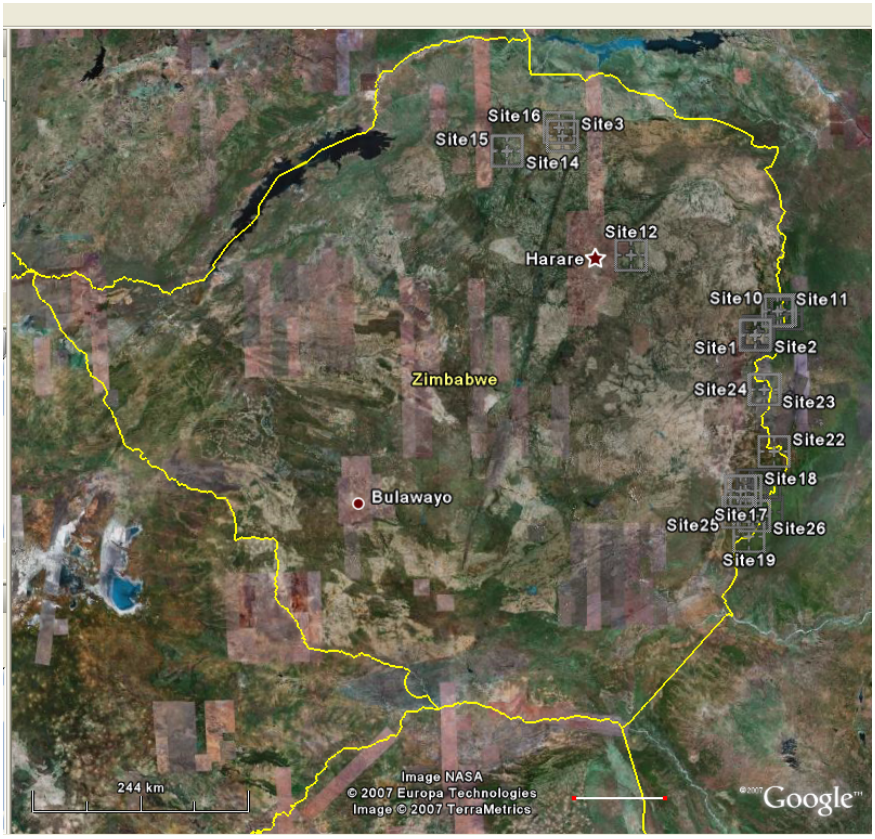
Variate: Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.03161	0.01054	0.61	0.628
Residual	8	0.13852	0.01732		
Total	11	0.17013			

Variate: Male_Female

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.04518	0.01506	0.70	0.580
Residual	8	0.17323	0.02165		
Total	11	0.21841			

APPENDIX 4. MAP SHOWING DISTRIBUTION OF ALTERNATIVE HOST SURVEY SITES IN ZIMBABWE (CHAPTER 6)



APPENDIX 5. RAW DATA ON ALTERNATIVE HOST SURVEYS (CHAPTER 6)

Ref no.	Area	Farming system	Site	Date	direction	Altitude	distance	Spp	Family
1	Honde	Smallholder	Mutamangira	02-Dec-03	West	1320	10	*Vangueria infausta*	Rubiaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	West	1320	10	*Vangueria apiculata*	Rubiaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	10	*Vangueria infausta*	Rubiaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	10	*Vangueria apiculata*	Rubiaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Ficus sycamorus*	Moraceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Ximenia americana*	Olacaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Rhoicissus tridentate*	Vitaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Diospyros lycioides*	Ebenaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Flacourtia indica*	Flacourtiaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Senna petersiana*	Caesalpiniaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Senna petersiana*	Caesalpiniaceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Vernonia amygadalina*	Asteraceae

1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Vernonia amygadalina*	Asteraceae
1	Honde	Smallholder	Mutamangira	02-Dec-03	North	1320	20	*Pittosporum viridiflorum*	Pittosporaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	South	1400	10	*Maesa lanceolata*	Myrsinaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	South	1400	10	*Vangueria apiculata*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	South	1400	20	*Dodonaea viscosa*	Sapindaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	South	1400	30	*Vangueria apiculata*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	North	1400	10	*Pavetta gardenifolia*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	North	1400	10	*Heteromorpha arborescens*	Apiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	North	1400	10	*Vangueria apiculata*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	west	1400	10	*Maesa lanceolata*	Myrsinaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	west	1400	10	*Vangueria apiculata*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	west	1400	20	*Celtis africana*	Ulmaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	west	1400	20	*Vangueria apiculata*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	west	1400	20	*Keetia venosa*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	west	1400	30	*Maesa lanceolata*	Myrsinaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	west	1400	30	*Vangueria apiculata*	Rubiaceae

2	Honde	Smallholder	Gwiriri 315	02-Dec-03	West	1400	30	*Dodonaea viscosa*	Sapindaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	10	*Lantana camara*	Verbenaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	10	*Leonotis sp*	Labiatae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	10	*Vangueria apiculata*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	20	*Vernonia amygadalina*	Asteraceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	20	*Maesa lanceolata*	Myrsinaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Vernonia amygadalina*	Asteraceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Maesa lanceolata*	Myrsinaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Vangueria apiculata*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Diospyros lycioides*	Ebenaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Bridelia macrantha*	Euphorbiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Erythrina lysistemon*	Fabaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Vangueria infausta*	Rubiaceae
2	Honde	Smallholder	Gwiriri 315	02-Dec-03	east	1400	30	*Ficus sycamorus*	Moraceae
3	Honde	Smallholder	Muranda	02-Dec-03	east	1224	10	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	east	1224	20	No trees	N/A

3	Honde	Smallholder	Muranda	02-Dec-03	east	1224	30	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	West	1224	10	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	West	1224	20	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	West	1224	30	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	South	1224	10	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	South	1224	20	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	South	1224	30	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	North	1224	10	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	North	1224	20	No trees	N/A
3	Honde	Smallholder	Muranda	02-Dec-03	North	1224	30	No trees	N/A
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Combretum molle*	Combretaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Parinari curatellifolia*	Chrysobalanaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Dodonaea viscosa*	Sapindaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Pittosporum viridiflorum*	Pittosporaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Vangueria infausta*	Rubiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Annona senegalensis*	Annonaceae

4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Faurea speciosa*	Proteaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Catha edulis*	Celastraceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	10	*Rhus longipes*	Anacardiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	20	*Brachystegia spiciformis*	Fabaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	20	*Uapaca kirkiana*	Euphorbiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	20	*Pittosporum viridiflorum*	Pittosporaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	East	1162	20	*Flacourtia indica*	Flacourtiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	10	*Parinari curatellifolia*	Chrysobalanaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	10	*Ficus sycamorus*	Moraceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	10	*Cussonia arborea*	Araliaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	10	*Senna petersiana*	Caesalpiniaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	10	*Vitex payos*	Lamiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	10	*Heteromorpha arborescens*	Apiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	10	*Catha edulis*	Celastraceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	20	*Catha edulis*	Celastraceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	20	*Parinari curatellifolia*	Chrysobalanaceae

4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	20	*Brachystegia spiciformis*	Fabaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	20	*Bobgunia madagascariensis*	Fabaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	30	*Rhus longipes*	Anacardiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	30	*Vangueria infausta*	Rubiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	30	*Uapaca kirkiana*	Euphorbiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	30	*Dichrostachys cinerea*	Fabaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	North	1162	30	*Lantana camara*	Verbenaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Uapaca kirkiana*	Euphorbiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Ficus sycamorus*	Moraceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Syzygium cordatum*	Myrtaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Vangueria infausta*	Rubiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Dodonaea viscosa*	Sapindaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Brachystegia spiciformis*	Fabaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Cussonia arborea*	Araliaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	10	*Rhus longipes*	Anacardiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	20	*Uapaca kirkiana*	Euphorbiaceae

4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	20	*Dodonaea viscosa*	Sapindaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	20	*Faurea speciosa*	Proteaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	20	*Lantana camara*	Verbenaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	30	*Brachystegia spiciformis*	Fabaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	30	*Uapaca kirkiana*	Euphorbiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	30	*Dodonaea viscosa*	Sapindaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	West	1162	30	*Psorospermum febrifugum*	Guttiferae
4	Honde	Smallholder	Muranganwa	02-Dec-03	South	1162	10	*Syzygium cordatum*	Myrtaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	South	1162	10	*Catha edulis*	Proteaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	South	1162	10	*Faurea speciosa*	Celastraceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	South	1162	10	*Uapaca kirkiana*	Euphorbiaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	South	1162	10	*Psidium guajava*	Myrtaceae
4	Honde	Smallholder	Muranganwa	02-Dec-03	South	1162	10	*Gardenia ternifolia*	Rubiaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	10	*Terminalia stenostachya*	Combretaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	10	*Heteromorpha arborescens*	Apiaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	10	*Lantana camara*	Verbenaceae

5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	10	*Cussonia arborea*	Araliaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	10	*Pittosporum viridiflorum*	Pittosporaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	10	*Bridelia macrantha*	Euphorbiaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	20	*Heteromorpha arborescens*	Apiaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	20	*Erythrina abyssinica*	Fabaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	20	*Catha edulis*	Celastraceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Psorospermum febrifugum*	Guttiferae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Psidium guajava*	Myrtaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Vitex payos*	Lamiaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Annona senegalensis*	Annonaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Syzygium cordatum*	Myrtaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Gardenia ternifolia*	Rubiaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Keetia venosa*	Rubiaceae
5	Honde	Smallholder	Charity Dumba	02-Dec-03	North	1219	30	*Psychotria capensis*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	10	*Albizia gummifera*	Fabaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	10	*Bridelia macrantha*	Euphorbiaceae

6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	10	*Markhamia obtusifolia*	Bignoniaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	10	*Uapaca nitida*	Euphorbiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	10	*Keetia guenzii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	20	*Keetia guenzii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	20	*Syzygium cordatum*	Myrtaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	20	*Ficus sycamorus*	Moraceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	20	*Anthocleista grandiflora*	Loganiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	30	*Keetia guenzii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	30	*Rothmannia manganjae*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	South	629	30	*Psychotria mahonii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Keetia guenzii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Bridelia macrantha*	Euphorbiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Albizia harveyi*	Fabaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Parinari curatellifolia*	Chrysobalanaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Uapaca nitida*	Euphorbiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Celtis africana*	Ulmaceae

6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Garcinia buchananii*	Guttiferae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	20	*Psychotria mahonii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	30	*Keetia guenzii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	30	*Parinari curatellifolia*	Chrysobalanaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	30	*Julbernardia globiflora*	Fabaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	30	*Psychotria mahonii*	Rubiaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	30	*Voacanga africana*	Apocynaceae
6	Honde	Estate	ARDA Katiyo1	02-Dec-03	North	629	30	*Antidesma venosum*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Parinari curatellifolia*	Chrysobalanaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Annona senegalensis*	Annonaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Combretum molle*	Combretaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Dalbergialla nyassae*	Fabaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Celtis africana*	Ulmaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Bridelia macrantha*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Keetia guenzii*	Rubiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	10	*Antidesma venosum*	Euphorbiaceae

7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Brachystegia utilis*	Fabaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Uapaca nitida*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Antidesma venosum*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Bridelia macrantha*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Albizia harveyi*	Fabaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Annona senegalensis*	Annonaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Garcinia buchananii*	Guttiferae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	20	*Syzygium cordatum*	Myrtaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Brachystegia utilis*	Fabaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Voacanga africana*	Apocynaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Bridelia macrantha*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Parinari curatellifolia*	Chrysobalanaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Dalbergialla nyassae*	Fabaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Antidesma venosum*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Cussonia arborea*	Araliaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Julbernardia globiflora*	Fabaceae

7	Honde	Estate	ARDA Katiyo	02-Dec-03	South	674	30	*Uapaca nitida*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Voacanga africana*	Apocynaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Celtis africana*	Ulmaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Uapaca nitida*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Bridelia macrantha*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Macaranga mellifera*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Syzygium cordatum*	Myrtaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	10	*Parinari curatellifolia*	Chrysobalanaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	20	*Keetia guenzii*	Rubiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	20	*Voacanga africana*	Apocynaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	20	*Syzygium cordatum*	Myrtaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	20	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	20	*Uapaca nitida*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	20	*Psidium guajava*	Myrtaceae

7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	30	*Keetia guenzii*	Rubiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	30	*Albizia gummifera*	Fabaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	30	*Bridelia macrantha*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	30	*Syzygium cordatum*	Myrtaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	30	*Voacanga africana*	Apocynaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	30	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
7	Honde	Estate	ARDA Katiyo	02-Dec-03	North	674	30	*Celtis africana*	Ulmaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	10	*Keetia guenzii*	Rubiaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	10	*Macaranga mellifera*	Euphorbiaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	10	*Senna simea*	Caesalpiniaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	10	*Erythrina lysistemon*	Fabaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	10	*Voacanga africana*	Apocynaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	20	*Ficus sycamorus*	Moraceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	20	*Bridelia macrantha*	Euphorbiaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	20	*Syzygium cordatum*	Myrtaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	30	*Macaranga mellifera*	Euphorbiaceae

8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	30	*Psidium guajava*	Myrtaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	West	688	30	*Anthocleista grandiflora*	Loganiaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	East	688	10	*Parinari curatellifolia*	Chrysobalanaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	East	688	10	*Albizia gummifera*	Fabaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	East	688	10	*Voacanga africana*	Apocynaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	East	688	20	*Anthocleista grandiflora*	Loganiaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	East	688	20	*Pteliopsis myrtifolia*	Combretaceae
8	Honde	Estate	Chikomba 2/En Hiighlands	02-Dec-03	East	688	20	*Markhamia acuminata*	Bignoniaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	10	*Aidia macrantha*	Rubiaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	10	*Keetia guenzii*	Rubiaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	10	*Parinari curatellifolia*	Chrysobalanaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	10	*Vangueria infausta*	Rubiaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	20	*Pteliopsis myrtifolia*	Combretaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	20	*Newtonia buchananii*	Mimosaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	20	*Aidia macrantha*	Rubiaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	20	*Parinari curatellifolia*	Chrysobalanaceae

9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	20	*Markhamia acuminata*	Bignoniaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	20	*Syzygium cordatum*	Myrtaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	30	*Keetia guenzii*	Rubiaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	30	*Albizia gummifera*	Fabaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	30	*Albizia harveyi*	Fabaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	30	*Combretum molle*	Combretaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	30	*Markhamia acuminata*	Bignoniaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	30	*Newtonia buchananii*	Mimosaceae
9	Honde	Estate	Chikomba 1/En Highlands	02-Dec-03	North	750	30	*Parinari curatellifolia*	Chrysobalanaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	10	*Vernonia coloratum*	Asteraceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	10	*Diospyros lycioides*	Ebenaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	20	*Vernonia coloratum*	Asteraceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	20	*Antidesma venosum*	Euphorbiaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	20	*Keetia venosa*	Rubiaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	20	*Spathodea campanulata*	Bignoniaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	30	*Harungana madagascariensis*	Clusiaceae

10	Honde	Estate	En Highlands	02-Dec-03	East	880	30	*Macaranga mellifera*	Euphorbiaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	30	*Antidesma venosum*	Euphorbiaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	30	*Pterocarpus angolensis*	Fabaceae
10	Honde	Estate	En Highlands	02-Dec-03	East	880	30	*Keetia venosa*	Rubiaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	10	*Ficus sycamorus*	Moraceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	10	*Combretum molle*	Combretaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	10	*Vitex payos*	Lamiaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	10	*Bridelia macrantha*	Euphorbiaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	10	*Celtis africana*	Ulmaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	20	*Albizia gummifera*	Fabaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	20	*Macaranga mellifera*	Euphorbiaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	20	*Combretum molle*	Combretaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	30	*Albizia gummifera*	Fabaceae
10	Honde	Estate	En Highlands	02-Dec-03	West	880	30	*Celtis africana*	Ulmaceae
11	Arcturus	Estate	Area 1-Lonlyl Farm	05-Dec-03	South	1313	10	*Acacia polyacantha*	Fabaceae
11	Arcturus	Estate	Area 1-Lonlyl Farm	05-Dec-03	South	1313	10	*Lantana camara*	Verbenaceae

11	Arcturus	Estate	Area 1-Lonlyl Farm	05-Dec-03	South	1313	10	*Lippia javanica*	Verbenaceae
11	Arcturus	Estate	Area 1-Lonlyl Farm	05-Dec-03	South	1313	10	*Toona ciliata*	Meliaceae
11	Arcturus	Estate	Area 1-Lonlyl Farm	05-Dec-03	South	1313	10	*Senna singueana*	Fabaceae
11	Arcturus	Estate	Area 1-Lonlyl Farm	05-Dec-03	South	1313	10	*Peltophorum africanum*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	10	*Julbernardia globiflora*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	10	*Uapaca kirkiana*	Euphorbiaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	10	*Combretum molle*	Combretaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	20	*Uapaca kirkiana*	Euphorbiaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	20	*Acacia caffra*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	20	*Julbernardia globiflora*	Fabaceae
11	Arcturus	Estate	Area 1-Lonlyl Farm	05-Dec-03	North	1313	30	*Acacia caffra*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	30	*Brachystegia spiciformis*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	30	*Gardenia resiniflua*	Rubiaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	30	*Julbernardia globiflora*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	30	*Uapaca kirkiana*	Euphorbiaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	30	*Peltophorum africanum*	Fabaceae

12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	North	1313	30	*Ziziphus mucronata*	Rhamnaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	East	1313	20	*Julbernardia globiflora*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	East	1313	20	*Parinari curatellifolia*	Chrysobalanaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	East	1313	20	*Strychnos spinosa*	Loganiaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	East	1313	20	*Combretum molle*	Combretaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	East	1313	30	*Uapaca kirkiana*	Euphorbiaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	East	1313	30	*Julbernardia globiflora*	Fabaceae
12	Arcturus	Estate	Area 2-Lonlyl Farm	05-Dec-03	East	1313	30	*Gardenia resiniflua*	Rubiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	10	*Julbernardia globiflora*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	10	*Diplorhynchus condylocarpon*	Apocynaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	10	*Gardenia resiniflua*	Rubiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	10	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	10	*Strychnos madagascariensis*	Loganiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	10	*Lannea discolor*	Anacardiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	10	*Gymnosporia senegalensis*	Celastraceae

13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	20	*Julbernardia globiflora*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	20	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	20	*Dichrostachys cinerea*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	30	*Bridelia carthatica*	Euphorbiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	30	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	30	*Faurea saligna*	Proteaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	30	*Catunaregam obovata*	Rubiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	30	*Ochna pulchra*	Ochnaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	East	1241	30	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	10	*Vangueria infausta*	Rubiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	10	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	10	*Brachystegia spiciformis*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	10	*Vangueriopsis lanciflora*	Rubiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	10	*Ozoroa reticulata*	Anacardiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	10	*Lannea discolor*	Anacardiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	20	*Julbernardia globiflora*	Fabaceae

13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	20	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	20	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	20	*Ochna pulchra*	Ochnaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	20	*Brachystegia spiciformis*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	20	*Diplorhynchus condylocarpon*	Apocynaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	30	*Julbernardia globiflora*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	30	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	30	*Brachystegia spiciformis*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	30	*Lannea discolor*	Anacardiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	30	*Monotes glaber*	Dipterocarpaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	West	1241	30	*Bobgunia madagascariensis*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	10	*Julbernardia globiflora*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	10	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	10	*Acacia amythethophylla*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	10	*Flacourtia indica*	Flacourtiaceae

13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	10	*Acacia mellifera*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	20	*Ozoroa reticulata*	Sapindaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	20	*Julbernardia globiflora*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	20	*Brachystegia boehmi*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	20	*Lannea discolor*	Anacardiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	20	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	30	*Acacia mellifera*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	30	*Diplorhynchus condylocarpon*	Apocynaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	30	*Lannea discolor*	Anacardiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	30	*Rhus longipes*	Anacardiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	30	*Senna spectabilis*	Fabaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	30	*Catunaregam obovata*	Rubiaceae
13	Chinhoyi	Estate	RSC PetrosvillPlot 9B	19-May-04	South	1241	30	*Diospyros lycioides*	Ebenaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	10	*Acacia amythethophylla*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	10	*Antidesma venosum*	Euphorbiaceae

14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	10	*Syzygium guineense*	Myrtaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	10	*Erythrina abyssinica*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	20	*Gymnosporia senegalensis*	Celastraceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	20	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	20	*Syzygium guineense*	Myrtaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	20	*Acacia amythethophylla*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	20	*Antidesma venosum*	Euphorbiaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	20	*Ziziphus mucronata*	Rhamnaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	20	*Ficus thonningii*	Moraceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Antidesma venosum*	Euphorbiaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Capparis tomentosa*	Capparaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Bauhinia galpinii*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Ziziphus mucronata*	Rhamnaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Erythrina abyssinica*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Flueggea virosa*	Euphorbiaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Combretum molle*	Combretaceae

14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Brachystegia spiciformis*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	West	1217	30	*Combretum zeyheri*	Combretaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Acacia ataxacantha*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Vernonia amygadalina*	Asteraceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Flueggea virosa*	Euphorbiaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Antidesma venosum*	euphorbiaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Acacia ataxacantha*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Bauhinia galpinii*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Acacia amythethophylla*	Fabaceae
14	Chinhoyi	Estate	Wilden Farm	19-May-04	North	1217	20	*Vangueria infausta*	Rubiaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	West	1313	20	*Julbernardia globiflora*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	West	1313	20	*Lantana camara*	Verbenaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	West	1313	30	*Acacia sieberiana*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	West	1313	30	*Julbernardia globiflora*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	West	1313	30	*Lannea discolor*	Anacardiaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	10	*Uapaca kirkiana*	Euphorbiaceae

15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	10	*Brachystegia spiciformis*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	10	*Brachystegia boehmi*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	10	*Combretum molle*	Combretaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	10	*Julbernardia globiflora*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	10	*Senna singueana*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	10	*Dodonaea viscosa*	Sapindaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	20	*Uapaca kirkiana*	Euphorbiaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	20	*Brachystegia spiciformis*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	20	*Brachystegia boehmi*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	20	*Lannea discolor*	Anacardiaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	30	*Julbernardia globiflora*	Fabaceae
15	Arcturus	Estate	Lonley Farm1	21-May-04	North	1313	30	*Brachystegia boehmi*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	10	*Julbernardia globiflora*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	10	*Uapaca kirkiana*	Euphorbiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	10	*Brachystegia boehmi*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	10	*Lannea discolor*	Anacardiaceae

16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	10	*Parinari curatellifolia*	Chrysobalanaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	10	*Combretum molle*	Combretaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	20	*Uapaca kirkiana*	Euphorbiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	20	*Brachystegia boehmi*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	30	*Uapaca kirkiana*	Euphorbiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	30	*Brachystegia boehmi*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	West	1313	30	*Acacia amythethophylla*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	10	*Gardenia resiniflua*	Rubiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	10	*Julbernardia globiflora*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	10	*Parinari curatellifolia*	Chrysobalanaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	10	*Combretum molle*	Combretaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	10	*Vernonia coloratum*	Asteraceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	10	*Fadogia urncilatum*	Rubiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	20	*Julbernardia globiflora*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	20	*Combretum molle*	Combretaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	20	*Lantana camara*	Verbenaceae

16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	20	*Cussonia natalensis*	Araliaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	30	*Acacia amythethophylla*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	30	*Julbernardia globiflora*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	30	*Diplorhynchus condylocarpon*	Apocynaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	North	1313	30	*Brachystegia spiciformis*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	10	*Parinari curatellifolia*	Chrysobalanaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	10	*Julbernardia globiflora*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	10	*Acacia amythethophylla*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	10	*Lannea discolor*	Anacardiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	10	*Brachystegia spiciformis*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	10	*Toona ciliata*	Meliaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	20	*Ficus sycamorus*	Moraceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	20	*Lannea discolor*	Anacardiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	20	*Parinari curatellifolia*	Chrysobalanaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	20	*Julbernardia globiflora*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	20	*Gardenia resiniflua*	Rubiaceae

16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	20	*Piliostigma thonningii*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	20	*Vangueriopsis lanciflora*	Rubiaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	30	*Parinari curatellifolia*	Chrysobalanaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	30	*Julbernardia globiflora*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	30	*Acacia amythethophylla*	Fabaceae
16	Arcturus	Estate	Lonley Farm2	21-May-04	East	1313	30	*Cussonia natalensis*	Araliaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	South	1200	10	*Acacia sieberiana*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	South	1200	10	*Faidherbia albida*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	South	1200	10	*Acacia polyacantha*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	South	1200	10	*Leucaena leucocephala*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	South	1200	10	*Azanza garckeana*	Malvaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	South	1200	10	*Spathodea campanulata*	Bignoniaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	South	1200	10	*Brachystegia boehmi*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Ficus sur*	Moraceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Acacia amythethophylla*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Senna singueana*	Fabaceae

17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Acacia amythethophylla*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Ziziphus mucronata*	Rhamnaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Lantana camara*	Verbenaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Lannea discolor*	Anacardiaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	East	1200	10	*Brachystegia boehmi*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	20	*Parinari curatellifolia*	Chrysobalanaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	20	*Bauhinia petersiana*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	20	*Lannea discolor*	Anacardiaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	20	*Flacourtia indica*	Flacourtiaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	20	*Brachystegia spiciformis*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	20	*Securidaca longipendunculata*	Polygalaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	30	*Strychnos madagascariensis*	Loganiaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	30	*Parinari curatellifolia*	Chrysobalanaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	30	*Brachystegia spiciformis*	Fabaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	30	*Lannea discolor*	Anacardiaceae

17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	30	*Vangueriopsis lanciflora*	Rubiaceae
17	Guruve	Estate	Amajuba Coffee Plantation	03-Jun-04	West	1200	30	*Burkea africana*	Fabaceae
18	Guruve	Estate	Nyamuseve Farm	03-Jun-04	East	1122	10	*Brachystegia spiciformis*	Fabaceae
18	Guruve	Estate	Nyamuseve Farm	03-Jun-04	East	1122	10	*Acacia amythethophylla*	Fabaceae
18	Guruve	Estate	Nyamuseve Farm	03-Jun-04	East	1122	10	*Combretum zeyheri*	Combretaceae
18	Guruve	Estate	Nyamuseve Farm	03-Jun-04	East	1122	10	*Steganotaenia araliacea*	Apiaceae
18	Guruve	Estate	Nyamuseve Farm	03-Jun-04	East	1122	10	*Bauhinia petersiana*	Fabaceae
18	Guruve	Estate	Nyamuseve Farm	03-Jun-04	East	1122	10	*Piliostigma thonningii*	Fabaceae
18	Guruve	Estate	Nyamuseve Farm	03-Jun-04	East	1122	10	*Ziziphus mucronata*	Rhamnaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	20	*Trema orientalis*	Ulmaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	20	*Philenoptera violacea*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	20	*Lantana camara*	Verbenaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	30	*Rauvolfia caffra*	Apocynaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	30	*Albizzia lebbeck*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	30	*Trema orientalis*	Ulmaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	30	*Trichilia dregeana*	Meliaceae

19	Chipinge	Estate	New Year's Gift	07-Jun-04	West	743	30	*Albizia amara*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	South	743	20	*Trema orientalis*	Ulmaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	South	743	20	*Lantana camara*	Verbenaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	South	743	20	*Rauvolfia caffra*	Apocynaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	South	743	30	*Acacia polyacantha*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	South	743	30	*Lantana camara*	Verbenaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	East	743	30	*Bauhinia galpinii*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	East	743	30	*Trichilia dregeana*	Meliaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	East	743	30	*Philenoptera violacea*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	East	743	30	*Ziziphus mucronata*	Rhamnaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	East	743	30	*Trema orientalis*	Ulmaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	East	743	30	*Lantana camara*	Verbenaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	20	*Trema orientalis*	Ulmaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	20	*Philenoptera bussei*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	20	*Peltophorum africanum*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	20	*Trichilia dregeana*	Meliaceae

19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	20	*Albizzia lebbeck*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	20	*Ficus sycamorus*	Moraceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	20	*Combretum mossambicensis*	Combretaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	30	*Trichilia dregeana*	Meliaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	30	*Trema orientalis*	Ulmaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	30	*Azanza garckeana*	Malvaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	30	*Rauvolfia caffra*	Apocynaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	30	*Pterocarpus rotundifolia*	Fabaceae
19	Chipinge	Estate	New Year's Gift	07-Jun-04	North	743	30	*Acacia polyacantha*	Fabaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Trema orientalis*	Ulmaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Lantana camara*	Verbenaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Acacia polyacantha*	Fabaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Pterocarpus rotundifolia*	Fabaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Ficus sycamorus*	Moraceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Adenia gummifera*	Passifloraceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Bridelia macrantha*	Euphorbiaceae

20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	10	*Heteromorpha arborescens*	Apiaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	20	*Trema orientalis*	Myrtaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	20	*Eucalyptus sp.*	Myrtaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	20	*Bridelia macrantha*	Euphorbiaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	East	944	20	*Lantana camara*	Verbenaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	10	*Lantana camara*	Verbenaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	10	*Annona senegalensis*	Annonaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	20	*Celtis africana*	Ulmaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	20	*Heteromorpha arborescens*	Apiaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	20	*Bridelia macrantha*	Euphorbiaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	20	*Dombeya burgessiae*	Sterculiaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	30	*Bridelia macrantha*	Euphorbiaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	30	*Acacia karroo*	Fabaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	30	*Rauvolfia caffra*	Apocynaceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	30	*Ficus sycamorus*	Moraceae
20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	30	*Trema orientalis*	Ulmaceae

20	Chipinge	Estate	Farfell Farm	08-Jun-04	South	944	30	*Lantana camara*	Verbenaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	North	766	10	*Lantana camara*	Verbenaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	North	766	10	*Psidium guajava*	Myrtaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	North	766	10	*Senna pendula*	Caesalpiniaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	North	766	10	*Trema orientalis*	Ulmaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	North	766	10	*Pteliopsis myrtifolia*	Combretaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	North	766	10	*Bridelia macrantha*	Euphorbiaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	North	766	10	*Albizia gummifera*	Fabaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	West	766	30	*Bridelia macrantha*	Euphorbiaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	West	766	30	*Albizia gummifera*	Fabaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	West	766	30	*Lantana camara*	Verbenaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	West	766	30	*Eucalyptus sp.*	Myrtaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	West	766	30	*Lippia javanica*	Verbenaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	West	766	30	*Pteliopsis myrtifolia*	Combretaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Celtis africana*	Ulmaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Lantana camara*	Verbenaceae

21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Lippia javanica*	Verbenaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Markhamia acuminata*	Bignoniaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Anthocleista grandiflora*	Gentianaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Psidium guajava*	Myrtaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Harungana madagascariensis*	Clusiaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	10	*Steganotaenia araliacea*	Apiaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	20	*Anthocleista grandiflora*	Gentianaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	20	*Bridelia macrantha*	Euphorbiaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	20	*Lantana camara*	Verbenaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	20	*Parinari curatellifolia*	Chrysobalanaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	East	766	20	*Annona senegalensis*	Annonaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Bridelia macrantha*	Euphorbiaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Markhamia acuminata*	Bignoniaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Psidium guajava*	Myrtaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Lippia javanica*	Verbenaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Lantana camara*	Verbenaceae

21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Albizia gummifera*	Fabaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Bauhinia galpinii*	Fabaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Vangueria infausta*	Rubiaceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Ficus sycamorus*	Moraceae
21	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Keetia venosa*	Rubiaceae
22	Chipinge	Smallholder	Tamandayi sithole	08-Jun-04	South	766	10	*Anthocleista grandiflora*	Gentianaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	North	1196	20	*Rauvolfia caffra*	Apocynaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	North	1196	20	*Psidium guajava*	Myrtaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	North	1196	20	*Trema orientalis*	Ulmaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	North	1196	20	*Macaranga capensis*	Euphorbiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	North	1196	20	*Heteromorpha arborescens*	Apiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Keetia venosa*	Rubiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Toddalia asiatica*	Rutaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Bridelia macrantha*	Euphorbiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Trema orientalis*	Ulmaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Albizia gummifera*	Fabaceae

22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Macaranga capensis*	Euphorbiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Lantana camara*	Verbenaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Heteropyxis natalensis*	Myrtaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Pittosporum viridiflorum*	Pittosporaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	20	*Oxyanthus latifolius*	Rubiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	30	*Erythrina lysistemon*	Fabaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	30	*Dodonaea viscosa*	Sapindaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	30	*Harungana madagascariensis*	Clusiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	30	*Bridelia macrantha*	Euphorbiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	South	1196	30	*Psidium guajava*	Myrtaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Oxyanthus latifolius*	Rubiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Maesa lanceolata*	Myrsinaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Bridelia macrantha*	Euphorbiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Psidium guajava*	Myrtaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Ficus sycamorus*	Moraceae

22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Harungana madagascariensis*	Clusiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Toddalia asiatica*	Rutaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Vangueria esculenta*	Rubiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	West	1196	20	*Heteropyxis natalensis*	Myrtaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Macaranga capensis*	Euphorbiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Ficus sycamorus*	Moraceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Heteropyxis natalensis*	Myrtaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Vangueria esculenta*	Rubiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Harungana madagascariensis*	Clusiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Bridelia macrantha*	Euphorbiaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Psidium guajava*	Myrtaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Prunus africana*	Rosaceae
22	Mutare	Intermediate	Bvumba Cooperative	10-Jun-04	East	1196	20	*Pavetta comostyla*	Rubiaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Piliostigma thonningii*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Vangueria infausta*	Rubiaceae

23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Vitex payos*	Lamiaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Terminalia sericea*	Combretaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Ziziphus mucronata*	Rhamnaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Lantana camara*	Verbenaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Combretum molle*	Combretaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Ehretia amoena*	Boraginaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Albizzia lebbeck*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Pterocarpus angolensis*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Euclea divinorum*	Ebenaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Psidium guajava*	Myrtaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	North	889	20	*Vernonia coloratum*	Asteraceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	20	*Terminalia sericea*	Combretaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	20	*Combretum coloratum*	Combretaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	20	*Ziziphus mucronata*	Rhamnaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	20	*Tabenamontana elegans*	Apocynaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	20	*Lippia javanica*	Verbenaceae

23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	20	*Lantana camara*	Verbenaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	20	*Vangueria infausta*	Rubiaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Terminalia sericea*	Combretaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Ziziphus mucronata*	Rhamnaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Tabenamontana elegans*	Apocynaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Lantana camara*	Verbenaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Bobgunia madagascariensis*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Acacia karroo*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Julbernardia globiflora*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Pterocarpus angolensis*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	West	889	30	*Pterocarpus rotundifolia*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	South	889	20	*Lantana camara*	Verbenaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	South	889	30	*Vangueria infausta*	Rubiaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	South	889	30	*Erythrina abyssinica*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	South	889	30	*Trema orientalis*	Ulmaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	South	889	30	*Lantana camara*	Verbenaceae

23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	South	889	30	*Julbernardia globiflora*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Dombeya rotundifolia*	Sterculiaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Terminalia sericea*	Combretaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Combretum molle*	Combretaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Lantana camara*	Verbenaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Piliostigma thonningii*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Acacia karroo*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Lippia javanica*	Verbenaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Ficus sycamorus*	Moraceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Burkea africana*	Fabaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Sclerocarya birrea*	Anacardiaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Trichilia dregeana*	Meliaceae
23	Chipinge	Estate	New Year's Gift Gombati	07-Jun-04	East	889	30	*Acacia sieberiana*	Fabaceae
24	Chipinge	Smallholder	Gwenzi area	08-Jun-04	East	817	30	*Albizzia lebbeck*	Fabaceae
24	Chipinge	Smallholder	Gwenzi area	08-Jun-04	East	817	30	*Acacia polyacantha*	Fabaceae
24	Chipinge	Smallholder	Gwenzi area	08-Jun-04	East	817	30	*Lantana camara*	Verbenaceae

24	Chipinge	Smallholder	Gwenzi area	08-Jun-04	East	817	30	*Annona senegalensis*	Annonaceae
24	Chipinge	Smallholder	Gwenzi area	08-Jun-04	East	817	30	*Acacia karroo*	Fabaceae
24	Chipinge	Smallholder	Gwenzi area	08-Jun-04	East	817	30	*Piliostigma thonningii*	Fabaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	10	*Trema orientalis*	Ulmaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	10	*Macaranga capensis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	10	*Steganotaenia araliacea*	Apiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	10	*Pittosporum viridiflorum*	Pittosporaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	20	*Macaranga capensis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	20	*Ficus sycamorus*	Moraceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	20	*Polyscias fulva*	Araliaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	20	*Trema orientalis*	Ulmaceae

25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	30	*Macaranga capensis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	30	*Trema orientalis*	Ulmaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	East	1076	30	*Bridelia macrantha*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	20	*Macaranga capensis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	20	*Steganotaenia araliacea*	Apiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	20	*Bridelia macrantha*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	20	*Vernonia amygadalina*	Asteraceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	20	*Ficus sycamorus*	Moraceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	30	*Clerodendrum glabrum*	Lamiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	30	*Macaranga capensis*	Euphorbiaceae

25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	30	*Steganotaenia araliacea*	Apiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	South	1076	30	*Dombeya burgessiae*	Sterculiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	10	*Rauvolfia caffra*	Apocynaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	10	*Ficus sycamorus*	Moraceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	10	*Maesa lanceolata*	Myrsinaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	10	*Shirakiopsis elliptica*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	10	*Diospyros lycioides*	Ebenaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	30	*Bridelia macrantha*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	30	*Macaranga capensis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	30	*Shirakiopsis elliptica*	Euphorbiaceae

25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	30	*Maesa lanceolata*	Myrsinaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	West	1076	30	*Heteromorpha arborescens*	Apiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	10	*Macaranga capensis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	10	*Trema orientalis*	Ulmaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	10	*Ficus sycamorus*	Moraceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	10	*Ricinus communis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	10	*Vernonia amygadalina*	Asteraceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	20	*Macaranga capensis*	Euphorbiaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	20	*Trema orientalis*	Ulmaceae
25	Chimanimani	Estate	ARDA Rusitu Valley Section 3	09-Jun-04	North	1076	20	*Ficus thonningii*	Moraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	10	*Vernonia amygadalina*	Asteraceae

26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	10	*Dichrostachys cinerea*	Fabaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	10	*Psidium guajava*	Myrtaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	10	*Lantana camara*	Verbenaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	20	*Syzygium guineense*	Myrtaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	20	*Syzygium cordatum*	Myrtaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	20	*Maesa lanceolata*	Myrsinaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	20	*Dissotis princeps* var. *princeps*	Melastomataceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	20	*Rhus longipes*	Anacardiaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	20	*Annona senegalensis*	Annonaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	30	*Syzygium guineense*	Myrtaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	30	*Acacia abbysinica*	Fabaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	30	*Ficus sycamorus*	Moraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	30	*Bridelia macrantha*	Euphorbiaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	North	1167	30	*Strychnos spinosa*	Loganiaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	East	1167	10	*Vernonia amygadalina*	Asteraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	East	1167	10	*Lantana camara*	Verbenaceae

26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	East	1167	10	*Vernonia amygadalina*	Asteraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	East	1167	10	*Lippia javanica*	Verbenaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	East	1167	20	*Acacia abbysinica*	Fabaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	East	1167	20	*Ficus sycamorus*	Moraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	East	1167	20	*Vernonia amygadalina*	Asteraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	10	*Vernonia amygadalina*	Asteraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	10	*Lantana camara*	Verbenaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	20	*Vernonia amygadalina*	Asteraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	20	*Acacia abbysinica*	Fabaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	20	*Rhus longipes*	Anacardiaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	20	*Psidium guajava*	Myrtaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	30	*Vernonia amygadalina*	Asteraceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	30	*Acacia abbysinica*	Fabaceae
26	Chimanimani	Intermediate	Steyn Farm	09-Jun-04	South	1167	30	*Steganotaenia araliacea*	Apiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	10	*Shirakiopsis elliptica*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	10	*Shirakiopsis elliptica*	Euphorbiaceae

27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	10	*Bridelia macrantha*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	10	*Diospyros lycioides*	Ebenaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	10	*Pterocarpus rotundifolia*	Fabaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	10	*Dodonaea viscosa*	Sapindaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	20	*Syzygium cordatum*	Myrtaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	20	*Bridelia macrantha*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	20	*Rhus longipes*	Anacardiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	20	*Heteromorpha arborescens*	Apiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	20	*Annona senegalensis*	Annonaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	20	*Dodonaea viscosa*	Sapindaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	South	1102	20	*Antidesma venosum*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	10	*Casimiroa edulis*	Rutaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	10	*Albizia gummifera*	Fabaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	10	*Ficus sycamorus*	Moraceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	10	*Bridelia macrantha*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	10	*Heteromorpha arborescens*	Apiaceae

27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	10	*Keetia venosa*	Rubiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	10	*Psychotria mahonii*	Rubiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	20	*Bridelia macrantha*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	20	*Shirakiopsis elliptica*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	20	*Erythrina lysistemon*	Fabaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	20	*Mangifera indica*	Anacardiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	20	*Markhamia obtusifolia*	Bignoniaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	East	1102	20	*Vangueria esculenta*	Rubiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	10	*Keetia venosa*	Rubiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	10	*Albizia gummifera*	Fabaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	10	*Markhamia obtusifolia*	Bignoniaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	10	*Oxyanthus latifolius*	Rubiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	10	*Vangueria esculenta*	Rubiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	10	*Syzygium cordatum*	Myrtaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	20	*Harungana madagascariensis*	Clusiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	20	*Albizia gummifera*	Fabaceae

27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	20	*Bridelia macrantha*	Euphorbiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	20	*Psychotria mahonii*	Rubiaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	20	*Toddalia asiatica*	Rutaceae
27	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	20	*Reissantia parviflora*	Celastraceae
28	Mutare	Estate	Crake Valley	10-Jun-04	North	1102	20	*Maytenus heterophylla*	Celastraceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Dichrostachys cinerea*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Julbernardia globiflora*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Burkea africana*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Vangueria infausta*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Pavetta gardenifolia*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Strychnos spinosa*	Loganiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Parinari curatellifolia*	Chrysobalanaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	20	*Combretum molle*	Combretaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Flacourtia indica*	Flacourtiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Canthium ngonii*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Julbernardia globiflora*	Fabaceae

28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Dichrostachys cinerea*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Vangueria infausta*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Diospyros lycioides*	Ebenaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Euclea divinorum*	Ebenaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Rhus longipes*	Anacardiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	West	1040	30	*Bersama abyssinica*	Melianthaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Brachystegia spiciformis*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Julbernardia globiflora*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Pseudolachnostylis maprouneifolia*	Euphorbiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Bersama abyssinica*	Melianthaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Antidesma venosum*	Euphorbiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Pterocarpus rotundifolia*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Parinari curatellifolia*	Chrysobalanaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Vangueria infausta*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Acacia sieberiana*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	North	1040	30	*Burkea africana*	Fabaceae

28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Pittosporum viridiflorum*	Pittosporaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Pavetta gardenifolia*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Vangueria infausta*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Brachylaena discolor*	Asteraceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Shirakiopsis elliptica*	Euphorbiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Antidesma venosum*	Euphorbiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Pterocarpus rotundifolia*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Euclea divinorum*	Ebenaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	10	*Pterocarpus lucens*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	20	*Vangueria infausta*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	20	*Brachystegia spiciformis*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	20	*Dichrostachys cinerea*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	20	*Pterocarpus angolensis*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	20	*Carissa edulis*	Apocynaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Brachystegia spiciformis*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Vangueria infausta*	Rubiaceae

28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Combretum molle*	Combretaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Pavetta gardenifolia*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Keetia venosa*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Albizia gummifera*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Pittosporum viridiflorum*	Pittosporaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	East	1040	30	*Coddia rudis*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Gardenia ternifolia*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Vangueria infausta*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Pavetta gardenifolia*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Keetia venosa*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Vitex payos*	Lamiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Uapaca kirkiana*	Euphorbiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Parinari curatellifolia*	Chrysobalanaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	20	*Antidesma venosum*	Euphorbiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	30	*Coddia rudis*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	30	*Carissa edulis*	Apocynaceae

28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	30	*Vangueria infausta*	Rubiaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	30	*Pittosporum viridiflorum*	Pittosporaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	30	*Brachystegia spiciformis*	Fabaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	30	*Parinari curatellifolia*	Chrysobalanaceae
28	Chipinge	Estate	Isis Farm	12-Jun-04	South	1040	30	*Bersama abyssinica*	Melianthaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Zanha africana*	Sapindaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Macaranga capensis*	Euphorbiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Albizia gummifera*	Fabaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Pteliopsis myrtifolia*	Combretaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Antidesma venosum*	Euphorbiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Rhus longipes*	Anacardiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Bobgunia madagascariensis*	Fabaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	20	*Vangueria infausta*	Rubiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	30	*Antidesma venosum*	Euphorbiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	30	*Vangueria infausta*	Rubiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	30	*Pteliopsis myrtifolia*	Combretaceae

29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	30	*Keetia venosa*	Rubiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	30	*Toddalia asiatica*	Rutaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	30	*Pavetta gardenifolia*	Rubiaceae
29	Chipinge	Estate	Stillfontein Estates	12-Jun-04	East	1021	30	*Harungana madagascariensis*	Clusiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	10	*Psidium guajava*	Myrtaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	10	*Vernonia amygadalina*	Asteraceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	10	*Ficus sur*	Moraceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	10	*Maesa lanceolata*	Myrsinaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	10	*Lippia javanica*	Verbenaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	20	*Erythrina lysistemon*	Fabaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	20	*Macaranga capensis*	Euphorbiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	20	*Trema orientalis*	Ulmaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	20	*Albizia gummifera*	Fabaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	South	1100	20	*Bridelia macrantha*	Euphorbiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	20	*Vangueria esculenta*	Rubiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	20	*Heteromorpha arborescens*	Apiaceae

30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	20	*Vernonia amygadalina*	Asteraceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	20	*Bridelia macrantha*	Euphorbiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	20	*Keetia venosa*	Rubiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	20	*Erythrina lysistemon*	Fabaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	20	*Maesa lanceolata*	Myrsinaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	30	*Toddalia asiatica*	Rutaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	30	*Macaranga capensis*	Euphorbiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	30	*Heteropyxis natalensis*	Myrtaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	30	*Shirakiopsis elliptica*	Euphorbiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	East	1100	30	*Erythrina lysistemon*	Fabaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	20	*Parinari curatellifolia*	Chrysobalanaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	20	*Maesa lanceolata*	Myrsinaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	20	*Vangueria infausta*	Rubiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	20	*Psidium guajava*	Myrtaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	20	*Keetia venosa*	Rubiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	20	*Shirakiopsis elliptica*	Euphorbiaceae

30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Macaranga capensis*	Euphorbiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Maesa lanceolata*	Myrsinaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Toddalia asiatica*	Rutaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Trichilia dregeana*	Meliaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Pittosporum viridiflorum*	Pittosporaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Ficus sur*	Moraceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Bridelia macrantha*	Euphorbiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Pavetta gardenifolia*	Rubiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Clerodendrum glabrum*	Lamiaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Pteliopsis myrtifolia*	Combretaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Cussonia zuluensis*	Araliaceae
30	Chimanimani	Estate	Arda Rusitu Valley	04-Sep-04	North	1100	30	*Albizia gummifera*	Fabaceae

APPENDIX 6: ANOVA FOR SPECIES DISTRIBUTION ACCORDING TO FARMING SYSTEMS AND DISTANCE FROM COFFEE FIELD (CHAPTER 6)

Analysis of variance

Variate: Count_of_Species

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Distance_from_field_edge stratum					
	2	398.2	199.1	0.26	
Distance_from_field_edge.*Units* stratum					
Farming_system	2	58883.6	29441.8	38.94	0.002
Residual	4	3024.4	756.1		
Total	8	62306.2			

Tables of means

Variate: Count_of_Species

Grand mean 95.4

Farming_system	1	2	3
	208.3	23.0	55.0

Standard errors of means

Table	Farming_system
rep.	3
d.f.	4
e.s.e.	15.88

Standard errors of differences of means

Table	Farming_system
rep.	3
d.f.	4
s.e.d.	22.45

Least significant differences of means (5% level)

Table	Farming_system
rep.	3
d.f.	4
l.s.d.	62.33

Combined estimates

No combined estimates (design orthogonal).

Stratum standard errors and coefficients of variation

Variate: Count_of_Species

Stratum	d.f.	s.e.	cv%
Distance_from_field_edge	2	8.15	8.5
Distance_from_field_edge.*Units*	4	27.50	28.8

Analysis of variance

Variate: Count_of_Species

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Farming_system stratum	2	58883.6	29441.8	38.94	
Farming_system.*Units* stratum					
Distance_from_field_edge	2	398.2	199.1	0.26	0.781
Residual	4	3024.4	756.1		
Total	8	62306.2			

Tables of means

Variate: Count_of_Species

Grand mean 95.4

Distance_from_field_edge	1	2	3
	88.3	104.3	93.7

Standard errors of means

Table	Distance_from_field_edge
rep.	3
d.f.	4
e.s.e.	15.88

Standard errors of differences of means

Table	Distance_from_field_edge
rep.	3
d.f.	4

s.e.d.	22.45

Least significant differences of means (5% level)

Table	Distance_from_field_edge
rep.	3
d.f.	4
l.s.d.	62.33

Stratum standard errors and coefficients of variation

Variate: Count_of_Species

Stratum	d.f.	s.e.	cv%
Farming_system	2	99.07	103.8
Farming_system.*Units*	4	27.50	28.8

Analysis of variance

Variate: Count_of_rubiaceae

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Distance_from_field_edge stratum					
	2	1.56	0.78	0.02	
Distance_from_field_edge.*Units* stratum					
Farming_system	2	726.22	363.11	10.44	0.026
Residual	4	139.11	34.78		
Total	8	866.89			

Tables of means

Variate: Count_of_rubiaceae

Grand mean 11.1

Farming_system	1	2	3
	23.3	2.0	8.0

Standard errors of means

Table	Farming_system
rep.	3
d.f.	4
e.s.e.	3.40

Standard errors of differences of means

Table	Farming_system
rep.	3
d.f.	4
s.e.d.	4.82

Least significant differences of means (5% level)

Table	Farming_system
rep.	3
d.f.	4
l.s.d.	13.37

Combined estimates

No combined estimates (design orthogonal).

Stratum standard errors and coefficients of variation

Variate: Count_of_rubiaceae

Stratum	d.f.	s.e.	cv%
Distance_from_field_edge			
	2	0.51	4.6
Distance_from_field_edge.*Units*			
	4	5.90	53.1

Analysis of variance

Variate: Count_of_rubiaceae

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Farming_system stratum	2	726.22	363.11	10.44	
Farming_system.*Units* stratum					
Distance_from_field_edge					
	2	1.56	0.78	0.02	0.978
Residual	4	139.11	34.78		
Total	8	866.89			

Tables of means

Variate: Count_of_rubiaceae

Grand mean 11.1

Distance_from_field_edge	1	2	3
	10.7	11.0	11.7

Standard errors of means

Table	Distance_from_field_edge
rep.	3
d.f.	4
e.s.e.	3.40

Standard errors of differences of means

Table	Distance_from_field_edge
rep.	3
d.f.	4
s.e.d.	4.82

Least significant differences of means (5% level)

Table	Distance_from_field_edge
rep.	3
d.f.	4
l.s.d.	13.37

Combined estimates

No combined estimates (design orthogonal).

Stratum standard errors and coefficients of variation

Variate: Count_of_rubiaceae

Stratum	d.f.	s.e.	cv%
Farming_system	2	11.00	99.0
Farming_system.*Units*	4	5.90	53.1