The Efficacy of Odour-Baited Bottom Board Trap for Controlling Small Hive Beetle, *Aethina tumida* (Coleoptera: Nitidulidae) In Honeybee Colonies

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my late sister, Jane. Thank you for all the support you gave me. You laid a good foundation that has seen me through to this level of education. May the Lord Almighty rest your soul in peace.

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LIST OF ABBREVIATIONS

GC	Gas Chromatography
HP	Hewlett-Packard
icipe	International Centre of Insect Physiology and Ecology
MS	Mass Spectroscopy
IS	Internal standard

ABSTRACT

Honeybees are of great value in Africa for both their economic and ecological importance. Economically, they are important pollinators of a great host of commercial crops as well as a source of food and livelihoods for thousands of small-scale beekeepers. Ecologically, they contribute to floral biodiversity and conservation through their pollination activity of both cultivated and wild plants. In Kenya, bee keeping as an income generating activity is being utilized as a tool to fight poverty in the rural arid and semi-arid areas. In the past, problems posed by honeybee pests were considered insignificant in Africa. More recently, however, an increasing number of exotic diseases like varroasis and pests *Varroa* mites as well as indigenous pests like wax moth and small hive beetles threaten honeybees, bee keeping and honeybee pollination in Africa. *Aethina tumida* Murray is considered a minor parasitic pest of African honeybee colonies within its native host range, and a serious exotic pest to European honeybees feeding on the honey, pollen and bee brood eventually causing honeybees to abscond the hives. The effectiveness of odour-baited bottom board trap for *A. tumida* was tested in a field apiary where eight traps were deployed in 24 honeybee colonies over a 32-week trapping period and the trap catches relative to the total population of *A. tumida* in the hives recorded. The bait consisted of commercial pollen dough conditioned by inoculation with yeast, *Kodamaea ohmeri* (NRRL Y-30722). The trap captured on average over 50% of the beetle population in the honeybee hives per trapping period although this varied between dry and rainy seasons with significantly more beetles

captured in the wet season. The trap nearly eliminated the beetles from the hives under trapping for a period of seven months. Laboratory bioassays using a dual choice olfactometer showed that *A. tumida* was significantly attracted to both worker honeybee and yeast-inoculated commercial pollen dough volatiles as compared to the controls, air and uninoculated pollen dough respectively while yeast-inoculated pollen dough volatiles attracted significantly more beetles compared to worker honeybee volatiles. The analysis of volatiles from worker honeybees and yeast-inoculated pollen dough showed that both odour sources contained some similar compounds with few fermentation related compounds being found in inoculated pollen dough only. This study showed that odour-baited bottom board trap is efficient not only as a monitoring tool but also as a management tool for *A. tumida* infestations in honeybee hives.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

1.1.1 Economic importance of honeybees

Honeybees (*Apis mellifera* Linnaeus) are of great importance in Africa and world over for both ecological and economic reasons (Allsopp, 2004). Ecologically, they offer pollination services to as many as 90% of the flowering plants which they visit (Damblon, 1987). *Apis mellifera* remains the most economically valuable pollinator of crops worldwide and yields of certain fruit, seed and nut crops could be reduced by 90% if they were not pollinated by honeybees (Klein *et al.*, 2007). They also play a key role in the maintenance of ecological balance and biodiversity.

Honeybees are well represented in Africa, being found almost throughout the continent and represented by ten different races (Ruttner, 1988). In Kenya, four honeybee races exist namely; *Apis mellifera litorea* Smith (in the coastal lowlands), *Apis mellifera scutellata* Lepeletier (in plains/grasslands), *Apis mellifera monticola* Smith (at higher altitudes of more than 2000 m above sea level) and *Apis mellifera nubica* Rutnure (in the arid Northern Kenya) (Mbae, 2006).

As befitting the continent with the richest archeological record of human association with honeybees, traditional beekeeping is practiced almost throughout the continent using a great variety of hives (Crane, 1983). Beekeeping in Kenya is a traditional art. In general, 80% of Kenya is suitable for beekeeping (Mbae, 2006). This is particularly so in arid and semi-arid areas. It is important to note that in these areas other agricultural activities like growing of crops are minimally carried out. *Acacia* kind of vegetation is found in abundance in these areas providing good flowers for honey production. There are also substantial apicultural activities in agriculturally high potential areas where good quality apicultural products may be found and improved production facilities are employed (Mbae, 2006).

Thousands of people in Africa depend on traditional and small scale beekeeping as part of their livelihood (Johannsmeier, 2001), with much of bee wax and honey produced being exported, earning valuable foreign currency. In the year 2004 for example, Kenya exported 0.5 and 1.5 metric tones of honey and bee wax respectively, earning 0.9 and 1.5 million Kenya shillings respectively (Mbae, 2006). Honey, which is produced by *A. mellifera,* is widely relished as human food, being a carbohydrate as well as having medicinal value (Patricia *et al.*, 2004). Kenya's honey potential is estimated at 80 – 100,000 metric tons with an equivalent of 10,000 metric tons of bee wax (Mbae, 2006). Bee keeping in Kenya is mainly focused on honey production and the government of Kenya has recognized the importance of bee-keeping in poverty eradication efforts (Raina, 2006). New hive products such as pollen, propolis, royal jelly, bee venom and

bee brood have found market in "apitherapy" or "apiotherapy" (treating health disorders using honeybee products) (Crane, 1983).

The contribution made by honeybees in maintaining and increasing biodiversity by virtue of their pollination of flowering plants is poorly researched in Africa (Hepburn and Radloff, 1998; Rodger and Balkwill, 2002), but could be substantial considering the numerical abundance of honey bee foragers. It has been reported that in some regions in Africa, as many as 90% of all flowering plants in the region are visited by honey bees (Damblon, 1987) and the 407 principal bee plant genera in Africa identified by Hepburn and Radloff (1998) represent approximately 40% of all plant genera on the continent.

1.1.2 Problems facing bee farming

Compared with the management of several wildbees, honeybees are prolific, versatile, cheap and convenient to manage (Roulston *et al.*, 1996; Klein *et al.*, 2007) and they have been extensively adapted for crop pollination (Roulston *et al.*, 1996). Lately honeybees have been declining at alarming rate due to unknown causes, a situation now termed Colony Collapse Disorders (CCDs) which is thought to be brought about by invasive pests and diseases (Kaplan, 2008).

Bee keeping is faced by a myriad of problems ranging from natural calamities like earthquakes, droughts to synthetic pesticides picked by bees when foraging on plants (Crane, 1990). Natural environmental hazards that can cause loss of human life and

economic loss such as fire and lightning, volcanic eruption and earthquake also cause damage to bees and hives in the region affected and the whole of beekeeper's livelihood may be endangered (Crane, 1983). Bee colonies also face problems such as bee diseases like American foul brood, European foul brood, sac brood, chalk brood, nosema disease, amoeba disease and melanosis (Crane, 1990). Bee colonies are also attacked by pests such as the parasitic mite *Varroa destructor* (Anderson and Trueman), various lepidopteron insects including wax moth *Galleria mellonella* (Linnaeus), several coleopterans like small hive and larger hive beetles and hymenopteran predators such as the Argentine ant, *Iridomyrnex humilis* (Mayr) and members of the vespidae family (Crane, 1990).

Since the 1990s, bee keeping in Africa, especially Sub-Saharan Africa has experienced an extended period of crisis, beset by a series of problems that have reduced honey production by half (Du Toit, 2001) and severely threatens the viability of the honey bee industry. Chief amongst these problems in countries South of Sahara are the Cape honey bee problem and the spread of the ectoparasitic mite *Varroa destructor* Anderson and Trueman (Allsopp, 2004), which was also recently discovered to have spread to East Africa (Frazier *et al.*, 2010). Both problems are currently important in Southern and Eastern Africa because of social, economic and ecological importance and both could ultimately be of importance throughout Africa (Allsopp, 2004; Frazier *et al.*, 2010). Among the coleopterans are the large hive beetles, *Oplostomus fuligineus* (Olivier), *O. haroldi* (Witte) and small hive beetle, *Aethina tumida* (Murray) (Torto *et al.*, 2010). Due

to its invasive nature and overwintering capacity, small hive beetle has spread world over and is posing a great challenge to the bee keeping industry through the feeding on honey, pollen and bee brood resulting to colony absconding (Neumann and Elzen, 2004).

1.2 Literature Review

1.2.1 Small hive beetle Aethina *tumida*, as a pest of honeybees

The small hive beetle was first described by Murray (1867) and is native to Africa (Lundie, 1940). It belongs to the coleopteran family Nitidulidae, which contains approximately 2800 described species in 172 genera worldwide (Habeck, 2002). The nitidulid beetle can be distinguished from other similar beetles by their transverse procoxal cavities, grooved metacoxae, dilated tarsal segments, small tarsi and three-segmented antennal club (Habeck, 2002). Most nitidulids feed on fresh, rotten and dried fruits, plant juices, carrion, crops and on flowers (Lin *et al.*, 1992; Fadamiro *et al.*, 1998; Hepburn and Radloff, 1998; Smart and Blight, 2000; Wolff *et al.*, 2001).

1.2.2 Life cycle of *Aethina tumida*

The natural history and morphology of *A. tumida* were described by Lundie (1940) and Schomolke (1974). Adult males and females enter honeybee colonies where they feed, mate and lay eggs (Lundie, 1940; Ellis *et al.*, 2002; Ellis and Hepburn, 2006). The eggs hatch into larvae that feed on bee brood, pollen or honey and develop through five larval

instars with the fifth larval instar entering the wandering phase where they drop into the soil to pupate (Steadman, 2006). Adults emerge from the soil and fly in search of a host, typically a honey bee colony (Figure 1.1). Alternative hosts of small hive beetles include wild bees and bumblebees (Neumann and Elzen, 2004).

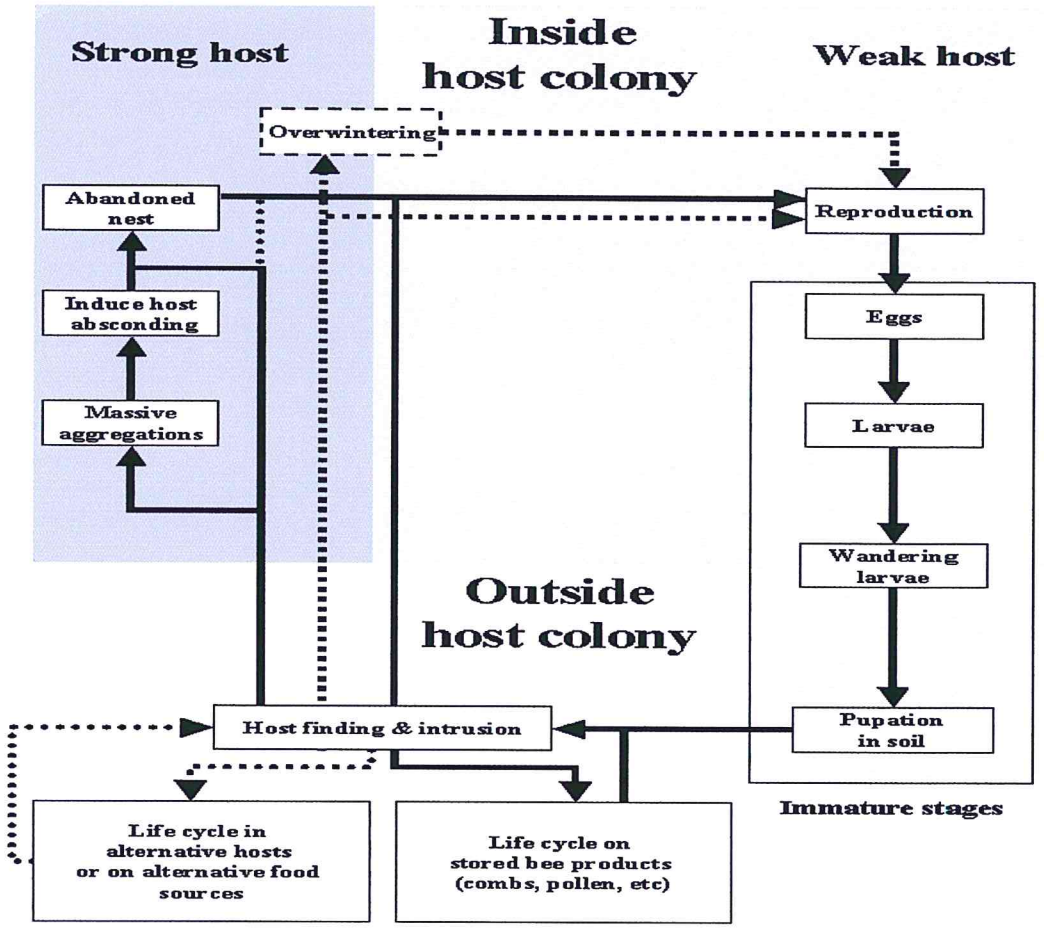


Figure 1.1: Putative life cycle of small hive beetle and interactions with honeybee colonies (dotted lines = rare events; dashed lines and dashed box = colonies of European honeybee subspecies only) (Neumann and Elzen, 2004)

1.2.2.1 Eggs of *Aethina tumida*

It is estimated that a female small hive beetle can lay between 1,000 – 2,000 eggs in her lifetime as reported by Lundie, 1940; Ellis *et al.*, 2002; Ellis and Hepburn, 2006. In situations where honeybee brood or pollen is left undefended, small hive beetle lay clusters of approximately 30 eggs per cell by: ovipositing on brood in open cells, tearing a small hole in capped brood and laying a cluster of eggs on or alongside pre-pupa or pupa or puncturing the sides of empty cells and ovipositing under the brood in adjoining cells. However, where brood and pollen frames are well defended, females will oviposit in cracks and crevices within the hive (Neumann and Elzen, 2004). Small hive beetle eggs are similar in shape and colour to honeybee eggs but are only two-thirds the length at 1.4 mm x 0.26 mm wide. Eggs are pearly white and tend to be laid in irregular masses (Plate 1.1). Egg hatch usually takes 2-4 days in the hive, but may take up to six days in cooler conditions such as when frames are stored during winter. Eggs are vulnerable to desiccation, although desiccation is unlikely to occur in viable hives (Stedman, 2006).

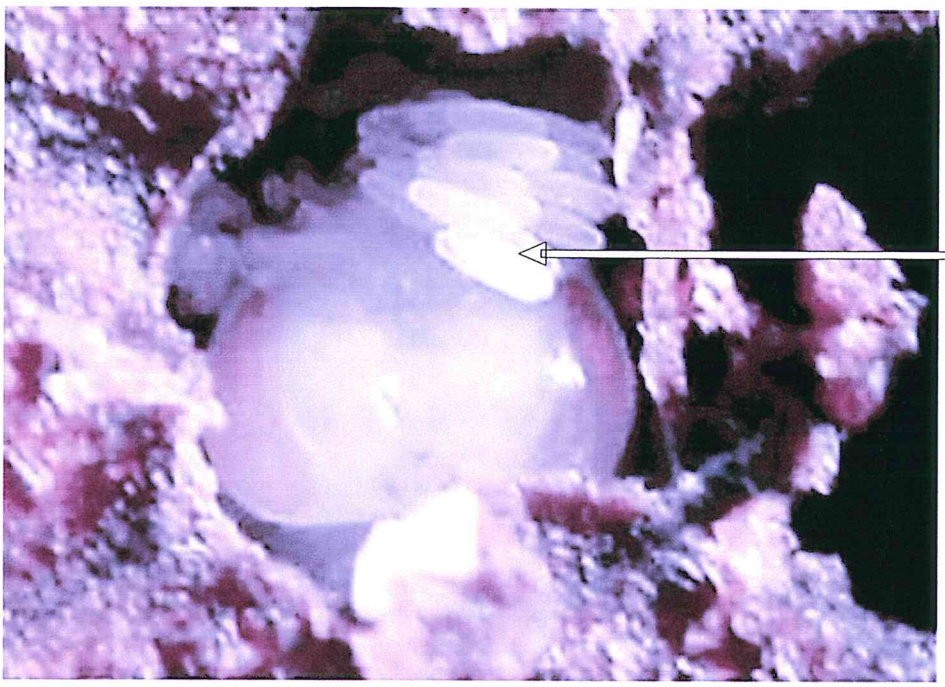


Plate 1.1: Eggs of *Aethina tumida*

1.2.2.2 Larvae of *Aethina tumida*

Once hatched the larvae begin feeding immediately on whatever food source is available including bee brood (preferred source), pollen or honey (Stedman, 2006). Larval development is five instars and takes 10-14 days to complete, although this may extend to 30 days depending on the quality and quantity of food resources and temperature (Lundie, 1940; Schomolke, 1974). Mature larvae vary in size depending on food sources but average around 11.1 mm long x 1.6 mm wide (7/16" x 1/16"). Larvae are cigar-shaped and pale yellow-cream in colour with a tan-brown head capsule (Plate 1.2).

The most distinctive diagnostic feature is the presence of a pair of raised dorsal spines on each larval segment, with the anterior and posterior pairs of spines being most pronounced (Stedman, 2006). Once larval feeding is complete, mature larvae enter a wandering phase, which may be triggered by increasing humidity. These larvae are attracted to light, migrating predominantly at dusk from colonies in search of a suitable pupation substrate. The majority of larval burrowing occurs within 90 cm of the colony entrance. However, larvae can wander over 200m in search of favourable conditions such as suitable substrate or moistened areas around plant irrigation zones or under mulch/leaf litter (Neumann and Elzen, 2004; Stedman, 2006).

Wandering larvae awaiting environmental triggers to pupate or unable to burrow into a suitable substrate appear to be able to arrest development for sometime. These wandering larvae are vulnerable to adverse weather conditions and predation, particularly by the generalist ant *Pheidole megacephala* and birds (Stedman, 2006, Torto *et al.*, in press).

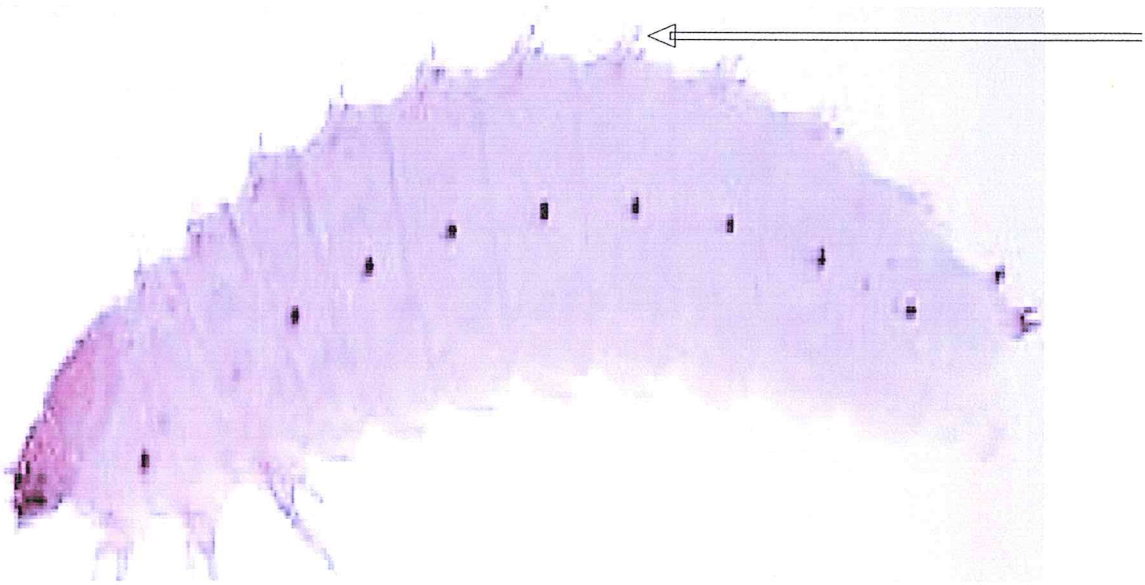


Plate 1.2: Larva of *Aethina tumida*

1.2.2.3 Pupae of *Aethina tumida*

Prior to pupation, larvae burrow 10-20cm into the soil and construct a pupal chamber. Soil moisture level appears critical to successful pupation and emergence, with moist loose soils (between 5-25% moisture by weight) being the most suitable substrate (Stedman, 2006; Torto *et al.*, in press). Although dry compacted soils, wet soils (at holding capacity) and certain soil types (for example, compacted) result in significant pupal mortality, pupation has been observed being initiated under gravel above a compacted driveway base in humid conditions, under dense leaf litter above red sand in hot dry conditions, and under in-hive debris within a dead sealed hive (Stedman, 2006). Pupae are pearly white, progressing to a light brown colour prior to emergence as adults (Plate 1.3).

Pupation time varies greatly depending on soil temperatures. Pupation over summer in United States of America usually takes between 15-60 days with most of adults emerging after 21-28 days. However, during cold periods (that is, less than 10°C) pupation may take up to 100 days (Schomolke, 1974; Stedman, 2006). Pupae are vulnerable to adverse weather conditions, soil-borne fungal infection, nematodes and soil cultivation (Stedman, 2006).

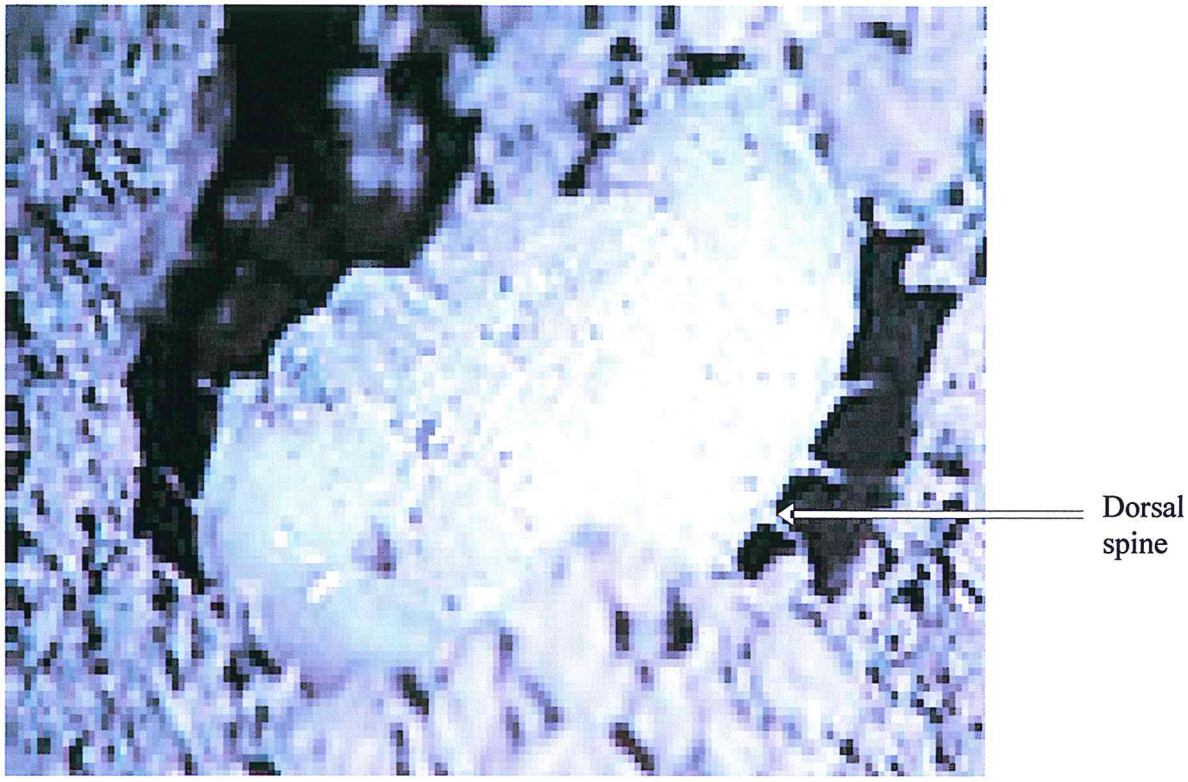


Plate 1.3: Pupa of *Aethina tumida*

1.2.2.4 Adult *Aethina tumida*

Adult small hive beetle darken after emergence to a brown-black colour dorsally and reddish brown-black ventrally. Adults are broad, flattened and oval in shape approximately 5-7 mm long x 2.5-3.5mm wide (Lundie, 1940) (Plate 1.4). The antennae are clubbed and the elytra are shortened exposing the last two abdominal segments, one segment of which is clearly visible (Habeck, 2002).

Anecdotal evidence suggests that younger adults are attracted to light; readily take to the wind around dusk and move between colonies. Older and presumably mated adults are less likely to move between colonies and are repelled by light (Stedman, 2006). Adults are able to fly up to 15 kilometres to locate colonies, and in colder areas, small hive beetle appear to migrate to stronger hives in autumn and back to weaker hives in spring (Stedman, 2006).

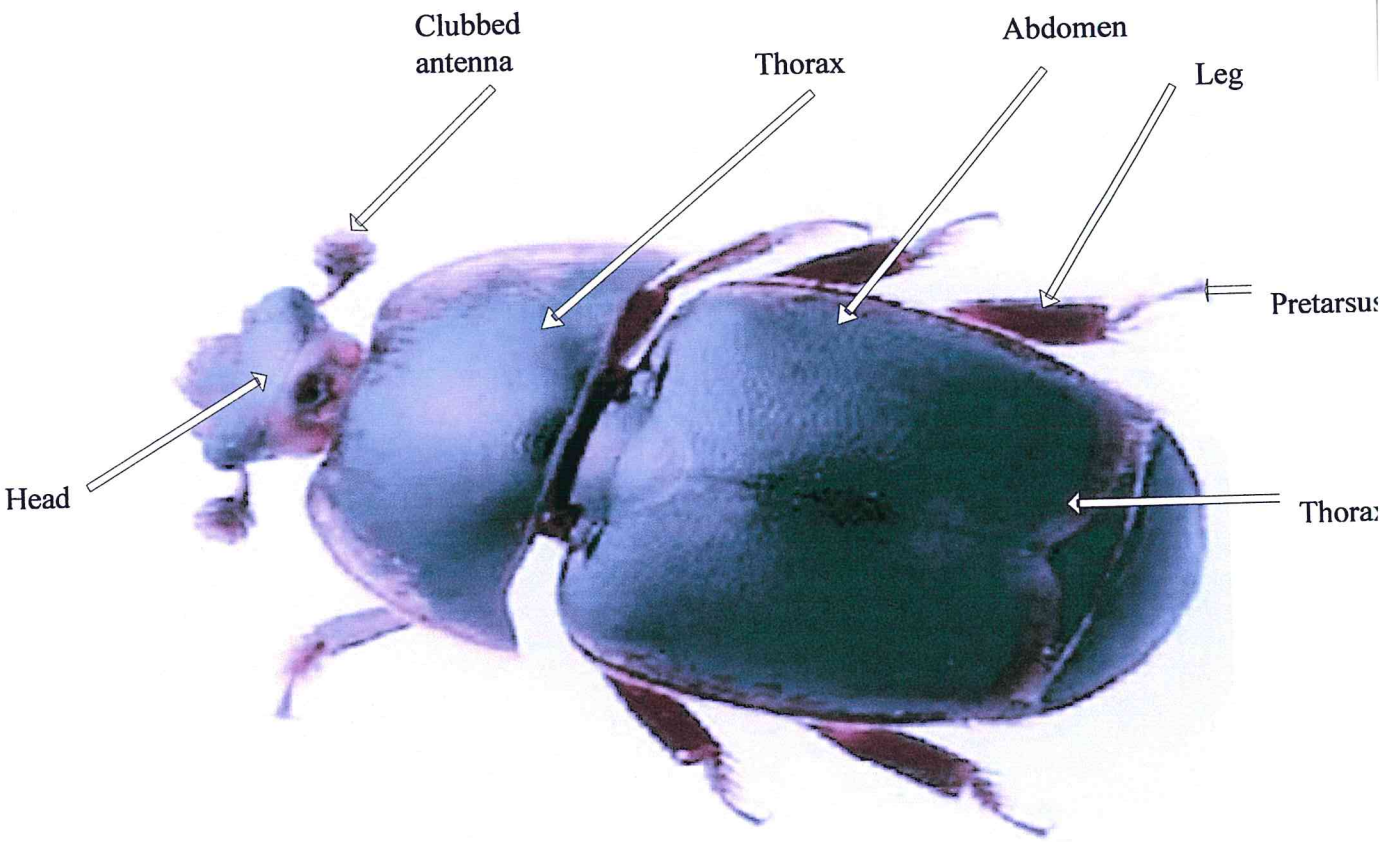


Plate 1.4: Adult *Aethina tumida*

1.2.3. Distribution of small hive beetle

The small hive beetle is native to Africa (Lundie, 1940; Neumann and Elzen, 2004), where they are found across the continent, and by 2003, the beetle had been identified in eighteen countries of Africa (Figure 1.2) (Neumann and Elzen, 2004). In Kenya, small hive beetles have been found in large populations in three provinces namely; Coast province (Watamu and Chawia-Taita), Eastern province (Matuu, Mwingi, Kitui) and Nairobi province (Kasarani, Kamiti) (Torto *et al.*, 2010).

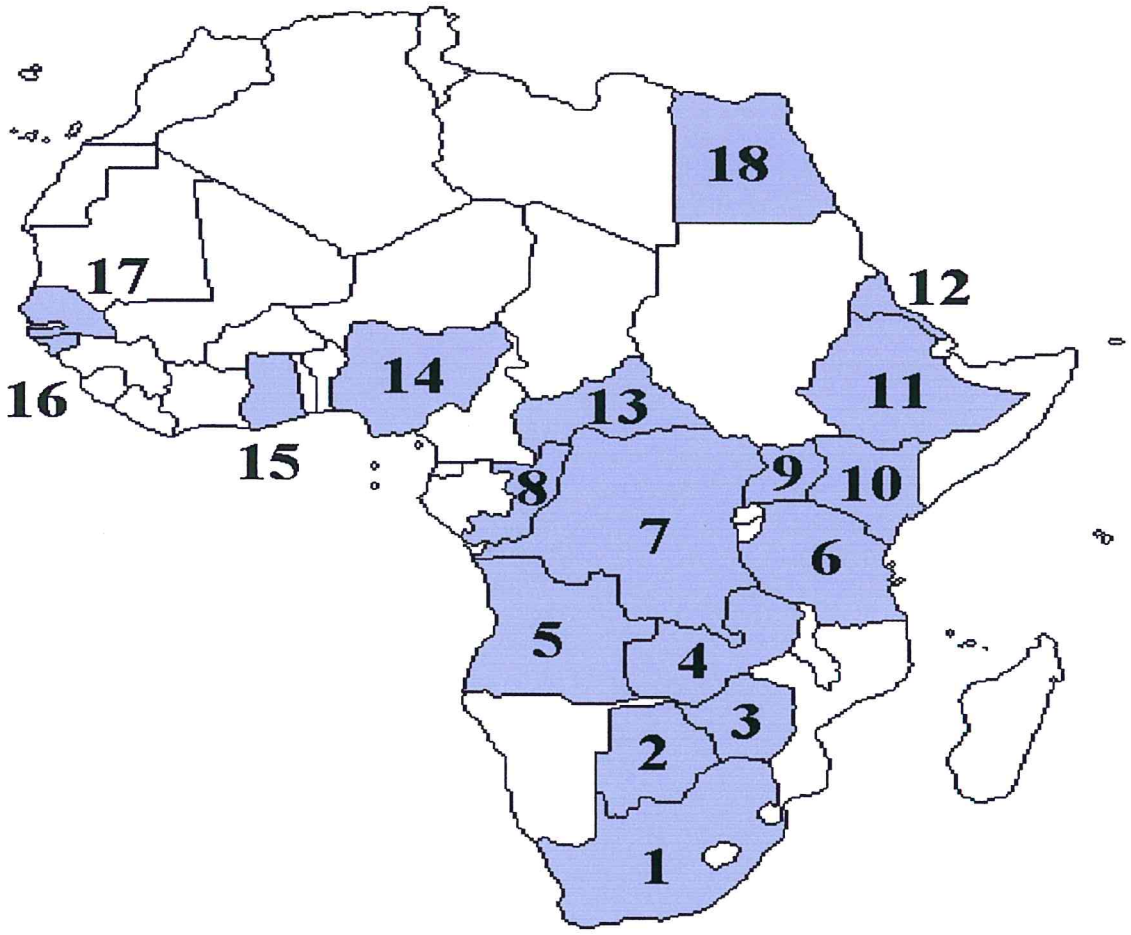


Figure 1.2: Distribution of the small hive beetle in Africa (Neumann and Elzen, 2004) each number represent a country

Currently, the small hive beetle has spread all over the world with detection in United States of America (USA) and Australia (Neumann, 2004). The first detection of small hive beetles in the United States was in St. Lucie, Florida in June 1998, as identified by the Florida Department of Agriculture and Consumer Services (Hood, 2000; Sanford, 2002). Earlier unidentified specimens were collected in Charleston, South Carolina, in

November 1996 (Hood, 1999). Since then, the small hive beetle has spread to virtually every state in the United States of America (Neumann, 2004) and more recently, it has been detected in Mexico and United Kingdom (www.defra.gov.uk/fera)

1.2.4 Damage caused by small hive beetle

In their native range, small hive beetles are better known for their damaging activities around honey houses and their effects on weak or stressed honey bee colonies (Lundie, 1940; Schomolke, 1974; Hepburn and Radloff, 1998). In Africa, the main problem associated with the beetles is in the destruction of stored bee products like honey (Lundie, 1940; Schomolke, 1974). Massive aggregations of small hive beetles and/or heavy infestations appear to induce colony absconding both in Africa and United States (Neumann and Elzen, 2004). The small hive beetle, recently introduced into the United States and Australia has become a devastating pest of resident European honey bees (Ellis *et al.*, 2002; Neumann and Radloff, 2004). The beetle is a threat to European honey bee pollinated crops worth $ 14 billion per annum in the United States (Torto *et al.*, 2007). The developing larvae are the most destructive stage, feeding on pollen and brood and contaminating honey with their faeces in the process (Lundie, 1940; Hood, 2004; Neumann and Elzen, 2004).

Adult small hive beetles are active flyers (Neumann and Elzen, 2004) and individuals or occasional swarms (Tribe, 2000) can infest honeybee colonies. It has been reported that small hive beetles can detect colonies under stress (Wenning, 2001), for example, due to

disease or management techniques such as splitting and that they detect such colonies from a distance of about 13-16 kilometres (Neumann and Elzen, 2004). Detection of stressed colonies might be adaptive in Africa, where reproduction is more likely in such colonies than in unstressed colonies (Hepburn and Radloff, 1998). Adult beetles can intrude strong honeybee colonies as well as weak ones with equal impunity (Lundie, 1940) despite presence of guard bees. Aggression by hosts, *A. mellifera*, is not very effective in killing the beetles (Neumann and Elzen, 2004). This is partly due to the hard exoskeleton (Lundie, 1940) but also due to defense tactics of the adult beetles such as turtle-like defense posture, running out of range of bees, dropping from comb to escape pursuit or hiding inside the nest cavity, in small cracks, under the bottom board of commercial hives or cells. Adult beetles also manage to come out of social encapsulations at night when bees are less active (Neumann and Elzen, 2004). Mating and cannibalism among the encapsulated beetles occur in the large encapsulations thus enhancing their survival in such conditions (Neumann and Elzen, 2004).

Large populations of small hive beetles can induce colony absconding in honeybee colonies (Neumann and Elzen, 2004). It can reduce colony efficiency in the long run because large scale infestations are continuous and major predatory pressure on honeybee nest have the worker bees take undertake the role of guarding the colony. Moreover, the occurrence of small hive beetle larvae and the resulting nest destruction and fermentation of honey (Lundie, 1940) are also likely to play a role in beetle-induced absconding (Neumann and Elzen, 2004).

1.2.5 Control methods for small hive beetle

A variety of control methods for the small hive beetle have been developed (Neumann and Elzen, 2004). These control measures range from biological, cultural, chemical to physical control methods (Hood, 2004; Teal *et al.*, 2006).

1.2.5.1 Biological control of *Aethina tumida*

Biological pest control relies upon other living organisms (parasites, predators, and pathogens) as pest control agents. These beneficial species are an important part of the ecological balance in every natural community. Biological agents, such as natural pathogens, may play an important role in small hive beetle control, especially in the beetles' endemic range of sub-Saharan Africa (Hood, 2004). Laboratory investigations identified several fungal pathogens from dead beetle larvae including soil dwelling fungi, *Aspergillus flavus* and *A. niger*, which are known for attacking other soil infesting insects (Ellis, 2004). Three other saprophytic fungi (*Clonostachys rosea, Gliocladium catenulatum* and *Mucor plumbeus*) were identified from the surface of dead small hive beetle larvae in experimental studies (Ellis, 2004), but their potential has not been determined. Other potential agents are soil infesting nematodes, parasitic wasps and flies, and predators such as ants that have been observed feeding on mature small hive beetle larvae as they enter soil to pupate (Hood, 2004; Torto *et al.*, in press). Fire ants may reduce activity in some areas but little is known about this predator-prey relationship (Hood, 2004).

1.2.5.2 Cultural control of *Aethina tumida*

Cultural or ecological control involves purposeful manipulation of the environment to make it less habitable for pest species. Methods include a broad range of normal management practices that can be modified or manipulated to manage one or more pest problems such as field sanitation. There are many cultural practices that beekeepers may use to minimize small hive beetle problems. Reducing colony stress conditions and maintaining strong productive colonies are highly recommended, especially in areas where beetles are problematic (Lundie, 1940; Schmolke, 1974). Good bee management practices that reduce the likelihood of brood disease, mite problems, wax moth activity, failing queens, excessive swarming, oversupering and colony starvation help minimize small hive beetle problem (Hood, 2004). Freezing dead or weakened colonies and emptying of supers that are infested help kill all life stages of the pest (Westervelt *et al.*, 2001). Good sanitation around honey storage houses helps prevent small hive beetle damage to stored combs (Lundie, 1940; Schmolke, 1974). Location of apiary in open, sunny sites with drier soil conditions helps to control the small hive beetle (Somerville, 2003; Ellis, 2004). However cultural methods only help reduce but do not drastically eliminate small hive beetles (Ellis, 2004).

1.5.3 Chemical control of *Aethina tumida*

Chemical products for small hive beetle control have been developed but varying results have been reported. Lundie (1940) reported the use of carbon disulfide as a fumigant to

control beetles in stored combs. Paradichlorobenzene has been suggested as a fumigant for small hive beetle control in comb (Mostafa and Williams, 2002). Household bleach was found to kill small hive beetle adults and larvae in honey houses (Park *et al.*, 2002). Soil treatment with materials such as benzene hexachloride (HCH), carbaryl, chlordasol and salt solution have been found to kill larvae when they enter the soil to pupate (Schmolke, 1974). However, as in the case of *V. destructor*, resistant strains may be developed due to continued use of chemicals (Spreafico *et al.*, 2001), as well as costs implications of procuring these chemicals to bee farmers and the environmental degradation consequences associated with these chemicals has made chemical control of small hive beetle to have some limitations, thus, development of sustainable alternative control methods is desirable to avoid chemical treatment in the long run.

1.2.5.4 Physical and Mechanical control of *Aethina tumida*

Physical and mechanical pest control includes a wide variety of devices that exclude, entrap, entangle, or electrocute pests. Several physical methods are available to beekeepers for controlling small hive beetle including removal of beetles from bee colonies using vacuum pump or by hand but these methods are time consuming and repeat visits to the same colonies are necessary especially when beetle infestations are high (Hood, 2004). Investigations involving trapping of small hive beetle have been reported (Schmolke, 1974; Elzen *et al.*, 1999; Hood and Miller, 2003; Sauzo *et al.*, 2003; Torto *et al.*, 2007; Arbogast *et al.*, 2009; Torto *et al.*, 2010).

Trapping can provide convenient and inexpensive method for beekeepers to manage small hive beetles but the traps deployed must be efficient enough for effective beetle control (Hood, 2004). Several small hive beetle traps have been developed including in-hive traps, flight traps and hanging traps (Hood and Miller, 2003; Torto *et al.*, 2007). Previous trappings have shown that in-hive traps baited with yeast-inoculated pollen dough capture more beetles compared to the flight and hanging traps with the same bait (Torto *et al.*, 2007). So far there is data to support the use of the in-hive traps in monitoring the presence of small hive beetle but no data is available to evaluate the efficiency of the baited in-hive trap for its potential in effective small hive beetle monitoring, control and management.

1.3 Statement of the problem

Despite the immense importance of honeybees both ecologically and economically as an income generating activity through bee keeping, an increasing number of exotic diseases and pests as well as indigenous pests such as small hive beetles threaten honeybees, bee keeping and honeybee pollination in Africa. The small hive beetles cause the fermentation of the honey both in the honeybee hives and honey storage houses due to the yeast, *Kodamaea ohmeri*, associatated with the beetle as it feeds on the honey. The developing larvae feed on the pollen and brood and contaminating honey with feaces in the process (Torto *et al.*, 2007). Aggression by hosts, *A. mellifera*, is not very effective in killing the beetles (Neumann and Elzen, 2004). This is partly due to the hard exoskeleton (Lundie, 1940) but also due to defense tactics of the adult beetles such as

turtle-like defense posture, running out of range of bees, dropping from comb to escape pursuit or hiding inside of the nest cavity, in small cracks, under the bottom board of commercial hives or cells. Large populations of small hive beetles can induce colony absconding in honeybee colonies (Neumann and Elzen, 2004). It can also reduce colony efficiency in the long run as worker honey bees take into guarding the colony.

Small hive beetles are efficient in long-range transportation and they can move over long distances. Moreover, they can establish populations in temperate regions due to their overwintering capacity in honeybee clusters. Host shifts to other bee species such as bumble bees may also occur. Thus, small hive beetles have the potential of becoming a global threat to apiculture and wild bee populations hence the need to put in place their effective control measures. Limitations of use of chemical control methods due to development of resistant strains and environmental degradation associated with these chemicals hinder effective control of this beetle.

1.4 Justification of the study

A variety of control methods for small hive beetle have been developed (Neumann and Elzen, 2004), which range from prevention through sanitation in apiaries and honey houses (Thomas, 1998), trapping of larvae using fluorescent lights and adult beetles using nucleus hives (Sanford, 1998) to chemical control in hive (Elzen *et al.*, 1999) and insecticide treatment of soil (Lafreniere, 2000). However, the use of chemicals has some negative impacts on the environment. As well, the small hive beetles have the potential

of developing some resistance to these chemicals (Spreafico *et al.*, 2001). Thus, development of sustainable control methods is desirable to avoid chemical treatment in the long run.

Currently, a number of traps have been designed. They include in-hive traps and flight traps with yeast inoculated pollen dough as an attractant (Torto *et al.,* 2007). In-hive traps have been shown to trap more small hive beetles compared to flight traps in America (Torto *et al.*, 2007). The beetles are lured to the traps by volatiles from fermenting pollen-dough conditioned either by feeding male or female adult beetles or by inoculation with yeast *K. ohmeri* (NRRL Y-30722) which is naturally associated with the beetles (Torto *et al.*, 2007). However, the use of the baited trap for monitoring *A. tumida* in its native home range is unknown. The efficacy of this baited trap in reducing populations of the small hive beetles in honey bee colonies is also unknown; hence the need to evaluate its efficiency as a potential tool for the management and control of the small hive beetle. In this study, the efficacy of the odour-baited bottom board trap was investigated.

1.5 Research hypotheses

1. Small hive beetles are not evenly distributed in different parts of the Langstroth hive.
2. Baited bottom board trap is not efficient in controlling small hive beetles in well managed honeybee colonies.
3. There is no difference in the attractiveness of volatiles emanating from worker honeybee and fermented pollen dough to small hive beetle.
4. Fermented pollen dough and worker honeybees do not produce the same volatiles

1.7 Research objectives

1.7.1 General objective

To determine the trapping efficacy of the baited bottom board small hive beetle trap

1.7.2 Specific objectives

1. To assess the distribution of small hive beetles in different parts of Langstroth hive
2. To quantify the trapping efficacy of the baited bottom board trap for small hive beetles in managed honeybee colonies.
3. To determine the response of *A. tumida* to volatiles from worker honeybee and fermented pollen dough
4. To identify and compare the volatiles released by fermented pollen dough and worker honeybees.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Study Site

Field studies were conducted at the International Centre of Insect Physiology and Ecology (*icipe*) apiary located at Duduville Campus, Nairobi. This campus is at S01°17'; E36°49'. The altitude is 1661 m above sea level and receives an average rainfall amount of 950 mm per year with two main rainy seasons; the short rains being between October and December and the long rains being between March to June. The temperature ranges between 16°C and 28°C (Nairobi History. http://www.city-data.com/.). Bioassays, collection and analysis of volatiles were done at this campus in the laboratories of the Behavioural and Chemical Ecology Department.

2.2 Distribution of small hive beetle in different parts of Langstroth hive and the effect of disturbance

Fifteen Langstroth hives were randomly selected from the *icipe* apiary. The small hive beetle population in each of the hives was surveyed. The number of the beetles found in each of the hive's parts namely top board, frames, hive box and the bottom board (Plate 2.1) were counted and recorded. The beetles were then returned into the hive. A subset of three colonies was checked daily for seven days to see when the beetles rebounded back at congregating at the bottom board of the hive.

2.3 Quantification of the trapping efficacy of the baited bottom board trap with attractant

Bottom board traps for small hive beetles previously designed by Torto (2007) were used (Plate 2.2). The upper part of the trap was resembled a typical Langstroth hive bottom board that was modified by cutting a rectangular opening (18 cm x 14 cm) in its centre. The bait, inoculated pollen dough was held in 500ml black plastic containers held by plywood on the underside of the trap. A soapy solution (150 ml) prepared from household dishwashing detergent (teepol) (0.25% in water) was placed in each container as a killing agent. The control traps did not have the bait but contained only the soapy solution.

Eight hives out of the total twenty two hives in the *icipe* apiary were randomly selected. The total number of small hive beetles in each of the hives was counted and recorded. The beetles were returned back into the hives before setting up the traps. Four baited traps were fixed into four hives, one trap per hive. The other four unbaited traps were also fixed into four other hives, one trap per hive. The trap catch was checked weekly and the bait was changed.

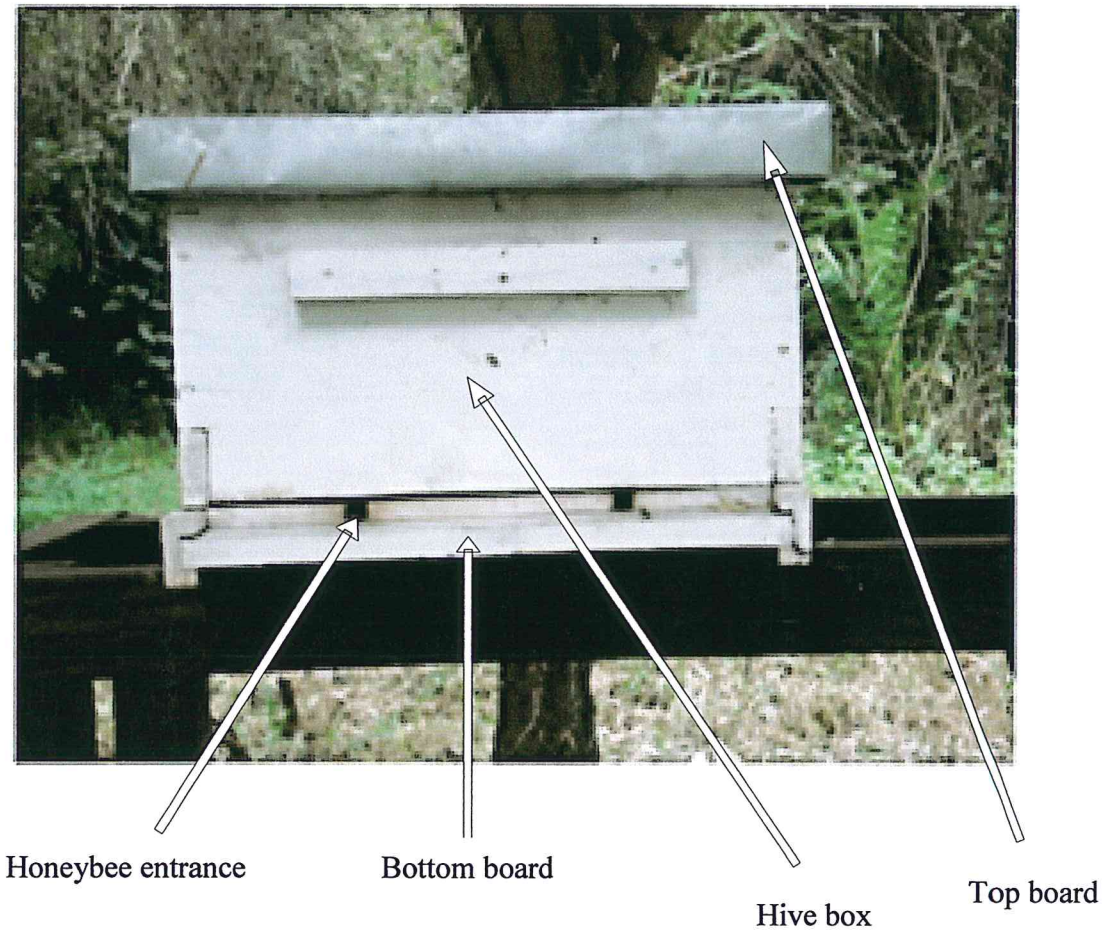
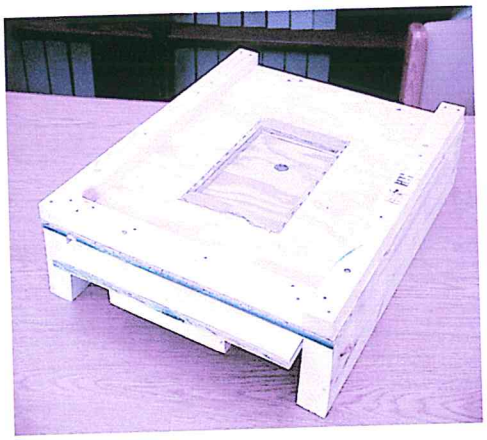
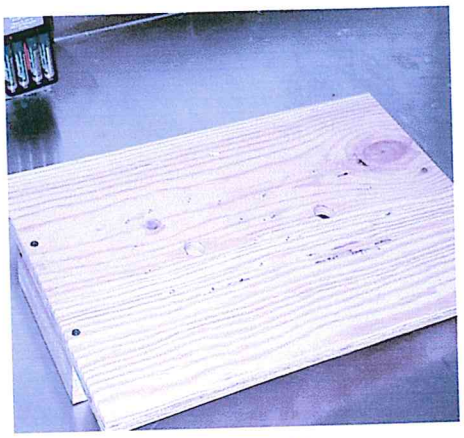


Plate 2.1: A Langstroth hive depicting various hive parts

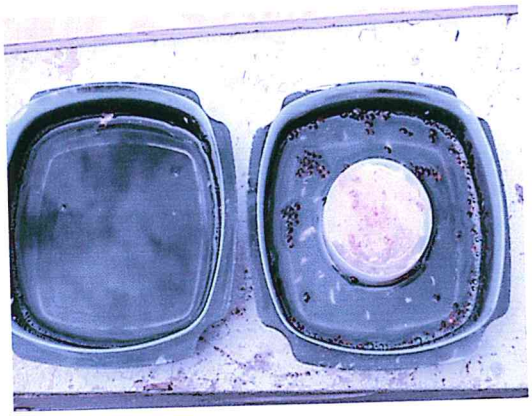


A



B

C



D

Plate 2.2: Components of the in-hive bottom board trap (A) a typical Langstroth hive bottom board, with a rectangular opening (18 cm x 14 cm) in its center and covered with a piece of aluminum screen, (B) trap slide with holes for entry of beetles (C) underside of trap slide with the lid of a Rubbermaid egg container and (D) control and baited containers for trapping the beetles.

2.4 Collection, identification and bioassays of volatiles from pollen dough and worker honeybees

Volatiles were collected from conditioned-pollen dough and worker honeybees (three replicates each) using a volatile collection instrument (Plate 2.3). Super Q adsorbent trap (30 mg, Alltech, Nicholasville, Kentucky) was used for adsorbing the volatiles for three hours at room temperature. Each filter was eluted with 200µl of GC/GC-MS-grade dichloromethane (Sigma-Aldrich, Gillingham-Dorset, England).

GC-MS analysis of the volatile extracts were carried out on an HP-7890 coupled to an HP-5973 mass spectrometer (EI, 70eV, Agilent, Palo Alto, California, USA) equipped with an HP-1column (30m x 0.25mm ID x 0.25 µm) (Agilent, Palo Alto, California, USA) (Plate 2.4). Butyl butyrate was used as the internal standard for the quantification of the volatiles. The components of the volatiles were identified by comparing their mass spectral data with those in the library of the mass spectrometer and by retention time analysis using authentic standards.

Bioassays were conducted in a dual choice olfactometer (100 cm x 31cm x 201 cm x 31 cm) constructed from glass and aluminum (Figure 2.1). The responses of 25 males and 25 females of *A. tumida* beetles (7-10 days old) to two odour sources; a) 300-400 worker honeybees collected from hive frames and b) 15g of pollen dough inoculated with the yeast *K. ohmeri* were each compared to a control of blank air and non-innoculated pollen dough respectively in the olfactometer. The responses of *A. tumida* between the two

odour sources were also tested. Test beetles were released singly into the centre of the olfactometer and after 10 minutes the odour to which the beetle responded by moving towards the side with that odour source was recorded.

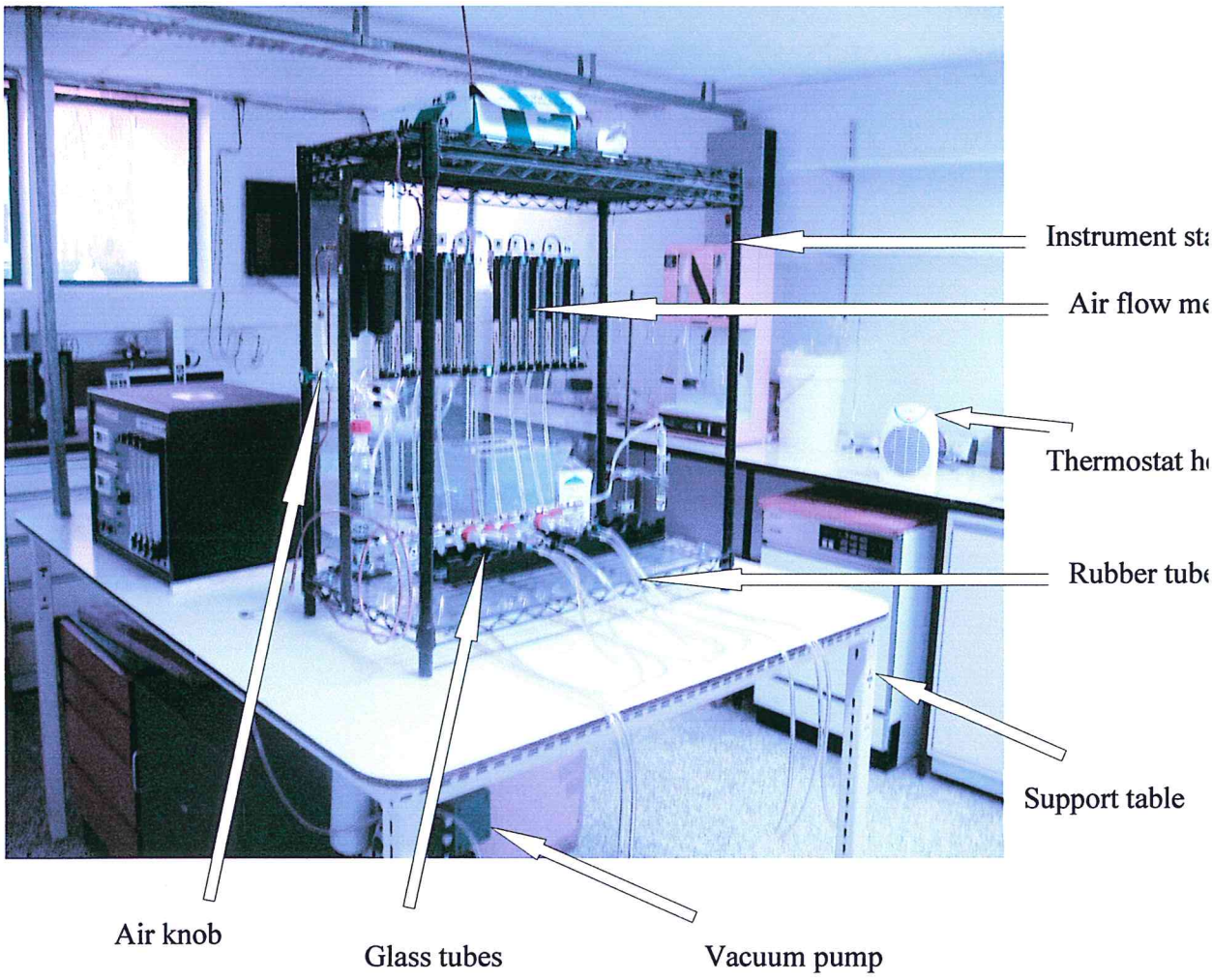


Plate 2.3: Volatile collection instrument

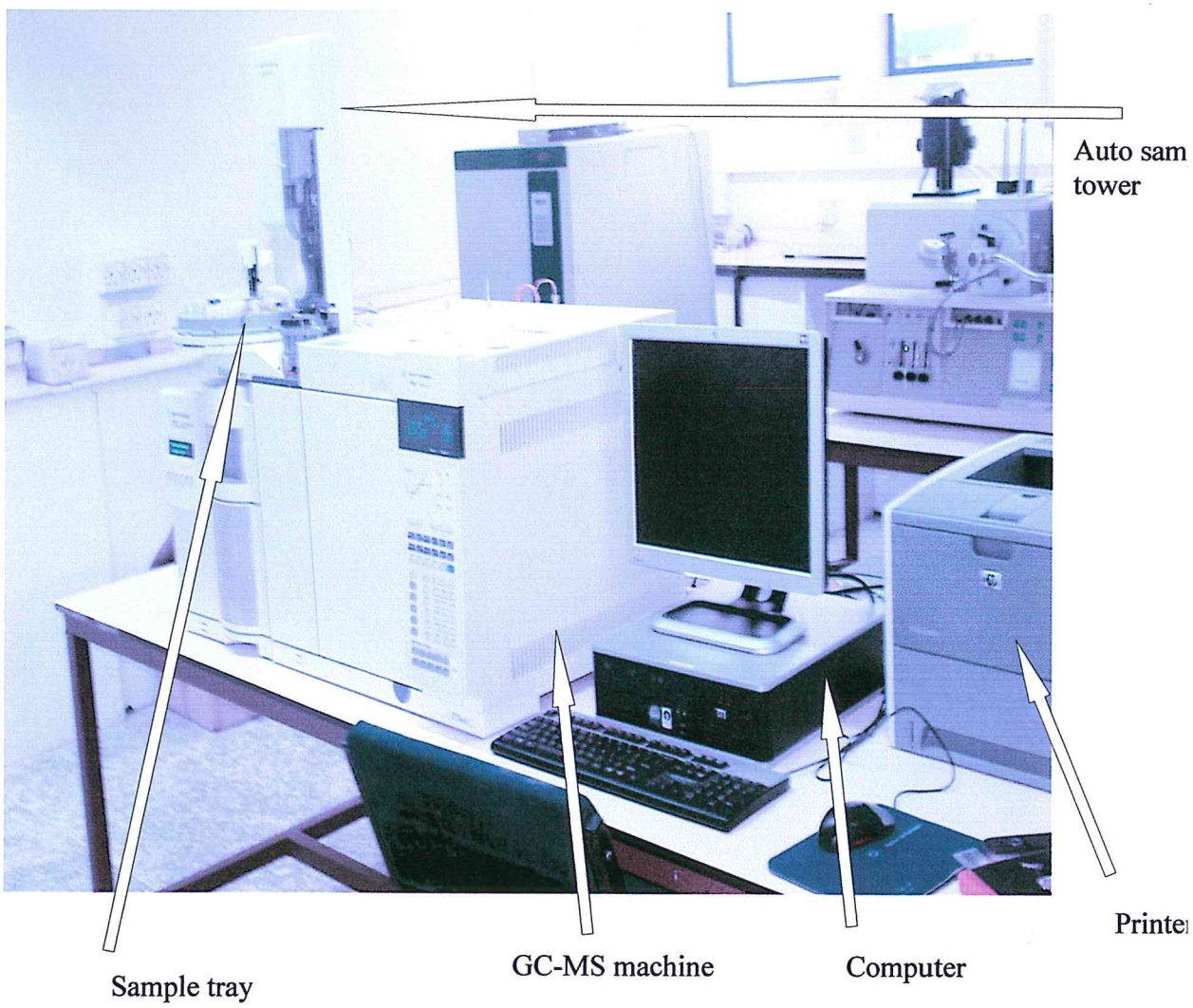


Plate 2.4: GC-MS instrument

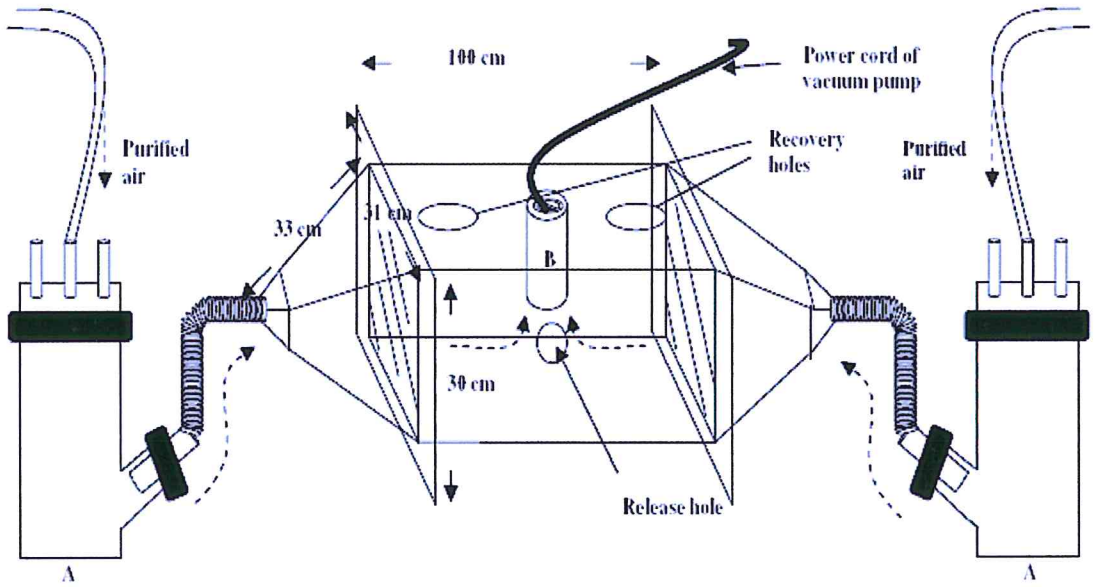


Figure 2.1: Dual choice olfactometer chamber (Torto 2010). (A) Glass jars (3 litres) that hold odor sources, (B) Vacuum pump; broken arrows represent the direction of flow of charcoal filtered air.

2.5 Data Analysis

Logistic regression analysis using SAS procedure GENMOD and pairwise orthogonal comparisons (SAS Institute, 2003) was used to analyze data on distribution pattern of *A. tumida* in different parts of Langstroth hive. T-test was used to analyze data on effect of colony disturbance to the distribution of *A. tumida* in Langstroth hive parts and also for the analysis of data on the quantification of the trapping efficacy of odour-baited bottom board trap for *A. tumida*. The proportion of beetles of both sexes responding to the different odour sources were subjected to a one -sample $\chi 2$ test at $\alpha = 0.05$.

CHAPTER 3

DISTRIBUTION OF *Aethina tumida* IN DIFFERENT PARTS OF LANGSTROTH HIVE AND EFFECTS OF DISTURBANCE TO THEIR DISTRIBUTION

3.1 Introduction

Adult small hive beetles are active flyers (Neumann and Elzen, 2004). Individuals or occasional swarms (Tribe, 2000) can infest honeybee colonies. It has been stated that small hive beetles can detect honeybee colonies under stress (Wenning, 2001), for example, due to disease or management techniques such as splitting and that they detect such colonies from a distance of about 13-16 km (Neumann and Elzen, 2004). Long range host finding adults (Wenning, 2001) require efficient cues.

Adult small hive beetles are attracted to volatiles of honey bees (Sauzo *et al.*, 2003; Torto *et al.*, 2005; Torto *et al.*, 2007) and the attraction is mediated by a blend of components dominated by the honey bee's alarm pheromone isopentyl acetate (IPA), 2-heptanone and methyl benzoate which account for ~ 70-80% of the blend (Torto *et al.*, 2007). Thus initial small hive beetle infestation of the honey bee colony is caused by the beetle detecting colony volatiles including bee alarm pheromones at thresholds lower than detected by worker honey bees. The beetles associate these chemicals with the presence of food resources in the colony. This initial attack also could be aided inadvertently by the honey bees themselves when they collect pollen from flowers contaminated by yeast spores deposited by flower-feeding nitidulids. Indeed, yeasts of

the genus *Kodamaea* have been found in certain ephemeral flowers, which serve as breeding and feeding sites for nitidulid beetles (Torto *et al.*, 2007). Behaviourally, host seeking adult small hive beetles enter bee hives mostly through the same entrance used by the foraging worker bees at the bottom of the hive. Congregation of *A. tumida* mainly at the bottom board of Langstroth hives has previously been reported (Lundie, 1940), but no data is available to support this observation and also to show the relative distribution of the beetles in the different parts of the Langstroth hives. It was against this background that a study was carried out to determine the relative distribution of *A. tumida* in different parts of Langstroth hive and the effects of disturbance to the beetle distribution by routine inspection of the hive.

3.2 Materials and Methods

3.2.1 Distribution of *Aethina tumida* in different sections of Langstroth hive

To establish the distribution of small hive beetles in different parts of Langstroth hive, fifteen Langstroth hives (Plate 2.1) were randomly selected from the *icipe* apiary. The small hive beetles were counted in each of the hive's parts namely top board, frames, hive box and the bottom board (Plate 3.1). The process of counting small hive beetles involved smoking the hive with a smoker to make honeybees less aggressive and opening the hive lid (top board) to separate different parts of the hive and expose the beetles. Using an aspirator, the beetles were sucked up from the lid and the inner cover counting them as they were being sucked. The crevices/cracks were checked carefully,

gently tapping the top board to flush out all the beetles hiding there and they were also counted. The top board was then placed upside-down on the hive stand next to the hive but not touching the other hive parts. The hive box was then separated from the bottom board by carefully removing the hive box and placing it in the upside-down lid, making sure that the hive box is set in the lid rather than placing it diagonally on the lid edges. The beetles on the bottom board were then counted as they were being sucked. The underside of the bottom board was checked for beetles that might have escaped through the cracks. The frames were carefully removed from the hive box, one at a time and checked for beetles while paying attention to the frame cracks and honey bee combs especially those with open brood, uncapped pollen or honey. The sides of the box were also checked as beetles were being sucked and counted. Frames were then returned into the hive box which was transferred back to the bottom board. The beetles on the upside-down lid that have fallen from the hive box were also counted. The counting procedure was carefully done especially in heavily infested hives to ensure all the beetles were sucked and counted. The total numbers of beetles caught and counted in different sections of the hive were recorded and the beetles were returned into the hive before closing the hive with the top board.

3.2.2 Comparison of *Aethina* tumida distribution before and after disturbance through colony inspection

The process of counting the small hive beetles in the honeybee hives and subsequent return of the beetles back to the hives caused the beetles to scatter all over the hive parts. It was therefore necessary to check after how long the beetles will congregate at the bottom board of the hive where they were found in large numbers. To determine this, a subset of three hives were checked daily for seven days and the distribution pattern before disturbance and after disturbance recorded.

3.2.3 Data analysis

Data obtained on the distribution pattern of small hive beetle on different parts of the Langstroth hive was subjected to logistic regression analysis using SAS procedure GENMOD and pairwise orthogonal comparisons (SAS Institute, 2003). The mean number of beetles recorded at the bottom board for each hive during the initial inspection was deducted from the mean number obtained during the second inspection and the difference in means noted. This difference in means was used as a measure of the effect of disturbance and subjected to a paired t-test.

Plate 3.1 Counting of *Aethina tumida*

3.3 Results

3.3.1 Distribution of *Aethina tumida* in different parts of Langstroth hive

Out of the 278 beetles counted in the 15 hives during the survey, 57.2% were found at the bottom board, 27.3% at the hive box, 8.6% at the frames and 6.8% at the top board as illustrated in Figure 3.1. Statistical analysis showed that bottom board had significantly higher number of small hive beetles compared to the other hive sections at $P < 0.05$ (Table 3.1).

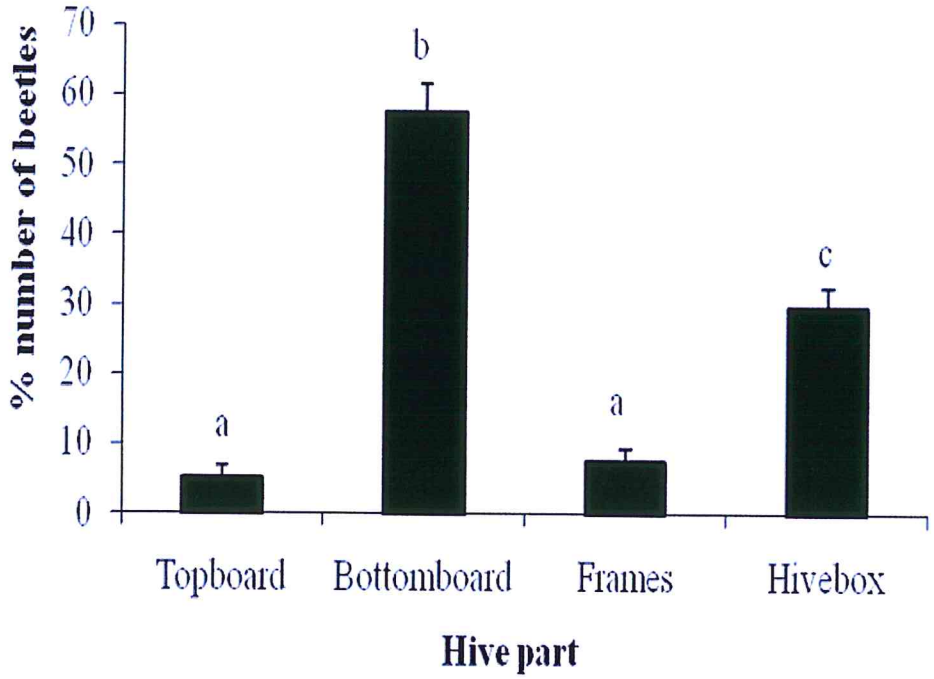


Figure 3.1: Distribution of *Aethina tumida* in different parts of a Langstroth hive

Table 3.1: Mean number ± S.E of *Aethina tumida* found in different parts of Langstroth hive

Hive part	Mean number ± S.E
Top board	19 ± 0.49 a
Bottom board	159 ± 1.95 b
Frames	24 ± 0.56 a
Hive box	76 ± 0.88 c

Same letters indicate no significant difference at P = 0.05

Table 3.2: Pairwise comparison of adult *A.tumida* distribution in different sections of Langstroth honey bee hive:

	$\chi 2$ value	P value	
Top board vs bottom board	18.12	0.0044 **	P < 0.05
Top board vs frames	1.68	0.1945 NS	P > 0.05
Top board vs hive box	62.17	< 0.0001**	P < 0.05
Bottom board vs frames	20.81	< 0.0001 **	P < 0.05
Bottom board vs hive box	31.11	< 0.0001**	P < 0.05
Frames vs hive box	50.40	< 0.0001**	P < 0.05

**-Highly significant at P = 0.05
NS – Not significant at P = 0.05

3.3.2 Comparison of *Aethina* tumida distribution before and after disturbance through colony inspection

There was no significant difference in the occurrence of beetles on the bottom board before and after disturbance except at day 4 (t = 2.7915, P = 0.0384), (Table 3.3). However, the least variation of mean difference from the assumed mean of zero was observed in days six and seven (Figure 3.2).

Table 3.3: Mean of difference ± S.E and paired-test analysis of counts of *Aethina tumida* at bottom board before and after disturbance of hive

Day	Mean difference ± S.E	t-value	P- value
1	0.5665 ± 0.575	1.3615	0.2315 NS
2	-0.0943 ± 0.0485	-0.2493	0.813 NS
3	0.8481 ± 0.412	1.4717	0.2011 NS
4	0.8653 ± 1.134	2.7915	0.0384 *
5	0.189 ± 0.2835	0.3625	0.7317 NS
6	0.2346 ± 0.1125	0.9553	0.3833 NS
7	0.2354 ± 0.1125	1.1815	0.2905 NS

* - Significant at P = 0.05
NS – Not significant at P = 0.05

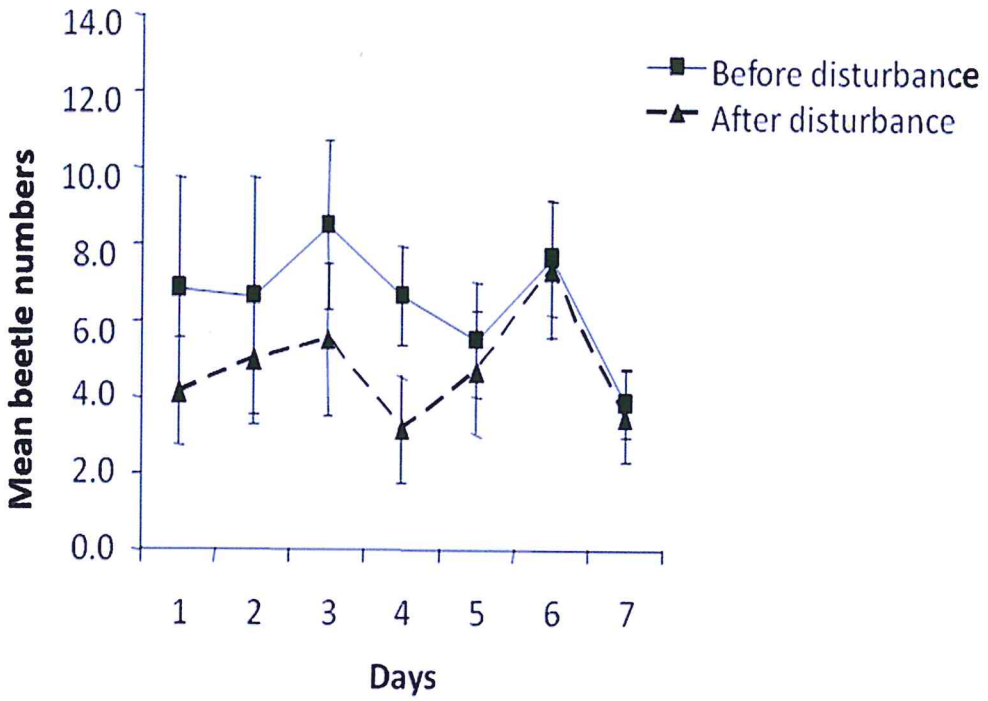


Figure 3.2: Mean beetle numbers recorded before and after hive inspection during a seven day inspection period. Error bars indicate S.E.

3.4 Discussion

Results on the distribution of *A. tumida* in Langstroth hive parts showed that most *A. tumida* congregated at the bottom board part of the hive. The congregation of most *A. tumida* at the bottom board of the Langstroth hive could be explained by the scavenging behaviour of the beetle and its preference to dark areas as reported by Lundie (1940). Usually the bottom board and the hive box accommodate very few bees and therefore provide sanctuary to the small hive beetles as they are protected from the aggressive

bees. The dark corners of the hive box could also provide hiding places which the beetle behaviorally prefers (Lundie, 1940).

The least number of beetles were found at the top board and the frames which showed no significant difference. Limited food resources at the top board, since it is just a cover for the hive with only propolis for sealing any opening at the hive could have contributed to this least number of beetles at this section. Although the frames hold the honey combs which contain the honey, pollen and bee brood, the presence of many bees around the combs and their aggressive and defensive behaviour and lack of possible areas to hide especially when frames do not have cracks might have contributed to having few beetles on the frames (Neumann and Elzen, 2004).

When the beetles were disturbed through the normal routine opening of the hive for the counting of beetles and when they were returned into the hive, they took about six to seven days to resume their initial distribution the one where they congregated at the bottom board of the hive. This was essential because it provided an estimate of the days to leave a bottom board trap fixed into the hive if maximum captures of beetles is intended after disturbing the infested honey bee colony.

3.5 Conclusion

Results from this study show about 57 % of the *A.tumida* congregate at the bottom board, with almost the other half of beetles being confined at other sections of

Langstroth hive. Based on previous studies where baited top and bottom board traps were compared in capturing *A. tumida* from European honey bee colonies managed in Langstroth hives in the USA, more of the beetles were captured by the bottom board trap than the top board trap (Torto *et al.*, 2007). Based on these results, it can be concluded most *A. tumida* are confined at the bottom board part and that for effective management of *A.tumida* using baited traps, a combination of baited bottom board trap and other traps which can conveniently capture beetles from the other parts of hive would be desirable.

CHAPTER 4

QUANTIFICATION OF TRAPPING EFFICACY OF ODOR-BAITED BOTTOM BOARD TRAP

4.1 Introduction

Small hive beetle traps have been developed by many researchers (Schmolke, 1974; Elzen *et al.*, 1999; Hood and Miller, 2003; Suazo *et al.*, 2003; Arbogast *et al.*, 2009; Torto *et al.*, 2010). Trapping can provide convenient and inexpensive method for beekeepers to manage small hive beetles but the traps deployed must be efficient enough for effective beetle control (Hood, 2004). Several types of small hive beetle traps have been developed including in-hive traps, flight traps and hanging traps (Hood and Miller, 2003; Torto *et al.*, 2007).

In Africa and United States, *A. tumida* has been found to carry a strain of the yeast named *Kodamaea ohmeri* that induces fermentation of pollen in the hive, producing volatiles such as isopentyl acetate, 2-heptanone and methyl benzoate that mimic bee alarm pheromones, which are potent attractants for the small hive beetle (Torto *et al.*, 2007). These beetles associate these volatiles with the presence of food in the hive. Other traps namely, top board traps, bottom board traps and flight traps for trapping small hive beetles have also been designed and are usually baited with pollen dough as lure (Torto *et al.*, 2007, Arbogast *et al.*, 2009). The pollen dough is conditioned either

being fed on by male or female adult small hive beetles or through inoculation with yeast, *K. ohmeri* (NRRL Y-30722).

Previous field trapping studies showed that baited traps with conditioned pollen dough captured significantly more small hive beetles than unbaited traps. Similarly traps positioned under the bottom board of a hive captured significantly more small hive beetles than traps located at the top of a hive (Torto *et al.*, 2007; Arbogast *et al.*, 2009). This showed the effectiveness of yeast-inoculated pollen dough as a lure and its potential as a monitoring tool in managing the small hive beetle. However, the number of beetles captured by these traps in relation to the total population of the beetles in the hive and the seasonal trap catch variability has not been established, which could help to establish the potential of these traps as a control tool for managing small hive beetle pests in apiaries. Therefore, the objectives of the current study were (1) To quantify the efficacy of baited bottom board trap in trapping small hive beetles and (2) To establish the seasonal variability in trap captures after using the baited bottom board trap.

4.2 Materials and Methods

4.2.1 Design of the bottom board trap

Bottom board trap (Plate 2.2) for trapping small hive beetles previously designed and described by Torto *et al.*, (2007) were used. The upper part of the trap was a typical bottom board part of Langstroth hive that was modified by cutting a rectangular opening

(18 cm x 14 cm) in its centre. This opening was covered with a piece of four-mesh aluminum screen, which allowed small hive beetles to pass but excluded bees. The modified bottom board was attached to a three-sided frame whose missing side was either toward the back or front of the hive. This opening permitted a plywood panel to slide beneath the bottom board on wooden runners that were attached to the side frame. Two holes were made in the plywood (each 2cm in diameter) 12 cm apart. These holes led to separate 500-ml plastic containers (Rubbermaid, Huntersville, North Carolina) attached beneath the plywood panel which had their conical tips cut off and were positioned with the tips extending downward.

The 500ml plastic containers which contained the bait and held trapped beetles, snapped tightly into this lid. The plastic containers had been sprayed with black paint (Rust-Oleum Corporation, Vernon Hills, Illinois) before use to enhance the trap efficacy since the beetles are attracted to dark shaded areas (Lundie, 1940), Each hole led to a triangular enclosure formed by three strips of wood (0.8 cm x 0.8 cm x 6 cm) attached to the underside of the plywood and covered with seven-mesh screen. Openings (0.5 cm wide) at the apices of each triangle allowed the beetles to enter the container below. Only one container, either baited or unbaited was fixed in each trap. In this case, one hole of the trap was sealed using aluminium foil. A Petri dish (6 cm in diameter x 2 cm high) for holding bait was glued to the bottom of the container. The Petri dish was covered with a tight-fitting lid perforated by approximately 60 pin-holes that allowed the release of odors into the honey bee colony. A soapy solution (150 ml) prepared from

household dishwashing detergent (teepol) (0.25% in water) was placed in each container as a killing agent. The control traps did not have the bait but contained only the soapy solution.

4.2.2 Bottom board trap bait

The bait consisted of conditioned commercial pollen dough (4% pollen with sugar, soy, yeast and water) obtained from Global Patties (Airdrie, Alberta, Canada) which was prepared in a room maintained at 26 ± 2°C by dissolving yeast (*Kadomaea ohmeri* (NRRL Y-30722), in distilled water and sprinkling it over the commercial bee pollen in the ratio of 1:100:1,000 by weight of yeast, distilled water and commercial bee pollen respectively and allowed to ferment for four days. The traps were then baited by placing inoculated pollen dough (50g in a piece of cotton stockinette) in the Petri dish on the bottom of the baited container and covering it with a tightly-fitting perforated lid.

4.2.3 Experimental set up and design

Eight hives out of the total twenty two hives at the *icipe* apiary were randomly selected. The total number of small hive beetles in each of the selected hives were counted, recorded and returned into the hive before setting up the traps. The counting of the small hive beetles and the experimental set up was done as described in section 3.2.1

Baited bottom board traps were installed into four hives selected randomly, each trap in one hive. Four unbaited traps were also installed into the remaining four hives, and each trap served as the control the experiment. Trap catch was checked weekly. Untrapped small hive beetles in the hives were also counted by opening the hive and using an aspirator to catch the beetles in all the parts of the hive. The live beetles were returned into the hive. To avoid frequent disturbance of the honeybees which could result in the experimental colony absconding, the experiment was carried out once every fortnight for seven months, with four replicates each for dry and wet season. The data was recorded. Rainfall and soil moisture were also recorded. Suitable gears including a bee suit and a smoker were used when handling honeybees.

4.2.4 Data analysis

Data was analyzed using one-sample t-test for efficacy determination with Ho: baited bottom board trap cannot trap 50% of total population in a hive per trapping. For the effect of season on trap catch, data was analyzed using t-test to check for mean differences between the seasons and also to compare trap catches between the treatment and the control at $\alpha = 0.05$. The weekly total in-hive beetle population was regressed with weekly trap catches by linear regression using SAS Proc REG (SAS Institute, 2003) to determine if any relationship existed between them.

4.3 Results

The baited bottom board trap captured significantly more beetles than the unbaited trap which acted as the control ($P < 0.05$) (Table 4.1). The mean percent trap catch for baited trap was 45.6 ± 4.2% while that one for the control was 4.4 ± 2.2 % (Table 4.1). The number of small hive beetles captured by the baited bottom board trap was significantly higher during rainy season as compared to the dry season ($P < 0.05$), with the baited trap trapping on average 31.3 ± 4.1% and 59.9 ± 5.5% during dry and wet seasons respectively, of total small hive beetle population in a honeybee hive per trapping (Table 4.1).

The selected honeybee colonies had a total of 250 small hive beetle infestation. This population was reduced to 23 beetles due to trapping during the trapping experiment although there were population upsurges following rainy season (Figure 4.3). The trap catch increased with an increase of the total monthly rainfall until the month of December 2009 when the beetle population in the study hives had been drastically reduced due to continuous trapping (Figure 4.1). The trap catch also increased with an increased with an increase in the population of the beetle in the hive (Figure 4.2)

Table 4.1: Comparison of percentage trap catch between control and baited trap, and between dry and wet seasons

Treatment	Mean % trap catch ± S.E	t-value	P- value
Control	4.4 ± 2.2 a		
Baited	45.6 ± 4.2 b	8.46	0.0001
Baited trap			
Dry season	31.3 ± 4.1 c		
Wet season	59.8 ± 5.5 d	2.04	0.0003

Different letters indicate significant difference at P = 0.05

Linear regression analysis of trap catches and total population of small hive beetles in the hive with the origin as the intercept were significant (F = 116.13, df = 1, 31, $P <$ 0.05, adjusted R = 0.7879) where R is the coefficient of determination. The intercept was set at the origin (x = 0, y = 0) because no beetles were expected to be trapped if there was none present in the hive (Figure 4.2). Consistent trapping using baited bottom board trap drastically reduced small hive beetle population in the hives over time, infact the trap nearly eliminated the small hive beetle population in the hives under study (Figure 4.3).

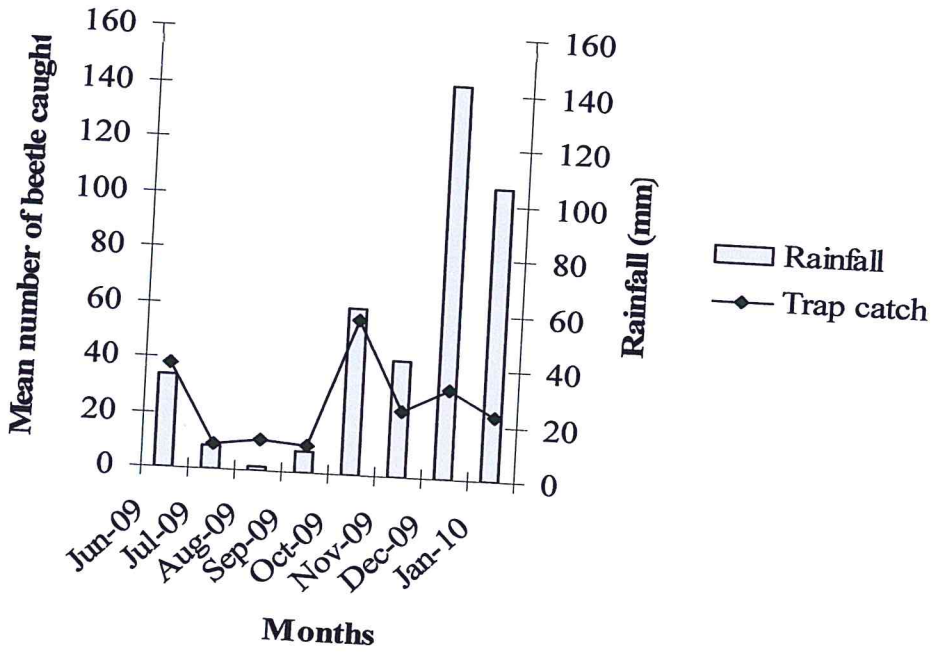


Figure 4.1: Relationship between mean number of beetle caught and rainfall

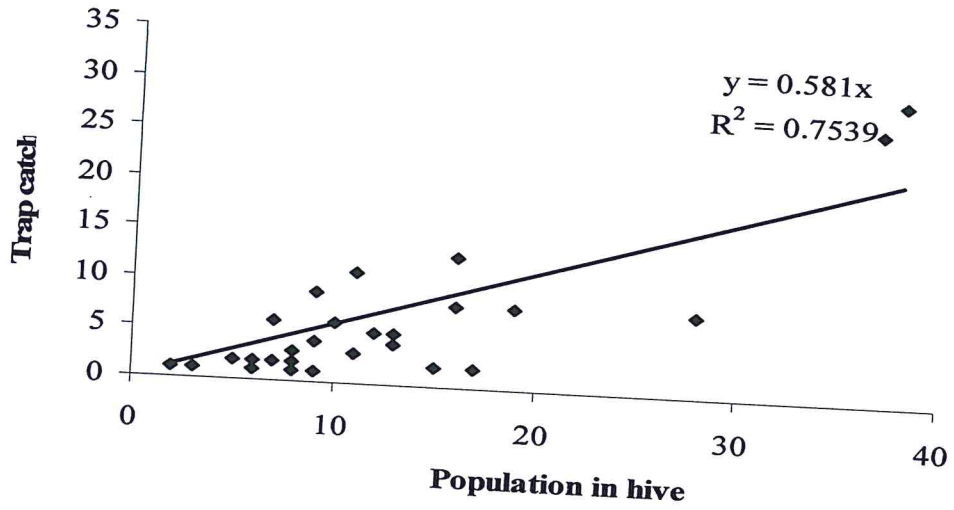


Figure 4.2: Correlation between total beetle population in hive and trap catch

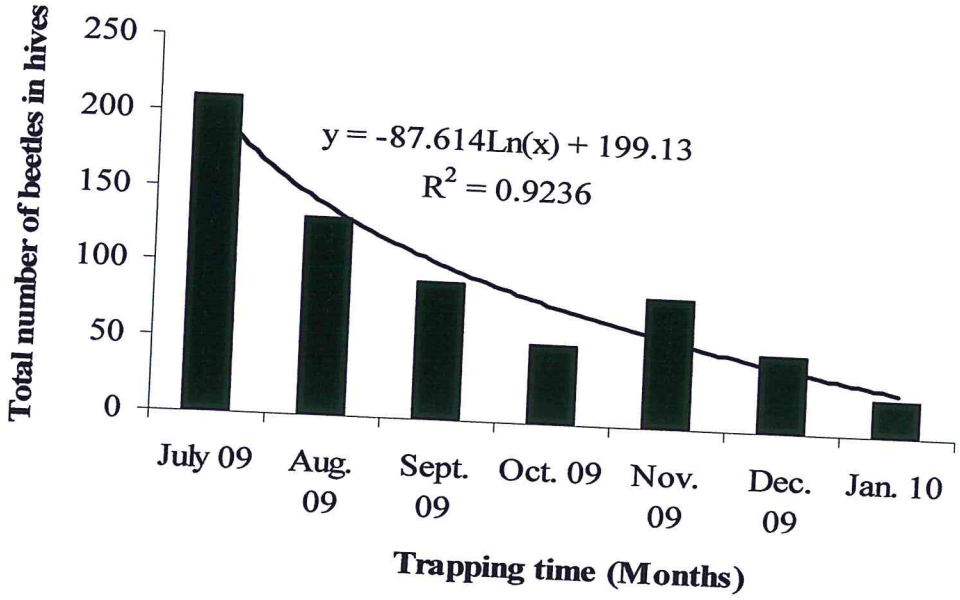


Figure 4.3: Trend of small hive beetle population in hives due to continued trapping using baited bottom board trap over time.

4.4 Discussion

The baited bottom board trap captured significantly more beetles than the unbaited control trap ($P < 0.05$). A total of 203 beetles were trapped by the baited traps while only five beetles were trapped by the control during the 32-week trapping period. These observations agree with other findings reported by Teal *et al.*, (2006); Torto *et al.*, (2007). This result showed that the bait made of inoculated pollen dough was an efficient lure for small hive beetles into the traps and could thus be used in monitoring the presence of small hive beetles in honeybee colonies.

The mean number of small hive beetles captured by the baited bottom board trap was significantly different between dry and rainy season, with the highest number of beetles being trapped during the rainy season, an indication that trap catch is influenced by rainfall. This observation concurs with findings of previous study by Torto *et al.*, (in press) where higher numbers of small hive beetle catches were recorded during the rainy season in a trapping experiment in Matuu, Kenya. This observation is of great significance since the beetles are known to multiply and increase in population when environmental conditions are favourable like adequate soil moisture (Lundie, 1940; Schmolke, 1974; Neumann and Elzen, 2004).

When the catches using the baited bottom board trap catch was related to the total population of small hive beetles in the hive in order to quantify its trapping efficacy, it was found out that the trap could catch on average 45.6% per trapping. However this again varied between the seasons with the highest percentage of 59.8% being recorded during the rainy season while in the dry season it was 31.3%. It was also evident for the current research that the trap could catch a higher percentage of beetles compared to the ones congregating at the bottom of the Langstroth hives especially during the rainy season. There was a strong correlation between the total beetle population and trap catch with a correlation coefficient of 0.89, an indication that the trap caught more small hive beetles in hives when the beetle infestation was high thus keeping the beetle population low in those hives.

The months of July to early October were dry and the small hive beetle population drastically reduced in the hives where traps were deployed. However, in the month of November, the small hive beetle population increased, and this could be attributed to the preceding favourable environmental conditions since it had started raining early in October but the presence of baited traps reduced the surging population. The data generated in the current study indicates that the baited bottom board trap is not only a good monitoring tool but also has a high potential in the control of *A. tumida* in well managed honey bee colonies.

4.5 Conclusion

Results from this study show that the yeast-inoculated pollen dough is an effective bait for luring small hive beetles into the traps. This is consistent with what was found out when the bait was used in apiaries in United States of America by Torto *et al.*, (2007). Further the results show that the baited bottom board trap is effective for eliminating more than 50% of the total population of small hive beetle in a managed honey bee colony per trapping period especially during the rainy season, of which it is suggested that it is during the rainy season when adult beetles have been reported to emerge from juveniles that have pupated in soil.

CHAPTER 5

COLLECTION AND IDENTIFICATION OF VOLATILES FROM POLLEN DOUGH AND HONEYBEES AND THE RESPONSE OF *Aethina tumida* TO THESE VOLATILES

5.1 Introduction

Adult small hive beetles are attracted to volatiles released by honeybees (Suazo *et al.*, 2003; Torto *et al.*, 2005) and that the beetles associate these volatiles with the presence of food in the hive. This is consistent with the fact that the beetles feed on pollen, honey and bee brood (Elzen *et al.*, 2000). The attraction is mediated by a blend of components dominated by the honeybee's alarm pheromones (Torto *et al.*, 2005), including isopentyl acetate (IPA), 2-heptanone and methyl benzoate. Acetaldehyde, ethanol, ethyl acetate and several ethyl esters have been identified in volatiles released by microbial cultures, and these volatiles either act alone as kairomones or synergize the activity of pheromones in various nitidulid species (Lin and Phelan, 1991; Bartelt *et al.*, 1993; Nout and Bartelt, 1998; Zilkowski *et al.*, 1999; Teal *et al.*, 2006). Small hive beetles, both from Africa and United States are vectors of a strain of yeast called *Kodamaea ohmeri*, which can cause fermentation of hive products such as pollen.

The attraction of nitidulids to volatiles released from fermenting food is well known (Phelan and Lin, 1991; Nout and Bartelt, 1998; Bartelt and Wicklow, 1999; Bartelt and Hossain, 2006; Torto *et al.*, 2007) and male-produced aggregation pheromones,

identified as part of these volatiles have been used to control some of these nitidulids (Torto *et al.*, 2007).

Small hive beetles show a stronger response to the volatiles of freshly-collected bee pollen (Suazo *et al.*, 2003) than those of commercially packaged bee pollen, alone or in combination with honey, as observed by Elzen *et al.* (1999) in a trapping study. Yeast-inoculated pollen has also been shown to attract small hive beetles (Torto *et al.*, 2007). However, whether worker honeybee volatiles attract more small hive beetles than yeast-inoculated pollen dough is unknown. The first aim of this experiment was therefore to determine which of these two sources of volatiles was more attractive to small hive beetles while the second aim was to collect and identify volatiles released by worker honeybees and yeast-inoculated pollen dough.

5.2 Materials and Methods

5.2.2 Response Aethina tumida to host odours

5.2.2.1 Test small hive beetles

Adult small hive beetles were collected from managed honeybee colonies maintained at the *icipe* apiary to start a laboratory colony. To ensure colony vigour, only small hive beetles from the first generation were used in bioassays while the rest of the colony was maintained for only four months and then restarted with freshly collected field small hive beetles for other experiments. Also, to ensure that the small hive beetles were virgins for use in assays, immature one-day adults were collected from the rearing soil,

sorted out into male and female and then put in different containers for each sex. The small hive beetles were reared on a pollen-honey diet (pollen dough) from Global Patties (Airdrie, Alberta, Canada), in a room maintained at 26 ± 2°C as described by Suazo *et al.*, (2003).

5.2.2.2. Odour sources

Two types of odour sources were tested: one consisted of worker honeybees (300 worker honeybees) and the other consisted of pollen, both yeast (*K. ohmeri*) inoculated and uninnoculatd pollen. Inoculated pollen dough was prepared by mixing yeast with distilled water and pollen dough in the ratio 1:100:1000 by weight and allowing the resulting dough to ferment for four days in a room maintained at 26 ± 2°C

5.2.2.3 Olfactometer bioassays

The response of one to two weeks old adult beetles (virgin males or females) to volatiles released by conditioned pollen dough and worker honeybees was tested by dual choice bioassays in a wind tunnel (1.33 x 0.31 x 0.30 m) (Torto *et al.*, 2010) (Figure 2.1). The beetles were deprived of food and water for one day prior to the bioassays. A stream of purified air was passed through each of the two glass tubes (30cm long x 5cm diameter) at a rate of 0.5L/minute and into the wind tunnel. One tube contained 10g of conditioned pollen dough and the other 10g of unconditioned pollen dough in the first set up. In the second set up, one tube contained 300 worker honeybees in a bee canister while the

other one contained air (empty glass tube) while in the third set up, one tube contained 300 worker honeybees in a bee canister while the other one contained 10g of conditioned pollen dough .

Twenty five virgin males or females (1-2 weeks old) were released each at a time into the wind tunnel through the release hole, observed for 10 minutes and then the side to which the beetle had moved was recorded. Each small hive beetle response was treated as a replicate. To avoid positional bias, the glass tubes were interchanged in between the experimental time with fresh air being passed over each arm of olfactometer while being pulled by a vacuum line powered fan at the centre to clean any residue odours for 15 minutes before the changeover of the glass tubes containing odor sources. A fresh sample for conditioned pollen and honeybees was used for each sex.

5.2.2 Volatile collection

Volatiles were collected from conditioned pollen dough and worker honeybee (approximately 300 worker honeybees). Three replicates of each source of volatiles were collected using a volatile collection system (Plate 2.3). This involved passing charcoal-filtered and humidified air at 0.35L/minute over 10g of pollen dough, 300 worker honeybees and through a Super Q adsorbent trap (30mg, Alltech, Nicholasville, Kentucky) separately for each replicate, for three hours at room temperature. Each filter was eluted with 200µl of GC/GC-MS-grade dichloromethane (Sigma-Aldrich,

Gillingham-Dorset, England), into a vial placed under ice. For analysis, 869ng of internal standard (butyl butyrate) were added to 40µl of each volatile extract and 1µl was injected into GC-MS (Plate 2.4) for analysis. The rest of the sample was stored at -40°C for further analysis. Mass spectral data for each of the sample was collected and the components identified by comparing the mass spectral data with those in the library of mass spectrometer and by retention time analysis using authentic standards.

5.3 Data analysis

For comparison of beetle responses to different odors, the proportion of beetles of both sexes responding to the different odor sources were subjected to a one -sample $\chi 2$ test at $\alpha = 0.05$ with H_0: the proportions of beetles responding to odor sources were not different.

GC-MS analyses of the volatile extracts were carried out on an HP-7890 coupled to an HP-5973 mass spectrometer (EI, 70eV, Agilent, Palo Alto, California, USA) equipped with an HP-1column (30m x 0.25mm ID x 0.25 µm) (Agilent, Palo Alto, California, USA), (Plate 2.4), where the oven temperature was held at 35°C for five minutes, then programmed to increase at 10°C/minute to 220°C and held at this temperature for five minutes. For quantification, 869ng of internal standard (butyl butyrate) were added to 40µl of each volatile extract and 1µl was analyzed. The components of the volatiles were identified by comparing their mass spectral data with those in the library of mass spectrometer and by retention time analysis using authentic standards.

5.4 Results

5.4.1 Response of small hive beetles to volatiles

Dual choice olfactometer bioassays showed that in general both sexes of small hive beetles were strongly attracted to yeast-inoculated pollen dough and worker honey bee volatiles compared to their controls. 72% males and 74% females were attracted to the worker honeybee odour while 28% males and 26% females responded to the control. Similar results were obtained when yeast-inoculated pollen dough was tested against control with 80% males and 84% females being attracted to yeast-inoculated pollen dough as compared to 20% of males and 16% of the females that were attracted by the non-inoculated pollen dough (Figure 5.1). When the yeast-inoculated pollen and honeybee odors were tested against each other, 60% of males and 64% of the females responded to the yeast-inoculated odors while 40% of the males and 36% of the females responded to the honeybee odors, (Figure 5.2). There were significant differences in the proportion of test beetles that responded to worker honey bee volatiles and to pollen dough inoculated with yeast compared to controls ($P < 0.05$, $\chi 2$ one sample test). However, the percentage of males and females responding to each of these odors were not significantly different ($P > 0.05$, $\chi 2$ one-sample test). When the beetle response between the volatiles of worker honey bee and yeast- inoculated pollen dough volatiles were compared, the proportion of beetles that responded to yeast-inoculated pollen dough volatiles was significantly higher than those that responded to worker honey bee volatiles response ($P < 0.05$, $\chi 2$ one-sample test).

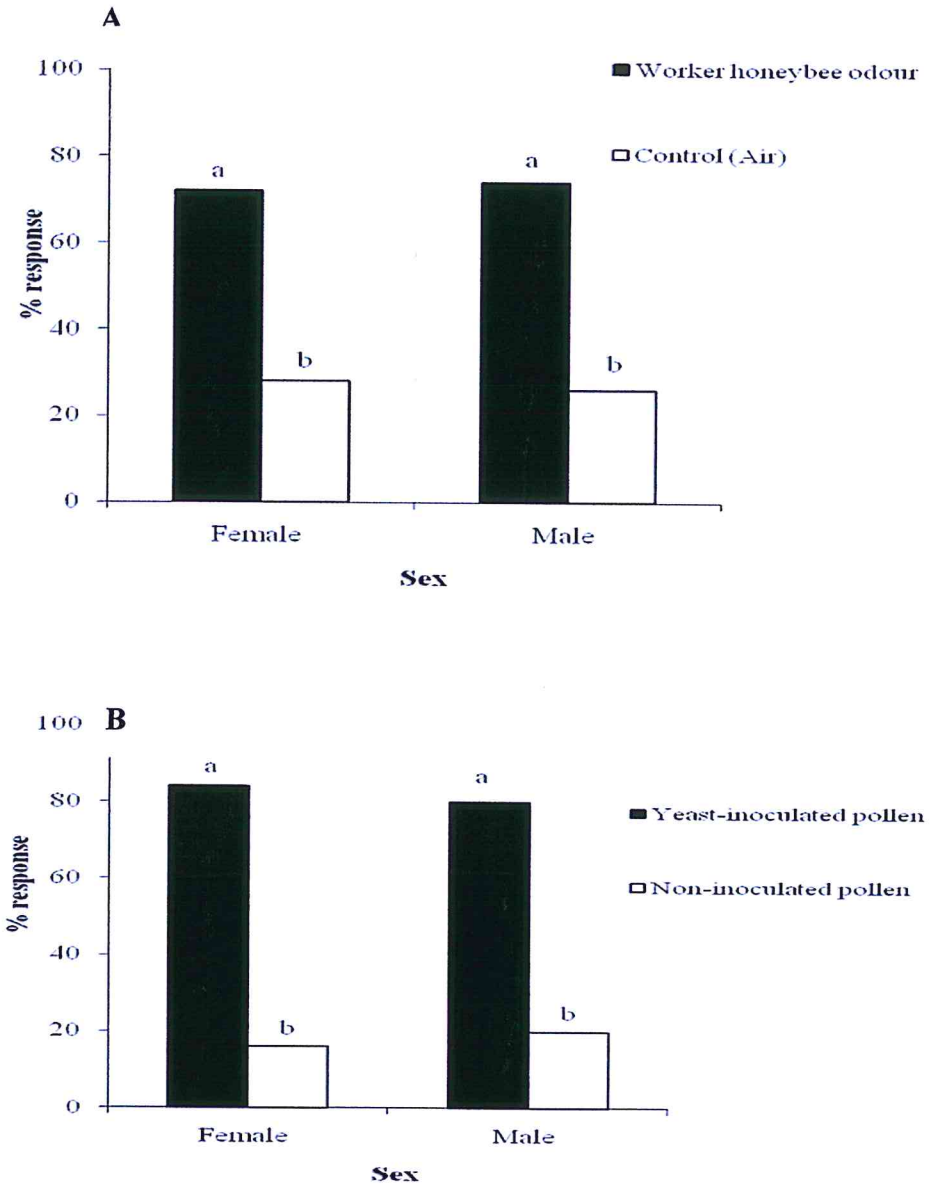


Figure 5.1: Responses of *Aethina tumida* to two hive odours. (A) Worker honey bee volatiles versus air, (B) yeast-inoculated pollen dough versus non-inoculated pollen dough. Pairs of bars with the same letter are not significantly different (chi-square one-sample test, $P < 0.05$).

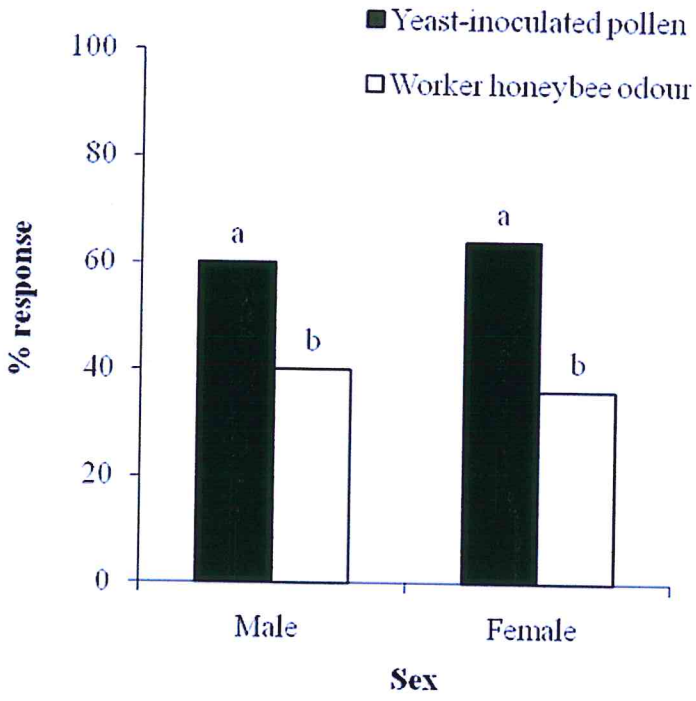


Figure 5.2: Responses of *Aethina tumida* to yeast-inoculated pollen versus worker honeybee odour. Pairs of bars with the same letter are not significantly different (chi-square one-sample test, $P < 0.05$).

5.4.2 Identification of volatiles

Several components mainly alcohols, aldehydes and ethyl esters were identified both in the worker honeybee and yeast-inoculated pollen dough volatiles GC-MS analysis (Figures 5.3 and 5.4). isopentyl formate was identified in both worker honeybee and yeast-inoculated pollen dough. However, the yeast inoculated pollen dough volatiles were compositionally and quantitatively richer than the honeybee volatiles (Tables 5.1

and 5.2). Noticeably, the inoculated pollen dough volatiles produced isopentyl alcohol and 2-phenyl ethyl alcohol which had large peak areas which were all absent in the worker honeybee volatiles.

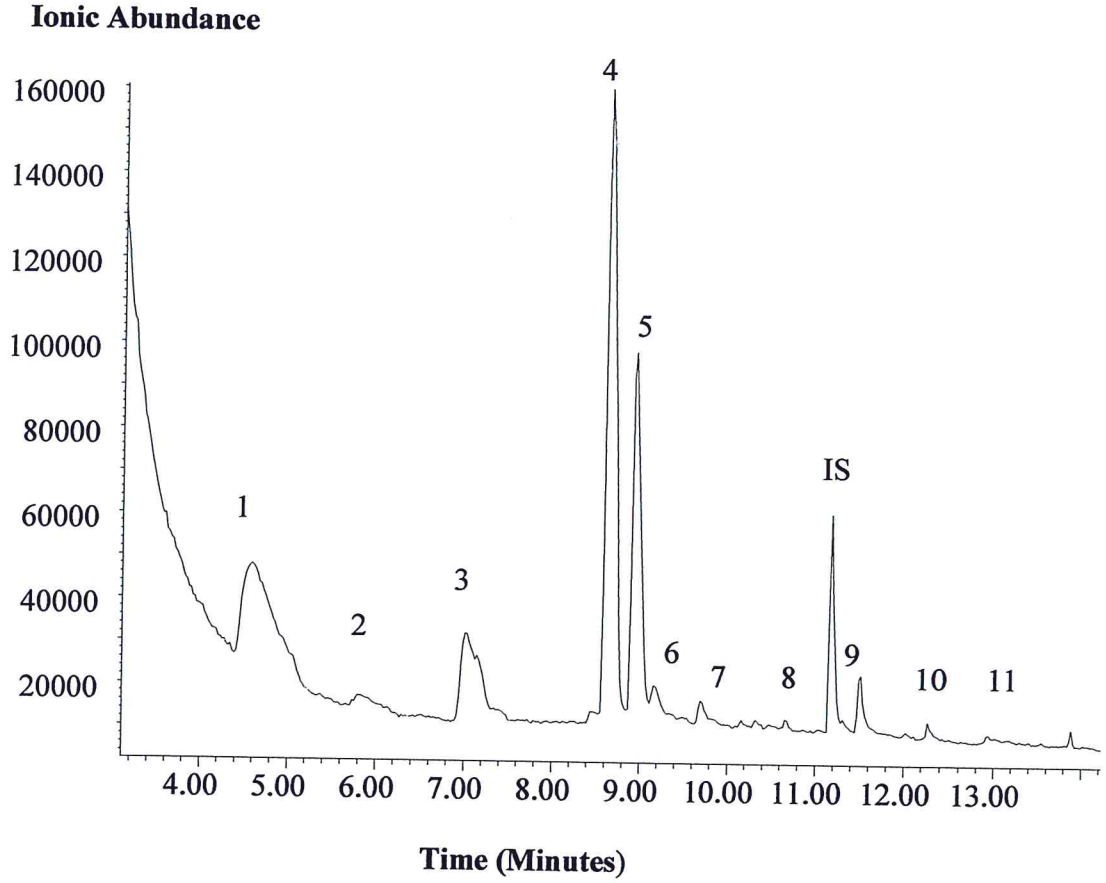


Figure 5.3: Total ion chromatograph showing GC-MS profiles of honey bee volatiles

IS = Internal standard (Butyl butyrate)

Table 5.1: Compounds from worker honeybee volatiles

Peak number	Retention time	Name of compound
1	4.597	Isopentyl formate
2	5.819	2-methylpropyl ester
3	7.027	Butyl acetate
4	8.634	Isopentyl acetate
5	8.937	2-Heptanone
6	9.176	2-Heptanol
7	9.710	2-Penten-1-ol acetate
8	10.676	1-Butanol, 3-methyl propanoate
9	11.509	Hexyl acetate
10	12.285	2-ethylhexyl ester
11	12.944	2-nonanone
IS (Internal Standard)	11,226	Butyl butyrate

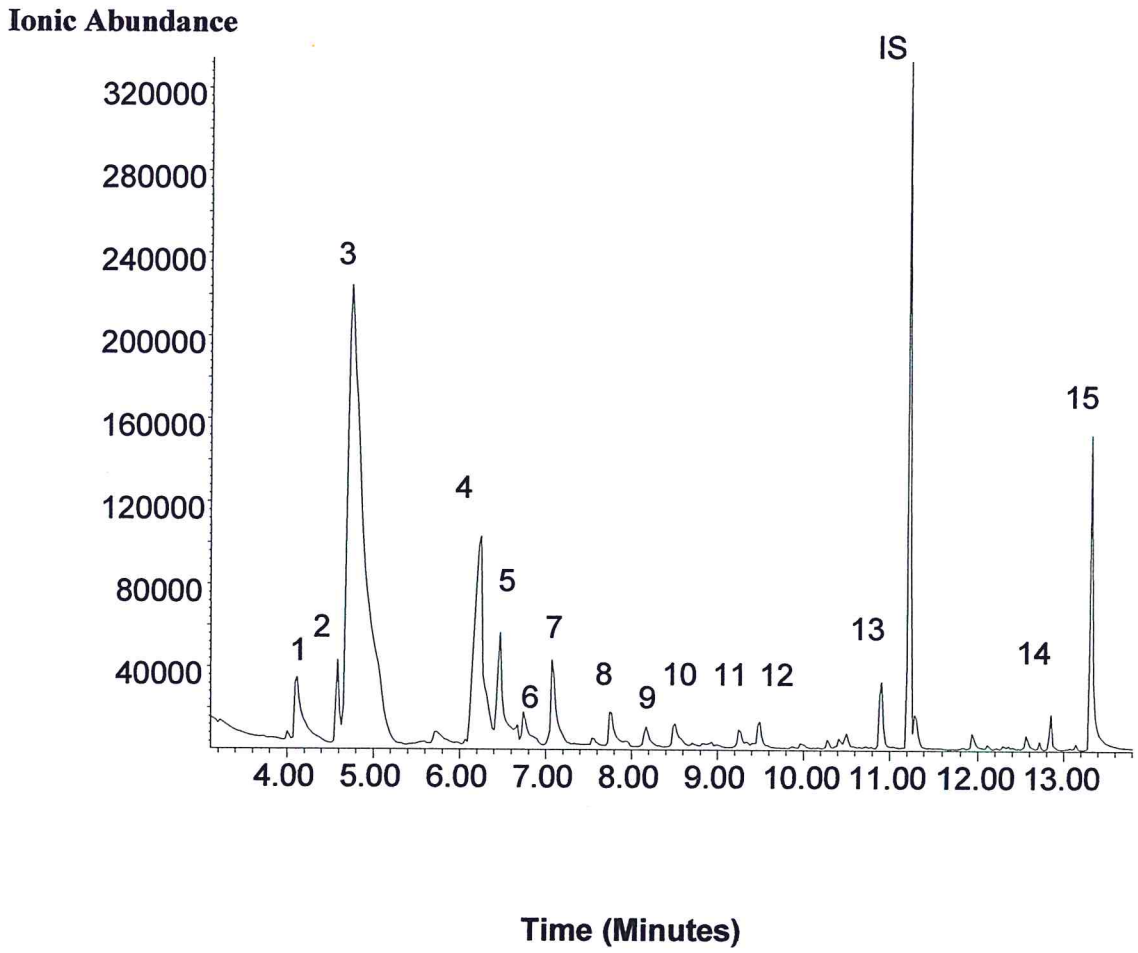


Figure 5.4: Total ion chromatograph showing GC-MS profiles of yeast inoculated pollen dough volatiles

IS = Internal standard (Butyl butyrate)

Table 5.2: Compounds from yeast inoculated pollen dough volatiles

Peak number	Retention time	Name of compound
1	4.121	3-Hydroxy-2-butanone
2	4.597	Isopentyl formate
3	4.754	Isopentyl alcohol
4	6.238	1, 3-Butanediol
5	6.474	2, 3 Butanediol
6	6.753	Ethyl butanpoate
7	7.09	Propanoic acid
8	7.768	3-ethoxy-1-propanol
9	8.181	Hexanoic acid
10	8.511	Hexanol
11	9.25	Ethyl pentanoate
12	9.494	Butyrolactone
13	10.906	Dec-1-en-ol
14	13.14	Nonanal
IS (Internal Standard)	11.226	Butyl butyrate

5.4 Discussion

There were significant differences in the proportion of test beetles that responded to worker honey bee volatiles and to pollen dough inoculated with yeast compared to controls ($P < 0.001$, $\chi 2$ one sample test). However, the percentage of males and females responding to each of these odors were not significantly different ($P > 0.05$, $\chi 2$ one-sample test). These results concur with previous findings by Wenning, (2001) that adult small hive beetles use host odor cues to find their host, honeybee colonies and wind tunnel bioassay experiments by Suazo *et al.*, (2003); Torto *et al.*, (2010), that found that small hive beetles were attracted to pollen dough and honeybee volatiles.

When the small hive beetle response between the volatiles of worker honey bee and yeast-inoculated pollen dough volatiles were compared, the proportion of small hive beetles that responded to yeast-inoculated pollen dough volatiles (60% for yeast-inoculated pollen dough and 40% for the honeybee) was significantly higher compared to honeybee volatiles response ($P < 0.05$, $\chi 2$ one-sample test). This explains why the bait, yeast-inoculated pollen dough, was able to attract more small hive beetles in baited bottom board trap in the presence of the honeybee colonies. This observation also explains why the baited bottom board trap could not eliminate all the small hive beetles in honeybee colonies at once. The trap required consistent deployment of the trap over a period of four months to almost eliminate the beetles in the honeybee colonies that were under study in the trapping experiment described in the previous chapter.

Significantly a large peak area of the alarm honeybee pheromone was detected in honeybee volatiles as compared to trace amounts that were found in the inoculated pollen dough. These results tally with previous studies on volatiles from honeybees by Suazo *et al.*, (2003) and Torto *et al.*, (2005) where a blend of components dominated by the honeybee's alarm pheromones, including isopentyl acetate (IPA), 2-heptanone and methyl benzoate were identified. Acetaldehyde, ethanol, ethyl acetate and several ethyl esters have been identified in volatiles released by microbial cultures (Lin and Phelan, 1991; Bartelt *et al.*, 1993; Nout and Bartelt, 1998). This concurs with the results that yeast-inoculated pollen produced more components compared to those produced by the honeybee volatiles meaning that growth of the yeast on the pollen dough caused fermentation of the pollen dough resulting into release of several fermentation-related compounds. Noticeably, the inoculated pollen produced isopentyl alcohol and 2-phenyl ethyl alcohol in relatively higher peak areas which were all absent in the honeybee volatiles. These results imply that the yeast-inoculated pollen dough is a better attractant to small hive beetles in honeybee colonies.

5.6 Conclusion

Results from this experiment show that small hive beetles are attracted to both honey bee and yeast-inoculated pollen dough volatiles and that there is no significant difference in attraction between the sexes. When the two volatile sources were compared, yeast-inoculated pollen dough attracted 20% more beetles in relation to those attracted by the honey bee volatiles. Both honey bee and yeast-inoculated pollen dough

volatiles produced similar compounds such as 3-hydroxy-2-butanone, isopentyl acetate and nonanal. However, different compounds such as isopentyl alcohol, 2-phenyl ethyl alcohol and some organic acids like propanoic acid, hexanoic acid which could have accounted for the 20% more attraction of small hive beetles to the yeast-inoculated pollen dough were produced by the yeast-inoculated pollen dough only.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussions

Small hive beetle, *Aethina tumida* has spread worldwide from its native home range of Africa and has become a devastating pest of both the managed honey bee colonies as well as the wild bees (Neumann and Elzen, 2004). Due to the invasive status of *A. tumida*, it has attracted a lot of attention from bee researchers who have made tremendous efforts in understanding various aspects of its biology (Elzen *et al.*, 2002; Neuman and Elzen, 2004; Ellis *et al.*, 2004; Suazo *et al.*, 2003; Torto *et al.*, 2005), and to develop management tools to curb its damage (Ellis *et al.*, 2003; Hood and Miller, 2005; Arbogast *et al.*, 2009; Torto *et al.*, 2010).

Results from this study showed that small hive beetles are confined to the bottom part of Langstroth hives where there are abundant food resources from pollen droppings coupled with the beetle's scavenging behaviour and its preference to dark areas as described by Lundie (1940) and the honeybee's defensive behaviour especially the African honeybee (Lundie, 1940; Schmolke, 1974; Neumann and Elzen, 2004; Torto *et al.*, 2007). However, when the honeybee colonies are disturbed the small hive beetles tend to scatter over in the other hive sections, an indication that if maximum trap captures are desired when using baited bottom board traps, colony disturbance through routine opening of the hives should be avoided once the traps are deployed. It was also

revealed that small hive beetles confine themselves at the bottom board part in a week's time after disturbance.

Yeast (*K. ohmeri*)-inoculated pollen dough was found to be an effective attractant of small hive beetles and thus it could be utilized in bottom board traps for monitoring and trapping small hive beetles in managed honeybee colonies. The strong positive correlation between the trap catch and rainfall as well as between the total in-hive beetle populations indicates that the baited trap is a useful tool for reducing the beetle population in highly infested honeybee colonies. The attractiveness of the yeast-inoculated pollen dough can be attributed to several compounds that were found in the inoculated pollen dough volatiles including aldehydes, alcohols, acetates and several ethyl esters similar to those which have been identified in volatiles released by microbial cultures and have been known to either act alone as kairomones or synergize the activity of pheromones in various nitidulid species (Lin *et al.*, 1992; Teal *et al.*, 2006).

6.2 Conclusions

1. Most *A. tumida* congregates at the bottom board as compared to the other sections of Langstroth hive.

2. Yeast (*K. ohmeri*)-inoculated pollen dough as well as worker honey bee volatiles produced compounds which comprised of fermentation-related products, floral volatiles and honey bee alarm pheromone and their mimics which are known to attract nitidulids,

with the yeast-inoculated pollen being richer in composition compared to the worker honey bee volatiles.

3. In olfactometer assays, the beetles were attracted both to the yeast-inoculated pollen dough volatiles and honey bee volatiles with more beetles being significantly attracted to the inoculated pollen dough volatiles demonstrating the effectiveness of yeast-inoculated pollen dough as an effective lure for small hive beetles. Trapping of *A. tumida* using baited bottom board trap nearly eliminated *A. tumida* in hives under trapping over the 32-week trapping period with the trap capturing over 50% percent of total small hive beetle population especially in the wet season. This demonstrates the potential of odor-baited bottom board trap not only as a monitoring tool but also for management of the small hive beetle. However, for effective management of the beetle, the trap has to be deployed consistently over several weeks.

6.3 Recommendations

6.3.1 Recommendations for honeybee farmer

1. Honeybee farmers can make use of yeast-inoculated bait in bottom board trap to monitor the small hive beetle infestation in their colonies and can deploy consistent trapping to manage the beetles in heavily infested honeybee colonies
2. Baited traps can be installed in weak and heavily infested colonies in apiaries that will act as a bait and attract the beetles outside the hives since the yeast-

inoculated pollen dough is more attractive to small hive beetles than worker honeybee volatiles

6.3.2 Recommendations for further studies

1. There is need for further trapping experiments especially in areas that experience different weather conditions from the weather conditions of the study site to compare the trap efficiency under different environmental conditions.

2. There is need for the development of better bait other than yeast-inoculated pollen dough that can attract small hive beetles more effectively in managed honeybee colonies.

3. Further studies should be carried out on isopentyl alcohol and 2-phenyl ethyl alcohols among other compounds produced in large quantities in yeast-inoculated pollen dough volatiles to establish their behavioural-activity to hive beetles which could provide methods for developing synthetic lures as an alternative to the use of yeast-inoculated pollen dough for management of hive beetles.

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