The possible role of stingless bees in the spread of Banana Xanthomonas Wilt in Uganda and the nesting biology of *Plebeina hildebrandti* and *Hypotrigona gribodoi* (Hymenoptera-Apidae-Meliponini)

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Dedication

To all banana farmers in Uganda

Abstract

In Uganda stingless bees were speculated to be the primary vectors of *Xanthomonas campestris pv. musacearum (Xcm)*, the causal agent of banana xanthomonas wilt which emerged in year 2001. *Xanthomonas* is speculated to enter the banana plant through moist scars of recently dehisced male flowers and floral bracts. However, no study had been done to support the hypothesis that stingless bees were the primary vectors. We therefore determined the probable role of stingless bees in the spread of *Xanthomonas* in Uganda. As there was no selective culture medium for *Xcm*, we applied indirect approaches to test whether stingless bees would get in contact and carry the bacteria during foraging. We documented the foraging behavior of colonies in wooden observation hives and in banana farms. We tested whether workers of *Hypotrigona gribodoi* Magretti and *Plebeina hildebrandti* Friese would take up banana sap, bacterial ooze and nectar offered at the nest entrance. Nectar of banana flowers has low sugar content. Therefore we investigated if *P. hildebrandti* workers would recruit nest mates to experimental feeders with 11%, 33%, 48% and 54% sugar solution. We further determined the distance the bees would fly to collect such solutions. In addition we documented nesting sites of stingless bees in the banana farms. The nest architecture of two most common species; *H. gribodoi* and *P. hildebrandti* was described.

We recorded four species of stingless bees, *Hypotrigona gribodoi*, *Plebeina hildebrandti*, *Meliponula ferruginea* Lepeletier and *Meliponula sp*. in the banana farms. They nested in termite mounds, cavities in trees, houses and other man made structures. The four species collected nectar from both male and female banana flowers. They did not collect banana and bacterial ooze from scars of recently dehisced male flowers and at the nest entrance. Pisang Awak, the banana variety which is most susceptible to *Xcm*, had mean nectar sugar concentration of 12.5%. The foraging distance of workers of *P. hildebrandti* decreased with decrease in sugar concentration. When 11% sugar solution was offered the bees stopped foraging at 1050m and for 33% sugar at 1215m. The foragers would therefore fly less than 1215m from the nest to collect Pisang Awak nectar.

If *Xcm* is present in nectar and incase the bees got accidentally contaminated with it, they would spread it over short distances. There is therefore need for studies on possible long distance vectors.

Kurzfassung

Stachellose Bienen wurden in Uganda als primäre Überträger von Xanthomonas pv. Musacearum (Xcm) vermutet, dem Verursacher der Bananen-Welke, die im Jahr 2001 auftauchte. Es wird vemutet, dass Xanthomonas durch feuchte Narben von kürzlich aufgeplatzten männlichen Blüten und Brakteen in die Bananenpflanze eintritt. Jedoch wurde bisher keine Studie durchgeführt um die Hypothese zu stützen, dass stachellose Bienen die primären Vektoren sind. Daher untersuchten wir die mögliche Rolle der stachellosen Bienen bei der Verbreitung von Xanthomonas in Uganda.

Da es kein selektives Kulturmedium für Xcm gab, wandten wir indirekte Methoden an um zu testen, ob stachellose Bienen bei ihren Sammlungsflügen in Berührung mit dem Bakterium kommen und es weitertransportieren. Wir dokumentierten das Verhalten der Völker in hölzernen Beobachtungs-Stöcken und ihr Sammlungsverhalten in Bananen-Plantagen. Wir testeten ob Arbeiterinnen von Hypotrigona gribodoi und Plebeina hildebrandti von Saft, Harz und Nektar angezogen werden und diese aufnehmen, wenn sie am Eingang des Beobachtungs-Stocks angeboten werden. Der Nektar der Bananenblüte hat einen geringen Zuckergehalt. Daher untersuchten wir ob Arbeiterinnen von P. hildebrandti andere Arbeiterinnen aus dem Nest zu experimentellen Futterstellen mit 11%, 33 %, 48 % und 54 % Zurckerlösung führt. Weiterhin bestimmten wir die Distanz, die Bienen fliegen würden (Sammelradius) um solche Lösungen zu sammeln.

In den Bananen-Plantagen wurden vier Arten der Stachellosen Bienen nachgewiesen: Hypotrigona gribodoi (Magretti), Plebeina hildebrandti (Friese), Melipona ferruginea (Lepeletier) und Meliponula sp.. Sie bewohnten Hohlräume in Bäumen, anthropogene Strukturen und Termiten-Bauten. Unsere Ergebnisse zeigen, dass alle diese vier Arten Nektar sowohl von männlichen als auch von weiblichen Bananenblüten sammeln. Die Bananensorte Pisang Awak ist am anfälligsten für Xcm. Sie hatte Nektar mit einem mittleren Zuckergehalt von 12.5 % (von 2 – 32 %). Der Sammelradius der Arbeiterinnen von P. hildebrandti verringerte sich mit einer Abnahme der Zuckerkonzentration. Als 11 %ige Zuckerlösung angeboten wurde, hörten die Bienen bei 1050 m Entfernung auf zu sammeln, bei 33 %iger Zuckerlösung bei 1215 m, bei 48 % bei 1220 m und bei 54 % bei

1230 m. Die Sammlerinnen würden daher weniger als 1215 Meter vom Nest fliegen um den Nektar der Sorte Pisang Awak zu sammeln.

Falls Xcm im Nektar vorkommt und im Falle, dass die Bienen versehentlich damit kontaminiert werden, verbreiten sie es wahrscheinlich über kurze Entfernungen und Sammlerinnen könnten es ins Nest eintragen. Durch eine alte Beziehung zwischen stachellosen Bienen und Bacillus-Arten, die antimikrobielle Stoffe produzieren, die andere Mikroorganismen in ihrem Wachstum hemmen, und durch die Anwesenheit von Propolis, welches antimikrobielle Stoffe enthält, wird Xcm erwartungsgemäß im Nest der stachellosen Bienen eliminiert. Desweiteren zeigte ein vorangegangener Test, dass Xcm nicht auf Propolis aus dem Nest von P. hildebrandti wachsen konnte.

Bemerkenswert ist, dass die Bienen niemals Saft oder Harz, der am Nesteingang angeboten wurde, sammelten; weder von gesunden noch von Bananen, die mit Xanthomonas infiziert waren. Falls sich Xcm durch den Nektar verbreiten kann, sind sowohl männliche als auch weibliche Blüten mögliche Eintrittspforten, falls die Verbreitung aber durch Narben erfolgt, die durch das Abfallen der männlichen Blüten und Brakteen entstehen, sind stachellose Bienen wahrscheinlich keine Überträger, da sie keinen Saft von den Narben sammelten.

Darüberhinaus besteht die Notwendigkeit weiterer Untersuchungen zu möglichen Langstrecken-Überträgern

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GENERAL INTRODUCTION

1. Bananas

1.1 What are Bananas?

Banana is a term used to refer to species and hybrids in the genus *Musa*, family Musaceae. Within the Musaceae there are two genera, *Musa* and *Ensete* (Simmonds 1966; Cobley & Steele 1976). The genus *Musa* contains 30-40 species and is further divided into five series. Most edible bananas usually seedless, originated from wild banana types; mainly from *Musa acuminata*, *M. balbisiana* and a hybrid *M. paradisica*. The edible bananas are classified according to their genotypes into groups, subgroups and clones.

1.2 Structure of the banana plant

Pseudostems

Banana plants which are wrongly referred to as trees are large herbs 2-9 metres. The pseudostem, which arises from a fleshy corm, is succulent and juicy made of leaf-petiole sheaths. Young pseudostems (suckers) grow to form a clump around the oldest sucker (mother sucker). They replace the mother sucker when it fruits and dies (Morton 1987)

Leaves

Bananas have about 4-15 fleshy leaves which are long, smooth, and oblong. They are arranged spirally around the pseudostem. They unfold as the plant grows normally at one leaf per week. Due to their tenderness, the leaves are always prone to shredding by the wind (Morton 1987).

Inflorescence

It is a transformed growing point which shoots out from the heart in the tip of the pseudostem. It's a purple bud which is initially long, oval and tapering and facing to the skies. The bud is composed of purple bracts (underside of the bracts is deep-red) beneath which the flowers are located. Once the bracts open, tubular white flower, clustered in whorled double rows along the floral stalk are exposed (Figure1).

Each cluster is covered by a bract. Normally the bract lifts and rolls from the first cluster of flowers in three to 10 days after emergence of the flower bud. Female flowers are the first to be exposed (Figure 1a and 2a). They occupy the lower three to 15 rows. Above them are a few rows of hermaphroditic flowers. Rows of male flowers follow the hermaphroditic rows (Figure 3). After opening of male flowers, it takes about one day before both the flowers and the bracts fall. This leaves the floral stalk bare, except at the tip where unopened bud remains containing the last formed of the male flower (Figure 1b and 2b). Some banana varieties have persistent male flowers and bracts which wither and remain filling the space between the fruits and the terminal bud (Morton 1987).

Fruits

They look like slender green fingers, hence referred to as fingers. As the fruits develop, the bracts are shed off and the fully grown fruits in each cluster become a hand of bananas (Figure 3). At this point the stalk droops with the weight until the bunch is upside down. Small (fruits) fingers form from the hermaphroditic flower but they do not grow beyond the finger stage. Bracts fall while the small fingers remain attached to the floral stalk (Figure 3).

Seeds

The common cultivated bananas are seedless with minute ovules at the centre of the fruit. Occasional cross-pollination by wild types results in a number of seeds in a normally seedless variety. Wild banana types are seeded.

Pollination

Edible bananas do not require pollination. They reproduce asexually by suckers from corm. Wild banana types may or may not require pollination as they reproduce both sexually or asexually (Purseglove 1972; Stover & Simmonds 1987). In the wild bananas are pollinated by bats, birds and tree shrews (Nur 1976). Generally bananas produce large quantities of nectar (Figure 2).

Figure 1: Banana plants (a) showing bracts rolling up (i) to reveal female flowers (ii) (b) Showing a bare floral stalk with scars (i), bract rolling up to reveal male flowers (ii) and terminal bud (iii)

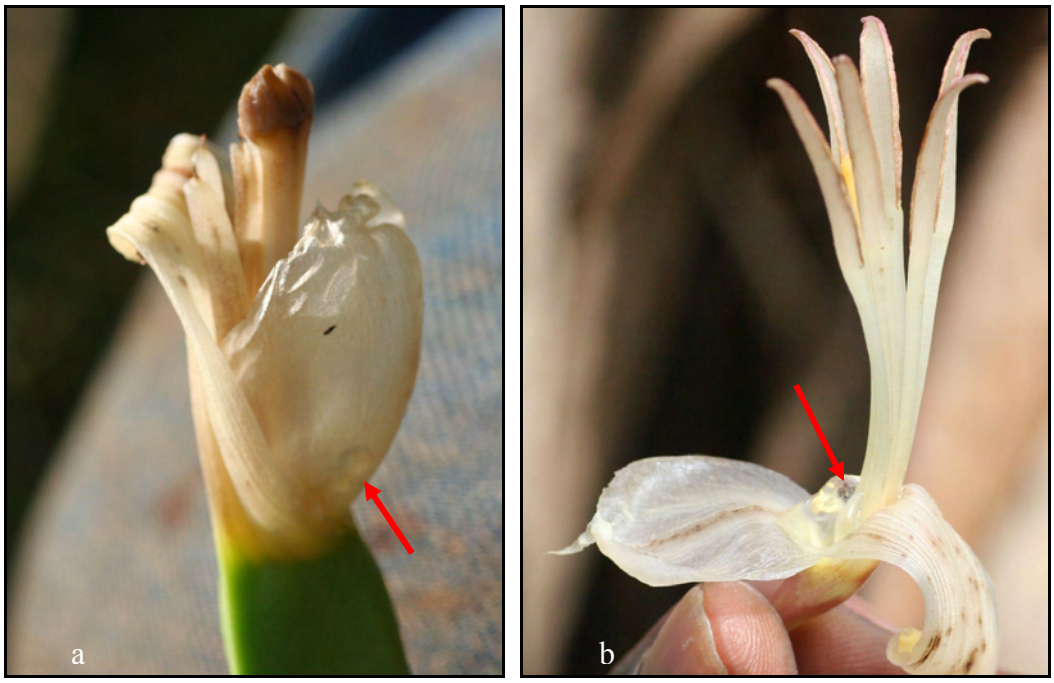

Figure 2: Banana flowers (a) female (b) male. Arrows in both flowers point to nectar

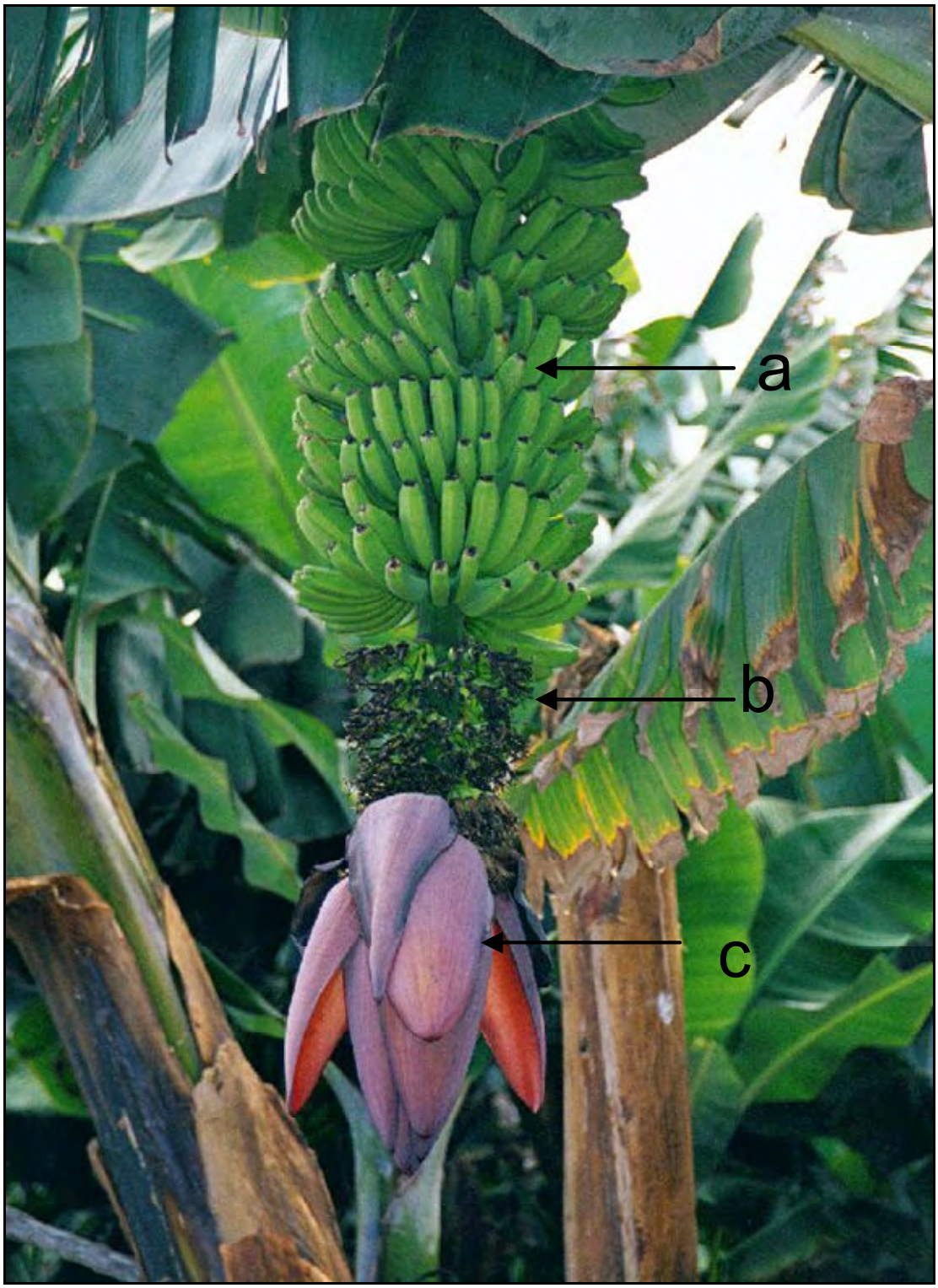

Figure 3: A banana plant showing (a) green fruits formed by female flowers (b) small never fully developed fingers formed by hermaphrodite flowers (c) purple bracts lifting to reveal male flowers

1.3 Distribution of bananas

Edible bananas are restricted to the tropics or near tropical regions. Bananas came originally from Southeast Asia, and then they spread out to the Indian Peninsula, Eastern Africa and the islands of the pacific. Today bananas are grown in every humid tropical region (Mexico, Panama, Jamaica, Ecuador, Colombia, West Africa, Central Africa, East Africa, India and China).

Ensete occurs wildly from West to Southern Africa. A few species are also found from northeast India to the Philippines and New Guinea. Enset (*Ensete ventricosum*) is cultivated in Ethiopia for fibre and for the stable food: flour derived from young shoot, base of the stem and corms (Lawrence 1951; Purseglove 1972; Morton 1987; Stover & Simmonds 1987).

1.4 Importance and uses of bananas

Bananas constitute the 4th largest fruit crop of the world after grapes, citrus and apples. They are 4th among the list of developing worlds most important food crops after rice, wheat and maize (Frison & Sharrock 1998). For developing countries, they are a precious source of revenue from both local and international trade.

Farmers in the tropics can intercrop bananas with legumes and can feed animals on by-products (peels and pseudostems) of the crops. The plant has been used for medicinal purposes (Wainwright 1953 in Karamura 1998), for celebrating marriage and for other rituals (Howes 1928 in Karamura 1998; Price 1994). Virtually all components of the plant have found use in the homesteads and many domestic industries like making baskets, carpets, shoes and a host of indoor decorations (Wainwright 1953 in Karamura 1998; Price 1994).

In Eastern Africa, cultivation of bananas has become woven into the socioeconomic life of the communities. The crop is a key component in both food security and agricultural sustainability of the region. It has an extended harvest period which ensures food and income throughout the year (Karamura 1992). Bananas reduce soil erosion on steep

slopes and are principal sources of mulch for maintaining and improving soil fertility (INIBAP 1986). This is because the giant herbs (2-9 metres high) with large leaves creates closed canopies which assist in arresting rain impaction and direct insolation, both of which are important in soil conservation (Karamura 1992). Rotten leaves and stems add organic matter and improve soil aeration. Environmentally, the banana is "a tropical forest" because once established, it enters a phase of continuous growth.

1.5 Production and consumption of bananas in Eastern and Southern Africa

About 26% of total world banana output is produced in the Eastern and Southern Africa. These regions are also the worlds leading consumer of bananas with an annual per capita consumption rate of 400-600kg (Karamura *et al*. 1998). Bananas are an all year round fruiting crop, and provide main staple food and source of income to millions of people living in the Great Lakes Zone of Uganda, Tanzania, Kenya, Rwanda and Burundi (Karamura *et al*. 1998; INIBAP 2004). Local trade is well developed although no export trade has developed. This is due to transportation problems and the fact that most of the export varieties selected for high rainfall, hot, humid conditions give poor results in the main growing areas of East Africa, which are cooler and drier (Acland 1971; Karamura 1998).

Green cooking bananas and table bananas are marketed for food while beer bananas are used for local brews. Beer bananas production is dominant in Rwanda and Burundi, though it's on the increase in Uganda and northern Tanzania where green cooking highland bananas have failed for various reasons (Karamura *et al*.1998). In Uganda the beer bananas (Pisang Awak) constitute 11% (Karamura *et al*. 1996) although it's increasingly replacing other types of bananas as its seen to be more hardy (Karamura 1998).

Banana production in Uganda

East Africa has a high diversity of bananas with Uganda on top with the highest level of cultivar diversity (Kyobe 1981). Varieties which are specially adapted to the highland conditions of Uganda "East African highland bananas" are widely cultivated (INPAB

2000). East African highland bananas constitute 85% of the bananas grown in Uganda, beer banana constitute 11%, dessert banana 3% and plantains 1% (Karamura *et al*. 1996). Bananas are grown as subsistence crops mostly mixed or intercropped with both perennial and annual crops (Karamura *et al*. 1998).

In the 1970s and 1980s, Uganda witnessed drastic banana yield declines in traditional growing areas of the east and central regions. Banana production declined from between 15-20 tonnes per acre to the current production of only 6 tonnes per acre and 9.5 million tones annually (Tushemereire 2004). Yield declines were due to population pressures, labour, competing activities, declining soil fertility, pests and diseases (Gold *et al*. 1993; Tushemereirwe 1996).

1.6 Diseases and pests of bananas

World wide, the main pests of bananas include nematodes, banana weevils, banana thrips and banana mites. The main diseases of bananas include Sigatoka, Panama disease, Moko disease, Black end, Cigar end, and Bunchy top disease.

In Uganda, weevils, nematodes, diseases like black Sigatoka and Fusarium wilt are a major constraint to indigenous and dominant highland cooking bananas (Tushemereirwe 1996). Fusarium wilt is also a major constraint to production of introduced banana cultivars. The wilt is prevalent in many other parts of Africa.

In 1993, symptoms similar to those of Fusarium wilt were observed in the Western Uganda highlands. The new disease affected all *Musa* cultivars grown in Uganda including plantains, which are normally not affected by Fusarium wilt (Kangire 1998; Kangire *et al*. 2000). The new disease and its causal agent were never identified (Gold *pers*. *com*.).

In 2001, a new disease, Banana Xanthomonas Wilt emerged in Central Uganda. This new disease is a subject of numerous investigations still going on to determine its origin and how to curb the spread.

1.6.1 Symptoms of Banana Xanthomonas Wilt (BXW)

BXW caused by *Xanthomonas campestris pv. musacearum*, was first reported in Uganda in year 2001. The first symptoms include discoloration at the tip of the flower and withering of the flower bracts. The banana bunch wilts and ripens before it's one month old. In heavily affected plants the male bud appears wilted and discolored. The discoloration on the male bud stalk progress from the base of the male flowers towards the bunch and there is cream colored ooze in the area close to the male bud. This suggests that the bacterium may be air or insect borne, although rain droplets and movement of planting material may also contribute to transmitting the infection (Kangire & Rutherford 2001; Tushemereirwe *et al*. 2003). The internal symptoms include discoloration of the corm of most affected plants, discolored central stalk that carriers the bunch, the banana fingers are stained reddish brown and the pulp is soft as when it is ripe, even when the bunch still need about one and half months to reach maturity (Kangire & Rutherford 2001; Tushemereirwe *et al*. 2003).

1.6.2 Distribution of Banana Xanthomonas Wilt in Uganda (BXW)

The disease emerged in Mukono district in central Uganda in year 2001. In January 2002, the disease was still restricted to a radius of five kilometer from the farm where the disease was first identified (Tushemereirwe *et al*. 2003). By June 2003, the disease had been reported in the districts of Kayunga, Lira, Apac and Kaberamaido (Figure 5) which are in northern and northeastern Uganda (Bao 2004; Tushemereirwe *et al*. 2003). The initial distribution was localized and patchy, but by year 2004, the disease was rapidly filling the gaps and moving southwards and westwards towards some of the most important banana growing areas (Eden-Green 2004). Since then the disease has spread into many other districts in Uganda and the neighboring countries (Figure 6) of Rwanda, Burundi, Tanzania and Kenya (Boa 2004).

Figure 5: Initial distribution of Banana Xanthomonas Wilt in Uganda (Boa 2004)

1.6.3 Banana Xanthomonas Wilt (BXW) in Democratic Republic of Congo (DRC)

Just like in Uganda, BXW emerged in DRC in year 2001. The symptoms and causal agent were like in Uganda, with the first symptoms always seen on flowers (Ndungo *et al*. 2004). However the spread in DRC was very slow compared to Uganda where the disease was spreading at a rate of 75km per year (Ndungo *et al*. 2004). In DRC infected flowers were much less common, leading to the suggestion that the mode of spread may have been different than the one in Uganda. It was thought that the disease in DRC may have originated just from within Congo; from wild enset plants (Ndungo *et al*. 2004).

1.6.4 Origin of Banana Xanthomonas Wilt in Uganda

The BXW affecting bananas in Uganda is speculated to have originated from Ethiopia (Figure 6). In Ethiopia the wilt caused by *Xanthomonas campestris pv*. *musacearum* was first reported in enset (*Ensete ventricosum*) by Yirgou and Bradbury (1968). Enset is a major source of food for the people and is grown widely in Southern Ethiopia for its pseudostem and corm which are used to make flour. Both cultivated and wild enset are affected by the bacterial wilt (Addies *et al*. 2004). As enset are cultivated for their pseudostems, they are not allowed to flower, hence insects transmission of the bacteria through flowers is not likely (Addies *et al*. 2004). Although there are bananas grown in Southern Ethiopia, no bacterial wilt had been reported in them (Addies *et al*. 2004). In South-Central Ethiopia, scattered mats of enset are present but no infections have been reported in bananas. However in western Ethiopia, it is reported that the wilt had been present in enset and bananas for about 20 years (Addies *et al*. 2004).

It has been argued that bananas grown in high altitude (over 1700m), and lower temperatures, such as in South-Central Ethiopia and North Kivu in DRC are not affected by the wilt, since high altitude and lower temperatures are not favorable to insect vectors. It has therefore been postulated that infections through male flowers in Uganda, is a primary cause of new infections since bananas are grown at altitudes of less than 1600m (Addies *et al*. 2004).

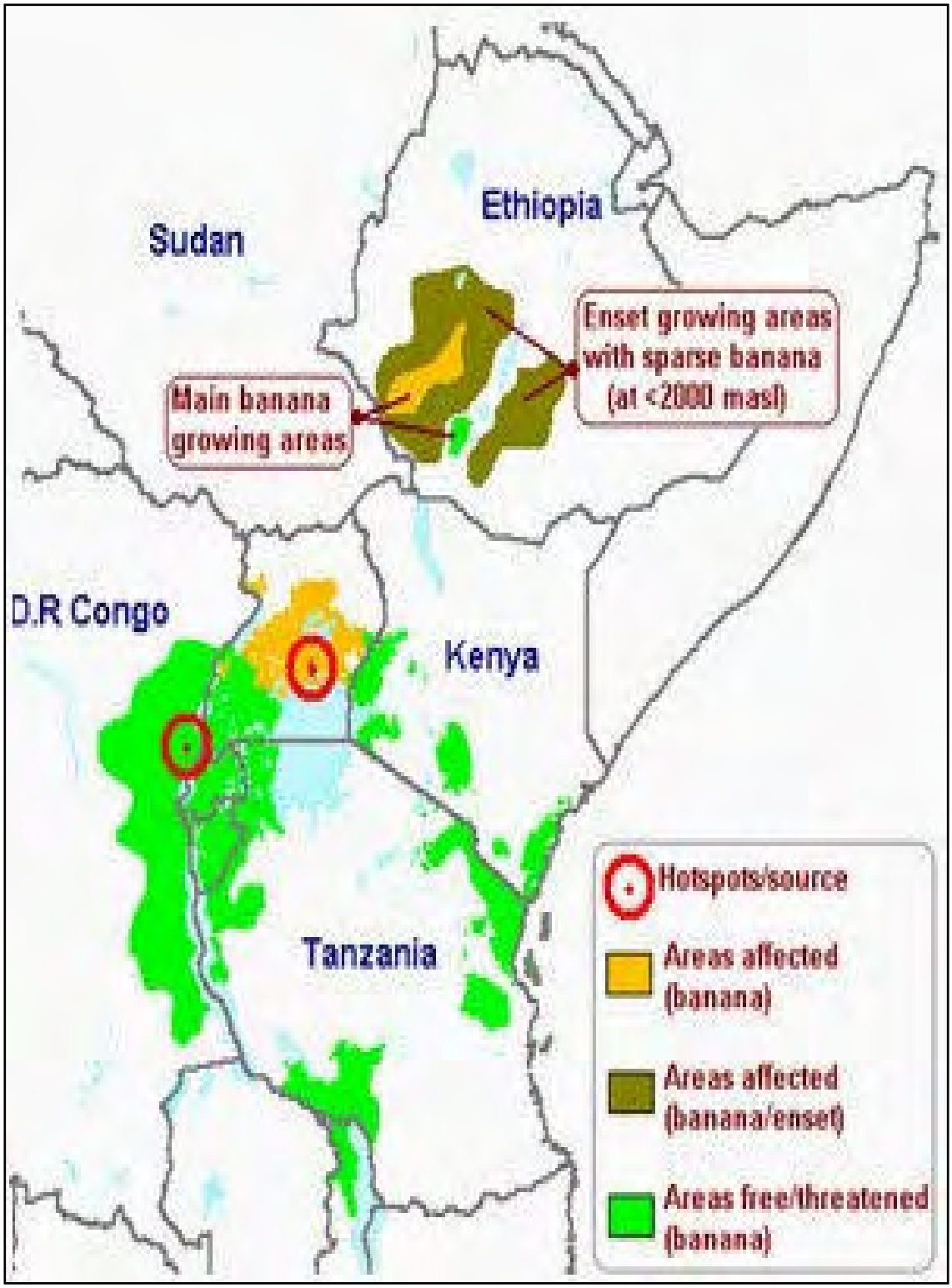

Figure 6: Origin and distribution of Banana Xanthomonas Wilt in Eastern Africa (Boa 2004)

1.6.5 Control of Banana Xanthomonas Wilt (BXW)

The control of BXW presents a unique demand since epidemiological studies such as transmission, insect vector, pathogen survival, factors that affect disease developments and even control measures are not readily available (Eden-Green 2004). The fact that its not yet known how the disease entered Uganda, in the affected areas of Mukono district, makes it difficult to come up with control measures. The disease was first reported in a plantation which was over seven years old suggesting that it had not been transmitted with planting materials. It is reported that the farmers with the wilt problem indicated that they had gotten their planting materials from local sources (Tushemereire *et al*. 2003).

Since BXW was reported to have many similarities to other banana bacterial wilts such as Moko, Blood and Bugtok diseases in other parts of the world, it was suggested that just like other wilt diseases, airborne infection via male flower parts, was the main mechanism driving the wilt epidemic in Uganda (Thwaites *et al*. 2000; Eden-Green 2004). It was speculated that infection via male flower parts was mainly through insects. In Uganda stingless bees were hypothesized to be the primary vectors (Gold *et al*. 2006; Tinzaara *et al*. 2006).

Removal of male flower buds as a measure of controlling the rapid spread of the disease to new areas through inflorescence infection was advocated (Eden-Green 2004). Removal of male buds had been effectively applied in Indonesia and Philippines in the control of Moko and Bugtok banana bacterial wilt diseases (Molina G.1996; Molina A. 1999). However there have been claims that the infection can occur through both the male and the female flowers. Other control measures advocated included: disinfection of tools and foot wear, no exchange of planting materials, uprooting and chopping into pieces all infected plant materials and burying them.

2. STINGLESS BEES

2.1 Introduction

Stingless bees are highly eusocial living in permanent colonies, composed of hundreds to thousands of individuals (Michener 1974). Due to their tropically confined distribution, the biology of stingless bees has been far less explored than that of honey bees. Most of the work done on biology of stingless bees has been reviewed by (Wilson 1971; Michener 1974; Sakagami 1982). Much of the biology is based on studies done in South America, Australia and Asia. Few studies have focused on the biology of stingless bees in Africa.

2.2 Stingless bee phylogeny and classification

There are different classifications of stingless bees by various authors (Michener 2007). The most widely used classification is by Michener (1990) and Carmargo and Pedro (1992). According to Michener's cladogram of genera of Meliponini, *Melipona* a Neotropical genus, is the sister group to all the other Meliponini. The African genera seem to have been derived from among New World genera. On the contrary Carmargo and Pedro cladogram shows a major division of Meliponini into African and non-African genera (Michener 2007).

2.3 Distribution of stingless bees

Stingless bees occur in the tropics and the subtropics around the world. To the South they extend up to 35^{o}S in Australia and South America, and 28^{o}S in Africa. To the north they extend to 23.5^{o}N (Michener 2007). The highest diversity of stingless bees is in tropical America, where they are also much studied compared to other parts of the world. There are 26 genera of stingless bees, with 15 genera belonging to the Neotropics, 3 to Asia and Sunda Island, 2 to Australia and New Guinea, 6 to Africa including Madagascar (Michener 2007). The six genera in Africa are: *Cleptotrigona*, *Liotrigona*, *Hypotrigona*, *Dactylurina*, *Meliponula* and *Plebeina* (Eardley 2004, Michener 2007). The number of stingless bee species in Africa remain unknown, with Kerr & Maule (1964; in Wilson 1971) noting 42 species while Eardley (2004) noted 19 species.

2.4 Nesting sites

Nesting sites in Stingless bees are species specific. Most Stingless bees species nest in pre-existing cavities in hollow trunks and branches of trees (e.g. *Hypotrigona,* Brasssindale 1955*)*, hollows between roots and other subterranean cavities (e.g. *Mourella,* Camargo and Wittmann 1989), deserted nests of ants or termites (e.g. *Axestotrigona*, Darchen 1971; *Paratrigona,* Vera Lucia 1972), cracks and holes in houses, or cavities of rocks (e.g. *Plebeia,* Wittmann 1989). Some species make exposed nests on tree branches, on walls or on cliff faces (Michener 2007).

2.5 Nest entrance and nest architecture

Nest entrances vary from species to species, ranging from simple holes to dome or trumpet shaped entrances. Generally there is concealment of entrance by stingless bees which nest in the soil as is the case of some African Meliponini (Brassindale 1955; Portugal-Araujo 1963; in Vera Lucia 1972).

Nests are constructed from a mixture of wax and propolis (resins and gums) called cerumen. Resin is a sticky material collected by stingless bees from plants used in nest construction (Ghilsalberti 1979). A basic meliponini nest consists of brood cells, compacted into combs or irregular clusters and storage pots for pollen and honey. The storage pots for honey and pollen may be the same shape or different. The brood cells may be surrounded by a soft sheath of cerumen (involucrum) while both the brood cells and the storage pots are surrounded by a hard protective layer of propolis called batumen (Wilson 1971; Michener 1974; Sakagami 1982). Brood cells are destroyed after use and cannot be reused as they are in *Apis*.

2.6 Nest defense

Stingless bees got their name from the fact that their stings are vestigial and cannot be used in defense. Ways through which the nest can be defended include: swarming over the intruder, biting using mandibles, ejection of a burning liquid from the mandibles camouflage of nesting sites, restricted nest entrances, walls of sticky resin around the

entrance tube, and positioning of guard bees in front of nest entrance (Wilson 1971; Michener 1974; Wittmann 1984).

2.7 Division of labor

The stingless bees have a well defined age dependent division of labor. The division of labor is correlated in part with exocrine gland activity and advanced systems of chemical alarm and recruitment communication. The recruitment is reinforced by a modulated sound signal similar to that found in the waggle dance of *Apis mellifera* (Wilson 1971). Normally a worker bee passes through four stages, callow, nursing, household and foraging, although there are variations in age ranges during which particular tasks are executed among different species (Sakagami 1982).

2.8 Provisioning and Oviposition

The process of provisioning and oviposition is highly sequential and complicated in stingless bees. The larval food is provisioned in mass before oviposition. The process of provisioning and oviposition in stingless bees has been studied in details by (Sakagami & Zucchi 1974; Sakagami 1982; Sakagami & Inoue 1990; Wittmann *et al*. 1991).

2.9 Foraging

In stingless bees colonies, foraging is mainly done by old workers. Once the workers have become foragers, they continue with this activity till death (Bego 1983). The patterns of foragers' visits and changes in standing crops of floral resources have been observed by various authors (Johnson & Hubbel 1975; 1974; Hubbel & Johnson 1978; 1977; Vorwohl 1979; Roubik 1978; 1979; 1980; 1981; 1982: In Inoue *et al*.1985) and Roubik & Aluja (1983). Foraging observations at nest entrance by Inoue *et al*. (1985), working on three species of *Trigona*, indicated that pollen, nectar and resin are the resources brought back by stingless bees to the nest.

Despite of all the above studies, there is still much to be learnt about stingless bees, compared to the wealth of knowledge available for honey bees, which is only one genus *Apis*. It's even more unfortunate that in some countries in Africa, there is very little

knowledge on the biology of stingless bees. Hence, the potential of utilizing stingless bees for income generation through honey, wax, propolis production, pollination, or their role in crop damage as pests or vectors of pathogens is far from being realized. Stingless bees collect resin and gums from plants which are mixed with wax for nest construction. Resin and gum collection may lead to injuries on plants creating entry points for pathogens.

Due to the implication that stingless bees are responsible for the BXW epidemic in Africa, there is an urgent need to study the behavior of African stingless bees in order to understand their role in the spread of the disease. A study on the foraging behavior of the bees is necessary to determine the likelihood and the means through which the bees can contract and spread the pathogen from a banana plant to another and back to the nest. The behavior of the bees within the nest is of special importance in determining the fate of the pathogen if brought back to the nest by the foragers. The possibility of the nest acting as a reservoir for the pathogen and a center for the disease spread require investigation.

Knowledge gained from the study will not only be useful in controlling the spread of the BXW epidemic, but also in the sustainable utilization of the stingless bees for meliponiculture and pollination.

We therefore aim to study the behavior of stingless bees found to visit bananas in Uganda, as a first step towards the control of BXW epidemic and understanding the biology of African stingless bees.

PART ONE

THE POSSIBLE ROLE OF STINGLESS BEES IN SPREAD OF *Xanthomonas campestris pv. musacearum (Xcm)* RESPONSIBLE FOR BANANA WILT IN UGANDA AND THE NEIGHBOURING COUNTRIES

1. Introduction

A number of insects are known to be vectors of plant pathogens. They transmit pathogens through various modes ranging from foregut borne, circulative, propagative and transovarial. Still, some insects may spread pathogens attached to their body as they move from plant to plant. For example honey bees, *Apis mellifera* spread the bacteria pathogen for fire blight (*Erwinia amylovoa*) through pollen (Johnson *et al*. 1993).

It is implied that the vectors of insect transmitted strains of Moko banana disease in Central America are most likely to be bees, wasps or flies (Buddenhagen & Elsasser 1962), while that of Bugtok disease are probably Thrips. In Uganda stingless bees were speculated to be the primary vectors in the spread of *Xanthomonas*. *Xanthomonas* is said to enter the banana plant through moist scars of recently dehisced male flowers and floral bracts (Gold *et al*. 2006; Tinzaara *et al*. 2006). The speculation is based on the grounds that Banana Xanthomonas Wilt (BXW) in Uganda has similar symptoms to other banana bacterial diseases such as Moko whose vectors are insects (Eden-Green 2007). In addition *Xanthomonas* was recovered from stingless bees body (Tinzaara *et al*. 2006). However no study has been done to show the role and mode of transmission of the bacteria by insects or to support the hypothesis that stingless bees are the primary vectors. Information is needed on the parts of bananas visited by stingless bees (especially the most susceptible type Pisang Awak), materials that the bees collect, their movement between banana plants, and between the bananas and their nests. Knowledge of the bees foraging range would be essential in determining the foragers' distribution radius from their nests, which would determine how far they could spread *Xanthomonas*.

We therefore did a study in central Uganda to determine the role of stingless bees in the spread of BXW.

1.2 Aims of the study

1. As stingless bees were said to be the primary vectors in the spread of *Xanthomonas campestris pv. musacearum*, we wanted to determine which species of stingless bees visited bananas and where they nested

2. To find out the likely ways through which stingless bees would get in contact with *Xanthomonas campestris pv. musacearum*, we determined stingless bees foraging behavior and the materials they collected from bananas by observation in banana fields and in colonies (*Plebeina hildebrandti* and *Hypotrigona gribodoi*,the most common stingless bees in the study area) reared in wooden hives

3. Since Pisang Awak banana variety was reported as the most susceptible to *Xanthomonas*, we determined the amount of nectar and the sugar concentration produced by male and female flowers which would be collected by visiting stingless bees.

4. Given that any moving object which comes into contact with diseased bananas had a likelihood of getting in contact with *Xanthomonas*, we wanted to establish how far stingless bees were likely to spread *Xanthomonas* if they got contaminated with it. We determined *Plebeina hildebrandti* foraging distance to sugar solutions of different concentrations and extrapolated the results to other species of stingless bees found visiting bananas

2. Study area and methods

Study area

The study was carried out from November 2005 to July 2006 in farmlands planted with bananas in four districts, Luwero, Mpigi, Mukono and Wakiso districts in Central Uganda (Figure 7)

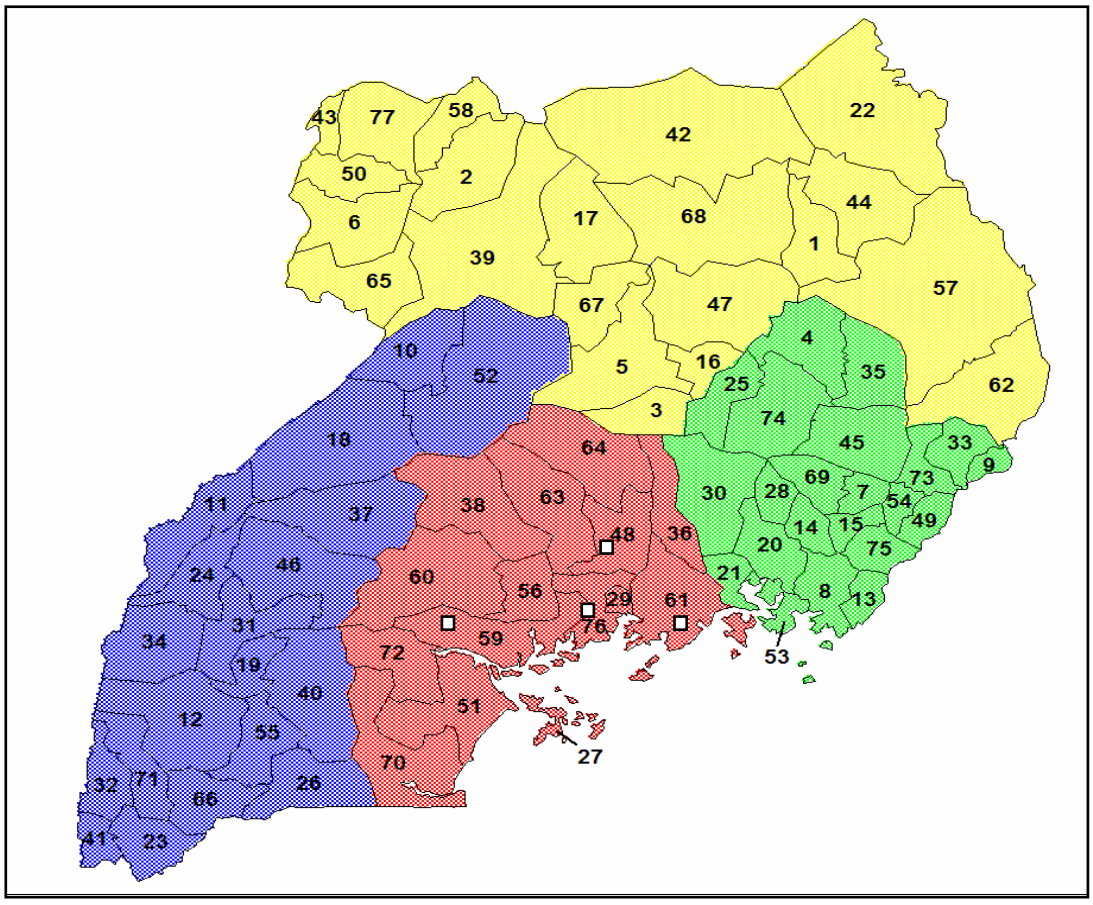

Figure 7: Image of Uganda showing district and regional boundaries. Each district is given a number: Number 48 = Luwero, 59 = Mpigi, 61 = Mukono, 76 = Wakiso districts.

Methods

As there was no selective culture medium for *Xanthomonas campestris pv. musacearum* (*Xcm)*, we applied indirect approaches to test whether stingless bees would get in contact and carry the bacteria. We documented the behavior of colonies in wooden observation boxes and their foraging behavior in banana fields.

2.1 Determining stingless bee species and their nesting sites in the farmland

A survey was done to determine the species of stingless bees visiting bananas and their nesting sites. The survey was done in selected farms in Luwero, Mpigi, Mukono and Wakiso districts (Figure 7). During random walks in the farms stingless bees were caught using a sweep net. Simultaneously we searched for nesting sites. Due to insufficient time and resources, no attempt was made to quantify the abundance of each species on bananas. Also no attempt was made to count the total number of nests in each farm. Identification of the bees was done at the National Museums of Kenya by Dr. Mary Gikungu.

2.2 Foraging behavior of stingless bees in banana farms and the materials they collected

2.2.1 Foraging behavior and foraging duration

Stingless bees foraging behavior on bananas was recorded in Mukono and Wakiso districts. In Mukono, the foraging behavior was recorded in eight Pisang Awak bananas (5 males, 3 females) for seven days. In Wakiso it was recorded on seven sweet bananas (6 male, 1 female) and one Highland (Matooke) banana for seven days. A forager was observed from the time it landed on the flowers until it left. The total time a bee spent on the flowers and the activities it did before flying away were recorded. When a forager flew away from the banana plant under observation, it was recorded if it landed on bananas within the vicinity. We also made observation to determine if the foragers visited the pseudostem, leaves, fruits and flower stalk of the bananas. When a forager flew away, the observer waited for the next stingless bee forager to arrive on the flowers and observations began. The species of the forager under observation was recorded. No

attempt was made to record the abundance of each species on the flowers. Other animal visitors to the flowers were recorded where possible.

2.2.2 Collection of sap and bacterial ooze in the field

On bananas in advanced *Xanthomonas* infection stages, we observed if stingless bees would take up bacterial ooze which was seeping out (Figure 8). To find out if stingless bees collected banana sap, green fruits, bracts, male and female flowers were cut to allow sap to seep out (Figure 9a, b, c), then we observed if the bees would take it up. We also observed whether the bees would collect sap from naturally occurring scars on the flower stalks caused by fall of male flowers and bracts.

Figure 8: Ooze (arrow) seeping out of a banana bunch infected with *Xanthomonas*. We observed if stingless bees took up the ooze

Figure 9a: Sap (arrow) seeping out of horizontally cut green bananas. We observed if stingless bees took up the sap

Figure 9b: Sap (arrow) seeping out of horizontally cut bracts. We observed if the bees took up the sap.

Figure 9c: Sap (arrow) seeping out of cut female flowers. We observed if the bees took up the sap.

2.2.3 Uptake of nectar, sap, and bacterial ooze offered at the nest entrance of colonies reared in observation hives

Three colonies of *Hypotrigona gribodoi* and four colonies of *Plebeina hildebrandti* were reared in wooden observation hives at Kawanda Agricultural Research Institute (KARI) in Uganda. The colonies served for further experiments to determine if stingless bees would collect fresh/old sap from healthy bananas, ooze from bananas infected with *Xanthomonas* and banana nectar provided at the nest entrance. Both sap and ooze were used in the experiment to determine if the bees would collect sap which was not contaminated with *Xanthomonas* or ooze which had *Xanthomonas*.

The observation hives were wooden with transparent glass cover. The hives for *P. hildebrandti* were 50cm long, 20cm wide and 25cm high, while for *H. gribodoi* were 20cm long, 10cm wide and 15cm high. The hives had holes drilled on one side in which silicon tubes were inserted for exit and entrance of the bees.

To hold nectar, sap and ooze at the entrance, a silicon tube, 3.8cm long and 2.5cm wide was cut open and placed at the edge of the stingless bees exit tube, such that it wrapped around it and left 1.3cm extending beyond the exit tube (Figure 10). Each at a time, two drops of nectar, sap, and ooze were placed within the 1.3cm extension of the silicon tube. The nectar was from Pisang Awak male flowers with 11% sugar concentration.

Immediately after placing either of the above substances, we counted for five minutes the numbers of bees that came to the entrance tube. That is immediately 0min, at 2.5min and at 5min. The bees that antenned or took up the substances provided were recorded with the aid of a digital video camera. We did not differentiate whether it was the same bees coming to the entrance tube from inside the hive and returning or different bees that we counted at 0min, 2.5min and 5min. The silicon tube was changed every time a new substance was under observation. Observations were done for *H. gribodoi* and *P. hildebrandti*. The experiment was repeated three times for both ooze and sap.

2.2.4 Offering banana sap within the hives

Since the sap provided to the bees at the nest entrance tube was fresh, we wanted to know if the bees would take up old sap or sap left exposed for sometime e.g. the sap which exudes from the scars caused by the fall (dehiscent) of male flowers and the flower bracts. To do this, three small containers were filled with banana sap and placed inside three *P. hildebrandti* hives for seven hours. The sap was squeezed from the base of male and female Pisang Awak flowers immediately after plucking them from the flower stalks. After seven hours we observed if the bees had taken up the sap.

The three small containers were then removed and placed adjacent to the hives to find out if the foragers would take up the substances within them.

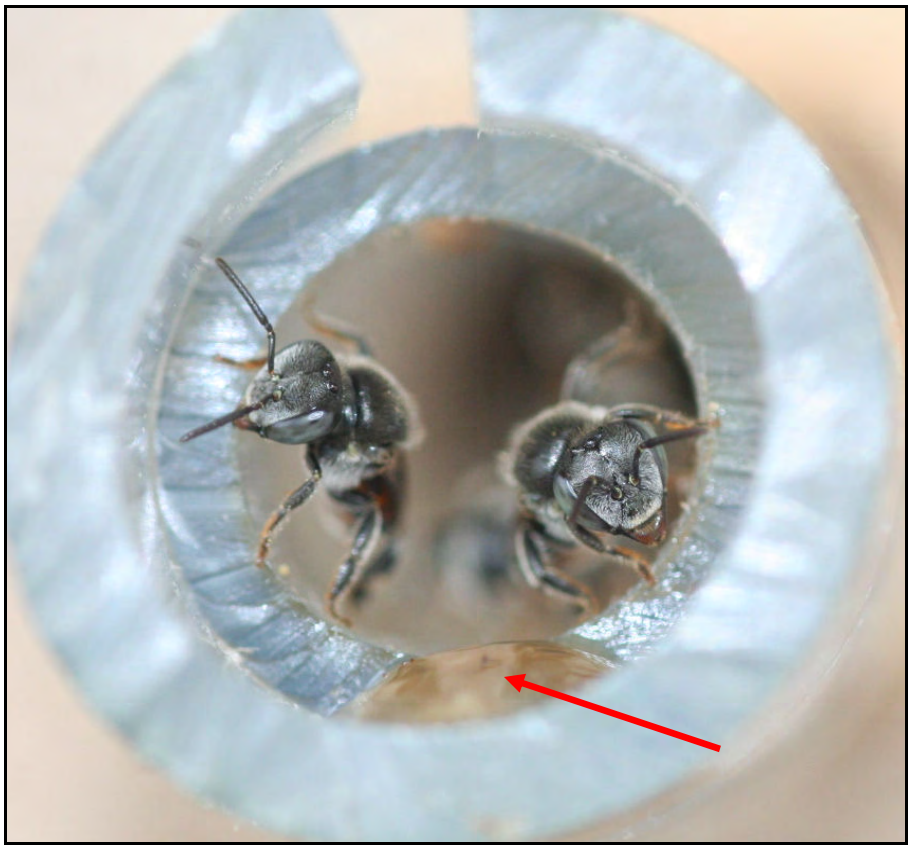

Figure 10: A 3.8cm x 2.5cm silicon tube cut open and placed at the edge of the hive exit tube, such that it wrapped around the exit tube and left 1.3cm extending beyond the tube. Drops of nectar, sap and ooze (arrow) were placed on the 1.3cm extension for uptake by stingless bees foragers

2.2.5 Provision of banana bunches with male flower buds 50cm in front of the hives

Three sweet banana bunches with at least two bracts with open male flowers were cut carefully from their mother pseudostems. The cut end was immediately covered with wet soil and wrapped with wet cotton wool and a plastic paper to prevent water loss hence withering and subsequent fall of the flowers. The bunches were suspended one meter from the ground and 50cm in front of *H. gribodoi* and *P. hildebrandti* hives. The fruits and the flower stalks were scratched with a metal object to allow sap to seep out. The bunches had a mean nectar sugar of 17%, 19% and 15%. We observed if *H. gribodoi* and *P. hildebrandti* collected sap and nectar from the bunches for 170 minutes.

2.3 Determining the amount of nectar and the sugar concentration produced by male and female Pisang Awak flowers

The number of open bracts per banana in eight Pisang Awak bananas was counted in Mukono district. The number of flowers under each open bract was also counted. The counting in the eight bananas was done within seven days. The flower buds were of different age.

Using micro-pipets (l µl, 2 µl, 5 µl, 10 µl, 100 µl), nectar was extracted and volume measured, from 29 male and 8 female flowers. With a hand held sugar refractometer, the nectar sugar in each flower was measured.

2. 4 *Plebeina hildebrandti* foraging behavior and foraging distance

2.4.1 Recruitment of nest mates to nectar sources

These experiments were done to determine whether, when a forager finds nectar at a certain source, nestmates would collect nectar from exactly that source. This would give an indication of how the bees are likely to spread *Xanthomonas* during foraging. Small containers with cotton wool soaked with sugar solutions were used as feeders. Cotton wool was provided to prevent bees from drowning in the sugar solution. The sugar solutions consisted of honey from *Apis mellifera*, sugar and water.

Using a feeder (feeder one), foragers were trained to come to the feeder up to a distance of 30m from the hive. To find out if bees scent marked feeder one which was used during the training, a second feeder (feeder two) was placed adjacent to feeder one at 30m from the hive. A third feeder (feeder three) was placed one meter from feeder one and two, but equal distances (30m) from the hive to determine if the bees would collect sugar solution from it (Figure 11). All the three feeders were identical small containers with cotton wool soaked with 40% sugar solution. Foragers arriving at the three feeders were counted and marked every five minutes for a period of 60 minutes. Bees arriving at feeder three were captured. Attention was paid on bees flying among the three feeders.

2.4.1.1 Training of the bees

A few drops of the sugar solution were put at the silicon exit tube of a hive in which *P. hildebrandti* colony was reared. After the foragers took up the solution, feeder one was placed in front of the exit tube. The feeder was in contact with the tube so that the bees which had previously taken up the sugar solution from the exit tube could find it. Foragers were allowed to take the sugar solution and return to the hive twice before the feeder together with the bees on it could be moved for some centimeters from the entrance and when the bees were back on the feeder they were moved stepwise for 1-3 meters up to 30m from the hive.

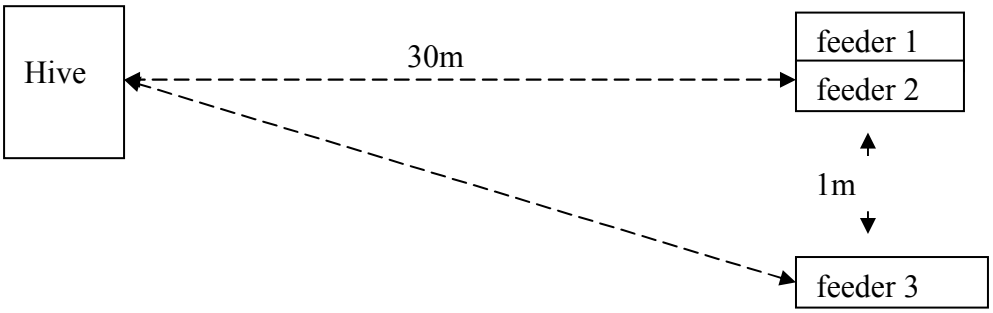

Figure 11: Feeder arrangement in an experiment to determine if *Plebeina hildebrandti* foragers recruited nest mates to a food source

2.4.2 Resource switching

2.4.2.1 Experiment to determine if sugar solution collectors would switch to either resin or sap collection when sugar solution was taken away

These experiments were done to determine if stingless bees collecting nectar from male banana flowers would switch to sap collection when the male flowers fell down leaving scars wet with sap, or when bananas in advanced disease stage had bacterial ooze seeping out. The resin used in the experiment was from within the stingless bee nests and was used as a control.

Using a small container with cotton wool soaked with a 40% sugar solution, bees were trained up to 30m from the hive. Training of the bees to the sugar solution was done as described in section 2.4.1.1. Nest mates recruited to the sugar solution every five minutes for a period of 60 minutes were counted and marked on the thorax. At the end of the 60 minutes, the sugar solution soaked cotton wool was removed from the container and replaced with banana sap. Bees arriving on the container were counted and marked every five minutes for a period of 60 minutes. We made observations on whether the bees would collect the sap. The experiment was repeated by replacing sap with resin extracted from a *P. hildebrandti* nest.

2.4.2.2 Experiment to determine if resin collectors would switch to either sap or sugar collection when resin was taken away

Another experiment (as in section 2.4.2.1) was done to determine if bees would recruit to resin and then switch to nectar or sap collection when resin was replaced with either of them. Training of the bees to resin was done as described in section 2.4.1.1.

2.4.3 Foraging distance to sugar solutions of different concentrations

To determine *P. hildebrandti* foraging distance and if sugar concentration influenced flight distance, bees were trained to 11%, 33%, 48% and 54% sugar solutions. The training was done as in section 2.4.1.1. The feeder together with the bees on it was moved step by step from the hive up to the furthest distance when recruitment diminished and no more bees arrived on it. At every step new recruits were counted and marked on the thorax.

The choice of 11% and 33% sugar concentrations was based on the knowledge that Pisang Awak, the banana variety most susceptible to *Xanthomonas* had nectar sugar concentrations of 0% to 32%, with a mean of 12.5%. The choice of 48% and 54% was based on the fact that stingless bees profit mostly from nectars with over 40% sugar concentration.

3. Statistical analysis

One way analysis of variance (ANOVA) was used to determine if there were differences in foraging duration among stingless bees' species collecting nectar on bananas from morning to evening, and to compare foragers to the three feeders used to determine nestmates recruitment. Student T-test was used to compare male and female flowers nectar volume and sugar concentration, and to compare stingless bees´ foraging duration on male and female flowers. Regression analysis was used to determine relationship between number of new foragers recruited to different sugar concentrations and the foraging distance. All the analysis were done using JMP (2005) statistical package.

4. Results

4.1 Stingless bee species and their nesting sites in banana farms

Four stingless bees' species, *Hypotrigona gribodoi*, *Plebeina hildebrandti*, *Meliponula ferruginea* (Lepeletier) and *Meliponula sp*. were recorded visiting banana flowers in the four districts. *Meliponula sp*. was recorded only in Mukono district.

Hypotrigona gribodoi nested in cavities in trees, and man made structures like walls of mud houses, blocked drainage pipes, electrical sockets and poles supporting electricity and telephone wires. *Plebeina hildebrandti* nested in inhabited termite mounds. The bees avoided termite mounds with no vegetation cover especially in areas with high human presence and activities. *Meliponula ferruginea* nested in cavities in trees with almost all the nests (10 nests) found in *Cupressus lucitanica*. *Meliponula sp* nests were not found. However about 50 workers were observed to repeatedly visit a crevice under a cemented pavement. They sealed the crack and removed soil from within the crevice. After a heavy downpour the workers never visited the site again.

4.2 Foraging behavior of stingless bees on bananas and the materials they collected

Foraging behavior

Four stingless bees' species visited banana flowers where they collected only nectar. There was no pollen visible in the bananas observed and no bees were seen collecting pollen.

Mostly the bees would land on top of the flowers then crawl inside. Occasionally the bees landed on the bracts then crawl to the flowers. More than one forager could easily access nectar in female flowers as the flowers opened widely (Figure 13a). On the other hand the bees had difficulties squeezing through the male flowers to reach the nectar, as the flowers were compressed together and did not open widely (Figure 13b). This was especially so in the inner rows in which case stingless bees would wait for honey bees to squeeze through the flowers to open them up. Occasionally some honey bees would get

stuck as they forced their way, backwards from inside the flower. In male flowers, normally only one forager would collect nectar at a time, except for the small *Hypotrigona gribodoi*. Sometimes the bees would crawl at the base of the flower on the outside below the spoon shaped petal to access nectar (Figure 13b).

As there were other animal visitors (honey bees, solitary bees, wasps, flies, beetles, drosophila, ants, weaver birds and sunbirds), competing for nectar or predating on other insects on/in flowers, a stingless bee forager visited several flowers under one or more open bracts before leaving a banana plant.

After collecting nectar, a bee would clean its wings and legs while seated on top of the flowers or bracts before flying away. Occasionally the bees would fly on top of the banana leaves clean the body and then fly away. At one time a bee was seen drying the nectar, passing it between the mouth and the front legs while seated on a banana leaf, it then flew back to the flowers on the same plant to collect more nectar.

In one occasion, a forager was observed fly from the banana plant under observation to a second banana in a separate stool hanging two meters away.

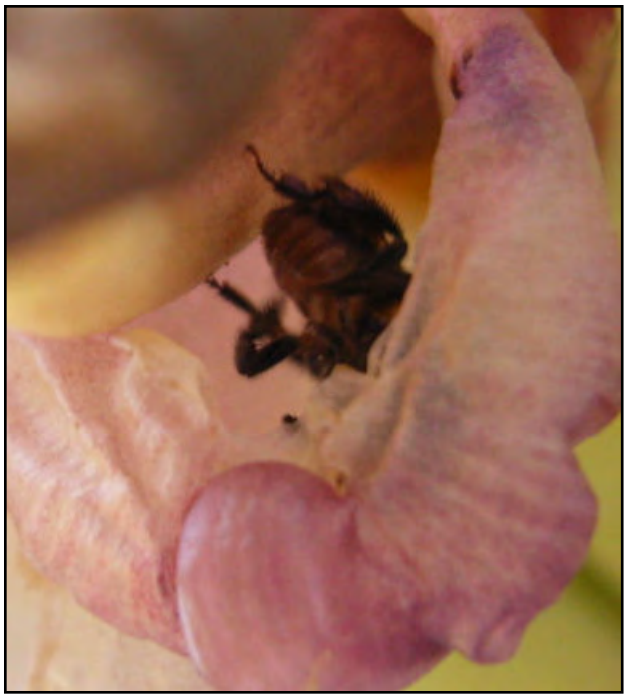

Figure 13a: A stingless bee forager collecting nectar from inside a female banana flower

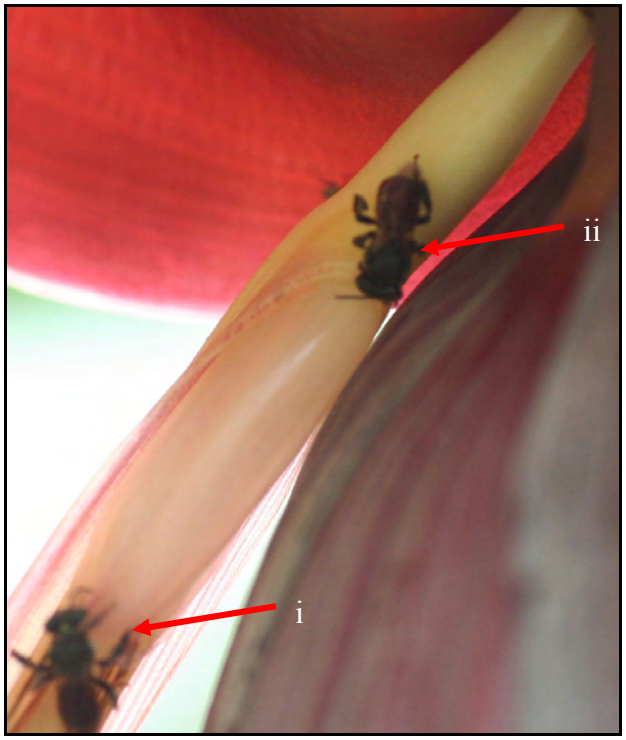

Figure 13b: Two stingless bees try to reach nectar in a male flower. One forager (i) tries to get inside the flower while another (ii) tries to access nectar outside, at the flower base

4.2.1.1 Foraging duration from the time a bee landed on the flowers till it left

Foraging time per visit to the banana flowers differed significantly among the four stingless bee species (one way ANOVA $F_{3,\ 257} = 10.77$, $P < 0.01$, Table 1) with *Meliponula ferruginae* spending more time per visit than the other three species (Contrast test $F = 25.43$, $P < 0.01$). By spending more time on the flowers, bigger species such as *M. ferruginae* had a higher possibility of getting contaminated with *Xanthomonas* than smaller foragers such as *Hypotrigona gribodoi*.

Table 1: Species of stingless bees observed collecting nectar from banana flowers, number of observations and mean time in seconds spent on flowers per visit

Species	Body size (mm) Eardley 2004	Average time (sec) spent on flowers per visit
Meliponula ferruginae	5.1-5.9	272 (n=54)
Meliponula sp.	*	174 (n=83)
Plebeina hildebrandti	3.3-5.2	119 (n=76)
Hypotrigona gribodoi	2.2-3.9	92 (n=44)

* Species not identified but approximately same size as *M. ferruginea*

4.2.1.2 Foraging duration from morning to evening

There was no significant difference on the duration that foragers belonging to the four species spent per visit to the flowers from morning to evening (One way ANOVA $F_{9, 247} = 1.13$ $P = 0.46$, Figure 15).

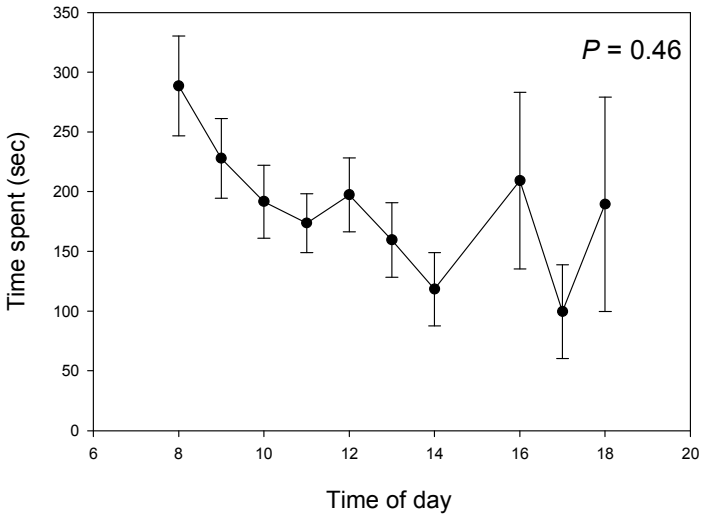

Figure 15: Mean (± S.E) time spent per visit by four species of stingless bees' foragers on banana flowers from 0800h to 1800h. One way ANOVA, P = 0.46

4.2.1.3 Foraging duration on male and female flowers

A comparison of time spent on male and female flowers by three species of stingless bees *Meliponula ferruginea, Meliponula sp.* and *Plebeina hildebrandti* in Mukono district, showed that foragers tended to spend more time on female flowers than on male flowers (t-Test = 2.5, DF= 108, P= 0.01 (Figure 16). By spending more time on female flowers, foragers had higher chances of getting contaminated with *Xanthomonas* on female than on male flowers: if *Xanthomonas* was present in female flowers, or through contacts with other foragers

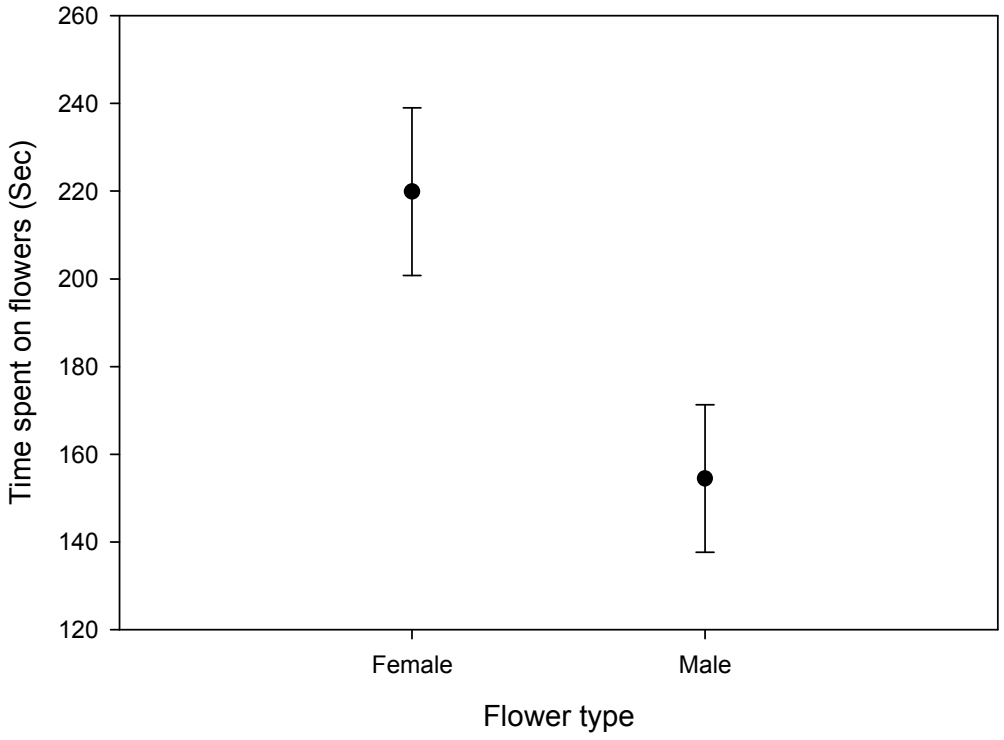

Figure 16: Mean (± S.E) time spent by three species of stingless bees' foragers on male and female banana flowers. N= 110 0bservations, 48 on female and 61 on male flowers

4.2.2 Collection of sap and ooze in the field

Stingless bees' foragers did not collect sap from green fruits, bracts, male and female flowers which were cut to allow sap to seep out. Also they did not collect sap from scars on the flower stalk caused by fall of male flowers and bracts. However in one instance a bee was found trying to collect some dry banana sap on a flower stalk (Figure 17). The bee pulled the dry sap with the mouth but left after a short time without packing any sap on the corbiculae. Would *Xanthomonas* be present in the dry sap? Stingless bees did not ooze from banana plants infected with *Xanthomonas*.

Figure 17: A stingless bee forager tries to collect dry banana sap

4.2.3 Uptake of nectar, sap, and ooze offered at the nest entrance

Both *Hypotrigona gribodoi* and *Plebeina hildebrandti* collected 11% Pisang Awak nectar provided at the nest entrance tube. During three (5 min) observation periods, five *H. gribodoi* and six *P. hildebrandti* collected the nectar. However there were bees at the nest entrance which did not collect the nectar, while others just antenned the nectar without taking it up (Table 2).The two species did not take up sap and ooze provided at the entrance. Some bees at the nest entrance antenned the sap and the ooze without taking it up (Table 3 and 4). After antenning the sap or ooze, the bees in the entrance tube became agitated.

Table 2: Mean number of *Hypotrigona gribodoi* and *Plebeina hildebrandti* workers observed at the nest entrance tube in the presence of 11% Pisang Awak nectar during three (5min) observation periods, and the number of workers that either antenned or collected the nectar

Species	Bees at the entrance tube			Bees antenning nectar	Bees collecting nectar
	0 min	2.5 min	5 min	In 5 min	In 5 min
H. gribodoi	1	5	8	4	5
P. hildebrandti	1	10	10	1	6

Table 3: Mean number of *Hypotrigona gribodoi* and *Plebeina hildebrandti* workers observed at the nest entrance tube in the presence of banana sap during three (5min) observation periods, and the number of workers that either antenned or collected the sap

Species	Bees at the entrance tube			Bees antenning sap	Bees collecting sap
	0 min	2.5 min	5 min	In 5 min	In 5 min
H. gribodoi	2	6	6	1	0
P. hildebrandti	4	8	2	2	0

Table 4: Mean number of *Hypotrigona gribodoi* and *Plebeina hildebrandti* workers observed at the hive entrance tube in the presence of ooze during three (5min) observation periods, and the number of workers that either antenned or collected the ooze

Species	Bees at the entrance tube			Bees antenning ooze	Bees collecting ooze
	0 min	2.5 min	5 min	In 5 min	In 5 min
H. gribodoi	2	5	8	3	0
P. hildebrandti	4	4	4	0	0

4.2.4 Treatment of sap introduced inside the hives

In one *Plebeina hildebrandti* hive the bees did not touch the sap that had been put inside. The sap had dried on top forming a thin layer but below the layer the sap was still fluid (Figure 18a). In the other two *P. hildebrandti* hives, the bees covered the sap with propolis collected from within the nests (Figure 18b). The sap below the propolis remained fluid.

Figure 18a: Sap in small containers introduced inside *P. hildebrandti* hive showing sap not touched by the bees. Sticks (arrow) put to prevent the bees from drowning in the sap

Figure 18b: Sap in small containers introduced inside *P. hildebrandti* hive showing propolis layer smeared on top of the sap by the bees

When the small containers were removed from *Plebeina hildebrandti* hives and placed next to them, the bees collected the propolis they had smeared on top of the sap but they did not collect the banana sap (Figure 19).Using mandibles, the bees scrapped propolis from the surface, and with the help of the forelegs pushed to the corbiculae in the hind legs. Both hind legs were loaded interchangeably.

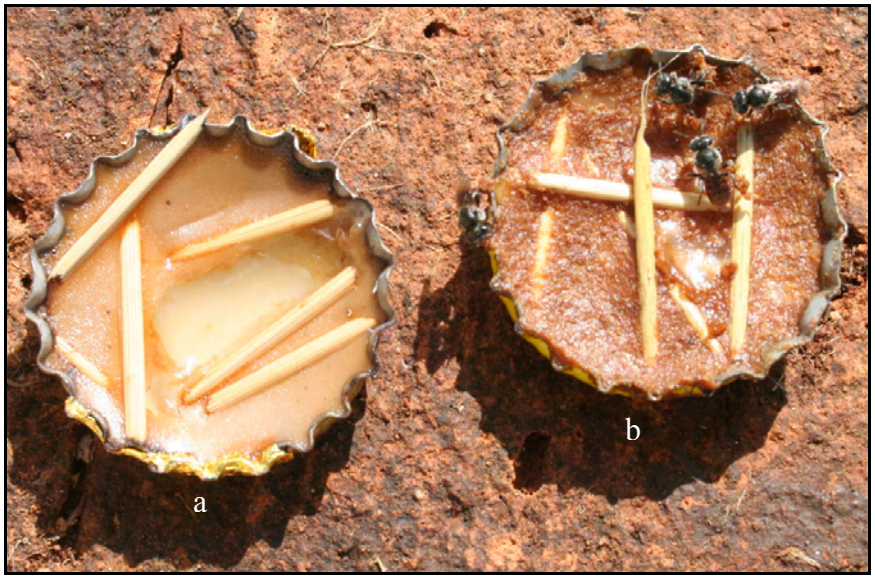

Figure 19: *P. hildebrandti* foragers did not visit the container with banana sap (a), but visited the container smeared with propolis, and collected the propolis (b)

4.2. 5 Reaction to banana bunches hung 50 cm in front of the hives

Both *H. gribodoi* and *P. hildebrandti* foragers flew from the nest to collect nectar from flowers on the banana bunches hang 50cm in front of the hive. They did not visit fruits and flower stalks which had been scratched to allow sap to seep out.

4.3 Determining the amount of nectar and the sugar concentration produced by male and female Pisang Awak flowers

Number of open bracts and flowers

On average only one bract was open in male flower buds per day, with a range of 1-3 open bracts. When there were three open bracts, two of them contained old flowers with no nectar but rotting and freely falling down leaving a bare stalk with scars. These old flowers were not visited by foraging bees. The average number of male flowers per bract was 19. Female flower buds had an average of eight open bracts, with a mean of 16 flowers per bract. Most stingless bees and other insect visitors were concentrated on flower clusters in the last three recently opened bracts. Flower clusters in older bracts had fewer visitors roaming over them.

Due to the higher number of bracts open in the female flower buds, there were more open flowers available for the bees than in the male flower buds. Consequently more stingless bees and other insect visitors visited female flowers than the male flowers.

Pisang Awak nectar volume and sugar concentration

On average Pisang Awak had nectar with 12.5 % sugar concentration with a range of 0-32%. The mean nectar volume per flower was 30.2µm with a range of 0.5-102 µm. There was significantly larger volumes and higher nectar sugar concentration in male flowers than in female flowers during the day (t-Test = -3.2, DF = 41, P =0.01 for nectar volume and t = -2.7, DF= 41, P= 0.01 for sugar concentration (Figure 12).

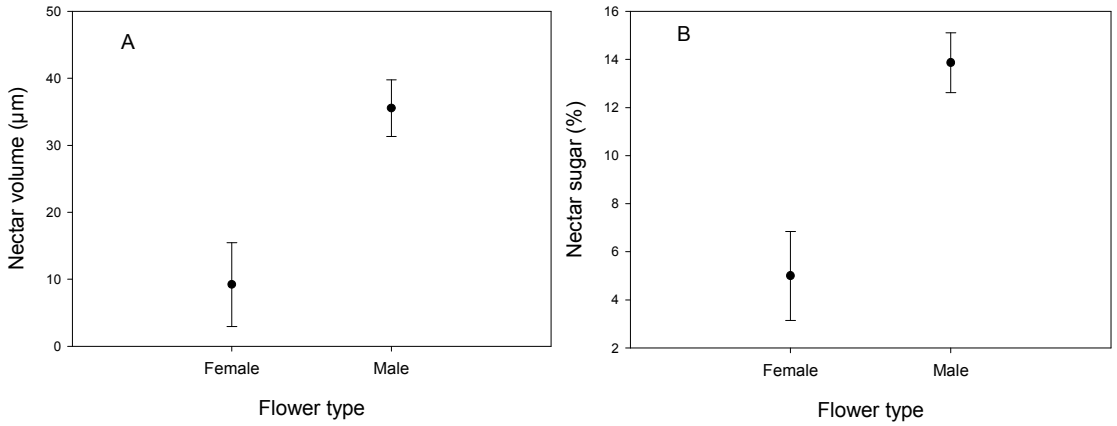

Figure 12: Mean (± S.E) in (A) nectar volume in µm and (B) nectar sugar (percentage) in male and female Pisang Awak flowers during the day. N = 43 flowers

4.4 *Plebeina hildebrandti* foraging behavior and forage distance

4.4.1 Recruitment of nest mates to food sources

Significantly more bees arrived on feeder one, than on feeders two and three ($F_{2, 33}$ = 17.05; P< 0.01) during the 60 minutes observation period. A total of 192 bees (93.2% of the total bees to the three feeders) arrived to feeder one, which had been used in training the bees from the nest entrance up to 30meters from the hive. Twelve bees (5.83%) arrived to feeder two, which had not been used in training the bees, but was placed adjacent to feeder one. Two bees (0.97%) arrived to feeder three, which had not been used in training the bees, but was placed one meter from feeder one and two (Figure 20). Of the 12 bees on feeder two, nine were directly from the nest while three had previously visited feeder one. The two bees that arrived to feeder three had previously visited feeder one.

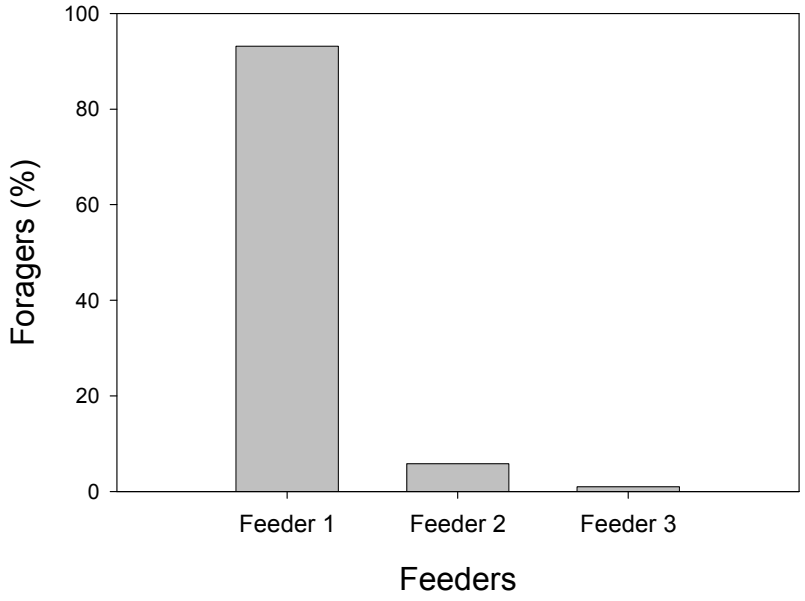

Figure 20: Percentage of *Plebeina hildebrandti* foragers arriving on three feeders with 40% sugar solution, placed 30m from the hive, for a period of 60 minutes. Feeder 3 placed one meter from the other two.

4.4.2 Resource switching

4.4.2.1 Replacing sugar solution with either sap or resin

Foragers steadily recruited new comers to the 40% sugar solution placed 30m from the hive, during a 60 minutes observation period (Figure 21).

When the sugar solution was replaced with banana sap, five bees landed on the feeder within the first five minutes but they did not collect sap. They either landed on the feeder, taste the sap with the proboscis, walk on and around the feeder or fly around the feeder up to one meter away. The bees inspected whether the sugar solution had been returned. By the end of the 60 minutes observation period, no bee was landing on the feeder, but flew around it (Figure 21).

When sap was replaced with resin, two bees collected the resin during a 60 minutes observation period (Figure 21).

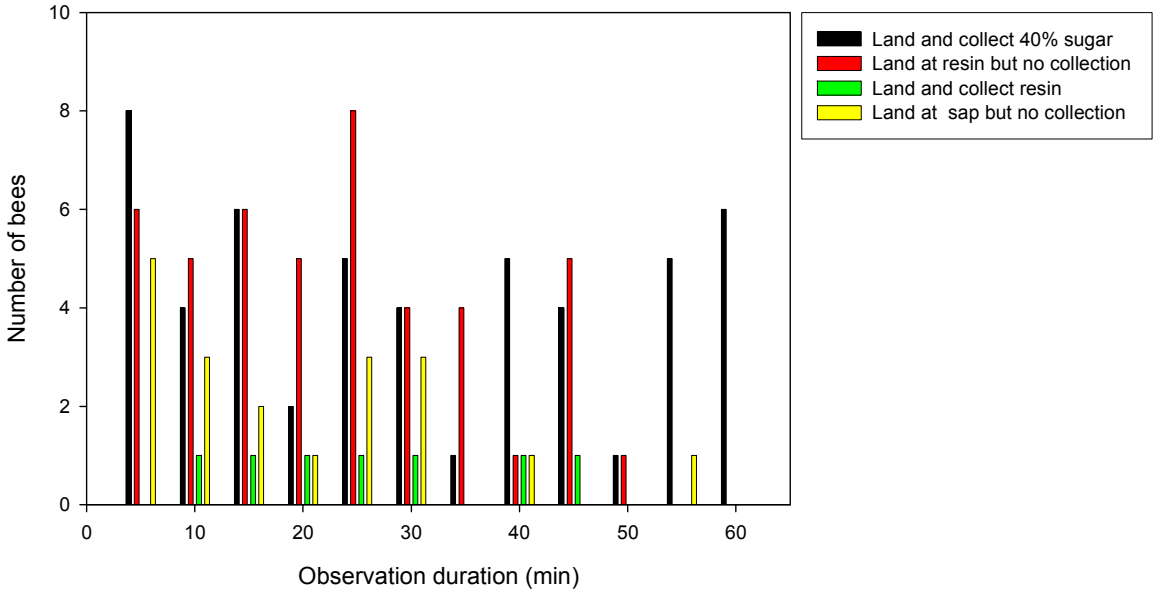

Figure 21: Number of *Plebeina hildebrandti* foragers that collected a 40% sugar solution, landed at banana sap without collecting, and those that landed at resin with or without collecting it after 40% sugar solution was replaced with either sap or resin

4.4.2.2 Replacing resin with either sap or sugar solution

Four foragers were trained and collected resin on a feeder placed 30m from the hive. The four foragers recruited three bees which collected resin during the 60 minutes observation duration (Figure 22).

When resin was replaced with banana sap, none of the bees that came to the feeder collected the sap (Figure 22).

When sap was substituted with 40% sugar solution, the number of foragers landing and collecting the sugar solution increased steadily (Figure 22).

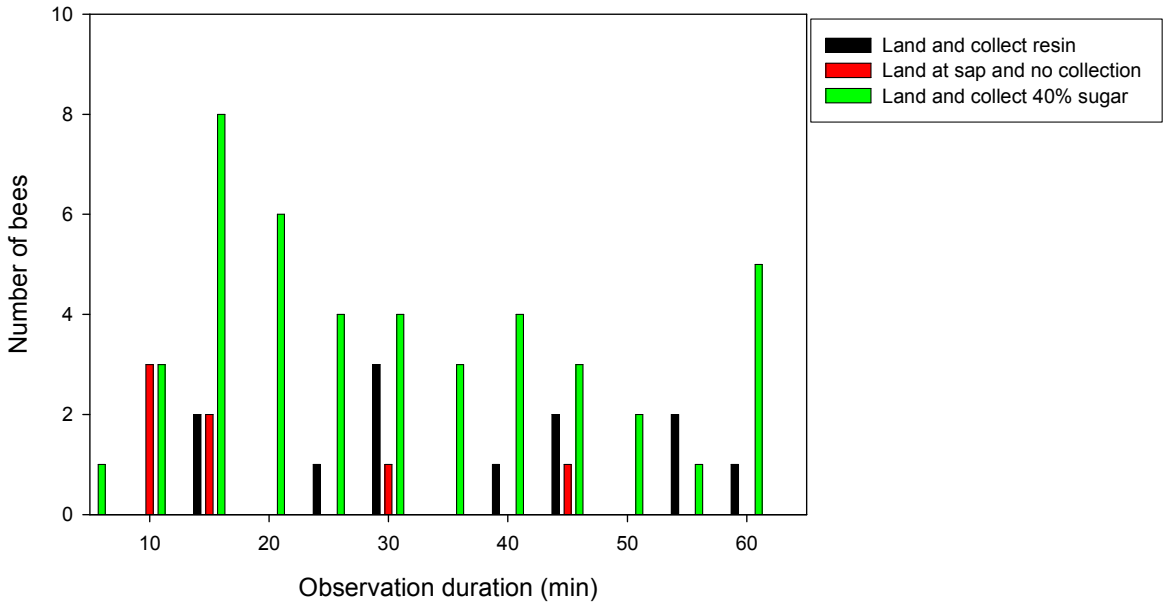

Figure 22: Number of *Plebeina hildebrandti* foragers that collected resin, landed at banana sap without collecting, and those that collected 40% sugar solution, after resin was replaced with either sap or sugar solution.

4.4.3 Foraging distance of *Plebeina hildebrandti* when 11%, 33%, 48% and 54% sugar solutions were offered

Bees recruited to the sugar solution declined with increase in distance. For example to 48% sugar solution, at the hive entrance (0m) 44 bees came to the feeder while at 1220m from the hive only one bee came to the feeder (Figure 23).

Recruitment of new comers stopped before the experienced foragers stopped foraging. Foraging distance was dependent on the concentration of the sugar solution, with bees flying shorter distances for low sugar concentration and longer distances for higher sugar concentration (Table 5).

Table 5: Distances from the hive at which recruitment of new nest mates and foraging by experienced *Plebeina hildebrandti* foragers stopped when provided with sugar solutions of varying concentrations

Sugar concentration of the solutions (%)	Distance (m), at which recruitment of new comers stopped	Distance (m), at which experienced foragers stopped collecting the sugar solutions
11	1030	1050
33	1160	1200
48	1185	1220
54	1215	1230

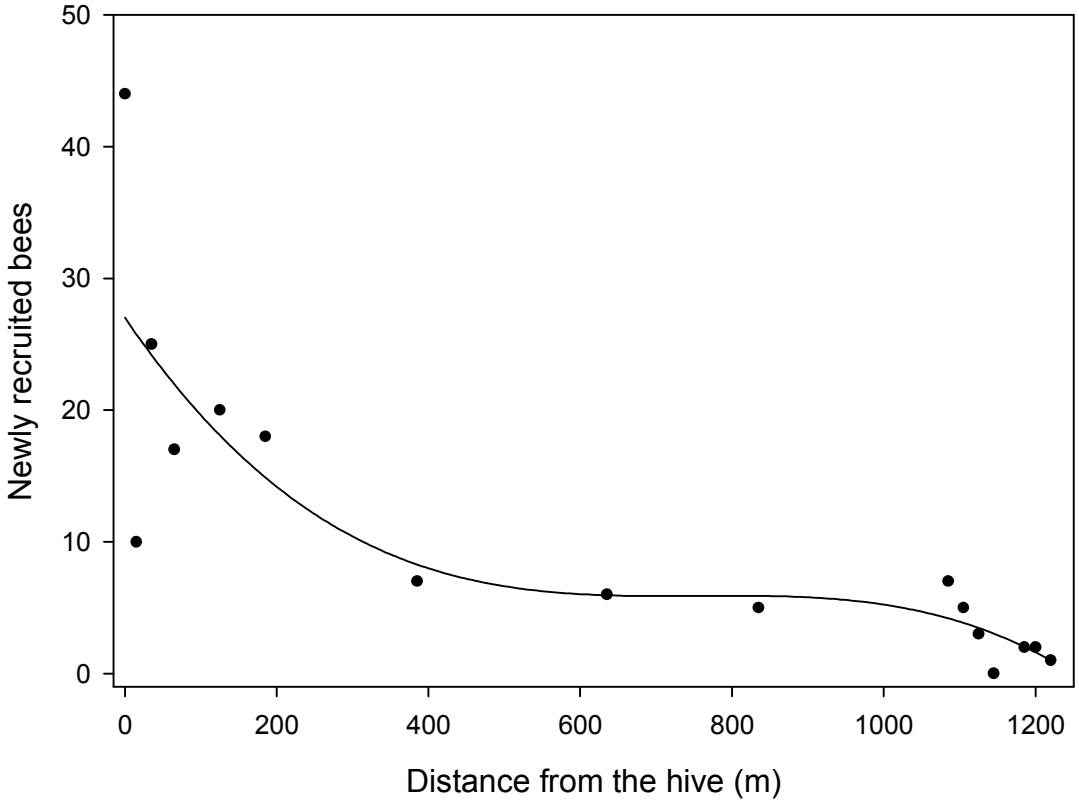

Figure 23: Number of *Plebeina hildebrandti* foragers to a feeder with 48% sugar solution placed various distances from the hive. Fitted is a linear regression, analysis y = 27; $r^2 = 0.69$; $F_{15} = 8.9$, $P = 0.002$

5. Discussion

Stingless bees foraging behavior and its implications to the spread of *Xanthomonas*

Four stingless bees species collected nectar from bananas in central Uganda. They collected nectar from both male and female Pisang Awak flowers. It was easier for the foragers to access nectar in female flowers as they opened widely in comparison to male flowers. This allowed more than one forager to simultaneously collect nectar in a female flower. On the other hand male flowers were narrow and so much compressed that it was difficult for the bees to get inside and only a single forager could get in at a time. As a result lager quantities of nectar were recorded in male flowers than in female flowers during the day. Due to nectar remaining in the male flowers for a longer time without being taken by insects, evaporation of water took place leading to higher sugar contents than in female flowers. Therefore female flower morphology encourages interactions of more than one forager which could enhance the spread of *Xanthomonas* as compared to male flower morphology.

The foragers neither collected ooze from bananas infected with *Xanthomonas* nor sap from injured parts and moist scars caused when male flowers dehisced. Provision of sap, ooze and nectar to *Hypotrigona gribodoi* and *Plebeina hildebrandti* reared in wooden observation boxes confirmed that stingless bees did not collect sap and ooze from bananas. In fact banana sap introduced inside the hives was embalmed with propolis. Embalming is normally done to predators and other intruders which die inside stingless bee nests to prevent bacteria growth (Ghilsalberti 1979).

In fact, in the absence of nectar foragers did not switch to collecting banana sap or ooze infected with *Xanthomonas*. Therefore if *Xanthomonas* is spread through scars left by fall of male flowers, stingless bees are not primary vectors as they did not visit or collect any material from the scars. Other animal vectors or modes of transmission may be more important in the spread of the disease. For example, since Banana Xanthomonas Wilt is a vascular disease (Biruma *et al*. 2007), biting and sucking insects may be more important vectors than stingless bees.

Possible accidental contamination of stingless bees with *Xanthomonas*

There is possible accidental contamination of stingless bees with *Xanthomonas* through contact with bacterial ooze. In this case, the bacteria would be on their surface and not inside their body. Also if *Xanthomonas* is present in the nectar, stingless bees are likely to get contaminated, either through contact with the nectary or imbibing the nectar into the nectar stomach. In this case *Xanthomonas* would be swallowed into the nectar stomach and also contaminate the outside of the foragers' body. Some studies have reported presence of *Xanthomonas* in nectar (Ssekiwoko *et al*. 2006), while others found no *Xanthomonas* in nectar (Mwangi *et al*. 2007). If *Xanthomonas* is present in the nectar, the bacteria would either be carried to the bee nest or transported to another banana plant.

Fate of *Xanthomonas* inside the stingless bee nest

In the nest, nectar from foragers may undergo several pathways. One, it is fed to nest mates (trophalaxis), in which case the nectar is partly eaten and digested. In this case *Xanthomonas* is likely to be destroyed or it may come out with the waste products. Waste products in stingless bee nests is stored in one corner from where its later carried out. Two, nectar is stored in nectar pots where it's dehydrated to honey with a high sugar concentration of about 70-80%. Nectar pots contain propolis (mixture of wax, plant resins and gums) as a constituent building material. Propolis has been shown to have antimicrobial properties (Ghilsalberti 1979; Mereste & Mereste 1988; Marcucci 1995; Gilliam 1997; De Campus *et al*. 1998. Therefore *Xanthomonas* would be eliminated from inside stingless bee nests by propolis and from honey with high sugar content through desiccation. Furthermore, the perennial colonies of stingless bees depend on food stores in humid tropical environments and species living in the soil employ mechanism for preservation and for protection of food stores from microorganisms (Gilliam *et al*. 1984; 1985). Honey bees and stingless bees in particular have an ancient relationship with *Bacillus* species which help to protect nest from microbial invasions. *Bacillus* species produce antimicrobial compounds that inhibit other microorganisms (Gilliam *et al*.1984; Cano *et al*. 1994; Gilliam1997)

Fate of *Xanthomonas* in the banana fields

Personal observations in the field showed that foragers visited a single banana plant per foraging trip. From recruitment experiments foragers (93.2%) persistently visited feeder one, which had been used in recruiting the bees from the nest entrance, while only 5.83% arrived on feeder two placed adjacent to feeder one at 30 meters from the hive. Persistency of foragers on feeder one for 60 minutes shows that, either the foragers were scent marking the feeder so that new comers only came to feeder one, or the foragers were guiding the new comers from the nest to the feeder. Therefore, even in banana plantations foragers from one nest would collect nectar from one banana plant and not each forager going to different plants. If *Xanthomonas* is present in a banana plant, it would be transported between the plant and the bee nest. Therefore the hypothesis that stingless bees are primary vectors as they live in large colonies, and are likely to spread *Xanthomonas* in mass need reexamination.

Further studies are required to determine if the 0.97% foragers, which moved from feeder one to feeder three placed one meter away), can carry bacteria load enough to make stingless bees the primary vectors.

***Plebeina hildebrandti* forage distance**

Plebeina hildebrandti foraging range decreased with decrease in sugar concentration. For example for 11% sugar solution foraging stopped at 1050m, for 33% at 1215m, for 48% at 1220m and for 54% at 1230m. Roubik *et al*. (1995) pointed out that the nectar gathered by Meliponini averaged 44% sugar concentration with mean range of 20-61%. An indication that although *P*. *hildebrandti* collected nectar from banana flowers, bananas may not be a preferred choice for optimal foraging, hence the bees may not be willing to fly for long distances to collect banana nectar. Roubik *et al*. (1995) predicted that a sizeable proportion of flowers must have nectar of optimal sweetness for bees, regardless of flower nectar volume. For instance Pisang Awak, the most susceptible banana variety to *Xanthomonas,* had copious nectar with a mean of 12.5 % sugar concentration, which is below optimal sweetness for the bees.

Extrapolating *Plebeina hildebrandti* foraging rage to the other stingless bees species

Three other species of stingless bees were found to collect nectar from banana flowers. *Hypotrigona gribodoi* (Magretti) is a small bee with a body size of 2-3mm, and likely to have a much shorter foraging distance than *P. hildebrandti,* which has a body size of 3.3-5.2mm. *Meliponula ferruginea* (Lepeletier) with a body size of 5.1-5.9mm, and *Meliponula sp* are slightly larger than *P. hilderbrandti*, hence likely to forage slightly further. Studies have shown that there is a correlation between the bee size and the maximum foraging distance (van Nieustadt & Iraheta 1996; Araújo *et al*. 2004). Maximal flight distance for medium sized stingless bees is estimated at 1159-1710m while that for larger stingless bees is estimated at 2km (Araújo *et al*. 2004). This shows that if stingless bees were the vectors of *Xanthomonas*, they could only spread it locally and long distance transmission would be through other means which require investigations.

Conclusion

Stingless bees are not the primary vectors of *Xanthomonas campestris pv. musacearum*. They are likely to get accidental contamination just like any other moving object or animal.

6. Summary

In Uganda, stingless bees were speculated to be the primary vectors in the spread of *Xanthomonas campestris pv*. *musacearum (Xcm)*, responsible for a banana wilt which emerged in year 2001, and rapidly spread in the neighboring countries. *Xanthomonas* is speculated to enter the banana plant through moist scars of recently dehisced male flowers and floral bracts (Gold *et al*. 2006; Tinzaara *et al*. 2006). The speculation is based on the grounds that Banana Xanthomonas Wilt in Uganda has similar symptoms to other banana bacterial diseases such as Moko whose vectors are insects (Eden-Green 2007), and that *Xanthomonas* was recovered from stingless bees body (Tinzaara *et al*. 2006). However, no study has been done to show the role and mode of transmission of the bacteria by insects or to support the hypothesis that stingless bees are the primary vectors. We therefore determined the probable role of stingless bees in the spread of *Xanthomonas* in Uganda.

As there is no culture medium for rearing *Xanthomonas*, we applied indirect approaches to test whether stingless bees would get in contact and carry the bacteria. We therefore documented the behavior of colonies in wooden observation boxes and their foraging behaviour in banana fields. We tested whether workers of *Hypotrigona gribodoi* and *Plebeina hildebrandti* are attracted to sap, ooze and nectar and would take them up when offered at the entrance of observation nests. Nectar of banana flowers has low sugar content. Therefore we investigated if *P. hildebrandti* recruit nest mates to experimental feeders with 11%, 33%, 48% and 54% sugar solution. Furthermore we determined the distance the bees would fly (forage range) to collect such solutions.

In the banana farmlands we recorded four species of stingless bees, *Hypotrigona gribodoi*, *Plebeina hildebrandti*, *Meliponula ferruginea* and *Meliponula sp*. They nested in cavities in trees, man made structures and in termite mounds. Our results show that all four species collected nectar from both male and female banana flowers. The banana variety (*Pisang Awak*) is the most susceptible to *Xanthomonas*. It had nectar with an average sugar concentration of 12.5% (range 1-32%). The foraging distance of workers of *P. hildebrandti* decreased with decrease in sugar concentration. When 11% sugar

solution was offered the bees stopped foraging at 1050m, with 33% sugar at 1215m, with 48% at 1220m and with 54% at 1230m. The foragers would therefore fly less than 1215 meters from the nest to collect *Pisang Awak* nectar.

If *Xcm* is present in nectar and incase the bees got accidentally contaminated with *Xanthomonas*, they are likely to spread it over short distances and foragers may carry it to their nest. However due to an ancient relationship between stingless bees and *Bacillus* species, which produce antimicrobial compounds that inhibit other microorganisms from growth and the presence of propolis which has antimicrobial compounds, *Xanthomonas* is likely to be eliminated in the stingless bees nest. Furthermore, a preliminary test showed that *Xanthomonas* could not grow on propolis collected from *P. hildebrandti* nest.

Noteworthy is that they never collected sap or ooze offered at the nest entrance, neither from healthy bananas nor from bananas infected with *Xanthomonas*. If *Xanthomonas* can be spread through nectar both male and female flowers are possible entry points, but if through scars caused by the fall of male flowers and flower bracts, stingless bees are unlikely vectors as they did not collect sap from the scars. There is therefore need for further studies on possible long distance vectors.

PART TWO

NESTING BIOLOGY OF *PLEBEINA HILDEBRANDTI* AND *HYPOTRIGONA GRIBODOI*

1. *Plebeina hildebrandti* (Friese)

Plebeina (Moure 1961) contains one variable species, *Melipona denoiti* (Type species Vachal, 1903*)*, which may be divisible into several closely related species (Michener 2007). Further synonyms found in Eardley 2004 are: *Trigona hildebrandti* (Friese 1900), *Trigona denoiti* (Vachal in Friese 1909), *Plebeina denoiti* (Vachal in Fletcher and Crewe 1981) *Meliplebeia denoiti* (Vachal in Moure 1961 and Michener 1990), *Trigona denoiti katagensis* (Cockerell 1934), *Plebeina denoiti katagensis* (Cockerell in Michener 1990), *Trigona clypeata* (Friese1909), *Trigona denoiti var. clypeata* (Friese in Cockerell 1920), *Plebeina clypeate* (Friese in Moure 1961), *Trigona zebra* (Strand 1911), *Trigona clypeata var. zebra Strand* (Friese 1912), *Plebeina zebra* (Friese in Moure 1961) and *Hypotrigona denoiti*.

Plebeina hildebrandti workers have a body size of 3.3-5.2mm (Eardly 2004). The species has been recorded in South Africa, Botswana, Zimbabwe, Tanzania, Kenya, Uganda, Rwanda, D.R. Congo and Nigeria (Cockerell 1934; Smith 1954; Fletcher and Crewe 1981; Eardly 2004). Nests have been described so far from Tanzania and South Africa. In Tanzania the nests were recorded either in occupied termite mounds or in the ground (Smith 1954), while in South Africa nests were recorded in the ground, although it is possible that the bees occupied cavities originally excavated by non-mound building termites (Fletcher and Crewe 1981).

General structure of the termite mounds in which *P. hildebrandti* was found

Termite mounds (termitaria) in Africa are normally constructed by termites belonging to the family Termitidae. The mounds have a subterranean and above ground part. They can be small half buried (Cubitermes) to large above-ground termitaria up to four meters high (*Macrotermes*). The mounds are constructed from subsoil of low organic content with added salivary secretions. Inside the mound is a nest within which royal cell and nursery are found. Macrotermitinae, a subfamily found only in Asia and Africa, construct “fungus garden”; spongy grey-brown combs constructed from chewed vegetable matter mixed with saliva and feacal pellets; that are scattered throughout the nest or surrounds the nursery. Various galleries run through the mound.

Humidity inside a mound is almost at saturation point (Hesse 1957; Krishnar & Weesner 1970; Darlington 1985; 1988, Bignell & Eggleton 2000).

2. *Hypotrigona gribodoi* (Magretti)

Hypotrigona gribodoi occurs widely in sub-Saharan Africa from Senegal to South Africa (Bassindale 1955; Tremblay & Halane 1993; Eardly 2004). In Eastern Africa the species is found in Kenya, Uganda, Tanzania, Somalia and Ethiopia. *Hypotrigona gribodoi* is a small bee with body sizes of workers ranging between 2-3mm (Eardly 2004; Kajobe 2007). Nests have been reported in cavities of *Ficus umbellatus, Heeria insignis, Pseudocadia zambesiaca*, *Spirostachys africana*, *Acacia horrida*, *A. senegalensis*, and in houses (Bassindale 1955; Tremblay & Halane 1993; Eardly 2004). The cavities are either slender tubular or planiform. *Hypotrigona gribodoi* is a cluster builder (Pooley and Michener 1969). Despite the many nesting possibilities, only a few nests of *H. gribodoi* have been described in Africa and from few nesting sites. The species of *Hypotrigona* are difficult to separate (Eardley 2004). Therefore if more nests of *H. gribodoi* and other species of *Hypotrigona* were described it would help not only in their identification but also in rearing them for honey and pollination purposes.

3. Objectives of the study

The aim of this study was to find the nesting sites, describe and compare the nests architecture of *Plebeina hildebrandti* and *Hypotrigona gribodoi* within Uganda

4. Study area and methods

Between November 2005 and July 2006, random searches of *P. hildebrandti* and *H. gribodoi* nests were carried out in farmlands and homesteads in Luwero, Mpigi, Mukono and Wakiso districts in central Uganda (Figure7). Vegetable crops, fruit crops, cereals, bananas, coffee, cassavas and sweet potatoes are grown within the study area. Livestock is also kept.

Different types of termite mounds ranging from small steep sided mounds up to 50cm high (subfamily Termitinae), wide dome-shaped up to 50cm high, to massive structures

above three meters high (subfamily Macrotermitinae) occurred within the farms (Figure 24a-d).

Ten nests of *P. hildebrandti* were dug out from termite mounds belonging to subfamily Macrotermitinae; *Macrotermes sp*. *Odontotermes sp*. and *Pseudocanthotermes sp*. The termite mounds were in Wakiso district at Kawada Agricultural Research Institute (KARI) farm, latitude 0^{o} 22' 39 N, longitude 32^{o}, 32'11 E, altitude 1193 meters. Kawanda receives annual rainfall of 1218mm and 2263 hours of sunshine. Detailed measurements of the architecture of the nests in their natural site were taken. To study the in-nest behavior, five colonies were kept in wooden observation hives placed on benches in a roofed shelter within KARI research farm. The hives had glass cover. They measured 50cm long, 20cm wide and 25cm high.

Six nests of *H. gribodoi* in Wakiso and Mpigi district were opened up and the architecture described. Four colonies were reared in wooden observation hives with glass cover. The hives measured 20cm long, 10cm wide and 15cm high.

5. Results: *Plebeina hildebrandti* nesting sites and nest architecture

5.1 Nesting sites

Nests of *P. hildebrandti* were found in inhabited termite mounds. The termite mounds in which the bees had built their nest had heights ranging from 40cm to over 200cm, and diameters ranging from 30cm to over 300cm (Figure 24a-c). One nest (without brood cells) was found in a small (up to 40cm high) steep sided mound, while another nest was in a low dome shaped mound of 300cm in diameter, and five small conical domes arising from it (Figure 24a). The mound had 12 stingless bee nests. All the other nests were in steep sided mounds with diameters of about 60cm and heights of about 150cm. Most of the mounds were covered with vegetation which was either scattered or dense. In a few cases, bushes and trees rooted from the sides of mounds. *Plebeina hildebrandti* nests were found at the core of the mounds adjacent to the termite nest (Figure 24d).

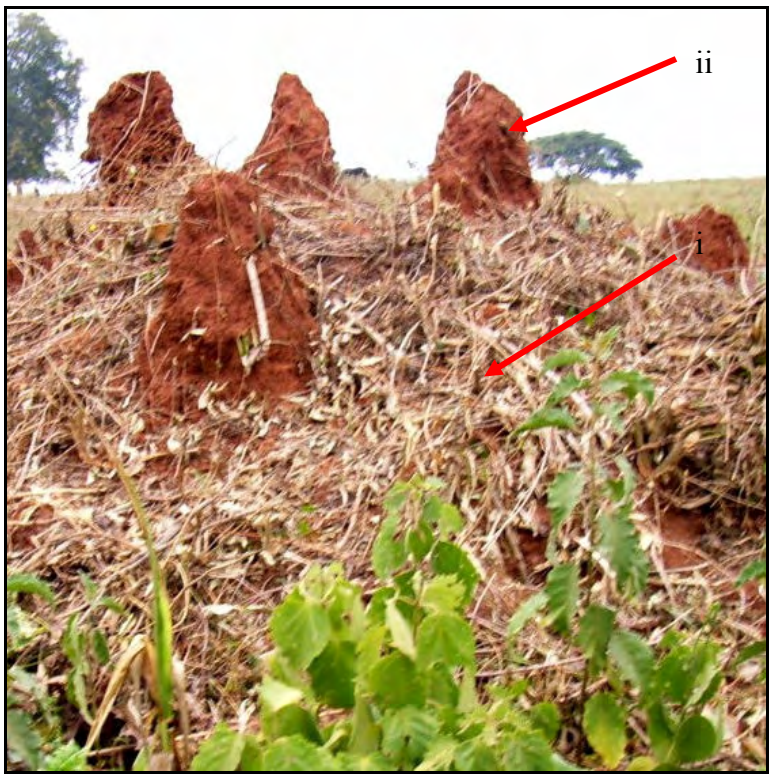

Figure 24a: A low dome shaped termite mound (i) with five small conical domes (ii). Vegetation cleared to reveal the mound.

Figure 24b: A vegetation covered low dome shaped termite mound

Figure 24c: A steep sided termite mound

Figure 24d: A stingless bees nest located within the core of a termite mound. Below the bee nest a termite queen (arrow) exposed in the royal cell

5.2 Nest architecture

The main nest structures and their measurements (Table 6) were:

1. external entrance tube
2. nest cavity
3. involucrum
4. storage pots
5. brood combs
6. drainage tube

5.2.1 Nest entrance

The nest entrances were composed of external tubes which projected above the surface of the termite mounds and ran through the mounds to connect to the nest cavity. The tubes did not enter into the nest cavity (to form internal entrance tube) but broadened so that their walls joined the wall of the cavities. The tubes were termite galleries but modified by the bees and lined with resin.

Table 6: Measurements of *Plebeina hildebrandti* nest structures in termite mounds

Structure	Length (cm)	Average length (cm)	Diameter range (cm)	Average diameter (cm)	Sample size
External entrance tube rising above the termite mound	0-25	9.5	1-1.5	1.1	50
Tube passing through the mound	43.5-120	72.3	1-1.4	1.1	5
Nest cavity	16.5-21.6	18.8	6.5-17	12.4	4
Lining batumen				0.15*	10
Batumen layer				0.3*	1
Involucrum				0.3*	10
Pollen pots	0.9-1.6	1.28	0.8-1.7	0.87	26
Nectar pots	1.1-1.3	1.23	1.0-1.9	1.37	5
Pillars connecting pots	1.0-8.8	4.4	0.1-0.6	0.2	13
Combs	0.3-0.5***[1]	0.45	8-16***[2]	11	6
Pillars between combs	0.5-0.8	0.6	0.1-0.6	0.3	3
Brood cells	0.3-0.5	0.45	0.2-0.3	0.26	1564
Queen cells	0.7-0.9	0.8	0.5-0.6	0.54	6
Drainage tube	10-82	38.3	1	1	3
Number of brood cells					3300-3775**
Adult bees (1 nest)					3091

* refers to thickness of lining batumen, batumen layer and involucrum layer

** counted in two nests

***[1] comb thickness, ***[2] comb diameter

External tube above the termite mound

The external tubes varied in length (Table 6), with some tubes less than a centimeter long in bare mounds (Figure 25a) to relatively long, sometimes to over 20cm in termite mounds covered with vegetation (Figure 25b). Measurements and observations were done for over 50 external entrance tubes. Tube concealment was either through color, where the tube color merged with the color of the surroundings (Figure 25c) or through addition of soil and plant material (Figure 25d). Intruders to the nest were barred by placing sticky resin droplets on and around the tube (Figure 25e) or smearing a layer of sticky resin on and around the tube (Figure 25f). Some tubes had perforations all round towards the apex (Figure 25g). These tubes were made of brittle cerumen. In some nests there were cases of two entrance tubes but only one in use; however a colony reared in a wooden observation hive was able to use two exit tubes simultaneously. All the tubes were cylindrical with mainly circular lips although irregular lips were also encountered (Figure 25f).

Figure 25a: A short entrance tube on a termite mound without vegetation cover

Figure 25 b: A long nest entrance tube on a termite mound covered with vegetation

Figure 25c: A nest entrance tube camouflaged by soil colour

Figure 25d: A nest entrance tube camouflaged by a mixture of mud and plant material

Figure 25e: A nest entrance tube with sticky resin droplets on and around it

Figure 25f: A nest entrance tube with a thin sticky resin layer smeared on and around it

Figure 25g: A nest entrance tube with perforations (arrows) around the tube apex

Tube passing through the termite mound

The tubes passing through the termite mound had walls lined with resin (Figure 26a, and b), with lengths ranging from 43.5cm to 120cm (Table 6). Pieces of resin structures (Figure 26b) were located at various positions within the entrance tubes (Table 7).

Table 7: Measurements and positions of resin structures located within stingless bees nest entrance tubes passing through termite mounds

	Resin pieces	Length of resin piece (cm)	Diameter of resin pieces (cm)	Entrance tube diameter and length at the point the resin piece was located (cm)	Distance from the surface of the termite mound to the position of the resin piece(cm)
Nest 1	a	*	*	1.5 x 1	7
	b	3	0.3	3 x 2	53
	c	6	3	4 x 3	59
	d	2	0.5		59.8
Nest 2	a	3	0.3	3 x 2	58
Nest 3	a	4.5	1.5	4.5 x 3.5	90
	b	*	*	10x6	101
	c	*	*	*	116
Nest 4	a	3	2	3.5 x 2	28.5
Nest 5	a	3	0.5		30

* Measurements of the resin piece not taken, due to the shape (amorphous structure)

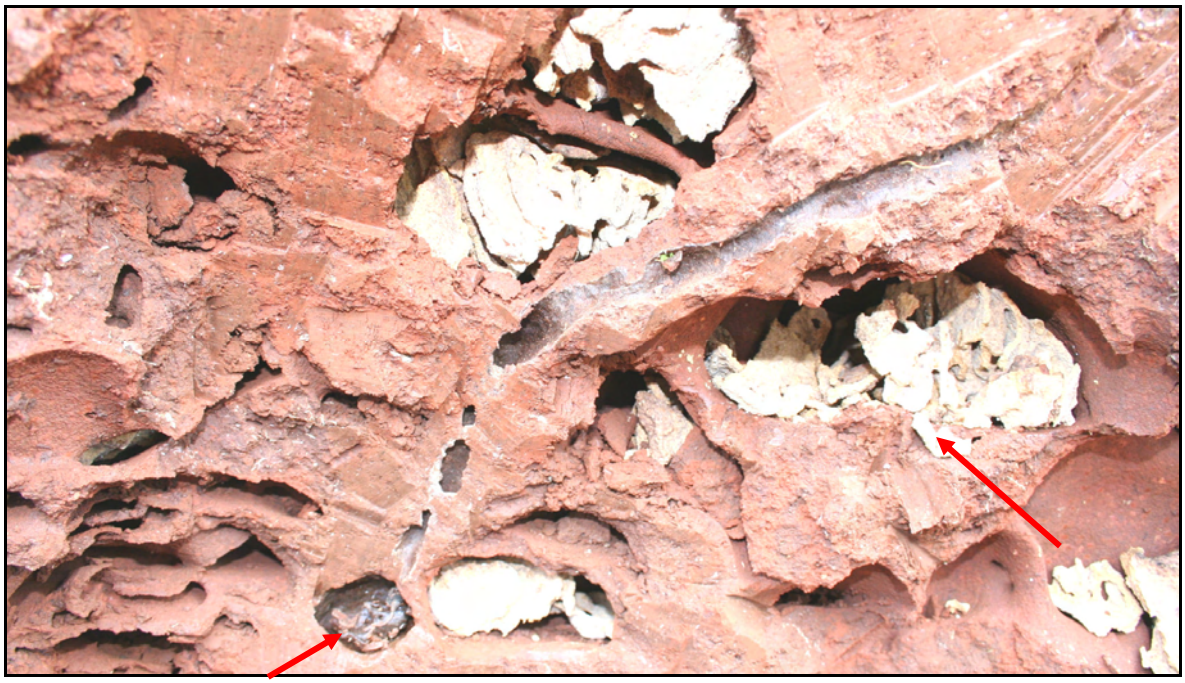

Figure 26a: A nest entrance tube lined with resin passing through a termite mound. On the lower left is an amorphous resin structure. Fungus garden (arrow on the right) visible

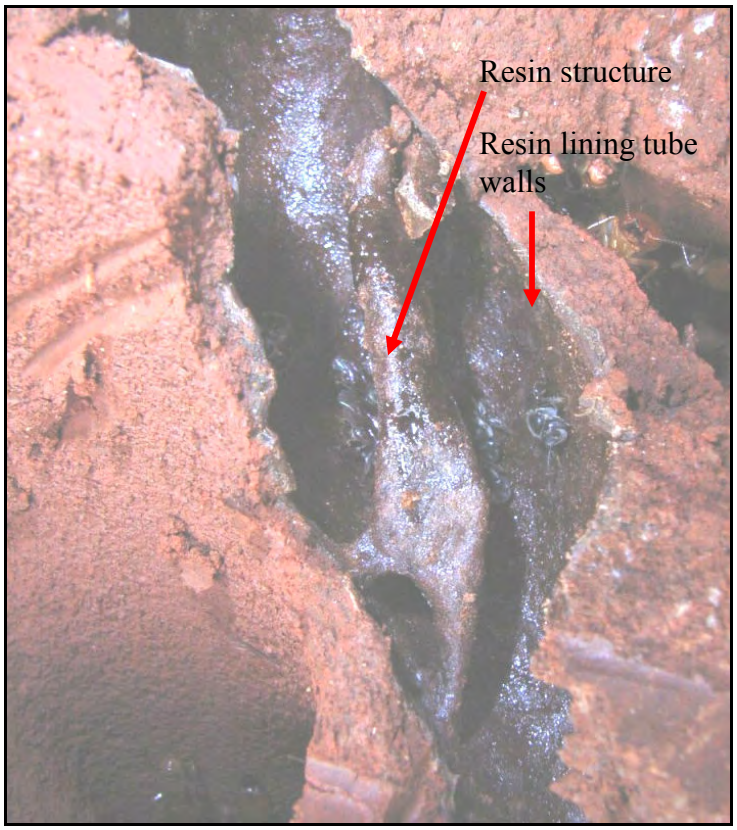

Figure 26b: A resin structure within a dilated part of the nest entrance tube passing through a termite mound

5.2.2 Nest cavity

The nest cavity ranged from 16.5 - 21.6cm long and 6.5 -17cm wide. The nest cavity was always within the core of the termite mound (Figure 24d). The cavities had been termite chambers but modified by the bees through digging and lining with 0.1- 0.2cm thick lining batumen. In one newly established bee nest, a heap of soil was found below the entrance tube and workers were bringing soil from inside the nest to the outside of the termite mound, an indication of bees digging.

Lining batumen on walls of the nest cavities

The color of the lining batumen on walls of the nest cavities was brownish (Figure 27a) to dark brown (Figure 27b). The thickness of the layer ranged from 0.1- 0.2cm. In some nests short pillars originated from the lining batumen to support storage pots (Figure 27a), while in other nests where the storage pots were surrounded by a soft batumen layer which separated them from lining batumen, there were no pillars (Figure 27b).

Figure 27a: A nest cavity with short pillars (arrows) emerging from lining batumen on the walls of the nest cavity

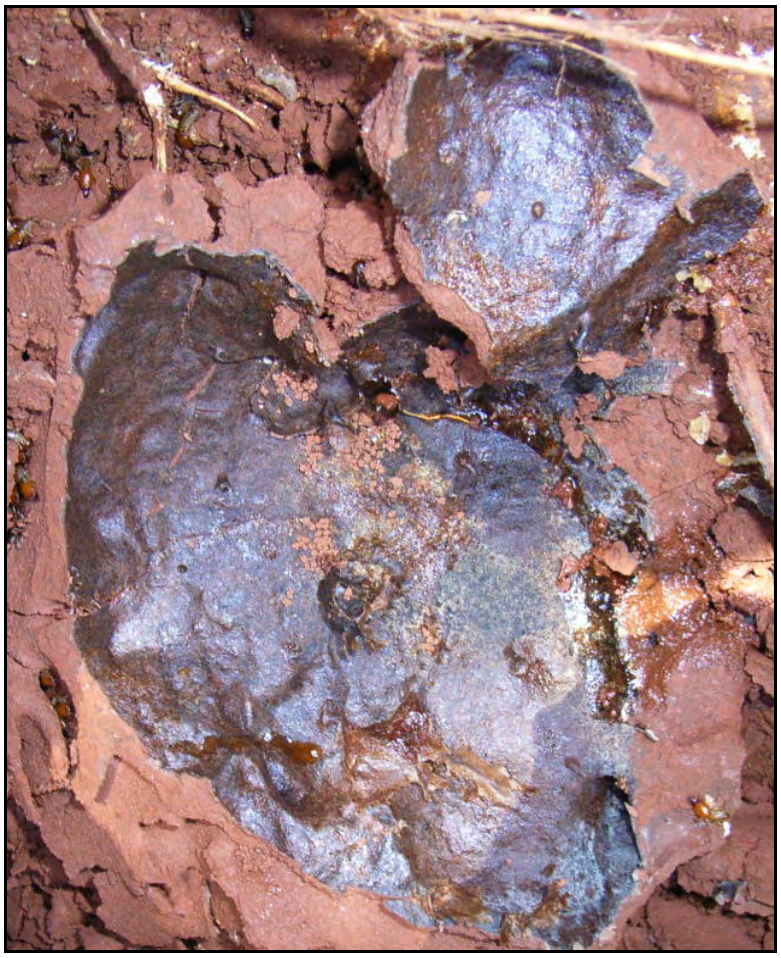

Figure 27b: A nest cavity with no pillars emerging from the lining batumen on the walls of nest cavity

5.2.3 Batumen layer separating the lining batumen and the storage pots

In some nests a soft batumen layer surrounded the entire nest separating it from the lining batumen on the walls of nest cavity (Figure 28a) while in others the batumen layer separated the nest from lining batumen only at the bottom. On the sides horizontal pillars joined storage pots and the lining batumen (Figure 29a). Still some nests did not have any batumen layer separating the nest and the lining batumen on the nest cavity. In this case short pillars separated storage pots and lining batumen. In colonies reared in wooden boxes, soft laminate batumen enclosed the nest with pillars and connectives originating from the batumen to the walls of the box (Figure 28b).

5.2.4 Involucrum separating brood combs from storage pots

In all the nests, a layer of soft involucrum surrounded the brood combs separating them from storage pots (Figure 28c).

Figure 28a: A soft batumen layer surround the nest separating it from the nest cavity

Figure 28b: Laminate batumen covering a nest in a *Plebeina hildebrandti* colony reared in a wooden observation hive. Pillars and connectives join the walls and top of the hive

Figure 28c: An involucrum layer (arrow) separate brood combs from storage pots. The involucrum is broken to reveal brood combs

5.2.5 Storage pots

Pollen and nectar pots surrounded the involucrum covering brood combs (Figure 29a), although in some nests the pots were lacking below the combs. The pollen and nectar pots were in groups interconnected by pillars. The pollen pots were ovoid with an average height of 1.2cm and width of 0.87cm while the nectar pots were circular to spherical with a mean height of 1.2cm and width of 1.3cm. Pots within a group had cerumen added on the outside between the walls. Pillars interconnecting groups of storage pots had a mean length of 4.4cm and width of 0.2cm (Table 6). A few storage pots contained waste materials from the nest (Figure 29b).

Figure 29a: Groups of pollen and nectar pots interconnected by pillars surround the involucrum which encloses the brood combs. On the sides pillars connect storage pots to lining batumen, while at the base a soft batumen layer (arrow) is present

Figure 29b: A half-way completed or half way destroyed storage pot used for waste deposition

5.2.6 Brood combs

The brood nest consisted of horizontal combs arranged into layers, enclosed by an involucrum sheet. The combs consisted of cells with a mean height of 0.45cm and width of 0.26cm (Table 6). There were 6-12 combs in the different nests studied, with each comb separated by short pillars. The pillars had a mean length of 0.6cm and width 0.3cm. Comb diameter and thickness varied in different nests (Table 6). The shape of combs and direction in which new cells were added varied from nest to nest.

Queen cells of various sizes (Table 6) were found in the hole at the center of the combs. In one nest there were 13 queen cells.

5.2.6.1 Brood comb architecture in different nests

Nest 1

In the first nest (Figure 30) horizontal circular combs were arranged into layers with short pillars separating them. The smallest and the youngest comb, with newly constructed cells, was at the top. New cells were added rotationally through an advancing edge of each comb to replace old cells. Although the advancing edge in each comb was not progressing at the same rate with the combs above and below, a side view of the nest from top to bottom showed one side of the combs to contain older cells with wax already removed and the other side to contain younger cells. There were no discernible queen cells.

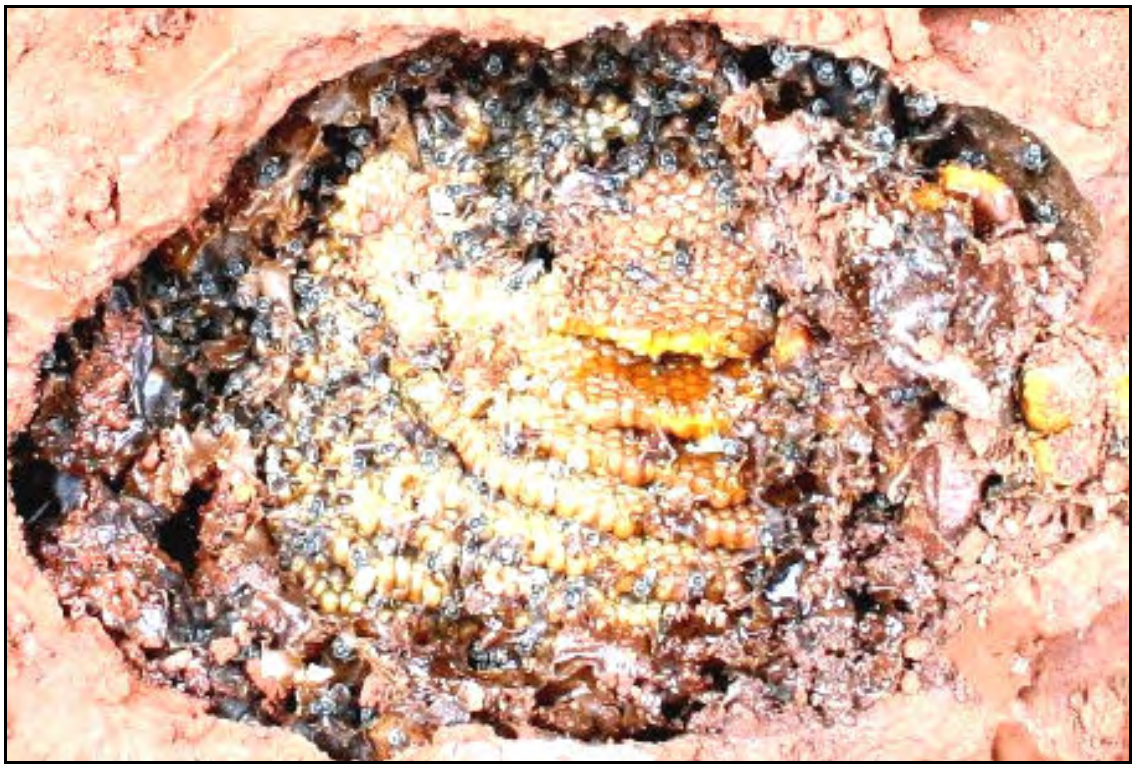

Figure 30: Nest 1: Circular combs arranged into layers. Cells on the right side of combs from top to bottom are younger than cells on the left side of the combs.

Nest number 2

In the second nest, there were horizontal circular combs in layers with a central hole and a radial gap which narrowed and extended to the edge of the combs. There were few

queen cells in the gap and workers used this space to move from top to bottom of the nest. New cells were added in an anticlockwise order replacing cocoons being removed.

Nest number 3

In the third nest (Figure 31), the center of the combs had a hole but no radial gap extending to the edge of the combs. In the central hole workers had constructed queen cells supported by pillars. The cells had a tendency towards spiral formation where cell progression was in the order of old cells at the beginning of the comb (Figure 31 arrow a) and younger cells forming an advancing edge (Figure 31 arrow b). However the spiral did not progress to form a new comb below or above the present comb, instead the advancing edge developed anticlockwise replacing the space left by cocoons being removed.

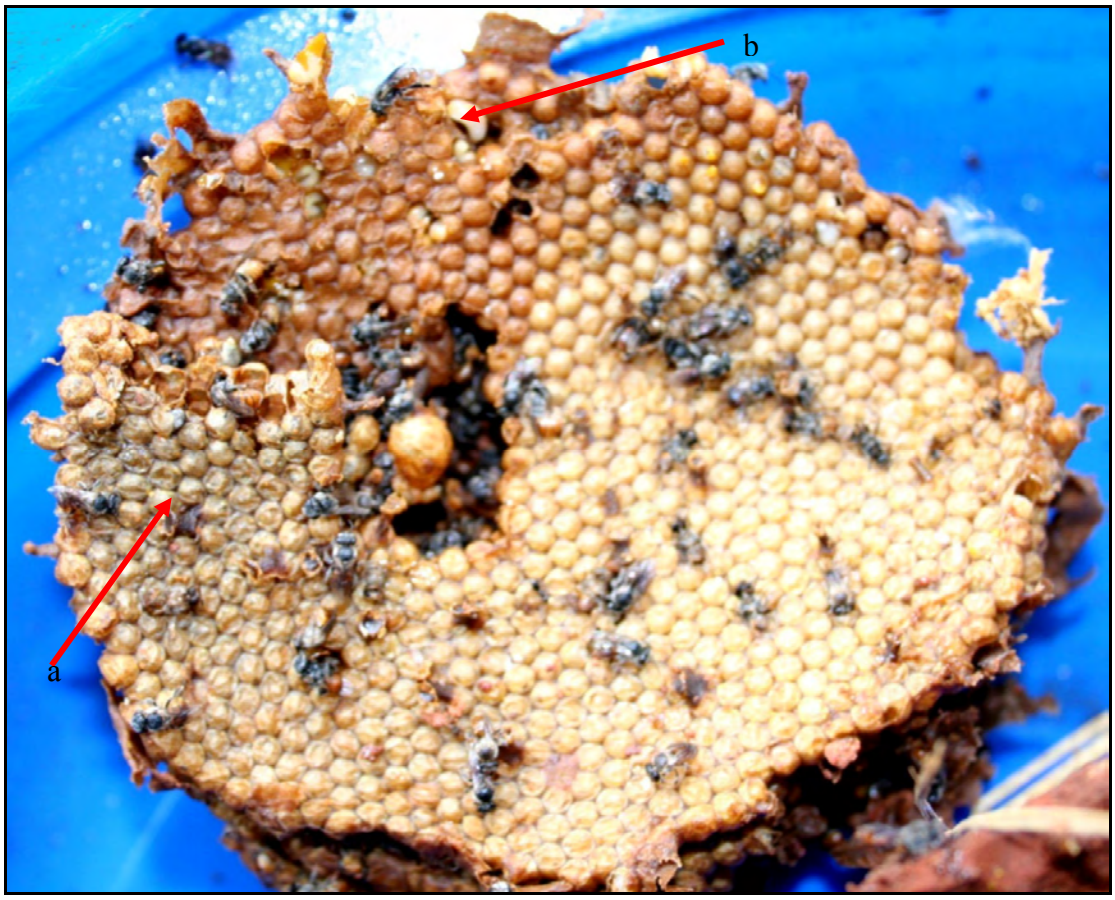

Figure 31: Nest 3: Circular combs with a central hole with queen cells. New cells added in an anticlockwise direction, (arrow a) indicates old cells marking the beginning of the comb, and (arrow b) indicates young cells on the progressing front

Nest number 4

In the fourth nest (Figure 32), the combs had a hole at the center filled with queen cells. There was no radial gap to the edge of combs. New cells were added to each comb to replace the ones removed in either clockwise (a) or anticlockwise direction (b)

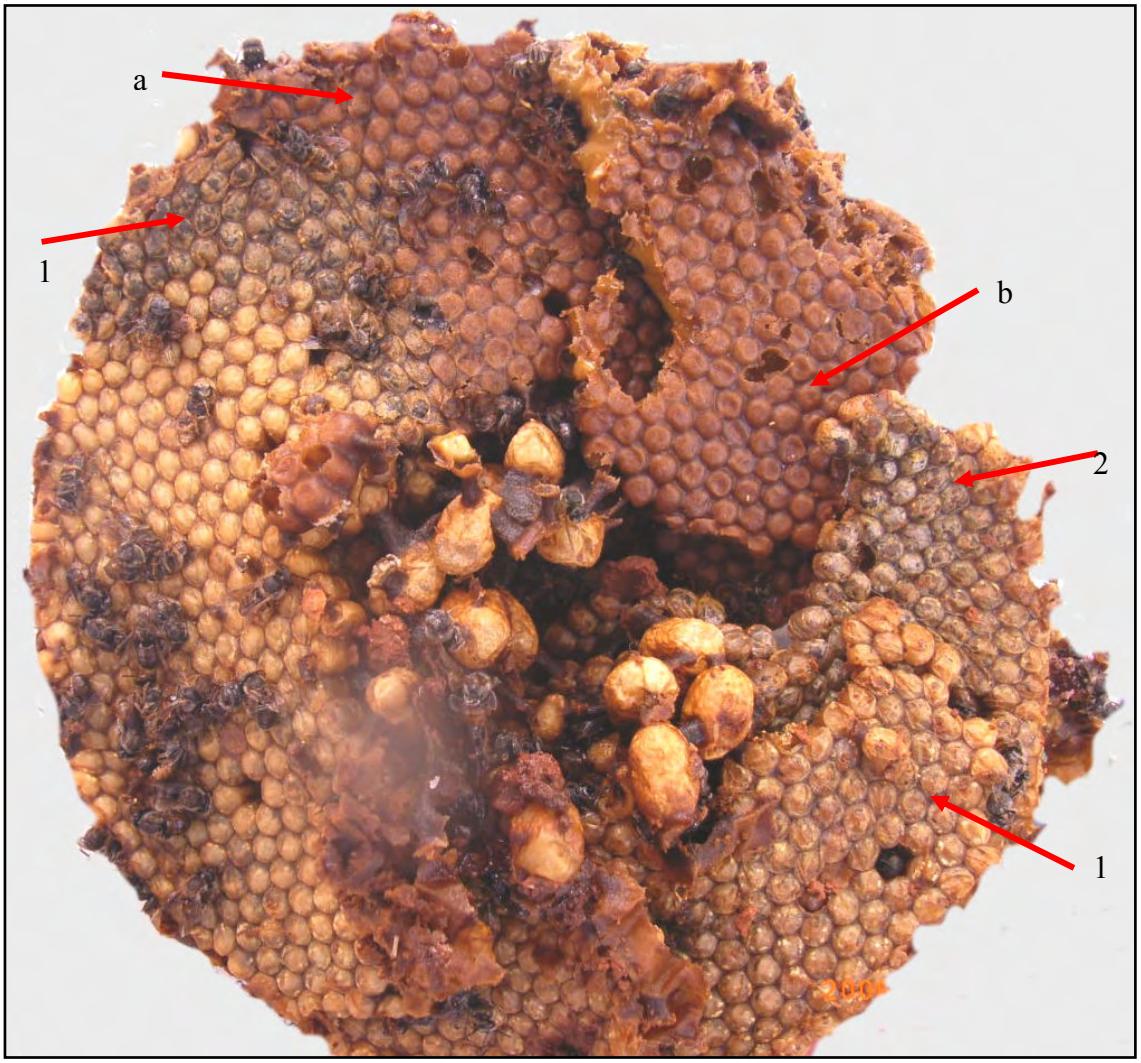

Figure 32: Nest 4: A central hole with queen cells: the top comb (1) showing new cells being added in a clockwise direction (arrow a), and the comb below it (2) new cells being added in anticlockwise direction. The new cells added were attached slightly below the older cells (arrow b)

Nest number 5

In the fifth nest (Figure 33), combs were arranged in two groups leaving a hole at the center with queen cells. There was no radial gap in these combs. One group on one side was composed of semicircular combs with cells containing old pupae and old larvae (Figure 33 arrow a) while the other group on the opposite side was composed of trapezoidal combs with cells containing young larvae and eggs (Figure 33 arrow b). For the trapezoidal combs with young cells, the top comb was the smallest and the comb size increased downwards. Addition of cells in the top trapezoidal comb seemed to had been started by a single cell supported by pillars from the comb below. In the second trapezoidal comb, attachment to the semicircular combs was on one edge. It was not possible to tell whether cells in the second trapezoidal comb had been added from the edge of the semicircular combs in a clockwise direction, or had been started by a single cell supported by pillar from the comb below it. The third trapezoidal comb was connected to the semicircular combs on both edges. It was also not possible to tell from which side the addition of cells had started

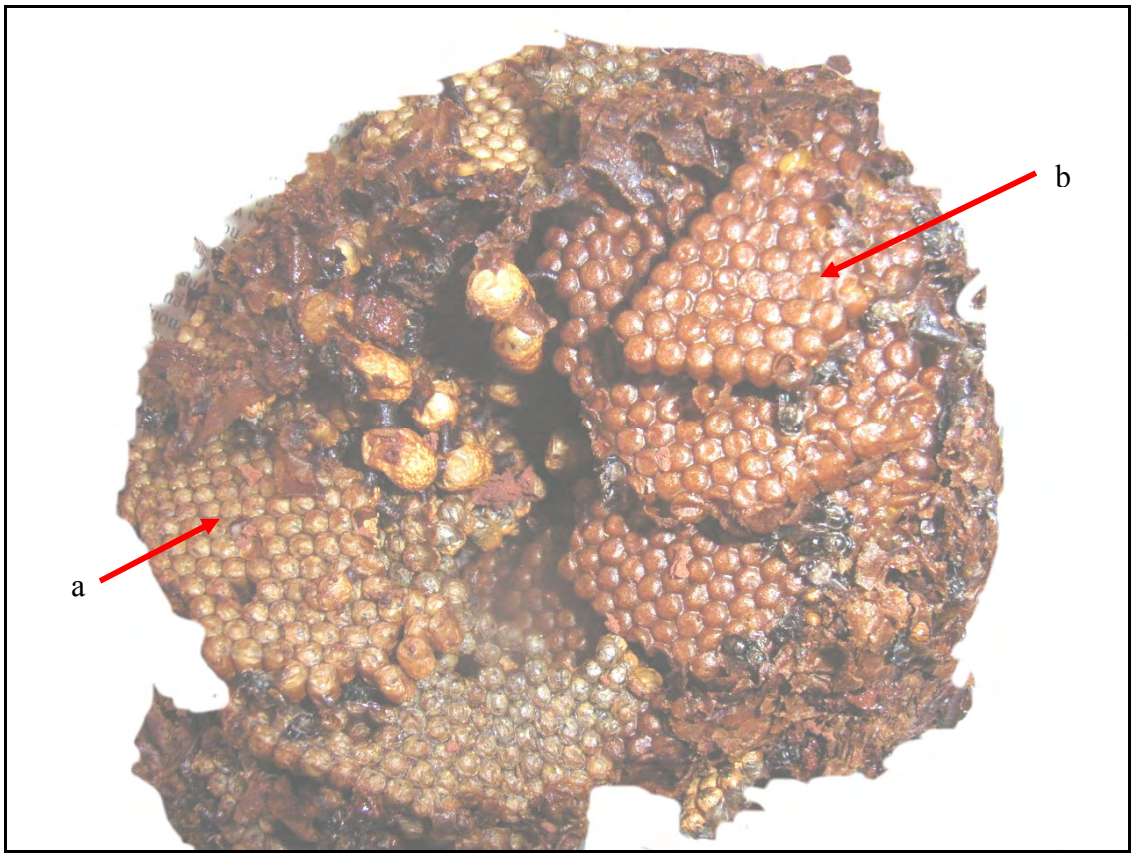

Figure 33: Nest 5: Two groups of combs: one group semicircular combs with older cells (arrow a) and the other group trapezoidal combs with younger cells (arrow b). At the center a hole with queen cells

Nest number 6

In the sixth nest (Figure 34) the cell and comb arrangement was as in nest 5, but the hole at the center was empty. There were no distinguishable queen cells.

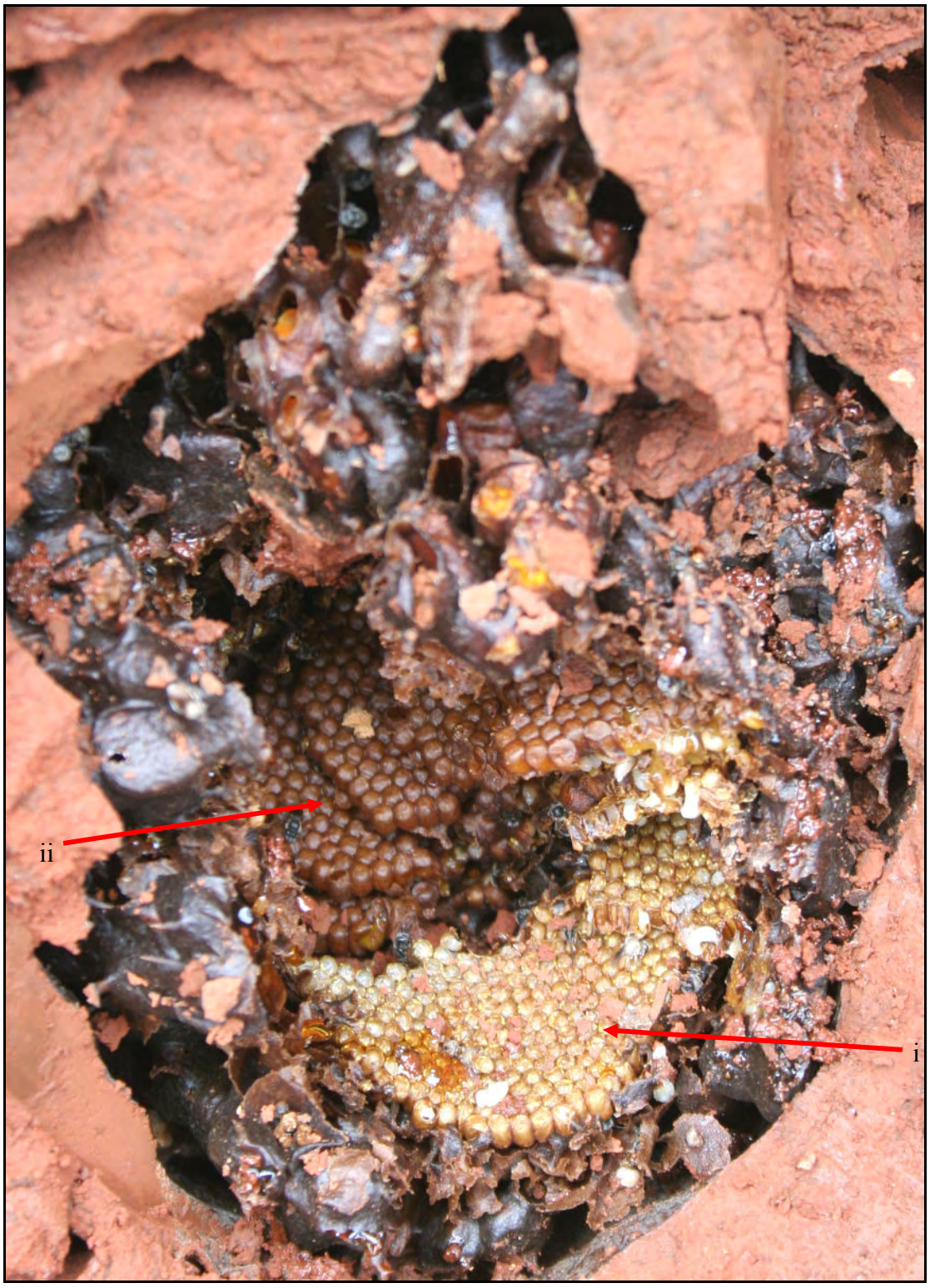

Figure 34: Nest 6: Showing cells in two groups: semicircular combs with older cells (arrow i) and trapezoidal combs with younger cells (arrow ii). A central hole without queen cells

Nest number 7

In the seventh nest (Figure 35) there was complete separation of old and new cells. A vertical gap through the center of the nest separated the combs into two parts. There were 12 combs with six combs composed of young cells forming a stairway on one side and the other six combs with old cells forming another stairway. The combs were trapezoidal and arranged slightly slanting with the smallest comb on top and the largest at the bottom. There were no distinguishable queen cells. Cerumen and pillars had been removed from the older cells making the stairway to collapse (Figure 35). The cells were not synchronically built.

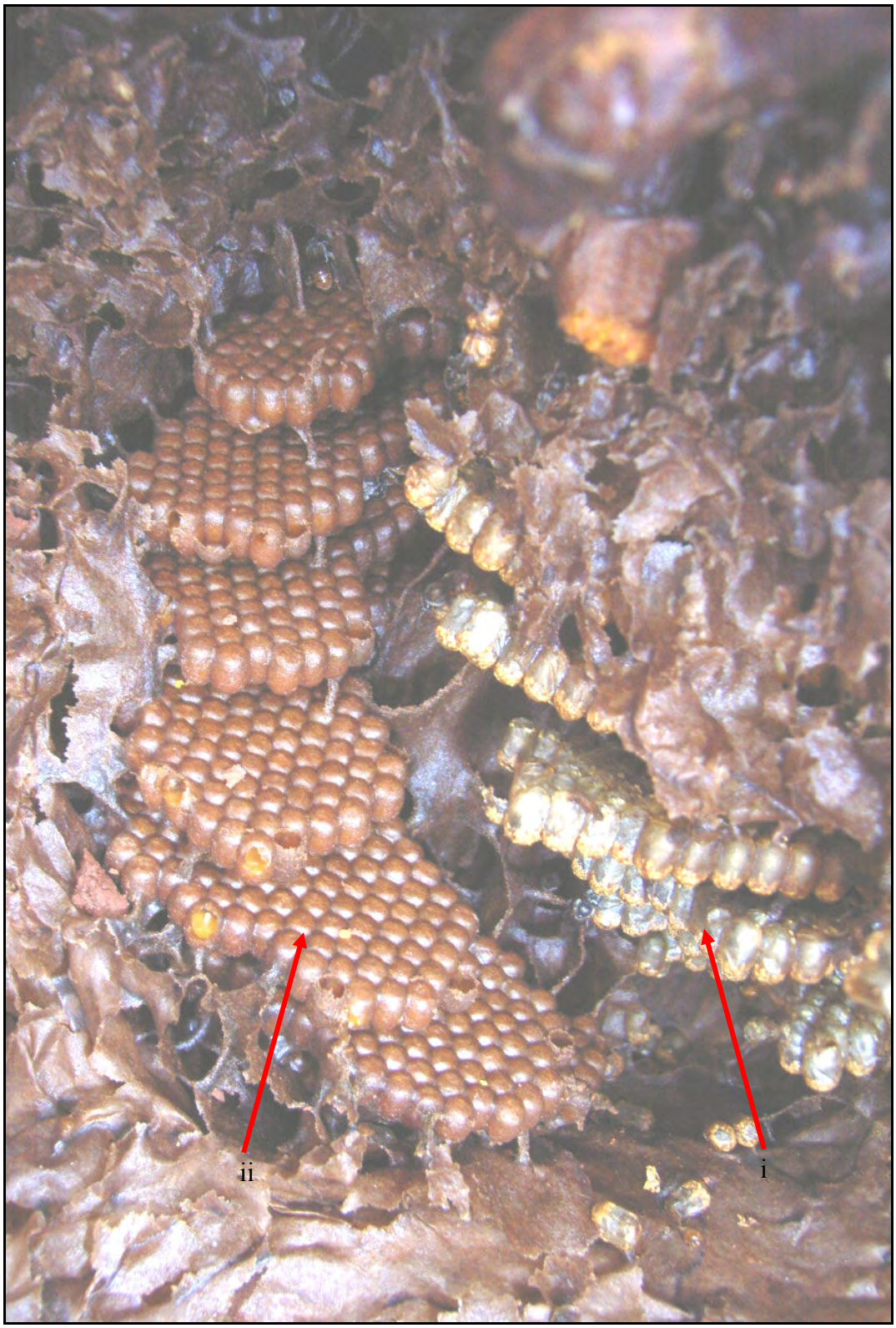

Figure 35: Nest 7: Trapezoidal combs with complete separation of old (arrow i) and new combs (arrow ii). The combs are slanting and forming a stairway.

5.2.7 Drainage tube

All the nests had drainage tubes originating from the base of the nest cavity. The tubes were lined with thin layer of cerumen. The tube either run vertically below the nest before slanting or slanted directly from the base (Figure 36)

Figure 36: A slanting drainage tube. A piece of grass indicates the tube

5.3 Behavior of the bees at the nest entrance

When the nests were opened within the termite mounds, workers did not show any aggressive behavior. During the day about four workers guarded the nest entrance. During the night the nest entrance was open and unguarded. The guards retreated into the nest when objects passed in front of the nest. When ants invaded the nest guards alerted nest mates. They exhibited two types of defense behavior. Those bees who carried pebbles of resin in their corbiculae deposited them at the rim of the entrance tube or around the base of the nesting box, thus creating a sticky barrier. Other bees brought a single pebble between their mandibles. These bees approached the ants and immobilized them by gluing their legs and antennae to the ground. We observed that such guards fought back termite soldiers by gluing various resin pebbles on their long mandibles so that they could not open them anymore (Figure 37 and 38).

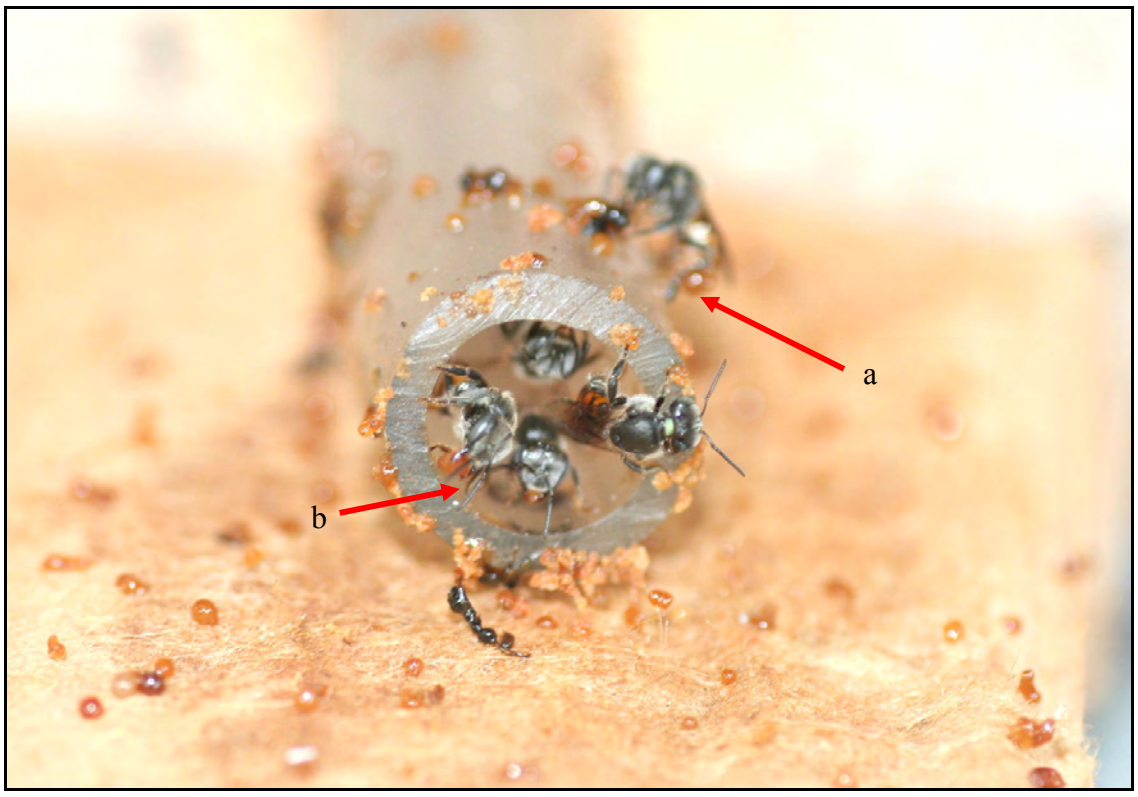

Figure 37: *Plebeina hildebrandti* workers respond to an invasion by ants at the nest entrance by carrying pebbles of resin on their corbiculae (arrow a) or between their mandibles (arrow b) and depositing it around the tube or sticking on the legs and antennae of the ants

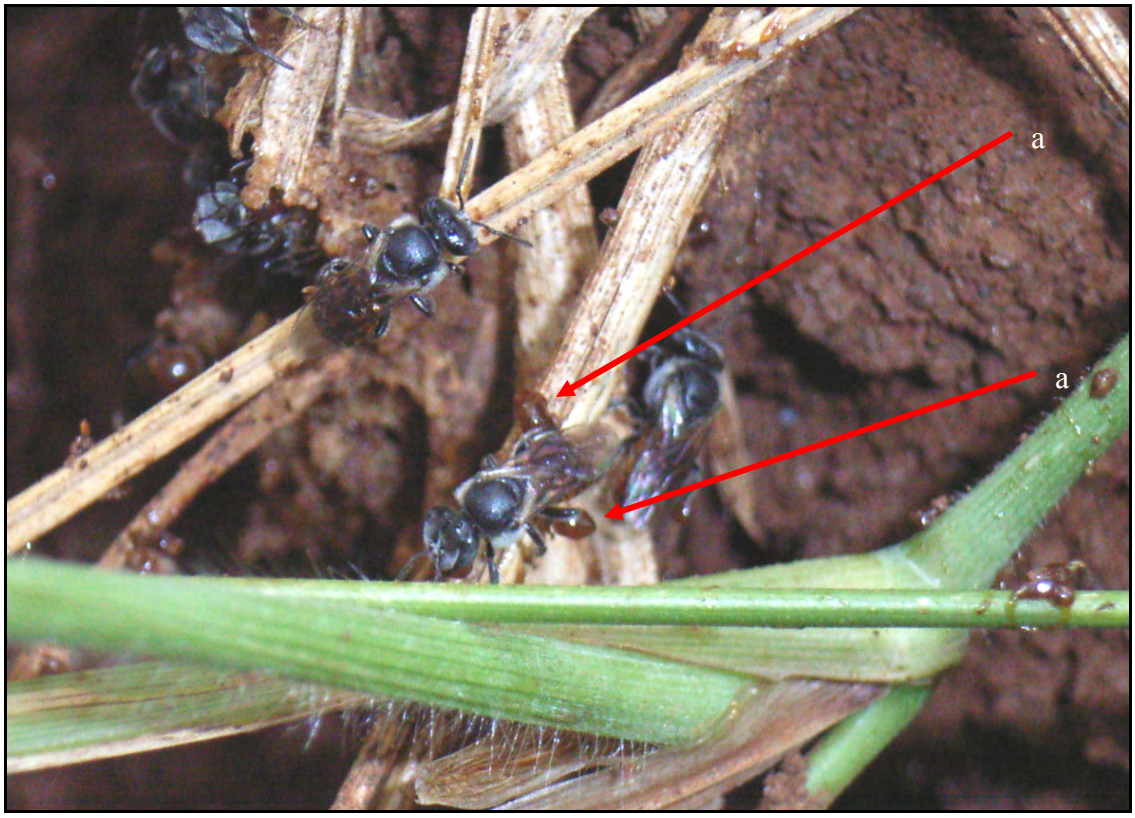

Figure 38: *Plebeina hildebrandti* workers respond to an invasion by ants at the nest entrance on a termite mound by carrying pebbles of resin on their corbiculae and depositing it on and around the tube. Some bees wait outside the entrance tube for invading ants with resin on both corbiculae (arrow a)

5.4 Discussion

Plebeina hildebrandti nests were found in occupied termite mounds at the core of the mound. The choice of the location may be advantageous to the bees in several ways. The mound shields the nest from rain, flooding, fire, and attack by large enemies. Nest location in the nursery part of inhabited termite mounds has additional advantages. One, termite nests are enclosed systems (Krishnar and Weesner, 1970), with the nursery maintained at a temperature of 36^{o}C and a relative humidity which does not drop below 96.2% (Howse, 1970). Therefore, the choice of the nursery is advantageous to the bees as it ensures a stable microclimate.

Nevertheless, the bees' nests have a thin lining batumen on the walls of the nest cavity which waterproofs the nest (Wille and Michener, 1973), preventing infiltration of moisture into or out of the nest. As a result, there is a danger of moisture saturation within the nest due to the bees' respiration and the dehydration of nectar to honey. Water would condense on the walls of the nest cavity if not removed, increasing humidity to saturation. To solve the problem of water accumulation, a long drainage tube at the bottom of the bee nest serves to get rid of excess water that would otherwise accumulate (Smith, 1954; Portugal-Araujo, 1963; Wille and Michener, 1973; Camargo and Wittmann, 1989). The lining of the drainage tube with resin, ensures that water is channeled a long distance away from the bee nest before it infiltrates the lower parts of the termite mound. Besides, the bees ensure that the run off water from their nest is conducted not only downwards but also side wards through slanting tubes. Otherwise this water may move up by capillary forces and pass back to the nest, especially when the termite mound is heated up during periods of high temperatures.

Secondly, nest location in the nursery part of inhabited termite mounds has an additional advantage to the bees in that they can easily dig and modify nest cavities. The nursery region of the termite mound is typically soft due to more organic material which is hygroscopic; hence high moisture absorbing capacity than the inner and the outer walls of the mound. The outer wall is made up of an impervious layer of sand and clay particles cemented together with the termites' salivary secretion (Howse, 1970).

Unlike the termite nests, the stingless bee nests had direct contact with the outside environment through the open external entrance tube. The tube may be used for thermoregulation especially during periods of high temperatures. A study by Fletcher and Crewe (1981) showed that *Plebeina denoiti* nesting in the ground was capable of thermoregulation through the entrance tube.

Variation in the size, shape, camouflage and firmness of the entrance tube indicated a defense role. This was more pronounced on termite mounds without vegetation cover, where all the entrance tubes were short and brown, or had mud added so that the color of the tube merged with the color of the mound. The color of the added plant material also merged with the color of the termite mound camouflaging the entrance tube. On termite mounds with vegetation cover, the tubes were dark at the base and only light colored at the apex, making it difficult to distinguish them from plant stems and branches. Addition defense strategies were gluing resin droplets on and around the tubes as well as depositing large quantities of resin in different parts inside the entrance tubes. The resin acted as a barrier to intruders and was also used to immobilize them.

In some nests a soft batumen layer separated the nest and the lining batumen, while in others the batumen layer was absent. In the latter, pillars connected the nest and the nest cavity. It was not clear why some nests had a batumen envelope while others did not. In colonies reared in wooden observation hives, soft batumen layer covered the entire nest with pillars originating from the layer to the walls of the hive. This is an indication that the batumen layer may serve three purposes: to support the nest, control microclimate within the nest and fill spaces within the nest for nest firmness. The brood combs were separated from the storage pots by an involucrum layer which served to probably protect the brood cells and control microclimate within the brood chamber.

High variations occurred in the arrangement of the brood combs with some nests having circular horizontal combs, others combination of semicircular horizontal combs and trapezoidal combs, while others had only trapezoidal combs. Although nest architecture conditions can be adaptations to various kinds of cavities, it is also a species specific trait

which can support identification of species. *Plebeina hildebrandti* may therefore require taxonomic re-examination to determine if it's one species or more.

Colonies of *P. hildebrandti* reared in wooden observation boxes did not adapt well. This was probably due to problems of temperature and humidity regulation in the hives, as the workers spent much time building soft involucrum sheets around the nest. No brood cells were constructed during this period, and the queen did not lay eggs, although foraging and construction of storage pots continued. As *P. hildebrandti* has a short forage distance, it can be reared for pollination purposes in plantations

6. Results: *Hypotrigona gribodoi* nesting sites and nest architecture

6.1 Nesting sites

More than a hundred nests of *Hypotrigona gribodoi* were found in cavities such as walls of mud houses between window frames, door frames, roofing timber, electric sockets, dry drainage pipes, electric and telephone poles, and cavities in trees. Most nests were found in walls of mud houses and in cavities in timber used for construction. In areas with stone or brick houses, nests were found between window frames, door frames and roofing timber. In areas without man made structures nests were found in cavities in trees.

6.2 Nest in walls of mud houses and in dry drainage pipes

6.3 Nest architecture

The main nest structures and their measurements (Table 8) were:

1. external entrance tube
2. nest cavity
3. involucrum
4. storage pots
5. clusters of brood cells

6.3.1 Entrance tube

The external entrance tubes had a mean length of 2cm and diameter of 0.4cm (Table 8). The shape of the tube apex varied from tampering (Figure 39a), circular (Figure 39b and c), to funnel shaped (Figure 39d). The tubes apexes were dark brown (Figure 39a and e), light brown (Figure 39b) milky white or transparent (Figure 39c and d). In one nest there were two tubes simultaneously in use (39a). Colonies nested in proximity (Figure 39e). Internal entrance tube was recorded only in one nest reared in a wooden observation hive (Figure 39f).

Table 8: Measurements of *Hypotrigona gribodoi* nest structure in mud walls and tree trunks.

Structure	Length (cm)	Average length (cm)	Diameter (cm)	Average diameter (cm)	Sample size
External entrance tube	0.5-2.5	2	0.3-0.5	0.4	32
Entrance tube through the cavity in trunks	4		0.3		1
Nest cavity in walls	5-15	10	5-10	8	5
Nest cavity in trunks	21		8.5		1
Resin lining nest cavity					6
Involucrum* surrounding brood combs					6
Pots	0.5-0.74	0.59	0.43-0.6	0.49	6
Pillars between clusters		0.2			
Brood cells	0.2-0.47	0.32	0.13-0.32	0.23	60
Queen cells	0	0	0	0	
Drainage tube	0	0	0	0	0
No. of brood cells					570-<1000**
Adult bees counted in					101-516**

* Absent

** counted in two nests

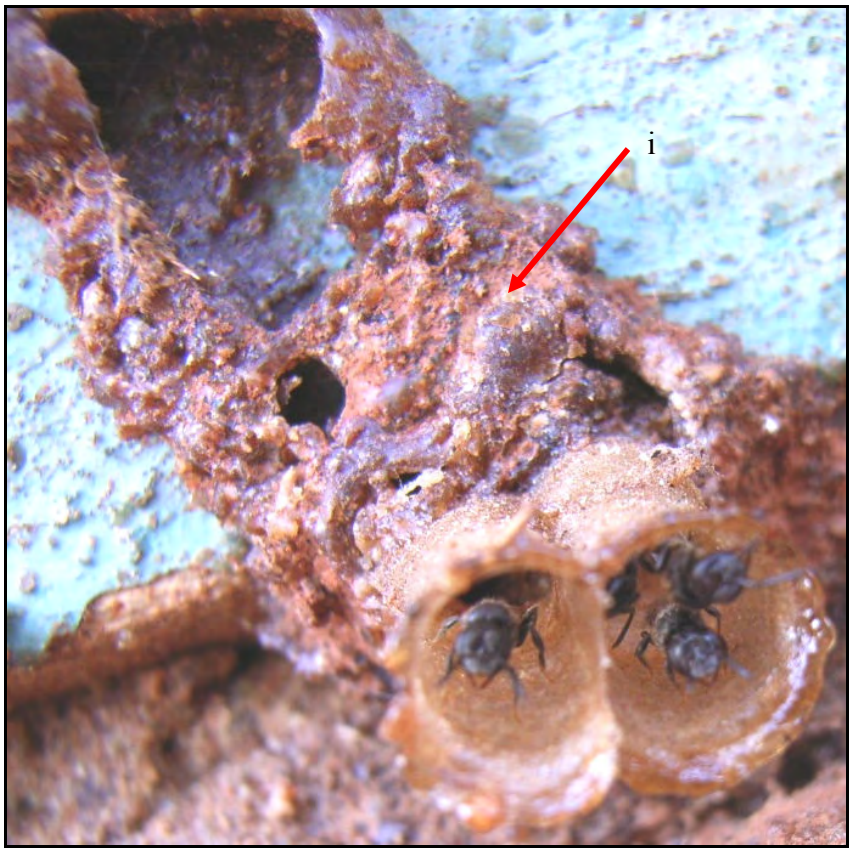

Figure 39a: *Hypotrigona gribodoi* double nests entrance tube with tampering shape. Brittle batumen (i) conceal the direction of the entrance tube into the nest

Figure 39b: *Hypotrigona gribodoi* funnel shaped nest entrance tube

Figure 39c: *Hypotrigona gribodoi* circular nest entrance tube with a milky white to transparent apex and a lower dark brown part

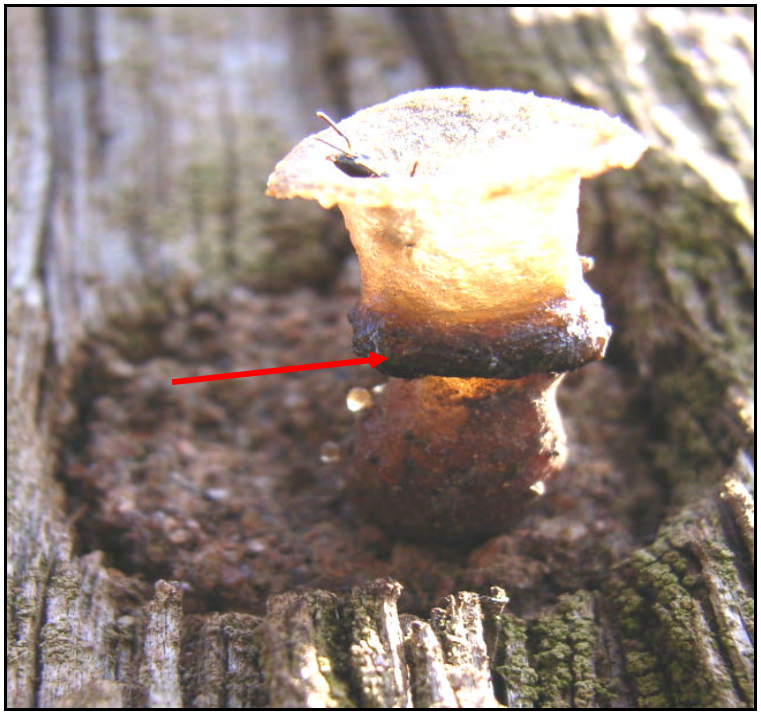

Figure 39d: *Hypotrigona gribodoi* funnel shaped nest entrance tube with a dark resinous ring (arrow) separating a transparent apex from the lower dark brown part

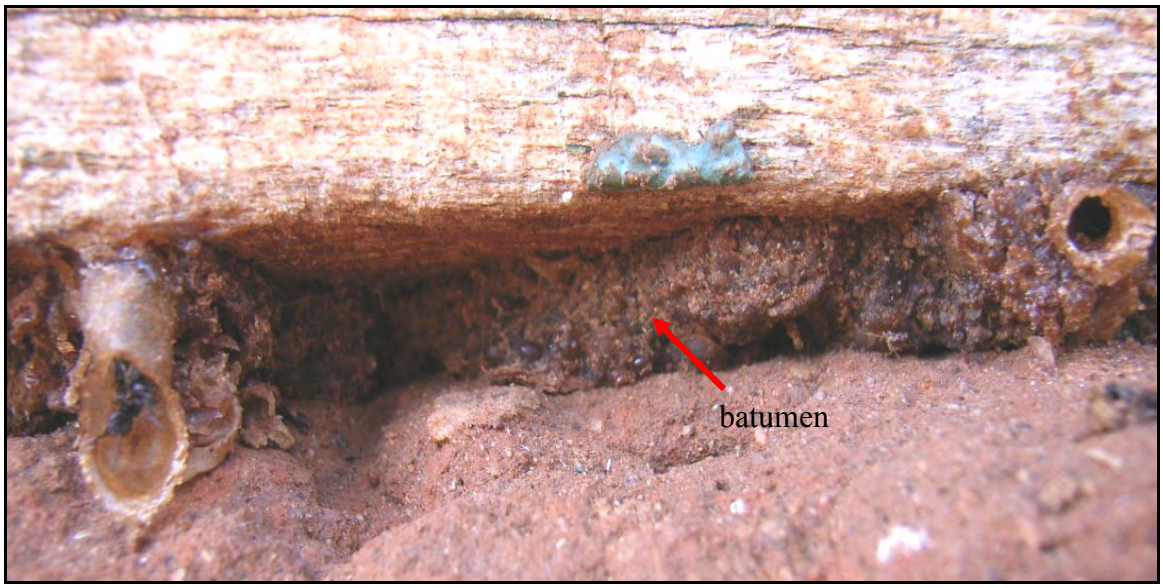

Figure 39e: Two colonies of *Hypotrigona gribodoi* nesting in proximity in a wall. In between the two entrance tubes, hard batumen mixed with soil used to fill the crevices.

Figure 39f: Internal entrance tube in a *Hypotrigona gribodoi* nest reared in a wooden observation hive

6.3.2 Nest cavity

Length and diameter of the nest cavities varied with means of 10cm and 8cm respectively (Table 8).

Lining batumen on the walls of the nest cavity

A less than 1mm transparent lining batumen lined the walls of the nest cavities (Figure 40a).

Figure 40a: Thin transparent shiny lining batumen on the walls of a *Hypotrigona gribodoi* nest cavity

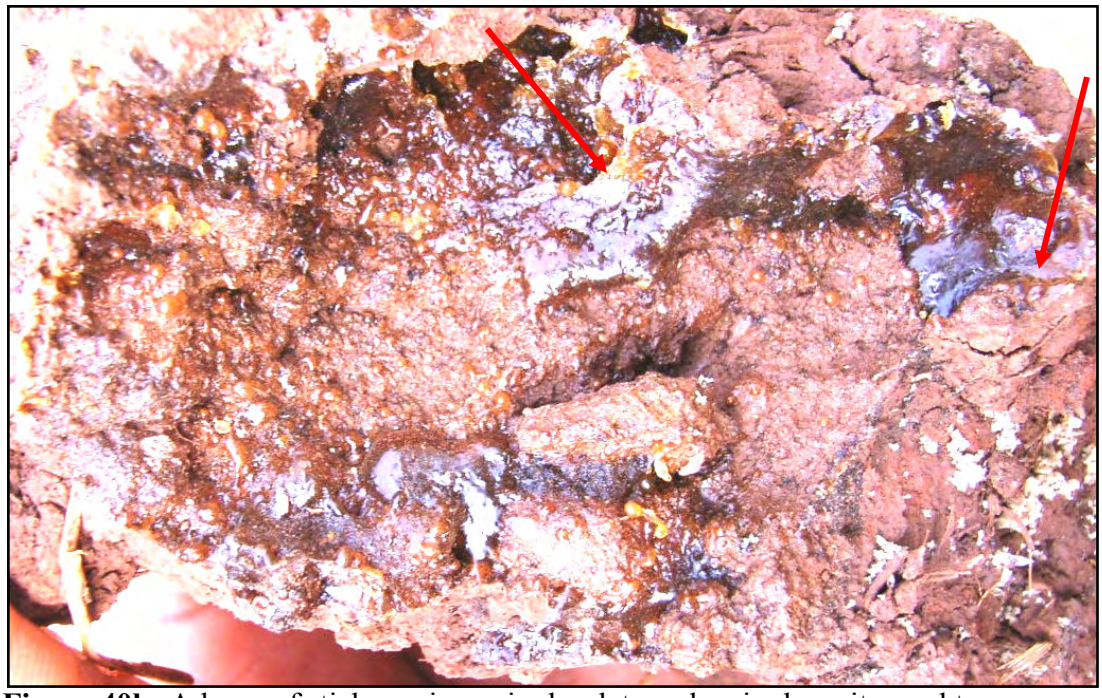

Figure 40b: A layer of sticky resin, resin droplets and resin deposits used to smear on ants which were attacking a *Hypotrigona gribodoi* nest

6.3.3 Batumen layer

In a nest constructed in a drainage pipe blocked by soil, a brittle batumen layer was constructed to seal the pipe after the soil blocking the pipe was removed exposing the colony. Pebbles of resin were added around the batumen layer (Figure 41a). As the bee nest occupied only a small portion of the pipe, on the opposite end of the pipe (away from the nest entrance tube) a mixture of resin, soil and sand was used to protect the nest by blocking entry of water, soil and intruders (Figure 41b).

Hard brittle batumen mixed with soil was also seen on the wall of mud houses, concealing the direction of the entrance tube into the nest (Figure 39a) and on crevices in the walls (Figure 39e).

Figure 41a: A brittle batumen layer constructed to protect a *Hypotrigona gribodoi* nest in a drainage pipe after the soil cover was removed exposing the colony.

Figure 41b: A batumen layer, with sad and soil added to protect a *Hypotrigona gribodoi* colony on the rear side of a drainage pipe

Involucrum

There was no involucrum in all the nests.

6.3.4 Storage pots

Pollen and nectar pots were in groups normally close to the exit tube (Figure 42). There were no distinct groups for pollen or nectar pots, but the pots were located randomly within the groups. There were no pillars between the pots; instead cerumen was added between the walls of the pots to hold them together. The color of the pollen and nectar pots was cream and opaque, an indication that they were not made of wax alone. The pots had a mean height of 0.6cm and width of 0.5cm (Table 8). Storage pots lay on the resin lining the nest cavity. No pots were used for waste storage. In the colonies reared in the wooden hives, waste from within the nest was collected and put into one or more heaps within the hive and later carried out from the nest by the workers. The waste consisted of fine powder, pollen remains and dead bees.

6.3.5 Brood cells

Brood cells were in clusters which lay on the resin lining the nest cavity (Figure 42). Two to three brood cells were built with their walls in contact to form a small cluster. The small clusters were then interconnected by short pillars to form bigger clusters. Addition of new cells was in all directions as long as space permitted (Figure 42). The cells had a mean height of 0.5cm and width of 0.3cm (Table 8). There were no recognizable queen cells. The color of the cells was cream indicating that the cells were not made of wax alone.

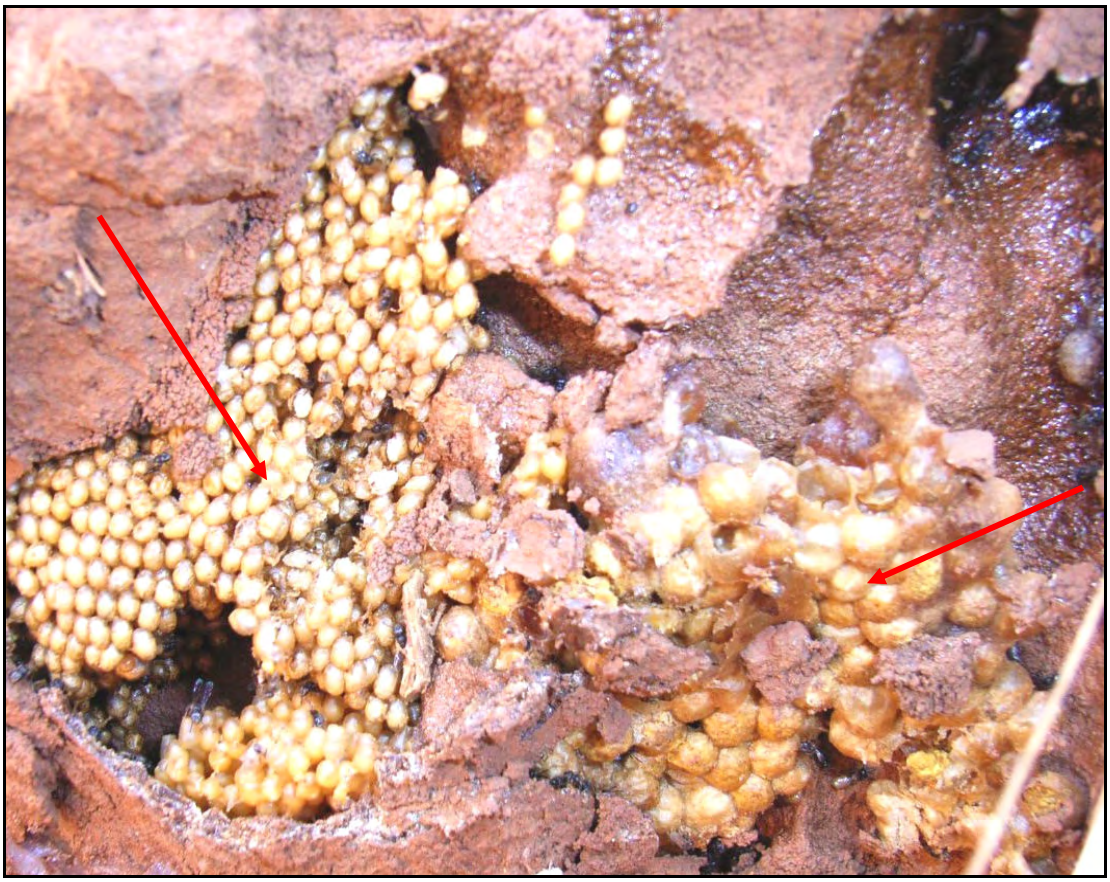

Figure 42: Clusters of cream colored *Hypotrigona gribodoi* brood cells on the left, and clusters of storage pots on the right

Drainage tube

There was no drainage tube in all the nests.

6.4 Behavior of the bees at the nest entrance

The nest entrance remained open during the day and at night. Two to four workers guarded the entrance. When the nest was opened, a few workers showed mild aggressiveness and attacked by biting hands and head. Intruders to the nest are smeared with resins and mud (Figure 43). In a nest which was under attack by ants nesting around the bee nest, the cavity was lined with a layer of resin and resin droplets were scattered all over the cavity. Also there were large resin deposits on the edges of the nest cavity (Figure 40b)

Figure 43: A wasp (arrow) smeared with a mixture of resin and mud on entering a *Hypotrigona gribodoi* nest when drilling its own nest.

6.5 Colony size

A *Hypotrigona gribodoi* nest can have about a hundred to thousands of workers and brood cells (Table 8). Newly constructed nests or unhealthy nests can have less than a hundred brood cells and workers, while an old and healthy nest can have thousands of cells and workers.

7. *Hypotrigona gribodoi* nest in cavities in tree trunks

Hypotrigona gribodoi nests were found in cavities in trunks of *Sapium ellipticum*

7.1 Entrance tube

The nests consisted of an external entrance tube with a length of 3cm above the surface of the trunk and a diameter of 0.3cm. The tubes were funnel shaped consisting of dark cerumen (Figure 44). Droplets of sticky resin were scattered on the outside of the tube in some nests. The tube into the tree trunk had a length of 4cm and a diameter of 0.3cm. It was lined on the edges with sticky resin. There was no internal entrance tube

Figure 44: A *Hypotrigona gribodoi* dark funnel shaped nest entrance tube in a tree cavity

7.2 Nest cavity

The nest cavity was in a trunk which had rotten and dried up parts. The cavity was long and shallow. The area covered by the bee nest was 21cm long and 8.5cm broad (Figure 45). The cavity was delimited by 0.2cm thick sticky resin.

Figure 45: Location of *Hypotrigona gribodoi* nectar pots (i), pollen pots (ii), and brood cells (iii), in a tree trunk. Note transparent pots

Batumen and involucrum layer

There was no batumen or involucrum layer seen.

7.3 Storage pots

Storage pots were in groups positioned close to the entrance tube. The pots were soft and transparent making the color of the pollen and nectar within them visible (Figure 45).This an indication that the pots were made from wax alone. There were distinct groups of pollen and nectar pots. The pot walls were in direct contact with one another and no pillars between the pots or between the groups of storage pots (Figure 45)

7.4 Brood cells

Brood cells were soft and arranged into clusters. New cells were transparent with the larval food visible, an indication that the cells were made from wax alone. Groups of two to three cells were interconnected by short pillars to form the clusters (Figure 45).The clusters lay on the nest cavity. There were no distinguishable queen cells

Drainage tube

The nests did not have drainage tubes.

7.5 **Discussion**

Nests of *Hypotrigona gribodoi* were found in areas like walls of houses protected by roofs, tree trunks protected by the canopy or in dry wood (poles) which insulates the nest. Probably due to nests being in protected areas, extreme temperature changes were not experienced, hence *H. gribodoi* did not require very well developed protective layers of batumen and involucrum. Although it could be that *H. gribodoi* can tolerate high temperature variations. For example colonies reared in wooden observation hives adapted easily and the colony multiplied within a short time. Unlike *Plebeina hildebrandti* colonies in wooden hives, *H. gribodoi* colonies did not build involucrum layers. However, in their study Moritz & Crewe (1988) reported that *H. gribodoi* brood is particularly sensitive to high nest temperatures. They showed by determining the fluctuations of oxygen concentration in the nest that *H. gribodoi* actively ventilate its nests.

Presence of transparent brood cells and extremely soft and transparent storage pots in nest in tree cavity is an indication that the pots and cells were constructed from wax with no other materials such as resins and gums added. It was not possible to determine why only wax had been used for pots and cells construction in tree cavity, while in walls a mixture of wax and other materials was used. In his study Bassindale (1955), reported that *H. gribodoi* uses only wax in nest construction while Pooley & Michener (1969) reported that they use cerumen. Pooley & Michener worked on *H. gribodoi* in Natal South Africa, whose range is from Natal to Ethiopia, while Bassidale worked on a conspecific species "braunsi" whose range is Angola to Ghana (and probably Senegal) Pooley and Michener (1969). Presence of nests with brood cells and storage pots made of pure wax and others made of a mixture of wax and other materials, shows that more nests need to be described to determine if *Hypotrigona gribodoi* in Uganda is one species or more.

Lack of drainage pipes, which serve to drain excess water and moisture from the nest, especially after dehydration of nectar (Camargo & Wittmann 1989), could be explained by the fact that *H. gribodoi* normally collects very concentrated nectar to reduce the

energy needed to evaporate the nectar to normal concentration (Kajobe 2007). However in our study *H. gribodoi* collected banana nectar which had low sugar concentration, sometimes less than 10%. It's therefore likely that, since the entrance tubes were short; 0.5-2.5cm on walls and 6cm on trunk, water loss through the entrance tube was possible.

Construction of nests in proximity especially on walls of houses is an indication that there was less aggression within the species. In their study Kirchner & Friebe (1999) found out that nest mates discrimination in *H. gribodoi* is non-aggressive.

GENERAL CONCLUSION

In Africa, there is insufficient information on the biology and the diversity of he African stingless bees. Due to the high medicinal value of their honey, stingless bees are highly sought after by local communities. As there are no developed Meliponaries in Africa, honey harvesting involves destruction of entire bee colonies and cutting of trees. Additionally the role of the bees as pollinators, pests or vectors of disease remains unknown. The speculation that stingless bees were the primary vectors of *Xanthomonas campestris pv*. *musacearum* (*Xcm*) responsible for Banana Xanthomas Wilt (BXW) in Uganda is an indicator of the urgent need to study and document the biology of the African bees.

It had been hypothesized that stingless bees spread *Xcm* from one banana plant to another and to the nest as they collected nectar and exudates such as sap and ooze from infected bananas. The nest was therefore considered to act as a reservoir for the bacteria. However from our study stingless bees did not collect banana sap and ooze. They were only interested in nectar. Banana nectar has low sugar concentration, which is not preferred for optimal foraging by stingless bees. In particular, Pisang Awak, the banana variety most susceptible to *Xcm*, had a mean nectar sugar concentration of 12.5% (range 0-32%), yet stingless bees gather nectar with an average of 44% sugar concentration. This means banana nectar is not a preferred choice for stingless bees optimal foraging.

Nevertheless if stingless bees got contaminated with *Xcm*, either through nectar or contact with bacterial ooze on bananas, they would spread it over short distances. We found out that the four species of stingless bees (*Hypotrigona gribodoi*, *Plebeina hildebrandti*, *Meliponula ferruginea* and *Meliponula sp*.) that collected nectar from bananas would fly up to 1220 meters from their nest to collect 33% nectar. This is because, there exist a relationship between foraging distance, a bee body size and nectar sugar concentration. There is therefore need for further studies on possible long distance vectors.

In case the bees carried *Xcm* to their nests, *Xcm* would be eradicated, as stingless bees have a relationship with *Bacillus* species. *Bacillus sp*. produces antimicrobial compounds that inhibit other microorganisms from growth. Furthermore *Xcm* is likely to be eliminated in the stingless bees nest due to the presence of propolis. Propolis is a constituent building material in stingless bee nests which has antimicrobial compounds. A preliminary test showed that *Xcm* could not grow on propolis collected from *P. hildebrandti* nest.

Nests of *P. hildebrandti* were found in inhabited termite mounds. The main structures were: external entrance tube with elongate bars of resin in the tubes running through the termite mound, brood chamber with circular, both circular and trapezoidal, or trapezoidal combs surrounded by an involucrum layer, groups of interconnected pollen and nectar pots surrounding brood combs, and slanting drainage tube.

Nests of *H. gribodoi* were found in man made structures such as walls of mud houses, in spaces in windows and door frames, roofing timber, electric sockets, dry drainage pipes, electric and telephone poles and cavities in tree trunks. The main nest structures were short entrance tubes, clusters of brood cells and masses of storage pots adjacent the brood cells, absence of involucrum and drainage tube. A unique feature in the nest in tree trunk was brood cells and storage pots constructed of only wax.

Although nest architecture conditions can be adaptations to various kinds of cavities, it is also a species specific trait which can support identification of species. There is a need for more research on the taxonomy of *Plebeina hildebrandti* and *Hypotrigona gribodoi*.

Both *P. hildebrandti* and *H. gribodoi* can be reared in wooden hives for honey production and for pollination purposes. However *P. hildebrandti* rearing is not easy and requires good hives to ensure stable microclimate.

OUTLOOK

Since the initial distribution of Banana Xanthomonas Wilt in Mukono and the neighboring districts was localized and patchy (Eden- Green 2004), and that although the disease emerged in year 2001, it was still restricted to a radius of five kilometer in year 2002 from the farm where the disease had been reported (Tushemereirwe *et al*. 2003), it would be interesting to find out what changes occurred in year 2004 when the disease started spreading rapidly and filling the gaps. Given that the stingless bees are not new to Uganda, and they had been there since the disease was first reported, it could be possible that other vectors were responsible for the entry of the disease in Mukono district, and in combination with various factors led to the rapid spread in year 2004.

To determine the long distance vectors, it would be important to find out what changes occurred in Uganda which facilitated *Xanthomonas* to cross from Ethiopia and establish in Mukono district in year 2001, yet *Xanthomonas* had been in Ethiopia since 1968. In addition it would be important to find out what happened in D.R. Congo in year 2001 that caused Xanthomonas to infect bananas from enset from within the country, yet before then *Xanthomonas* did not infect bananas.

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