NATIONAL BEEKEEPING CALENDAR, HONEYBEE PEST AND DISEASE CONTROL METHODS FOR IMPROVED PRODUCTION OF HONEY AND OTHER HIVE PRODUCTS IN UGANDA

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Executive summary

Beekeeping is recognised as being a very important sector in the recent government programmes for development, poverty alleviation and conservation and sustainable use of forest resources. Apiculture industry provides enormous potential for income generation and socio-economic development. There is an expanding international market niche for special flavoured and organic honey which could be exported. In 2005, Uganda was licensed to export honey to the EU market, thus creating an immense opportunity. The most important service the honeybees render to mankind is pollination of agricultural and forestry crops. Despite the substantial attention is given to the importance of beekeeping in Uganda, little research in apiculture has been carried out. There is inadequate information about a national beekeeping calendar that relates flowering of honeybee forage plants to honey flow and harvesting seasons. There is also no national honeybee pest and disease control system to eradicate and create pest and disease free export zones. This study aimed to develop a national beekeeping calendar that relates flowering of honeybee forage plants to honey flow and harvesting. In addition, the study was to develop an appropriate control system for honeybee pests and diseases. Data was collected from 6 of the 10 different agro-ecological zones of Uganda. These are classified on the basis of distinct vegetation type, elevation and climatic conditions. One district was selected from each zone. The study was conducted from February 2007 to June 2009 using socio-economic (Interviews, PRA and discussions with key informants) tools and experimental methods.

This report is divided into three different parts. Chapter 1 is on development of a national beekeeping calendar to promote planning of appropriate interventions to boost production of honey and other hive products. Chapter 2 is on development of honeybee pest and disease control methods to improve the quality of honey and other hive products in Uganda. While Chapter 3 is on molecular detection of a viral pathogen in Ugandan honey bees: occurrence, prevalence and distribution patterns. Findings from Chapter 1 showed that a number of trees, shrubs, herbs and agricultural crops were used by the bees for nectar and pollen. Most of the plants flowered during the rainy seasons with exception of plants such as *Calliandra calorthusus*, Bananas and Bottle brush, which flowered throughout the year. There are mainly two major honey-harvesting periods depending on the rainfall seasons in the year (April-June and October-December). On average farmers harvested about 6-10 kg of honey from the traditional hives and 10-15 kg from top bar hives.

In Chapter 2, farmers documented at least 12 honeybee pests that affect the production of honey and other bee products in Uganda. The most important being ants, small hive beetles and wax moths. The important pest control methods include; frequent smoking of hives (to drive out beetles), keeping the apiary tidy and clean from bushes, avoiding throwing/scattering combs and honey around the apiary and housing the apiary Application of ash near the hives at the apiary was considered to be effective while fencing off the apiary was considered to be less effective method of organic pet control. In Chapter 3, bee viruses, larval, pupal and adult *A. mellifera* were collected from 63 beekeeping sites in nine of Uganda's ten agroecological zones for screening. Using real-time RT PCR, samples were screened for seven different viral pathogens: *Black queen cell virus* (BQCV), *Chronic bee paralysis virus* (CBPV), *Sacbrood virus* (SBV), *Deformed wing virus* (DWV), *Acute bee paralysis virus* (ABPV), *Apis iridescent virus* (AIV) and *Israeli acute paralysis virus* (IAPV). No samples tested positive for DWV, SBV, CBPV, ABPV, IAPV or AIV, but BQCV was found in approximately one third of samples. BQCV was only found in adult and larval bees. Infected material came from seven of the nine zones sampled. BQCV was most prevalent in the Western Highlands, accounting for over 40% of positive results for BQCV nationally. It was comparatively less widespread in the Eastern zone, and only present in single sites elsewhere.

Background

Beekeeping is one of the important undertakings in the current government programmes of poverty alleviation (MAIF, 2000). It provides enormous potential for income generation, poverty alleviation, sustainable use of forest resources and diversifying the export base (Common wealth 2002, FAO 1990). Compared to other agricultural projects such as fish farming (aquaculture), poultry and livestock, beekeeping is a relatively low investment venture that can be undertaken by most people (women, youths, disabled and elderly). Beekeeping does not compete for resources used by other forms of agriculture. It is environmentally friendly and can be productive even in semi-arid areas that are unsuitable for other agricultural use (FAO 1990). The market for bee products is locally and internationally available. Pharmaceutical and cosmetic industries use bee products such as honey, propolis, royal jelly and beeswax (UEPB 2005). Honey and bee brood are sources of carbohydrate and protein food that farmers can obtain at minimal cost. There is an expanding international market niche for special flavoured and organic honey which could be exported (UEPB 2005). In 2005, Uganda was licensed to export honey to the EU market, thus creating an immense opportunity. The President of Uganda's Prosperity for all Programme identified beekeeping as a major enterprise for income generation in rural areas. The most important service the honeybees render to mankind is pollination of agricultural and forestry crops. However, farmers have little knowledge on the importance pollinators. Incidentally, Uganda has not adequately harnessed the relationship between honeybees and agricultural crops. However, according to Commonwealth Secretariat (2002) honeybees in Uganda are currently used for pollination of coffee, Tea, Cotton, Pulses, Oil seeds, Maize, sorghum, mangoes, oranges, peas, beans and spices.

Despite the fact that considerable attention is given in reports and documents to the importance of beekeeping in Uganda, little research and development in apiculture has been carried out. The annual honey production in Uganda is estimated to be 5,000 tonnes (MAAIF, 2008) which is only 1% of the national annual production potential estimated to be 500,000 tonnes (Horn, 2004). Efforts to increase production are however, curtailed by various constraints related to production, processing, packaging, storage and marketing (UEPB, 2005). There is inadequate information about a national beekeeping calendar that relates flowering of honeybee forage plants to honey flow and harvesting seasons. A calendar for beekeeping is a time-table that indicates to the beekeepers the approximate date and duration of the blossoming periods of the important honey and pollen plants in their area. All apiary management practices are related to the bee colony cycle and understanding which stage the colony is in. Honeybees collect nectar and pollen from different flowering plants but little is known about the species of plants used by honeybees to process nectar into honey in Uganda. There is also no national honeybee pest and disease surveillance system tailored for their eradication and creation of pest and disease free export zones. Effective control or eradication of pests and diseases requires an efficiency pest and disease control system in place. The overall objective of this project was to develop a national beekeeping calendar that relates flowering of honeybee forage plants to honey flow and harvesting. In addition, the study was to develop an appropriate surveillance system for honeybee pests and diseases for improved production of honey and other hive products in Uganda.

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CHAPTER 1

NATIONAL BEEKEEPING CALENDAR FOR PLANNING OF APPROPRIATE INTERVENTIONS TO BOOST HONEY PRODUCTION AND OTHER HIVE PRODUCTS IN UGANDA

1.1 Introduction

A calendar for beekeeping is a time-table that indicates to the beekeepers the approximate date and duration of the blossoming periods of the important honey and pollen plants in their area. The experienced beekeepers will have acquired much of this information over the years, but published charts are important for all beekeepers to use (FAO 1990). The beekeeping calendar is one of the most useful tools of the apicultural extension workers. It enables them to inform the beekeepers on what to expect in bee-forage availability, and when, so that they can manage their colonies in the most rational manner. Beekeeping in any specific area cannot develop without an understanding of the calendar, and for migratory beekeeping, special calendars for the different foraging zones along the migration route are required. Assembling a beekeeping calendar for any specific area requires complete observation of the seasonal changes in the vegetation patterns and/or agro-ecological zones, the foraging behaviour of the bees, and the manner in which the honeybee colonies interact with their floral environment. The accuracy of a beekeeping calendar, and hence its practical value, depend solely on the careful recording of the beginning and end of the flowering season of the plants and how they affect the bees.

Although the Commonwealth Secretariat (2002) report on strategy to develop beekeeping in Uganda highlights the importance of the industry, little research has been done in this area. There is a lack of information about a national beekeeping calendar that relates flowering of honeybee forage plants to honey flow and harvesting seasons. Seasonal weather impacts upon nectar and pollen resources, which in turn impact upon the colony population. Reduced food means that the queen lays less eggs and the population of the hive falls. Increased food means increased laying and the population increases. Since more bees means more food can be collected the colonies with small populations will emphasize brood rearing (FAO, 1990). Alternatively the colonies reaching a certain size will emphasize honey production. It is important to understand how the bee colony changes throughout the year because manipulating the colony to be at the peak strength at the right time is fundamental to good beekeeping (Bees for Development, 2000). Conditions for bees can vary widely throughout the country and the management of the bees depends on where they are found (Commonwealth Secretariat. 2002). Nevertheless when managing bees for honey production, the aim is to have the maximum colony population during the nectar flow. Providing the nectar flow is good and the weather conditions are right a good honey crop can result (Horn, 2004).

All apiary management practices are related to the bee colony cycle and understanding which stage the colony is in. There are three periods during a cycle and these may occur more than once in a year: 1) Dearth- not much nectar is being collected due to bad weather and poor forage. 2) Build up- there are many bee forage plants and the weather is favourable the colony expands 3) Honey flow- many plants provide nectar and flower at the same time (FAO, 1990). Honeybees collect nectar and pollen from different flowering plants but little is known about the species of plants used by honeybees to process nectar into honey in Uganda. Answering the following questions will give a good overview of the honey year and helps the beekeeper to prepare for the honey flow: Which plants are important sources of forage for honeybees? When do they flower? When are the swarming seasons? Which trees or plants give the best honey? When are the right months of the year to expect honey and which are the signs of harvesting? What factors such as rainfall and temperature affect plant flowering and nectar secretion?

1.2 Justification

In the apiculture sub-sector, the national goal is to enhance the production and marketing of honey and other hive products. In order to fulfil this, the country has to maintain a large enough national honeybee population to sustain the supply of honey and other hive products for the domestic, regional and international markets. Qualitative information about bee forage plants, their flowering period and type of blossom can be used for planning of appropriate intervention to boost production of honey and other hive products. Wrong timing in harvesting of honey and other hive products has led to severe unquantified losses in the beekeeping industry in the country. In this study, the important honeybee forage plants, their flowering and nectar harvesting periods will be determined. The flowering periods will be correlated to the climatic factors and brood production.

1.3 Objectives

1.3.1 Overall objective

The purpose of the study was to develop appropriate national beekeeping calendar that relates flowering of honeybee forage plants to honey flow and harvesting seasons

1.3.2 Specific objectives

1. To determine the bee forage plants that give the best quality honey
2. To establish the flowering periods of important bee forage plants
3. To determine how environmental factors such as rainfall, temperature and humidity influence flowering of bee forage plants and nectar production
4. To relate flowering periods of major bee forage plants to honey flow seasons
5. To establish the right months of the year when to harvest ripe honey in each agro-ecological zone

1.4 Materials and methods

1.4.1 Study area

Data was collected from 6 of the 10 different agro-ecological zones of Uganda (Table 1.1 and Figure 1.1). These are classified on the basis of distinct vegetation type, elevation and climatic conditions. One district was selected from each production zone (Mbarara in Southern Dry lands; Mukono in Lake Victoria Crescent; Soroti in Eastern; Hoima in Lake Albert Crescent, Lira in mid northern and Kabarole in Western Highlands. The selection of the zones was guided by the number of active bee farmers groups in those zones and the records of honey productivity in those zones.

Table 1.1 Agro-ecological zones of Uganda

No.	Agro-ecological zone	Districts in the zone
1	Eastern	Pallisa, Tororo, Kumi, Kaberamaido, Katakwi, Soroti Mbale, Sironko, Kapchorwa
2	Lake Albert Crescent	Masindi, Hoima, Kibale
3	Lake Victoria Crescent	Masaka, Mpigi, Luwero, Kampala, Mukono, Kayunga, Wakiso, Kiboga, Nakasongola, Kalangala, Mubende
4	Mid Northern	Lira, Apac, Kitgum, Gulu, Pader
5	South East	Jinja, Iganga, Bugiri, Busia, Kamuli, Mayuge
6	Southern Dry lands	Rakai, Sembabule, Mbarara, Ntungamo
7	Southern Highlands	Kisoro, Kabale, Rukungiri, Kanungu
8	West Nile	Arua, Moyo, Nebbi, Adjumani, Yumbe, Koboko
9	Western Highlands	Bushenyi, Kasese, Bundibugyo, Kamwenge, Kyenjojo, Kabarole
10	Karamoja Drylands	Moroto, Kotodo, Nakapiripiri

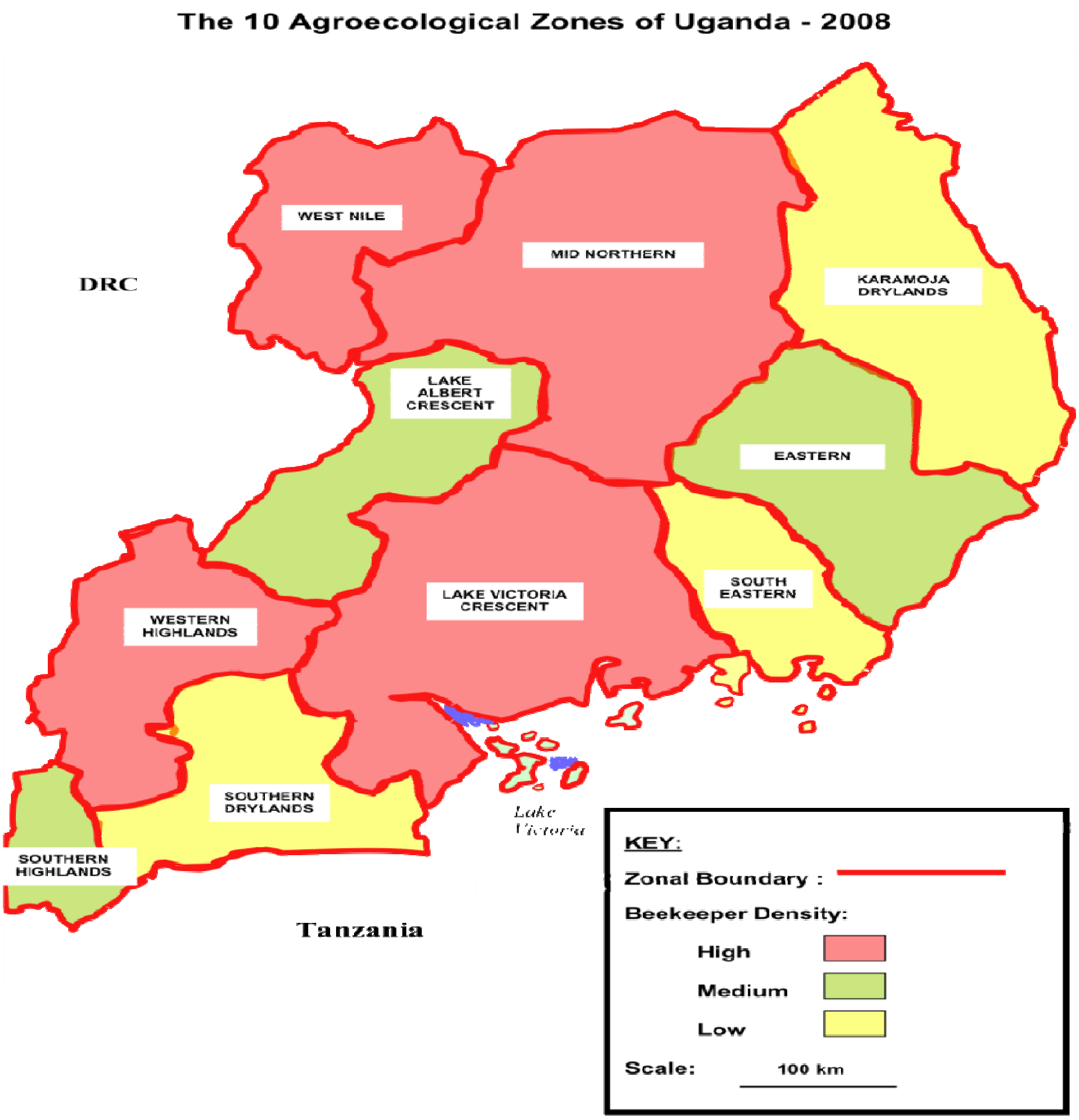

Figure 1.1 Map of Uganda showing level of beekeeping in the agro-ecological zones

1.4.2 Socio-economic study

The socio-economic data were collected from February 2007 to June 2009 in the different agroecological zones.

1.4.2.1 Interviews and PRA

The beekeepers were visited either individually at home or met in their respective groups (Figure 1.2). For the group meetings, each group consisted of 25-35 members. The discussions were carried out in the local languages and responses translated and written in English. The discussions began with interacting with the beekeepers to find out the general problems limiting beekeeping production, problems they face in bee colony management and to understand the context of beekeeping in their agro-ecological zone. The second step involved gathering local knowledge about important sources of forage for honeybees, when they flower, when the right months of the year to harvest ripe honey and what factors such as rainfall and temperature affect plant flowering (Figure 1.3). Discussions were also held on production practices, duration between different stages of honey production, number of beekeepers harvesting honey at different months/seasons, availability of indigenous knowledge about beekeeping calendars. These steps involved use of Participatory Rural Appraisal (PRA) tools.

Figure 1.2 Focus group discussion session with beekeepers in Western Highlands, Uganda.

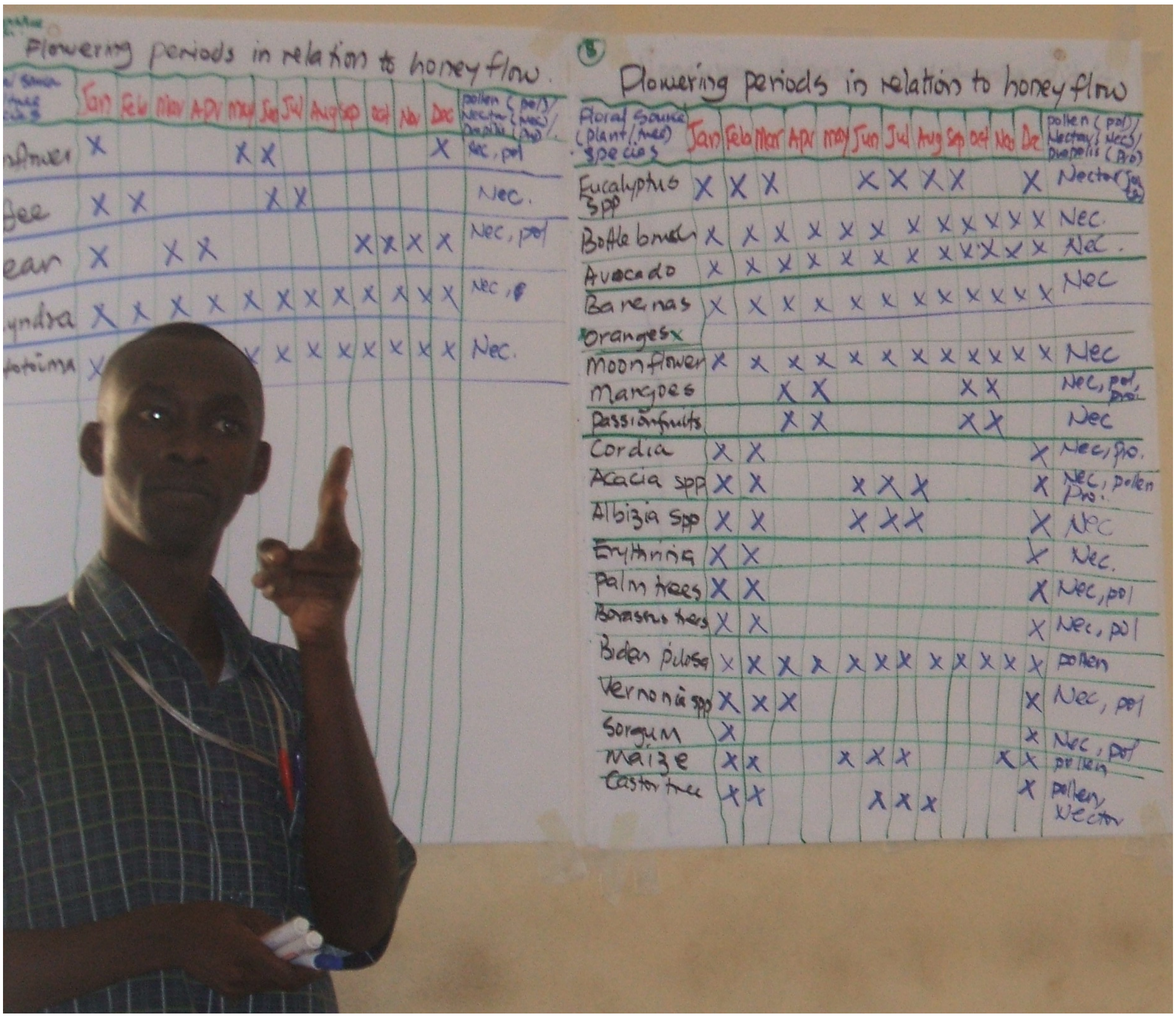

Figure 1.3 Local knowledge on important forage plants for honeybees and when they flower in relation to honey flow.

1.4.2.2 Discussions with key informants

Key informant interviews were also used. The key informants were identified on the basis of their organisational and community positions, reputations, and knowledge of the issues under study. Their insights, recollections, and experiences provided an important and logical starting point for the compilation of data about the social reality of beekeeping sector. Gender considerations were carefully made while conducting the PRA exercises. The key informants included beekeepers, district entomologists, local leaders and elders who have lived in the area for about 20-30 years. In addition, the beekeeping NGOs provided information on the status of beekeeping in each zone (Figure 1.4). Discussions focused on plants that are important sources of forage for honeybees, when they flower, when the swarming seasons are, which plants give the best honey, the right months of the year to expect honey and the signs of harvesting.

Figure 1.4 Discussions with Kabarloe Beekeepers Association.

1.4.2.3 Observations

Observations such as in Figure 1.5 were made during the field visits to help confirm and crosscheck information from other sources. Plant species in flower, the methods of harvesting, processing, and storage were observed and noted.

Figure 1.5 Research team checking whether honey from log hive is ready for harvesting.

1.4.3 Experimental study

A general survey was undertaken from each agro-ecological zone. A list of flowering plants known to be visited by the bees was drawn up. Only plants found within a 1 km^2 of the apiary of the foraging honeybees in an area were sampled because honeybees effectively utilise the plant resource within 1 km radius (Steffan-Dewenter & Tscharntke 1999). Most of the forage plant samples were identified by the farmers. Observations were made to determine whether the plants were visited for nectar or for pollen. Pollen-foragers had pollen pellets attached to their hind legs. To determine whether the bees visited flowers for nectar the observer squeezed the abdomen of captured individual bees to obtain a drop of regurgitated nectar and tasting it for sweetness.

1.5 Results

1.5.1 General problems affecting beekeeping

Farmers mention different problems affecting the beekeeping industry in their respective agro-ecological zones. Some of the problems cut across the different zones while the others are specific to particular zones. The beekeepers in Western Highlands zone generated at least 11 problems that hinder the development of beekeeping in the region (Table 1.2). These problems were ranked according to what the farmers perceived were the most important factors affecting beekeeping development. The most important factors were loss of bee forage due to deforestation and presence of Banana Bacterial Wilt (BBW) which beekeepers suspect to be transmitted by the bees (more scientific studies need to urgently be undertaken to ascertain these claims). The other important problems include bee diseases and the multi-sectoral policy contradictions and conflicts within the Ministry of Agriculture Animal Industry and Fisheries e.g. use of agricultural chemicals. Thefts of bee hives, conflicts between the beekeepers and their neighbours and poor handling (management) of apiaries were considered to least important in hindering beekeeping development. In the Eastern agro-ecological zone, the most important problems faced by beekeepers include inadequate bee keeping equipment, lack of knowledge and skills for beekeeping, pests and disease and inadequate bee forage (Table 1.3).

Table 1.2 Pair-wise ranking of problems affecting beekeeping in Western Highlands agro-ecological zone

	Beekeeping problem	DF	BBW	Conf.	BD	Pests	LME	SC	BB	LCo	Theft	Ph	LC	Total	Rank
1	**Deforestation (DF)**	X	DF	DF	BD	DF	DF	DF	DF	DF	DF	DF	DF	10	1
2	**Banana Bacterial Wilt (BBW)**		X	BBW	BBW	BBW	BBW	BBW	BBW	BBW	BBW	BBW	BBW	10	2
3	**Bee diseases (BD)**				X	BD	BD	SC	BD	BD	BD	BD	BD	09	4
4	**Bee pests (Pests)**					X	LME	SC	BB	LCo	Pests	Pests	LC	03	9
5	**Lack of modern equipment (LME)**						X	SC	LME	LCo	LME	LME	LC	05	7
6	**Sectoral conflicts within MAAIF (SC)**							X	SC	SC	SC	SC	SC	09	3
7	**Bush burning (BB)**								X	LCo	BB	BB	LC	04	8
8	**Low colonisation (LC)**									X	LCo	LCo	LC	06	6
9	**Theft (Theft)**										X	Theft	LC	02	10
10	**Poor handling (Ph)**											X	LC	00	11
11	**Lack of capital (LC)**												X	07	5

Table 1.3. Pair-wise ranking of problems affecting beekeeping in Eastern agro-ecological zone

No	Problems	BE	LK	FP	P	M	D	LF	LW	BA	T	IB	F	Total	Rank
1	**Bee keeping equipment (BE)**	X	BE	FP	BE	BE	BE	BE	BE	BE	BE	BE	BE	10	2
2	**Lack of knowledge (LK)**		X	FP	LK	LK	LK	LK	LK	LK	LK	LK	LK	9	3
3	**Financial problem (FP)**			X	FP	FP	FP	FP	FP	FP	FP	FP	FP	11	1
4	**Pests (P)**				X	P	P	P	P	P	P	P	P	8	4
5	**Markets (M)**					X	D	LF	M	M	M	M	M	5	7
6	**Diseases (D)**						X	D	D	D	D	D	D	7	5
7	**Lack of forage (LF)**							X	LF	LF	LF	LF	LF	6	6
8	**Lack of water (LW)**								X	LW	LW	IB	F	2	10
9	**Bad attitude (BA)**									X	T	IB	F	0	12
10	**Thieves (T)**										X	IB	F	1	11
11	**Inadequate bee stock (IB)**											X	IB	4	8
12	**Changing weather flooding (F)**												X	3	9

1.5.2 Beehive types, honey production and profitability

The beekeepers use three types of hives (Figure 1.6): traditional hives, top bar and Langstroth hives. Most of the traditional hives are either made locally by the beekeepers or made by local carpenters and sold in local markets. Many of the traditional hives were not in appropriate sizes and shape for bees to maintain them. Most of them were either larger or smaller than required. Many beekeepers had beehives that are empty of bees. As a result, these beekeepers fail to supply sufficient honey for the market. Apiary colonisation rates are estimated to be as low as (30-60%), hence there is need to devise methods of increasing apiary colonisation capacities. Few beekeepers use the modern (Langstroth hive). The likely reasons for limited use of the Langstroth hive may include the fact that it has relatively advance hive technology and is expensive (100,000 – 140,000 UGX per hive). The use of Langstroth hive necessitates the need for centrifugal honey extractor, which is expensive (about 2,500,000 UGX). Figure 1.7 shows some of the honeybee products in Uganda (Honey and beeswax) while Figure 1.8 shows a well developed fresh comb from a top bar hive

Traditional (Log hive)

Top bar hive (Mordern)

Langstroth hive (Mordern)

Figure 1.6 Types of beehives that are being used for honey production in Uganda

In order to demonstrate the profitability of beekeeping to a household in Uganda, we developed a simple analysis. For the analysis, we assume that each beekeeper acquires 10 modern (Langstroth) and 10 traditional hives. For both types of hives, colonisation is expected to be 70% in the first year and increases up to 100% in the fourth year (Table 1.4). Honey yield in the Langstroth hive is estimated to be 25 kg per season and there are two harvest seasons in a year. Honey yield per traditional (log) hive is 10 kg. For every 10 kg of honey produced, 1 kg of beeswax is produced. The farm gate price of honey is estimated to be Shs 3,000 per kg. Beeswax is only extracted from the traditional hives. According to MAAIF (2008) there are 1,200,000 beekeepers in Uganda. In the first year of production, we expect that all the beekeepers in the country (if well supported and trained) can contribute up to 504,000 tonnes (UGX 865 billion) to the economy. In the second and third year, the same number of beekeepers would contribute up to 576,000 tonnes (1.0854 trillion) and 648,000 tonnes (UGX 1.3134 trillion) respectively to the GDP. We assume that funds to purchase the inputs are borrowed from a Micro finance institution such as Prosperity-for-All (Bonnabagaggawale) and are expected to be paid back in equal instalments over a period of 4 years.

Figure 1.7 Some of the honeybee products in Uganda (Honey and beeswax)

Figure 1.8 Well developed fresh comb from a top bar hive

Table 1.4 Simple profitability analysis for a beekeeping household in Uganda

Input cost	**Units**	**Year 1**	**Year 2**	**Year 3**	**Year 4**
Langstroth hives					
Langstroth hives at Shs 100,000 per hive	10	1,000,000			
Barbed wire for fencing apiary at Shs 70,000 per roll	2	140,000			
Airtight, food grade buckets at Shs 25,000 per bucket	4	100,000			
Hive stands at Shs 20,000 per stand	4	80,000			
Inspection/harvesting equipment					
- hive tool at Shs 2,000	1	2,000			
- smoker at Shs 20,000	1	20,000			
- bee suit at Shs 80,000	1	80,000			
Total input cost for Langstroth hive		**1,422,000**			
Total cost apportioned over four years		355,500	355,500	355,500	355,500
Labour for mgt of hives (Shs 20,000 per month)		240,000	240,000	240,000	240,000
Stationary		20,000	20,000	20,000	20,000
Total annual costs for Langstroth hive		**615,500**	**615,500**	**615,500**	**615,500**
Revenue					
Langstroth hive					
Percentage yield per year		70%	80%	90%	100%
Honey production: 25 kg per hive, 2 harvests per year		350	400	450	500
Total sales of extracted honey at farm gate price (Shs 3,000 per kg)		1,050,000	1,200,000	1,350,000	1,500,000
Profit in Shs		**434,500**	**584,500**	**734,500**	**884,500**
Profitability in Shs per hive (10 hives)		***43,450***	***58,450***	***73,450***	***88,450***
Input cost for traditional (log) hives					
Traditional (log) hives at Shs 5,000 per hive	10	50,000			
Revenue					
Traditional (log) hives					
Percentage yield per year		70%	80%	90%	100%
Honey production: 10 kg per hive, 2 harvests per year		70	80	90	100
Total sales of extracted honey at farm gate price (Shs 3,000 per kg)		210,000	240,000	270,000	300,000
Sales of beeswax at Shs.10,000 per Kg. (10kg of honey produce 1kg of beeswax)		**70,000**	**80,000**	**90,000**	**100,000**
Profit in Shs		**280,000**	**320,000**	**360,000**	**400,000**
Profitability in Shs per hive (10 hives)		***28,000***	***32,000***	***36,000***	***40,000***

1.5.3 Flowering periods of important bee forage plants in relation to honey flow seasons

The beekeepers produced a list of important bee forage plants in the region (Table 1.5 and 1.6). A number of trees, shrubs, herbs and agricultural crops were mentioned to be used by the bees for nectar, pollen and propolis. The flowering period of bee plants varied with the different seasons of the year. Most of the important bee plants flowered during the rainy seasons (March-May and August-November) with exception of plants such as *Bidens pilosa*, *Calliandra calorthusus*, Bananas and Bottlebrush, which flowered throughout the year. Flowering periods of agricultural crops depended on the date when the crops were planted. There was some variation among the plant species with respect to length of flowering time. Some of the individual species produced a few flowers for a few days but some plant species bloomed for longer periods, lasting from several weeks to several months. Figure shows the Bottlebrush which was planted as a forage source for a stingless bee colony in the homestead of one of the beekeeper in Kabarole district, Uganda. Under the tree is a colonised hive of a stingless bees made from banana leaves and sheath.

Figure 1.9 Bottlebrush planted as a forage source for a stingless bee colony in the homestead of one of the beekeeper in Kabarole district, Uganda.

Table 1.5 Flowering periods of important forage plants in relation to honey flow seasons in the Western Highlands agro-ecological zone

	Floral sources	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Pollen/Nectar/Propolis
1	*Eucalyptus* spp.	X	X	X			X	X	X	X			X	Nectar
2	Bottle brush	X	X	X	X	X	X	X	X	X	X	X	X	Nectar
3	Avocado	X	X	X	X	X	X	X	X	X	X	X	X	Nectar
4	Bananas	X	X	X	X	X	X	X	X	X	X	X	X	Nectar
5	Oranges													
6	Moon flower	X	X	X	X	X	X	X	X	X	X	X	X	Nectar
6	Mangoes			X	X					X	X			Nectar Pollen
7	Passion fruit			X	X					X	X			Nectar
8	*Cordia* sp.	X	X										X	Nectar Pro.
9	*Acacia* spp.	X	X			X	X	X					X	Nectar Pollen
10	*Albizia* spp.	X	X			X	X	X					X	Nectar
11	Erythrina	X	X										X	Nectar
12	Palm trees	X	X										X	Nectar Pollen
13	Borassus tree	X	X										X	Nectar Pollen
14	*Bidens pilosa*	X	X	X	X	X	X	X	X	X	X	X	X	Pollen
15	*Vernonia* spp.	X	X	X									X	Nectar Pollen
16	Sorghum	X											X	Nectar Pollen
17	Maize	X	X			X	X	X			X	X		Pollen
18	Castor tree	X	X				X	X	X			X		Nectar Pollen
19	Sunflower	X				X	X						X	Nectar Pollen
20	Coffee	X	X				X	X						Nectar
21	Beans	X		X	X					X	X	X	X	Nectar Pollen
22	*Calliandra calorthusus*	X	X	X	X	X	X	X	X	X	X	X	X	Nectar
23	Orutotoima	X	X	X	X	X	X	X	X	X	X	X	X	Nectar

Table 1.6 Flowering periods of important forage plants in relation to honey flow seasons in the Eastern agro-ecological zone

	Floral sources	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Pollen/ Nectar/ Propolis
1	Acacia spp.	X	X			X	X	X					X	Nectar Pollen Propolis
2	Bananas	X	X	X	X	X	X	X	X	X	X	X	X	Nectar
3	Beans	X		X	X					X	X	X	X	Nectar Pollen
4	Bottle brush	X	X	X	X	X	X	X	X	X	X	X	X	Nectar
5	Coffee	X	X				X	X						Nectar
6	Cotton													
7	Erythrina	X	X										X	Nectar
8	Eucalyptus spp.	X	X	X			X	X	X	X			X	Nectar
9	Ground nuts													
10	Maize	X	X			X	X	X			X	X		Pollen
11	Mangoes			X	X					X	X			Nectar Pollen Propolis
12	Oranges													
13	Passion fruit			X	X					X	X			Nectar
14	Potatoes													
15	Simsim													
16	Sunflower	X				X	X						X	Nectar Pollen

1.5.4 Agroforestry trees found on gardens of farmers in Mukono district

Beekeeping requires the presence of appropriate crops and plants to favour foraging. Many agroforestry tree species were found in gardens of farmers in the Lake Victoria Crescent agro-ecological zone (Figure 1.9 and 1.10) and were good sources of forage for the beekeepers. Most of such trees were planted either at plot peripherals or in the thicket, marginal lands or woodlot. However, the presence of these tree species could not have necessarily been caused by the desire to develop apiculture.

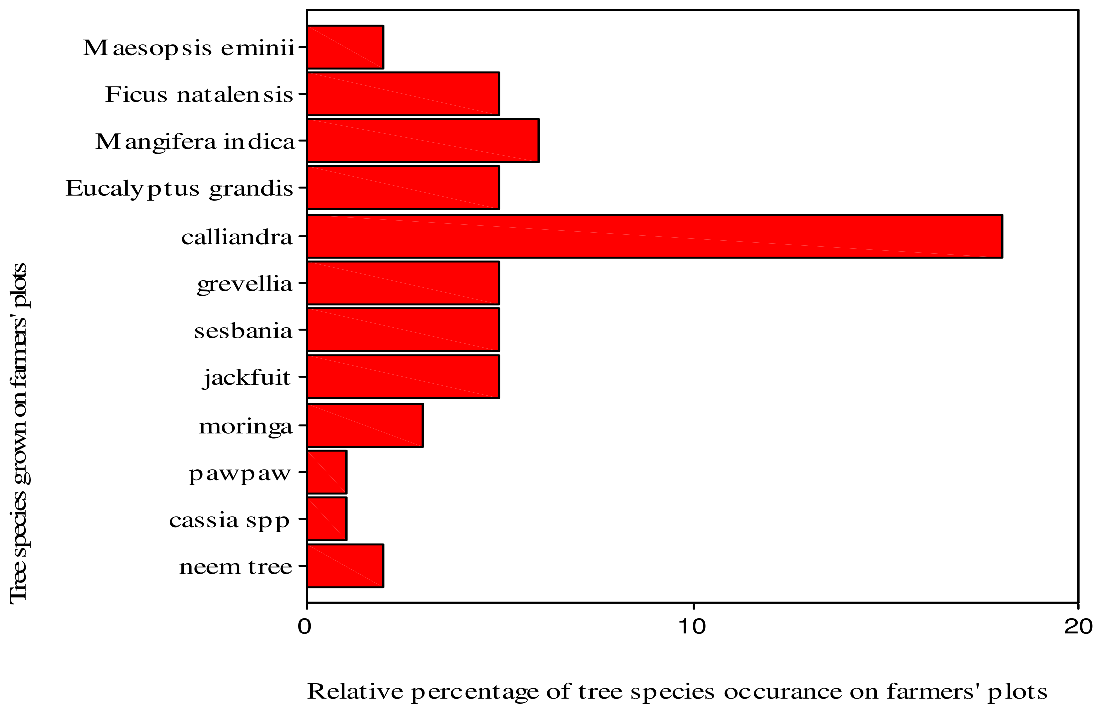

Figure 1.10 Some of the bee forage plants on gardens of farmers in Mukono district.

Figure 1.11 Some of the important bee forage plants

1.5.5 Frequency of honey harvesting according to hive type in one production year

In the Western Highlands agro-ecological zone, there are mainly two major honey-harvesting periods from both traditional and top bar hive (April-June and October-December; Figure 1.11). The flowering periods were immediately followed by honey flow seasons. On average farmers reported that they harvest about 10 kg of already processed honey from the traditional hives and 15kg from top bar hive in the zone. The frequency of harvest and amount of honey produced per production season varied with for the Langstroth hive. The variation depends on management of the hive (Table 1.7). In the Eastern agro-ecological zone there are 2-3 seasons per year depending on the rainfall and skill of the beekeeper (April-May, October-September and December – January; Table 7). Many beekeepers did not have accurate records of honey yields per season per year. Most of the honey yield records are per house hold per year. Apart from producing honey, at least 73% of the beekeepers produce beeswax and 27% produce propolis.

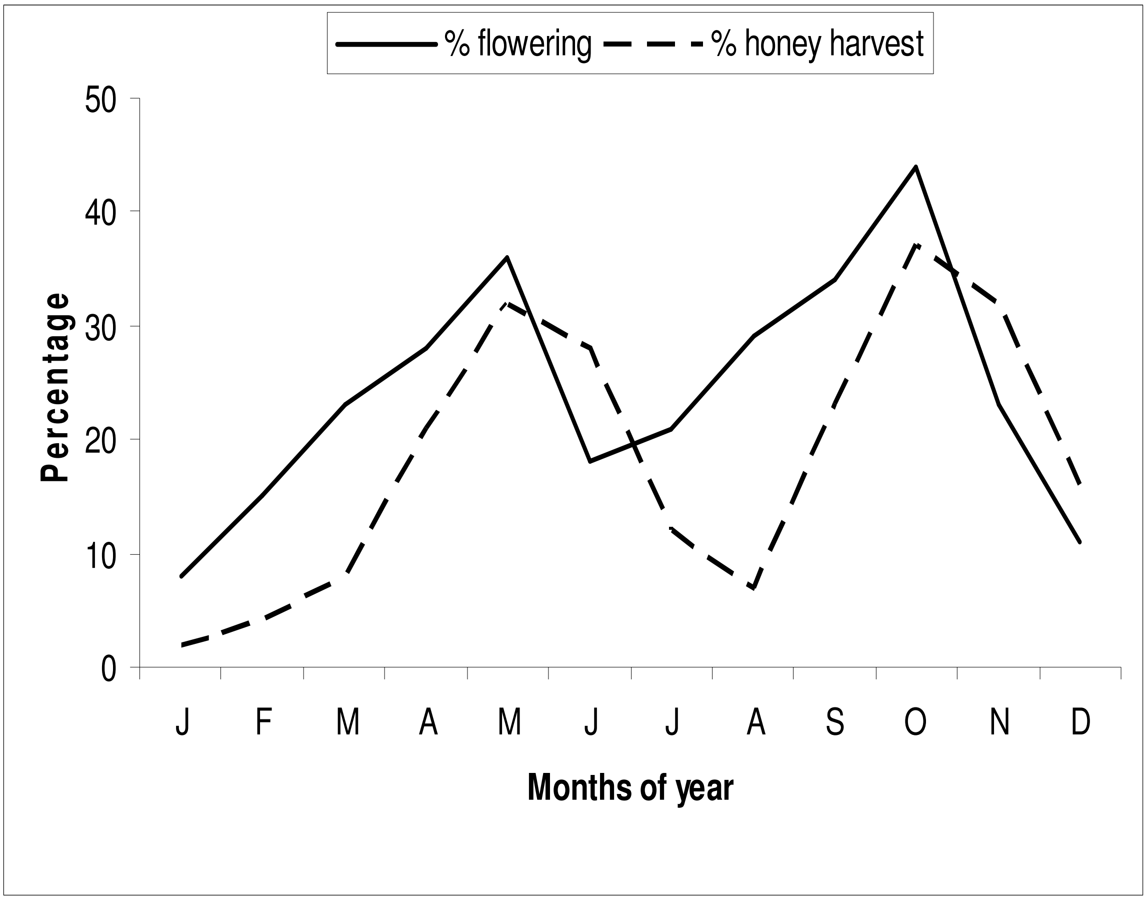

Figure 1.12 Flowering of bee plants and number of beekeepers who harvest honey at different seasons.

Note: The honey-harvesting period lagged behind flowering period.

Table 1.7 Frequency of honey harvesting and amount harvested according to hive type in one production year in the Western Highlands and Eastern agro-ecological zone

Type of hive	Frequency of harvest per year	Amount of kg per harvest	Remarks
Western Highlands			
Traditional Hive	2	10	Varies according to mgt of hive
Top bar hive	2	15	Varies according to mgt of hive
Langstroth hive	4-8	8-13	Varies according to mgt of hive
Eastern agro-ecological zone			
Traditional Hive	2-3	5-10	Varies according to mgt of hive
Top bar hive	2-3	15-20	Varies according to mgt of hive
Langstroth hive	2-3	15-20	Varies according to mgt of hive

1.6 Discussions

The main principles of beekeeping include knowing the area, the plants that bees like, when they flower, understanding the colony cycle and aiming for strong colonies at the same time as the nectar flow for maximum honey yield. The floral calendar is one of the most useful tools of the beekeeper. The calendar enables the beekeepers on what to expect in an apiary so that they can manage their colonies in the most rational manner (FAO, 1990). Beekeeping in any specific area cannot develop without an understanding of the calendar, special calendars for the different agro-ecological zones. Assembling a beekeeping calendar for any specific area requires complete observation of the seasonal changes in the vegetation patterns and the agro-ecosystems of the area, the foraging behaviour of the bees, and the manner in which the honeybee colonies interact with their floral environment (Croat, 1969). The accuracy of a beekeeping calendar, and hence its practical value, depend solely on the careful recording of the beginning and end of the flowering season of the plants and how they affect the bees.

In order to survive, prosper and be productive, honeybee colonies must have a supply of both nectar and pollen in adequate quantities (Sommeijer et al., 1997). Our result showed that not all plant species are equally good for beekeeping. Some supplied both nectar and pollen when in bloom and these are often called honey plants, because they are best suited for honey production. Plants producing nectar but little or no pollen are also considered to be honey plants. Other plants, however, may yield pollen but little or no nectar. These pollen plants are also important in beekeeping, especially at the time of colony build-up, when the bees need large amounts of the protein contained in pollen for their brood-rearing (FAO 1990). A good beekeeping area is one in which honey and pollen plants grow abundantly and with a relatively long blooming season. Such areas are however, not always available or easy to find (Sommeijer et al., 1997). Our results showed that beekeepers used their skill in colony management in order to provide their bees with good and productive foraging environments. Some of them know the time and duration of the blossoming season of the major honey plants. However, very few beekeepers in the study area know the environmental factors affecting flowering of bee plants and can make reasonable assessment of the supporting capacity of an apiary, e.g. the number of colonies that can be put to productive work in an area. Since the practice of modern beekeeping is relatively new in Uganda, the compilation of economic bee forages and the identification of areas suitable for beekeeping are still far from complete.

Productive beekeeping depends on good colony management and good beekeeping areas. In order to promote beekeeping as a profitable agricultural enterprise, places with a good potential for beekeeping should be located and assessed. Uganda is rich in places inhabited by feral swarms of native honeybees, and this fact often makes people think that beekeeping can be promoted almost anywhere in the country where native bees are found (Commonwealth Secretariat. 2002). The problem is that most feral colonies of Ugandan honeybees have high tendency of swarming, moving with the seasons and the availability of forage. Thus, the temporary presence of a few feral swarms of honeybees in a place for short periods does not necessarily indicate that there is enough forage in the area to support year round beekeeping.

Our results showed that there are mainly two major rainfall seasons in a year. The flowering periods were immediately followed by honey flow seasons. Therefore there are also two major honey harvesting seasons in the year. When many plants start to bloom, bees collect alot of nectar and pollen, and the young workers eating more pollen, produce more bee milk. As a result, the queen lays more eggs, and the nurse bees feed and rear more brood, the amount of brood increases gradually to cover all the combs. As the availability of pollen and nectar increases, the population of the colony thus increases with it (FAO, 1990). The crowded hive may then be provoked to swarm. During the main honey flow, bees collect large amounts of nectar. The other hive bees are engaged in evaporation, ripening honey and sealing combs. At that time, the bees require many empty comb cells to store the nectar they collect which is later transformed into honey. This is why more empty cells must be available in the hive during the main honey flow than are necessary for storing honey. After the main honey-flow season ends, the nectar sources diminish and later stop completely.

In this preliminary study, the hives were not weighed; otherwise in a comprehensive assembling of floral calendars, weighing the hives is one of the most accurate ways of assessing the suitability and supporting capacity of an area. The preparation of an accurate, detailed calendar requires several years of repeated recording and refinement of the information obtained. In order to build a comprehensive floral calendar, the beekeeper should make a general survey of the area, drawing up a list of flowering plants found, special attention being paid to plants with a high floral population density per unit area or per tree. The beekeeper should place several strong honeybee colonies in the area, inspecting the hives regularly and observing changes in the amount of food stored within the hive to determine whether it is depleted, stable or increasing. Any food gains or losses are monitored accurately by weighing the hives. During the time of monitoring the food stores of the hive, the beekeeper surveys areas in the vicinity of the apiary and within the flight range of the bees, to record the species of plants that the bees visit. The beekeeper then determines whether the plants are visited for nectar or for pollen. Pollen foragers will have pollen pellets attached to their hind legs.

To determine whether the bees visit flowers for nectar the observer squeezes the abdomen of individual bees to obtain a drop of regurgitated nectar, tasting it for sweetness or measuring the nectar concentration with a hand refractometer. The beekeepers then studies the frequency with which the bees visit each flower species, in relation to changes in the level of the colonies' food stores. If there is a continuous increase in food stores, in direct response to the availability of the plants visited, the plants are good forage sources. When the food stores remain stable, the plants can be depended upon to meet the colonies' daily food requirements, but they cannot be classified as major honey sources. The beekeeper should carefully record all the changes in the blossoming of the plants visited. When the colonies begin to lose weight, the flowering season is finished for all practical purposes. Once all the data on forage species have been assembled and repeatedly verified, they should be judged as they relate to the actual performance of the honeybee colonies. The calendar can then be drawn up in the form of circular or linear charts, showing the weekly or monthly availability of each plant and their flowering sequence.

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CHAPTER 2

HONEYBEE PEST AND DISEASE CONTROL METHODS FOR IMPROVED PRODUCTION OF HONEY AND OTHER HIVE PRODUCTS IN UGANDA

2.1 Introduction

In the apiculture sub-sector, the national goal is to enhance the production and marketing of honey and other hive products. In order to fulfil this, Uganda has to maintain a large healthy national honeybee population to sustain the supply of honey and other hive products for the domestic, regional and international markets. In addition, to attain certification for export of honey and other bee products, the country has to prepare a Monitoring Plan for Residues in Honey as required by international markets, especially the European Union. The plan has to demonstrate that there are no residues in our honey. The country also needs to draft the Standard Operating Procedures and Code of Practice for the apiculture sub-sector. The entire country should be free of major honeybee pest and diseases, which affect the honeybees and quality of their products. The important honeybee pests causing economic losses in the apiculture industry in Uganda include ants, wax moths, hive beetles, termites and lizards. All types of ants (driver, tailor, black, red, brown, large or small) are dangerous to the hive. They eat sweets such as nectar, honey, sugar and the bee's body. They like to live in hollows like the bee, and the same empty beehive produced by man for bees can also be a good home for them (FAO, 1990). Wax moths (*Galleria mellonella* and *Achroia grisella*) attack colonies during the warm periods of the year. Strong colonies are able to repel them, but weak ones are susceptible to attack. The moth itself does no harm to adult bees but does harm the larvae. The female, which is slightly smaller than the honeybee, enters the hive freely and lays her eggs in the combs. The eggs hatch in three days, and the emerged larvae begin to eat the wax, tunnelling through and destroying the comb cells, and spinning web-like cocoons about themselves for protection against the bees. They are capable of destroying all the combs in a hive. The bees may leave the hive and cluster on a support near the apiary. Hive beetles may enter through gaps and cracks but also through large entrance holes. The large black beetle feeds on brood and is most numerous during the rains. Others feed on small amounts of honey and pollen. The smaller hive beetles lay eggs in pollen cells, which can be turned into a stinking mess by the maggots within a few days. Termites do not attack the bees themselves but destroy hives and equipment. Lizards sometimes stay very close to the hive or accommodate themselves between the lid and the hive body, if they can find an entrance. From that convenient spot, they may feed indefinitely on the bees. Even lizards not living near the hive will feed on the bees once they can locate the apiary.

The brood diseases the beekeeper in Uganda must watch against are American foulbrood (AFB), European foulbrood (EFB) and chalk brood. American foul brood causes heavy losses to the colony's population. It can wipe out not only a single colony but all the colonies in an apiary, and it can easily spread quickly from one apiary to another. The disease is caused by Bacillus larvae. The organisms attack the larva, which dies after it has been capped (i.e. pre-pupa). The dead insect becomes brown and finally dries up into a hard scale which is difficult to remove from the cell. The European foul brood is caused by bacterium *Melissococcus pluton*. The young larva is infected by taking in food containing the bacteria, which multiply in its gut; the larva dies on the fourth day, and the worker bees may leave the cell containing the dead larva uncapped. The regular laying pattern of the queen is lost, and different age groups are scattered throughout the comb. Chalk brood is also an important brood disease. The name "chalk brood" derives from the chalky appearance of the dead brood. This fungal disease, caused by *Ascophaera apis*, may cause serious problems to bee colonies in humid areas. Spores of the fungus are ingested in the brood food. The spores germinate in the gut, and the growth of the fungus causes the death of the brood, which occurs in the pre-pupal stage. Effective control or eradication of these pests and diseases requires an efficiency pest and disease surveillance system in place. Unfortunately, there is no operational surveillance system in Uganda tailored to the eradication of these pest and diseases and creation of pest and disease free export zones. Eradication of honeybee pests and diseases needs an efficient system of monitoring of bee swarming/absconding movements, honeybee hive transfers and proper honey harvesting methods. This can only be achieved if supported by an efficient surveillance system. In this study, an appropriate surveillance system for major honeybee pest and diseases will be developed for the apiculture sub-sector.

2.1 Research problem

There is a dearth of information about honeybee pests and diseases existing in the agro-ecological zones of Uganda. Factors affecting honeybee population density and honey production like geographical location and natural enemies are not known (Commonwealth, 2002). There is no available information pertaining to the populations of these pests and diseases, their prevalence, geographical location in Uganda. Unsubstantiated reports show that there is decline in colony population in most beekeeping areas due to nest habitat destruction, nutritional imbalance, pests, diseases, and / or predators. Also, beekeepers near the flower farms around Lake Victoria and the tea estates in western Uganda have complained of decline in bee colony populations which they attribute to insecticides / pesticides poisoning. No studies have been undertaken to ascertain such claims. Safe, effective, and environmentally acceptable forms of pest and disease control are lacking for the beekeeping industry. New and effective antibiotics for control of many of honeybee pests and diseases have not yet been approved for use. Control measures for these pests and diseases that are efficacious over a range of agro-ecological conditions are needed. The economic impact of honeybee pests and diseases on the production of honey and other bee products is poorly understood, partly because many of the pests and diseases purported to attack bees do not appear to cause overt harm. Cost-effective methods of pests and disease detection and control are lacking.

2.3 Objectives

2.3.1 Overall objective

The overall objective of the study was to develop appropriate honeybee pests and diseases control method for improved production of honey and other hive products in Uganda

2.3.2 Specific objectives

i. To document pests and diseases of honeybees existing in Uganda's agro-ecological zones.
ii. To assess the prevalence of pests and diseases affecting honeybee colonies in the agroecological zones.
iii. To determine the effect of honeybee pests and diseases on the production of honey and other hive products and develop diagnostic tools for their identification.
iv. To examine and document organic (bee-safe) methods of insect pest control using local herbs and integrated vegetable growing techniques to prevent bee poisoning.
v. To engage beekeepers and other stakeholders in dialogue for experience sharing in honeybee pests and diseases

2.4 Materials and methods

2.4.1 Study area

Sampling was based on the 10 different agro-ecological zones of Uganda (Table 2.1 and Figure 2.1). These are classified on the basis of distinct vegetation type, elevation and climatic conditions. One district was selected from each zone (Mbarara in Southern Dry lands; Kampala in Lake Victoria Crescent; Mbale in Eastern; Hoima in Lake Albert Crescent, Kamuli in South Eastern, Lira in mid northern and Kabarole in Western Highlands. The selection of the production zones for the study was guided by the number of active bee farmers groups in those zones and the records of honey productivity in those zones.

Table 2.1 Agro-ecological zones of Uganda

No.	Agro-ecological zone	Districts in the zone
1	Eastern	Pallisa, Tororo, Kumi, Kaberamaido, Katakwi, Soroti Mbale, Sironko, Kapchorwa
2	Lake Albert Crescent	Masindi, Hoima, Kibale
3	Lake Victoria Crescent	Masaka, Mpigi, Luwero, Kampala, Mukono, Kayunga, Wakiso, Kiboga, Nakasongola, Kalangala, Mubende
4	Mid Northern	Lira, Apac, Kitgum, Gulu, Pader
5	South East	Jinja, Iganga, Bugiri, Busia, Kamuli, Mayuge
6	Southern Dry lands	Rakai, Sembabule, Mbarara, Ntungamo
7	Southern Highlands	Kisoro, Kabale, Rukungiri, Kanungu
8	West Nile	Arua, Moyo, Nebbi, Adjumani, Yumbe, Koboko
9	Western Highlands	Bushenyi, Kasese, Bundibugyo, Kamwenge, Kyenjojo, Kabarole
10	Karamoja Drylands	Moroto, Kotodo, Nakapiripiri

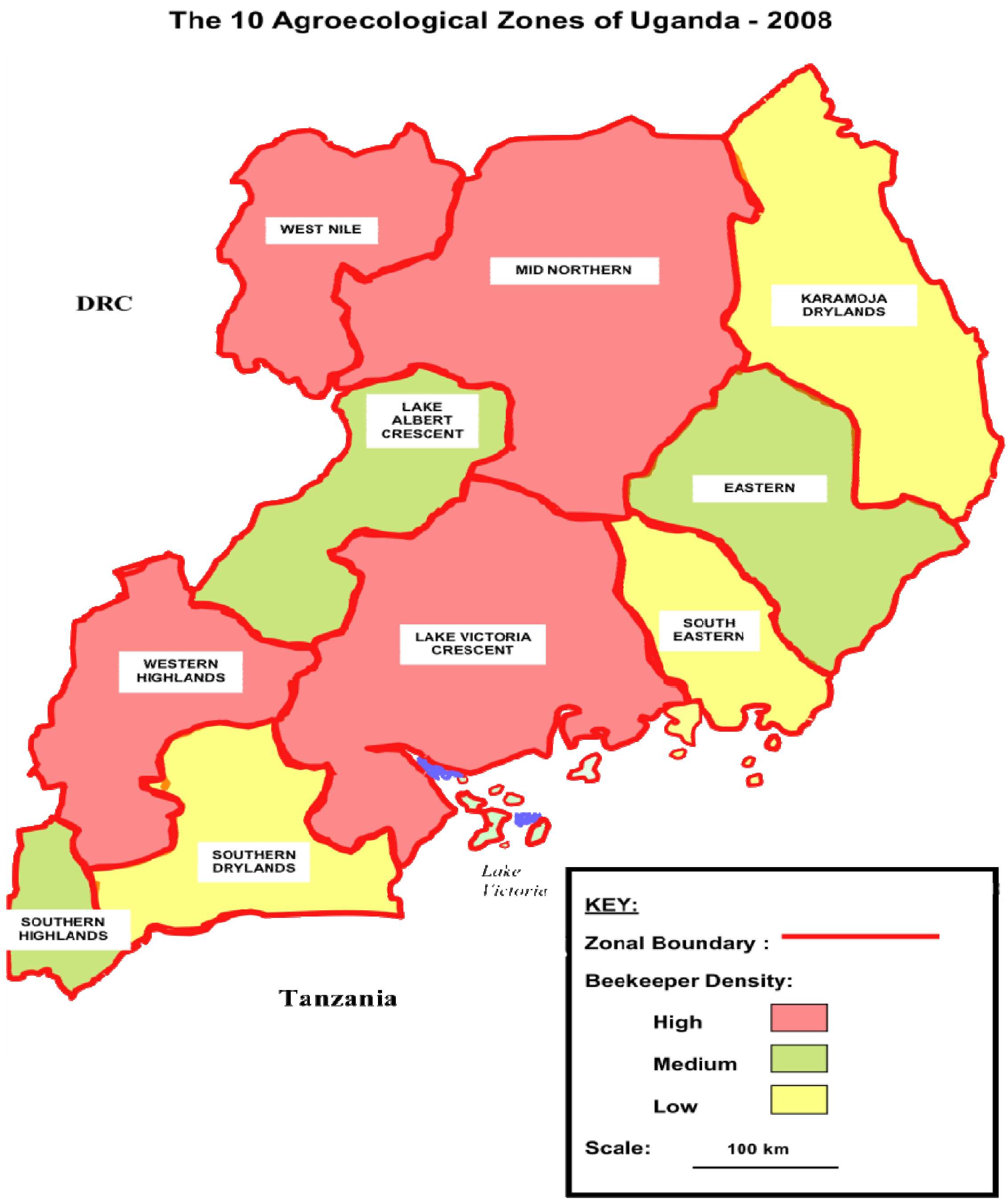

Figure 2.1 Map of Uganda showing level of beekeeping in the agro-ecological zones

2.4.2 Research design and methodology

The study took the form of 'participatory action research. The step-wise approach was to use social mobilisation to motivate farmers to participate in the research and capacity building of TUNADO and (grassroots) community based organisations to enable local people to conduct participatory research and disseminate successful technologies to farmers in the future.

Step 1 began by interaction with beekeepers to find out the general factors limiting beekeeping production, problems they face in bee colony management and to understand the context of beekeeping in the existing farming livelihoods. The second step involved gathering indigenous knowledge from the farmers about honeybee pests and diseases; disease and pest prevalence. These two steps involved use of Participatory Rural Appraisal (PRA) tools such as pair-wise preference ranking (Saville 2001). Figure 2.2 shows one the pair-wise preference ranking that was undertaken. Key informant interviews were also used. Key informants were identified on the basis of their organizational and community positions, reputations, and knowledge of the issues under study. Their insights, recollections, and experiences provided an important and logical starting point for the compilation of data about the social reality of beekeeping sector.

Figure 2.2 Ranking of honeybee pests that affect colonies in Kabarole district, southwestern, Uganda

Selection of respondents and or key informants was accomplished by the use of a modified "snowball" procedure. First, a small number of initial respondents/key informants was identified with the guidance of TUNADO, beekeepers, district entomologists, beekeeping NGOs and CBOs, local leaders and elders who have lived in the area for about 20-30 years. Individuals to act as informants in the general groups were identified on the basis of stakeholder mapping or through informal conversations with local residents. More informants were added during interviews basing on the advice of those being interviewed until redundant information was collected and after a fairly comprehensive account of the issues and problems was compiled. Gender considerations were carefully made while conducting PRA exercises (Mosse 1993, Gurung 1995). The issues of inequity in workload and decision-making power between women and men that very often make it difficult to access women's opinions as easily as men's was addressed.

Third step was quasi-experimental. It involved determining the effect of honeybee pests and diseases on the production of honey and other hive products. In this step, records on production were taken. Samples of bees collected from beehives in different agro-ecological zones were preserved in 70% ethanol for morphologically examinations. Dead bees found in the colony or under the bee hives (Figure 2.3) were also collected to ascertain their cause of death. Some samples were dissected and examined under microscope to detect parasites and pests. Samples of infected combs and other bee products were also collected for laboratory analysis. The fourth step involved simple feasibility trials or testing of local materials and techniques for pest control methods. This made farmers and researchers to work as partners in the technology development process. It has the advantage that the more often and the earlier that farmers are involved in the technology development process, the greater the probability that the practice will be adopted. The participatory trials also helped in ascertaining farmers' assessments of a practice and their ideas on how it may be modified and for observing their innovations.

Figure 2.3. Dead bees found under the beehives in Western Highlands, Uganda.

2.4.3 Secondary data and observations

Secondary data was collected on the study area (physical, social and economic factors), weather conditions (rainfall, temperature and humidity) and on the past beekeeping practices. Beehives were also inspected for any presence of pests as in Figure 2.4.

Figure 2.4 Research team checks for the presence pests in a colonised Kenya Top bar hive

2.5 Results

2.5.1 Honeybee pests that affect production in colonies

Farmers documented at least 12 honeybee pests that affect the production of honey and other bee products in Uganda (Table 2.2). The pair-wise ranking of the pests was based on what beekeepers perceived were the most important pests leading to greater losses in the apiculture sector. The most important pests were man, small hive beetles, small black ants and wax moths. Termites eat and destroy beehives that are not well managed. Spiders, rats and snakes were the least important honeybee pests causing economic loses to beekeepers. Traditional hives were found to be most vulnerable to pest attack (52% of cases) especially by the black ants and wax moths followed by the top bar hives (35% of cases. The Langstroth hives were the least attacked by the pests (5% of cases). Figure 2.5 shows some of the honeybee pests causing economic losses to beekeepers.

Table 2.2 Pairwise ranking of honeybee pests that affect colonies

	Pests	Li	Wm	Be	Ra	Ba	B	Sn	Hb	M	S	R	T	Total	Rank
1	**Lizard (Li)**	X	Wm	Be	Ra	Ba	Li	Li	Hb	M	Li	R	T	04	8
2	**Wax moth (Wm)**		X	Be	Wm	Ba	Wm	Wm	Wm	M	Wm	Wm	T	07	4
3	**Beetle (Be)**			X	Be	Be	Be	Be	Be	M	Be	Be	Be	10	2
4	**Red ants (Ra)**				X	Ba	Ra	Ra	Ra	M	Ra	Ra	T	06	6
5	**Small black ants (Ba)**					X	Ba	Ba	Ba	M	Ba	Ba	Ba	09	3
6	**Birds (B)**						X	B	Hb	M	B	B	T	03	9
7	**Snakes (Sn)**							X	Hb	M	S	R	T	00	12
8	**Honey badger (Hb), Fox, Chimpanzees**								X	M	Hb	Hb	Hb	06	6
9	**Man (M)**									X	M	M	M	11	1
10	**Spiders (S)**										X	S	T	02	10
11	**Rat (R)**											X	T	01	11
12	**Termites (T)**												X	07	4

	
Honeybee pests – Small black ants	Honeybee pests - red ants
	
Honeybee pests - Small hive beetles	Honeybee pests - Wax moth
	
Honeybee pests – birds (honeyguides)	Honeybee predator - Man
	
Honeybee pests – Honey badger	Honeybee pests - Termites

Figure 2.5 Some of the honeybee pests causing economic losses to beekeepers

2.5.2 Prevalence of honeybee pests affecting colonies

The beekeepers categorised the prevalence of honeybee pests according to how they perceived these pests to be most common (very frequent), common (frequent), not common (rare), or very rare (Table 2.3). The most common (very frequent) honeybee pest mentioned was the beetle. The common (frequent) pests include the wax moth, red ants, small black ants and the termites. Lizards, birds, snakes and rats were said not to be common (rare) pests.

Table 2.3 Prevalence of honeybee pests affecting the colonies

Pests	Most common (very frequent)	Common (frequent)	Not common (rare)
Lizards			X
Wax moths		X	
Beetles	X		
Red ants		X	
Small black ants		X	
Birds			X
Snakes			X
Honey badger, foxes, chimpanzees		X	
Man (theft)			X
Spiders			X
Rats			X
Termites		X	

2.5.3 Traditional methods used by beekeepers to diagnose honeybee pests and diseases

Traditional methods used by beekeepers to diagnose honeybee pests and diseases were documented and are shown in Table 2.4. Symptoms that beekeepers use to diagnose presence of pests include very frequent in-and-out flights without any loads, presence of pests in the apiary or in hives, reduction in hive occupancy rate and absconding. Beekeepers diagnose diseases by death of many bees, inactivity in comb construction, presence of moulds in the hives etc.

Table 2.4 Traditional methods used by beekeepers to diagnose honeybee pests and diseases

Honeybee pests	Honeybee diseases
1. Unstable flights (very frequent in and out flights)	1. Death of many bees
2. Presence of pests in the hive when inspected	2. Inactivity in flights
3. Unusual zooming in the apiary sites	3. Inactivity in comb construction
4. Empty hives during harvest time	4. Presence of moulds in the hives
5. Absconding of bees	
6. Bees invading people's homes	
7. Prevalence of pests at the apiary	
8. Decreasing size of the bee colonies	
9. Most bees stay outside the hives	

2.5.4 Local organic (bee safe) methods for pest control

The traditional (bee safe) methods used by beekeepers for pest control were documented and categorised according whether the beekeepers considered them to be most effective, effective, less effective and not effective (Table 2.5). The methods that were considered to be most effective include; frequent smoking of hives (to drive out beetles), keeping the apiary tidy and clean from bushes, avoiding throwing/scattering combs and honey around the apiary, and housing the apiary (building apiary house was considered to be expensive). Application of ash near the hives at the apiary was considered to be effective while fencing off the apiary was considered to be less effective method of organic pet control.

Table 2.5 Traditional/local organic bee safe methods for pest control

	Methods	Most effective	Effective	Less effective	Not effective
1	Frequent smoking of hives (many pests)	X			
2	Ash application at the apiary		X		
3	Keeping the apiary tidy and clean from bushes	X			
4	Avoiding throwing/scattering combs and honey around the apiary	X			
5	Sensitisation of man/ bye laws				
6	Fencing of the apiary			X	
7	Housing the apiary (expensive)	X			

2.5.5 Problems faced by farmers in controlling pest and diseases

Several problems that hinder beekeepers from effectively controlling and managing pest and diseases were reported. The most important were lack of adequate information (reported by 34% of respondents) and inadequate time needed for management of colonies. Some of the problems include night dwelling pests, inadequate techniques for management and control of the pests (Table 2.6).

Table 2.6 Problems faced by farmers in control of pest and diseases

Problems	% responses
Lack of adequate information.	34
Inadequate time for management of colonies.	16
Flying pest (e.g. birds).	16
Night dwelling pests.	13
Inadequate techniques for management and control.	11
Labour intensity.	10

2.5.6 Effect of pests on quantity of honey produced

At least 41 % of the farmers reported that pests and diseases led to the absconding of the colonies. Honey production with the traditional hives was most affected by the pest with a 35% loss, followed by the top bar hive with a 21% loss. Langstroth hives where the least affected by the pests with a 15% (Table 2.7).

Table 2.7 Effect of pests on quantity of honey produced

Hive	Honey production per hive per harvest		
	Without pests (Kg)	With pest (Kg)	% loss
Traditional (local)	4.5	2.1	53.3
Top bar	8.8	5.9	33.0
Langstroth	16.6	12.1	27.1

2.6 Discussions

2.6.1 Common honeybee pests and diseases in Uganda

Introducing an appropriate pest and disease control method requires proper recognition of the causal organism. Apart from mechanical control methods of dealing with the obvious insect pests, rational means of control could only arise after the cause of the injury has been known (Barasa, 2005). In this study at least 12 pest and predators that attack honeybees and the hives in Uganda were documented. These include insects, mammals, reptiles and birds. A wide variety of pests and predators (besides infectious microorganism) are known to attack adult honeybees, bee brood stages, materials stored in the hive and even the hive itself (Dewey (1999). Some of these organisms may simply use the hive as a place to live or as a shelter for their own young/ nest, but some may cause harm by feeding on honey, pollen, brood remains or beeswax. Almost all hives where attacked by the pests but the severity of the pest attack varied from hive to hive and from apiary to apiary. The traditional hive where most attacked by the hives probably because of the materials they are made of. Such materials include all types of grasses which are habitats for the pests. In addition, traditional hives make it difficult to control the pests because of the limited accessibility to the hives. The Langstroth hives where the least attacked by the pests because the beekeepers can easily inspect the hives and control the pests.

The black ants are one of the most important honeybee pests causing economic losses to beekeepers. They suck out the honey and kill the pupae and eggs. They are too small to be stopped by beehive guards and in many cases make the bees leave the hive. The large hive beetle (*Oplostomus fuligineus*) (20mm long) and the small hive beetle (*Aethina tumida*) are common pests. They cause damage to combs and setup fermentation of stored honey. Both types of hive beetles eat pollen and honey, but the Oplostomus species also eats brood. Bees can be seen trying to remove Aethina but its hard slippery carapale makes it impossible to grasp. Aethina lays eggs on occupied combs and in cracks and crevices (MAAREC, 2000). The greater wax moth (*Galleria mellonella*) and the lesser wax moth (*Achroia grisella*) are opportunistic pests and will quickly lay eggs on older, abandoned honey combs where a weak colony is in residence. Both types of wax moths are generally found wherever there are honey bees and particularly in warm conditions. In the wild, the wax moth plays a part in completely destroying old, useless combs and bees will eventually reoccupy the clean nesting site and build new combs. Beekeepers however, suffer great economic loss when frames of drawn combs built on foundation wax are infected by wax moth. Bees in a strong colony are able to remove small wax moth larvae from the hive. Strong colonies are therefore the best defence against infestation of wax moth. When the moths do succeed in gaining entry to a beehive they lay their eggs on the combs and in cracks in the hive body. The wax moth larvae develop in eight growth stages when hatched, the larvae burrow their way through the wax leaving trails of silken tunnels and black faecal droppings. Their preferred food is found in old brood combs where they eat the pupal cases living brood cells. Between each successive pupation stage, the growing larvae become more voracious until the comb is destroyed and all the remains are a mass of silken webbing and faeces (Marchard, 2005). The mature larvae of the greater wax moth cause great damage to wooden frames and hive bodies. They curve out depressions in the wood, settle into the depressions and spin their final cocoons. When they do this by the hundred, frames can be weakened to the extent that they have to be discarded. These frames are difficult to clean, but they are worth saving, pulling out the bulk of the silk and then scalding them briefly with the frame of a blow torch will clean them up. Bird feed on the bees especially those that forage for nectar. These birds strategically position themselves near the hives and capture the bees that are flying out or into the hive. This greatly reduces on the population of the bees in the hives hence resulting in the reduction in the production of the hives.

The termites feed on the equipments used in beekeeping especially those made out of wood for example the beehives. The termites do not eat the honey or the bees themselves, but they are agents of biodegradation of wood since many of the beehives are made typically of wood. FAO (1990) noted that termites are only after the wood and may not be classified the pest. Hives placed on the ground or bee equipments left lying on the ground or stacked directly on the ground may be subject to termite infestation. If termites destroy the bottom boards, the bee may not have a bottom entrance and the colony could be more difficult of move. Lizards where reported by the respondents that they eat the bees and the honey. Lizards stay very close to the hive or accommodate itself comfortably between the lid and the hive body, if they can fine an entrance and may feed indefinitely on the bees. Bees are also eaten by the lizards as they locate the apiary.

2.6.2 The local management practices used to control the pests and diseases

The experienced beekeepers demonstrated some good knowledge of local pest control methods, especially pests like black ants, red ants and termites. Various parts of plants and plant extracts are known to be either toxic or repellent to pests of crops and trees and are widely used by small scale farmers. For example, extracts from plants such as neem (*Azadirachta indica*, red pepper, *Tithonia* sp., *Tephrosia vogelii*, wood ash, cow dung and urine have been used to control termites in the field (Wardell, 1987; Logan et al., 1990). The extent of the local methods used by the beekeepers to control and manage the pest and diseases varied from individual to individual and they also ranged from mechanical methods to chemical methods. Most of the pests, especially the crawling pests where controlled by siting the hives on wires and was considered as effective method in controlling the crawling pests. Nsubuga (2000) reported that the beekeeper in Luweero district controlled ants by siting the hives on wires that where greased. The grease made the movement of these pests on the wire difficult thus such wires were avoided by the pests. Hive stands where also used in the control of the crawling pests, whose legs were placed in old engine oil container or a grease ring between the hive and the ground. The hive stands were alternatively treated with used engine oil which is mostly quite effective (FAO, 1990 and MAAREC, 2000). Metal plates can also be placed on the stands to prevent lizard from reaching the hives. Nsubuga (2000) also suggested that a combination of ash and grease can be used to control ants.

Many of the beekeepers also made bio-pesticides which they used in the control of most of the pests. The major ingredients in the manufacturing of these pesticides included, red pepper, neem tree (*Azaradichta indica*) and *Tephrosia* sp. Most of the bio-pesticides are mixed with ash and urine then sprinkled directly on the pests or in their path. Nkunika (2002) reported that some extracts from plants such as Neem tree, wild tobacco, *Tephrosia* spp and dried red pepper have been used to control termites in the field and storage. Most of these bio-pesticides are not harmful to the bees as compared to the inorganic pesticides. Most of the ingredients used in the control of the pests and diseases where locally available and most of them affordable to the farmers. Most of the beekeepers had planted the Neem tree and *Tephrosia vogelii*, on their farmers since most of them where practicing agro-forestry where by the trees in turn provided forage for the bees. The beekeeper also reported that good hive management can be an effective method of controlling pests and disease in the bee colonies. According to MAAREC (2000), good hive management, including regular changes of wax foundation in the brood chambers can control the hive beetles. Good hygiene in the hives can also control the wax moth, hive beetles since these thrive in damp hives. Ntenga and Mugongo (1991) emphasized that good hive management is important in colony development where by a stronger colony can easily defend itself from the pests. This coupled with regular hive manipulation and inspection can be used to control most of the pests and diseases. With regular manipulation and inspection of the hives, the beekeeper can be able to notice any pests or symptoms of the disease hence are able to mount an early control on these pests and diseases.

2.6.3 Problems experienced by farmers in controlling honeybee pests

Control of honey bee pests is not an easy task because there are many huddles involved. Farmers reported numerous problems that limited their efforts in controlling pests and diseases. Inadequate information about the pests and their control measures was a major problem that the farmers experienced. According to Agnes et al., 1993, subsistence farmers in Africa are generally poorly educated and very poorly served by overstretched extension systems. Therefore, extension services about honey bee pests and thus their biology and ecology, identification depending on the symptoms or damage provides farmers with a more objective basis for making decisions about the pests. Time constraint was another problem experience by the farmers since most of them where not full time beekeepers. Therefore, farmers inspected their hives occasionally. Also some of the methods used by the farmers to control the pest for example the physical removal and killing the pests such as the wax moth where effective at night because the moth flies away during the day. Since the black ants are too small, a lot of time is usually spent in their control. Pests that are mobile and others that attack the bees at night (lizards, birds and other mammals) cause massive reduction in the colony populations. They cause serious problems during their control because they were unpredictable. Understanding and using the different techniques for pest control also cause confusion to the beekeepers. Most of the beekeepers do not have adequate knowledge about bio-pesticides and sitting hive on the wires. As a result of inadequate pest control, some colonies absconded while other hives where seriously damaged.

2.6.4 Effects of the honeybee pests and diseases on hive production

Pests and diseases have a significant effect on the quality and quantity of honey produced (MEERAC, 2000). Pests like the black ants that feed on the honey can reduce on the quantity of the honey produced. Other pest like lizards, birds, mammals that feed on the bees reduce on the colony population hence reducing the quantity of honey produced. The wax moth, small and large hive beetles that lay eggs on the honey combs (Marchand and Marchand, 2003), contaminate the honey and other bee products such as was which consequently affects the quality of the hive products. The effects of the pests were detrimental in the traditional hives and least with the Langstroth hives. This is because the traditional hives are more susceptible to the pests since these hives can easily harbour the pests; besides traditional pests are more difficult to manipulate in a bid to control the pests and diseases than the Langstroth hives. Absconding of the colony is one of the effects of the pests and diseases on the honey bees. African bees often depart from their hives by absconding instead of the normal swarming process. There are many factors that contribute to the absconding of the African bees and among them are the frequent attacks by diseases and pests such as lizards, mice, wax moths, ants or animals among others. Absconding reduces the production of the hives because the hive become empty. Pests especially the termites destroyed the hive since they feed on the wood which in turn reduced on the number of hives and led to the absconding of the bees where the bottom of the hive was eaten out by the termites (MAAREC, 2000). The destruction of the hives forced the farmers to replace the hives frequently which was very expensive for the farmers. Some of the farmers decided not to replace the hive which they said was worthless. This consequently affected the production of the hives.

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CHAPTER 3

FIRST MOLECULAR DETECTION OF A VIRAL PATHOGEN IN UGANDAN HONEY BEES: OCCURRENCE, PREVALENCE AND DISTRIBUTION PATTERNS

3.1 Introduction

Beekeeping is a widespread practice throughout Uganda (Kajobe, 2006). Several different types of bees are utilised in Ugandan apiculture, but the most common native hive species is *Apis mellifera* L. These bees are of immense economic significance, not just as producers of honey and beeswax, but also because of the high value of those commercial crops that depend on their foraging activities for pollination (e.g. coffee, cotton and sunflowers). Furthermore, as a pollinator, *A. mellifera* also plays a crucial role in the conservation of biodiversity and sustainable use of land and forest resources in many ecosystems (Adjare, 1990; Crane, 1999; Commonwealth Secretariat, 2002). For these reasons beekeeping is an important undertaking in the recent development programmes for poverty alleviation and rural development in Uganda (MAAIF, 2000). In order to realize the full potential of a new and expanding apiculture sector, it is vital that Uganda maintains a robust national bee population, strong enough to achieve and sustain required supplies of honey, other hive products, and essential pollination services (UEPB, 2005). Although the need to have healthy bees is paramount, very little is known about the current pest and disease status of Ugandan honey bee stocks. Honey bees are prone to a range of viral diseases, which vary in their symptoms and the severity of their effects (Aubert et al., 2008), but there is no available literature pertaining to their prevalence or geographical distribution in Uganda. Since Uganda is directly bordered by five other sub-Saharan African countries; Rwanda, Kenya, Tanzania, Sudan and the Democratic Republic of the Congo (DRC), where certain diseases are reported as being/suspected to be present, there is no safe assumption that the causative pathogens are absent in Uganda (Dubois and Collart, 1950; Rosario-Nunes and Tordo, 1960; Bauduin, 1966; Kigatiira, 1984, 1988; Ntenga and Mugongo, 1991; Kumar et al., 1993; Matheson, 1993; Mostafa and Williams, 2002; Ellis and Munn, 2005). The present study therefore sought to establish the occurrence of honey bee viruses in Uganda and, where present, to map their incidence in different regions.

3.2 Objective

To establish the occurrence of honeybee viruses in Uganda and, where present, to map their incidence in those areas.

3.3 Materials and Methods

3.3.1 Sample collection

Uganda is divided into 10 agroecological zones (Table 3.1), each of which is characterised by its vegetation, elevation, weather and proximity to water bodies. These environmental factors influence the density of beekeeping practiced in each respective zone. Between February 2007 and May 2008, *A. mellifera* were collected from beekeeping sites in nine of Uganda's ten agroecological zones. Within each zone the intensity of sampling reflected floral diversity and the extent to which apiculture is practiced therein: most samples were collected in Western Highlands; least were collected in Southern Drylands. With the exception of one site, in the Western Highlands (Kabarole/Bwenderwe), where dead adult worker honeybees were found, samples were randomly collected from non-problematic colonies that were not suffering from any overt signs of disease or infestation, such as weakness, depopulation, or sudden collapse of the colony. In total, 138 different colonies were sampled from 63 sites across Uganda. Since viruses infect all developmental stages of bees (Chen et al., 2006a), larvae, pupae and adult specimens were harvested from each site. In line with existing United Kingdom (UK) recommendations for pest and disease monitoring regimes (Ball, 2007; Byham, 2007a, 2007b), every effort was made to collect an appropriate number of individuals to allow a high (95%) probability of disease detection. For our purposes, the minimum required sample size/site was fifteen, based on the assumptions that colonies were relatively small (< 10,000 bees) and that disease prevalence was intermediate (20% of the population affected) (Cannon and Roe, 1982). In a few cases, however, sample sizes were lower due to the considerable restraints placed on brood access by the traditional beekeeping methods practiced in some rural areas of Uganda (e.g. the destructive nature of harvesting honey and larvae for food leaves little surviving brood). All samples were stored in 70% alcohol, prior to their import to the UK for the purposes of pests and disease screening (Defra Import Authorisation no. POAO/2008/373). All molecular diagnoses were carried out using real-time PCR (TaqMan) in the Molecular Technology Unit of The Food and Environment Research Agency (Fera), York, UK (Figure 3.1).

Figure 3.1 Dr. Kajobe and a colleague in the Molecular Technology Unit of the Food and Environment Research Agency (FERA), York, UK.

Table 3.1 Number of sites and bee samples from at least one district representing each agro-ecological zone

Agro-ecological zone	Estimated density of beekeepers in district	Sites sampled (District/ village or beekeeper)	No. of sites sampled
Eastern	Medium (1,000 – 5,000 beekeepers)	Mbale/Bufumbo	1
		Mbale/Municipality	1
		Mbale/Mutoto village	4
		Tororo/Kwappa	1
		Tororo/Rugbongi	2
Lake Albert Crescent	Medium (1,000 – 5,000 beekeepers)	Hoima/Bulindi	2
		Masindi/Nyabyeya	3
Lake Victoria Crescent	High (5,000 – 10,000 beekeepers)	Kampala/Kawempe	2
		Kampala/Mengo	1
		Luwero/Bututumula	3
		Luwero/Katikamu	1
		Mubende/Mubende	1
		Nakasongola/Nakasongola	2
Mid Northern	High (5,000 – 10,000 beekeepers)	Kitgum/NAADS apiary	1
		Lira/Adingo	1
		Lira/Amone	1
		Lira/DickAmone	1
		Lira/Eswapu	1
		Lira/Ogur	1
South East	Low (< 1,000 beekeepers)	Busia/Busia	1
		Kamuli/Kisozi	3
Southern Drylands	Low (< 1,000 beekeepers)	Mbarara/Rwampara	3
Southern Highlands	Medium (1,000 – 5,000 beekeepers)	Rukungiri/Bujumbura	5
		Rukungiri/Bwambara	1
West Nile	High (5,000 – 10,000 beekeepers)	Arua/Katrini	3
		Arua/Ayivu county Pajulu	1
		Yumbe/Kechi	1
Western Highlands	High (5,000 – 10,000 beekeepers)	Kabarole/Bukuku	3
		Kabarole/Bwenderwe	1
		Kabarole/Fort Portal	5
		Kabarole/Karambi	1
		Kabarole/Katurru	1
		Kabarole/Mugusu	3
		Kyenjojo/Kasamba	1
Karamoja Drylands	Low (< 1,000 beekeepers)	Not sampled	
Total	**63**		

3.3.2 RNA Extraction

RNA was extracted on a Kingfisher ML magnetic particle processor (Thermo Electron Corporation) using the default RNA protocol and including the optional 5 minute heating step at 65°C prior to elution. Samples, comprising of 5 individual bees, were placed into 5 ml of Guanidine lysis buffer (5.25 M guanidiniumthiocyanate, 50 mM Tris HCl Buffer (pH6.4), 20 mM EDTA, 1.3% (wt/vol) Triton X-100 (Boom et al., 1990)) and ground to homogeneity on a Kleco 4-96 beater using 7/16 inch ball bearings. The macerated samples were centrifuged for 2 minutes at 6,000g to pellet the debris and produce a clear lysate for extraction. Clarified extract was transferred to the first well of the Kingfisher ML extraction strip containing 50 µl of Paramagnetic Particles (Promega: MD1441). The sample was passed through three wash stages; one wash with Lysis Buffer B (Promega: Z3191), two washes with 70% ethanol before elution in 200 µl of 1 x TE buffer. Blank extraction controls were also completed to monitor contamination. Eluted RNA was stored at –25°C prior to use in real-time RT-PCR.

3.3.3 Real-time RT PCR

For the purposes of this study, samples were screened for the presence of seven honey bee viruses using real-time RT-PCR: *Black queen cell virus* (BQCV), *Chronic bee paralysis virus* (CBPV), *Sacbrood virus* (SBV), *Deformed wing virus* (DWV), *Acute bee paralysis virus* (ABPV), *Apis iridescent virus* (AIV) and *Israeli acute paralysis virus* (IAPV). This particular panel of candidate viruses was chosen because of their known associations with honey bee colonies, the damaging effects of the diseases they can cause, and because of their high or potentially high economic significance in international beekeeping outside Africa. Real-time RT-PCR testing was completed using assays and cycling conditions as described by Chantawannakul et al. (2006). The primers for IAPV were modified from Palacios et al. (2008) to detect all known sequence variants of IAPV (Jeffrey Hui, Centre for Infection and Immunity, Columbia University, USA, *pers. comm.*) (F Primer CGA ACT TGG TGA CTT GAR GG; R Primer RCR TCA GTC GTC TTC CAG GT) and IAPV Taqman® Probe (5’FAM-TTG CGG CAA TCC AGC CGT GAA AC-3’TAMRA) was designed using Primer Express 2 (Applied Biosystems). All real-time RT-PCR protocols were initially validated using pure viral cultures and virus infected bees (Ward et al., 2005). An internal control, designed to detect *Apis mellifera* 18S rRNA was included in the study to compare extraction efficiencies between samples and to allow interpretation of negative results (Ward et al., 2007). The C_T value for each reaction was assessed using Sequence Detection Software v2.2.2 (Applied Biosystems). Samples giving the lowest C_T values were selected for confirmatory testing using direct sequencing.

3.3.4 Confirmatory testing using conventional RT-PCR and direct sequencing

In order to validate our findings, samples that tested positive for the presence of virus (BQCV) using real-time RT-PCR were subjected to further confirmatory screening, using conventional RT-PCR coupled with direct sequencing. Published primers *BQCVF* GGACGAAAGGAAGCCTAAAC, *BQCVR* ACTAGGAAGAGACTTGCACC, designed to amplify a 424 nucleotide region of the RNA-dependant RNA polymerase (RdRp; Tentcheva et al. 2004a), were used to confirm BQCV positives. To avoid any possible contamination, 1-step RT-PCR was performed using 0.3 μM of each primer, 2 mM dithiothreitol (DTT), 20 U RiboLock™ Ribonuclease Inhibitor (Fermentas), 2X ReddyMix™ PCR Master Mix (1.5 mM MgCl2; Thermo Scientific), 100 U Superscript III™ Reverse Transcriptase (Invitrogen), 0.4 U BIO-X-ACT™ Short DNA Polymerase (Bioline) made up to a final reaction volume of 25 μl using RNASE free water. Samples were retrotranscribed by heating for 5 min at 25°C followed by 90 min at 50°C. The reaction mixture was then heated for 15 min at 94°C followed by 40 amplification cycles of PCR (30s at 94°C, 30s at 54°C, and 1 min at 72°C) and a final extension step of 10 min at 72°C. PCR products were visualised by electrophoresis on a 1% agarose gel. Virus positive and no template controls were also performed for each virus. To ensure provenance of nucleotide data from Ugandan virus, RT-PCR products from Ugandan and BQCV positive controls were sequenced directly. Initial sequence identity was confirmed by identifying the closest sequence matches from all the published sequences on the EMBL database using the BLASTN search algorithm. Alignments and phylogenetic analyses of closely related sequences were completed using the default setting of MEGA 4.0 (Tamura *et al.*, 2007). The nucleotide and the translated protein sequences were analysed.

3.4 Results

No samples tested positive for DWV, SBV, CBPV, ABPV, IAPV or AIV. However, initial screening with RT-PCR suggested that Black Queen Cell Virus (BQCV) was present in a number of Ugandan bee samples (Table 3.2). It was not possible to confirm the majority of real-time RT-PCR positive samples using conventional RT-PCR coupled with sequencing, due to their typically high C_T values. However, a product of the correct size was produced from one sample collected from the Mbale district in the Eastern zone (C_T 21) using BQCV primers (Figure 3.2), and direct sequencing of the RT-PCR product confirmed the presence of BQCV (Accession number FJ495181). The phylogram shown in Figure 3.3 depicts the relationship between the BQCV sampled in the current study (FJ495181 Uganda 1) and other bee viruses. The similar sequences were found by doing a Blast search on the whole nucleotide database. The sequence then was trimmed for the alignment according to the shortest sequence found (ABPV DQ434969), giving a 177nt section within the polymerase region, corresponding to nt 4741–4909 of the BQCV AF183905 genome (Leat et al., 2000), using *Solenopsis invicta* virus-1 (SnIV-1) as outgroup. Isolates were named according to their strain and geographic origin. The original phylogram was constructed by MEGA-4 (Tamura et al., 2007), using Minimum Evolution criteria and the default parameters. The statistical strength of the nodes is shown as the percentage of correct partitions in a 1000 replicate bootstrap analysis. Branches with less than 60% bootstrap support were collapsed. The resulting phylogram demonstrates that BQCV from Uganda clusters with other BQCV isolates from South Africa and the European Union and was distinct from isolates of other RNA viruses.

BQCV was found in 32 of the 90 samples that were tested (i.e. 35.6% occurrence) (all blank extraction controls tested negative for all seven honey bee viruses). It was found in adult and larval samples, but not in any of the pupae that were screened during this study, even those from BQCV positive colonies. Virus was detected far more often in adults (87.5% of samples), than it was in immature bees (12.5 %). C_T values for BQCV were between C_T 21 and 35, and no differences between levels of infection were detected between adult and larval bees. Figure 3.4 maps the geographical incidence of BQCV in Uganda, as suggested by samples obtained in the current study. BQCV-infected material came from seven out of the nine zones that were sampled, the two exceptions being the South East and the Southern Highlands. It was most prevalent in samples from the Western Highlands, where it was found in seven separate sites (accounting for over 40% of positive results for BQCV nationally). It was comparatively less widespread in the Eastern zone (found at three sites), and only present in single sites elsewhere.

Table 3.2 The incidence of BQCV in different bee development stages from nine agro-ecological zones of Uganda

Agroecological zone	District	Site no.	Bee lifestage	Mean C_T values + (±SD)	
				BQCV +ve	Internal control 18S rRNA
Eastern	Mbale	1	Adult	38.48 (±2.143)	15.05 (±0.102)
	Mbale		Adult	30.91 (±0.044)	15.17 (±0.020)
	Mbale		Adult	29.97 (±0.125)	16.98 (±0.128)
	Mbale	2	Adult	34.77 (±0.071)	15.71 (±0.124)
	Mbale		Larva	32.16 (±0.320)	13.97 (±0.038)
	Mbale	3	Larva	21.56 (±0.312)	14.60 (±0.053)
	Mbale		Larva	28.12 (±0.007)	13.87 (±0.082)
Lake Albert Crescent	Hoima	1	Adult	33.59 (±0.342)	16.05 (±0.007)
	Hoima		Adult	32.10 (±0.195)	15.32 (±0.227)
	Hoima		Adult	33.71 (±0.575)	15.56 (±0.034)
Lake Victoria Crescent	Mubende	1	Adult	31.52 (±0.583)	22.70 (±0.367)
	Mubende		Adult	33.70 (±0.068)	28.77 (±0.115)
Mid Northern	Lira	1	Adult	38.27 (±0.395)	18.45 (±0.072)
South East	No samples tested positive for BQCV				
Southern Drylands	Mbarara	1	Adult	27.47 (±0.428)	16.96 (±0.060)
Southern Highlands	No samples tested positive for BQCV				
West Nile	Yumbe	1	Adult	30.22 (±0.468)	14.31 (±0.071)
	Yumbe		Adult	32.17 (±1.316)	14.37 (±0.062)
	Yumbe		Adult	30.54 (±0.171)	14.38 (±0.105)
	Yumbe		Adult	32.75 (±0.222)	15.20 (±0.132)
	Yumbe		Larva	36.97 (±4.279)	14.31 (±0.225)
Western Highlands	Kabarole	1	Adult	35.84 (±0.122)	22.76 (±0.079)
	Kabarole	2	Adult	33.17 (±0.948)	17.45 (±0.364)
	Kabarole		Adult	30.34 (±0.027)	15.96 (±0.042)
	Kabarole		Adult	28.74 (±0.566)	16.75 (±0.020)
	Kabarole	3	Adult	35.72 (±2.277)	18.10 (±0.005)
	Kabarole	4	Adult	35.62 (±0.676)	16.90 (±0.365)
	Kabarole	5	Adult	32.83 (±0.500)	20.13 (±0.198)
	Kabarole		Adult	29.82 (±0.046)	16.04 (±0.030)
	Kabarole		Adult	30.00 (±0.075)	18.16 (±0.050)
	Kabarole		Adult	28.76 (±2.083)	17.02 (±0.027)
	Kabarole	6	Adult	38.36 (±2.326)	16.00 (±0.036)
	Kabarole		Adult	34.32 (±0.246)	16.87 (±0.167)
	Kyenjojo	7	Adult	32.03 (±0.199)	16.16 (±0.186)

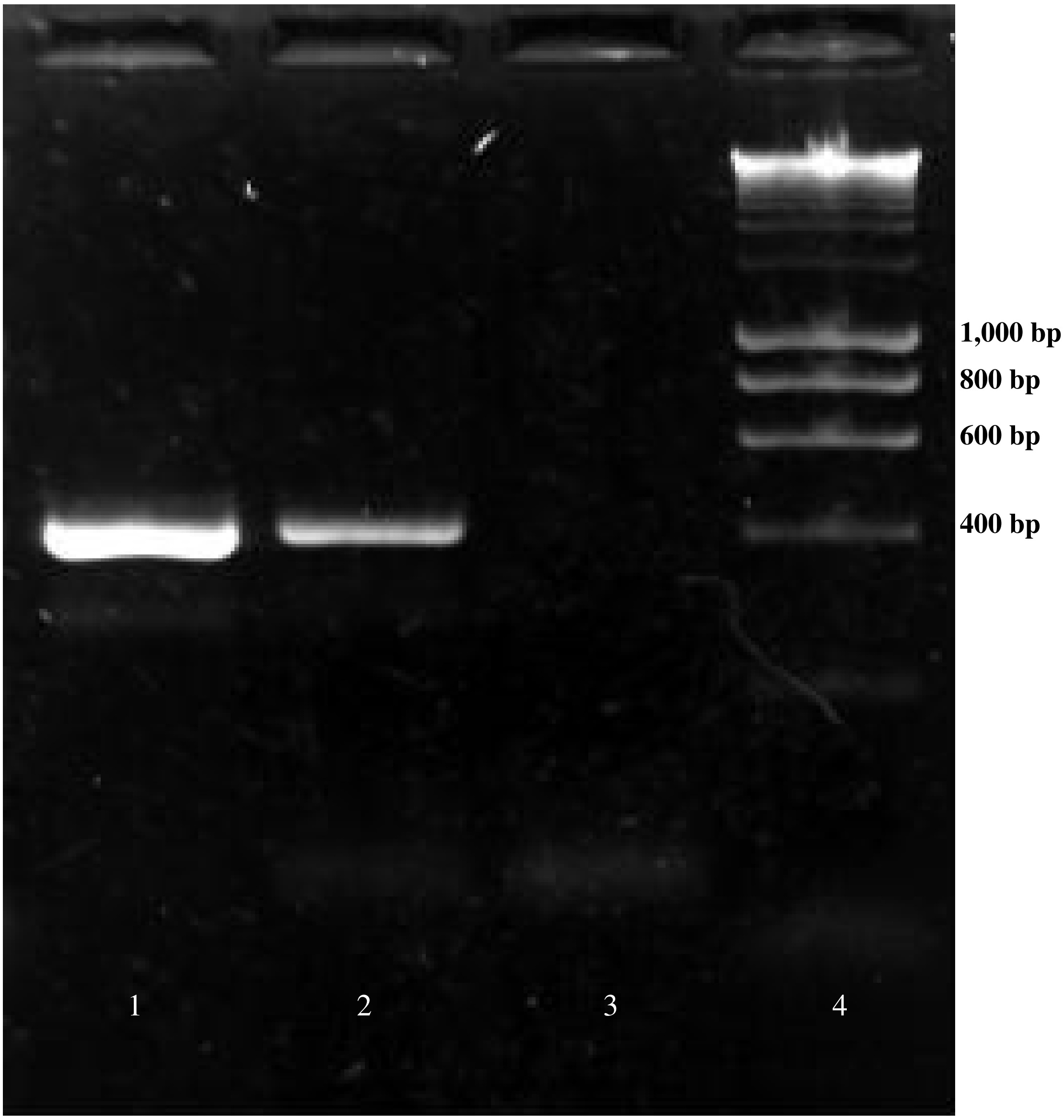

Figure 3.2 Visualisation of the amplified 424 nucleotide region of the RNA-dependant RNA polymerase of BQCV using conventional RT PCR (Tencheva et al., 2004b).

Lane 1: BQCV positive control; Lane 2: sample 33B; Lane 3: negative control; Lane 4: hyperladder 1 (Bioline).

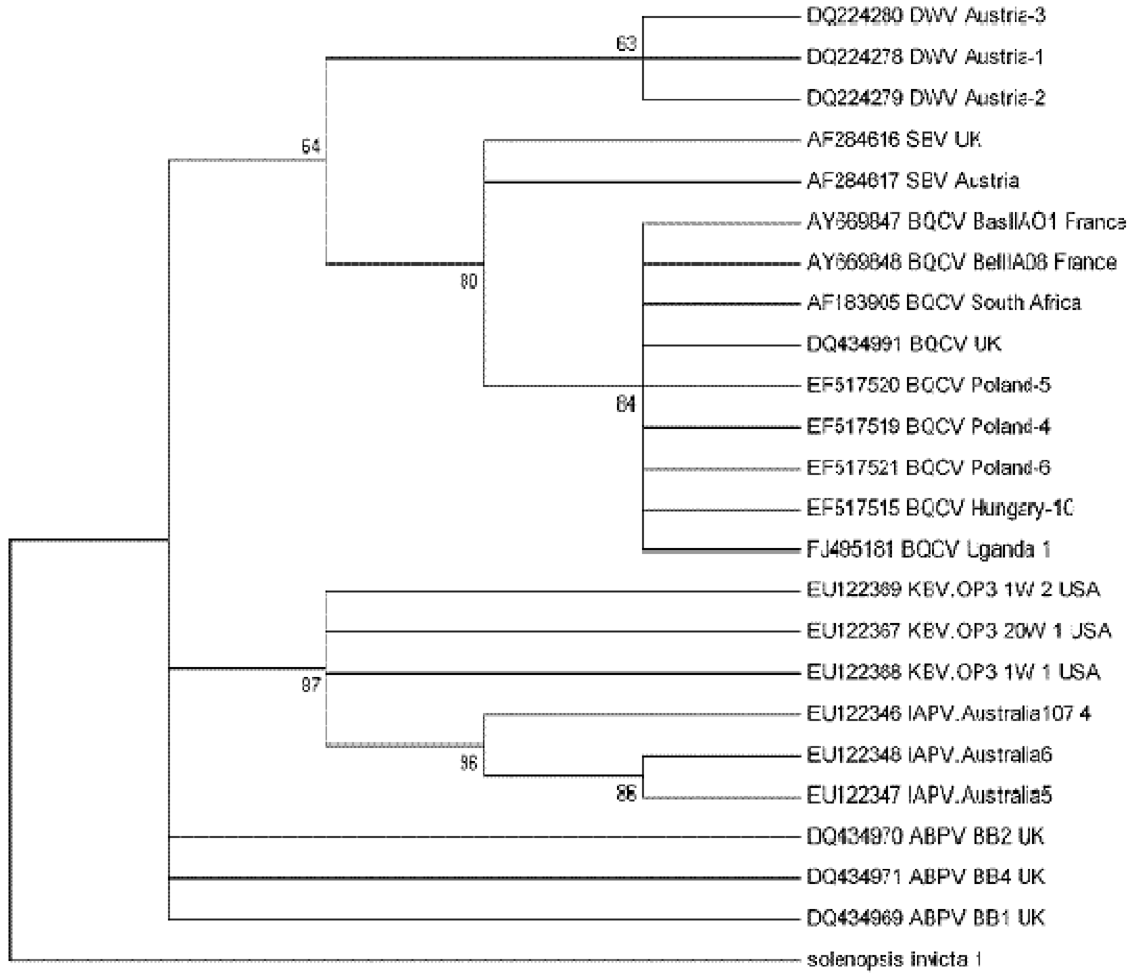

Figure 3.3 Phylogram depicting the relationships between BQCV FJ495181 Uganda 1 with other bee viruses.

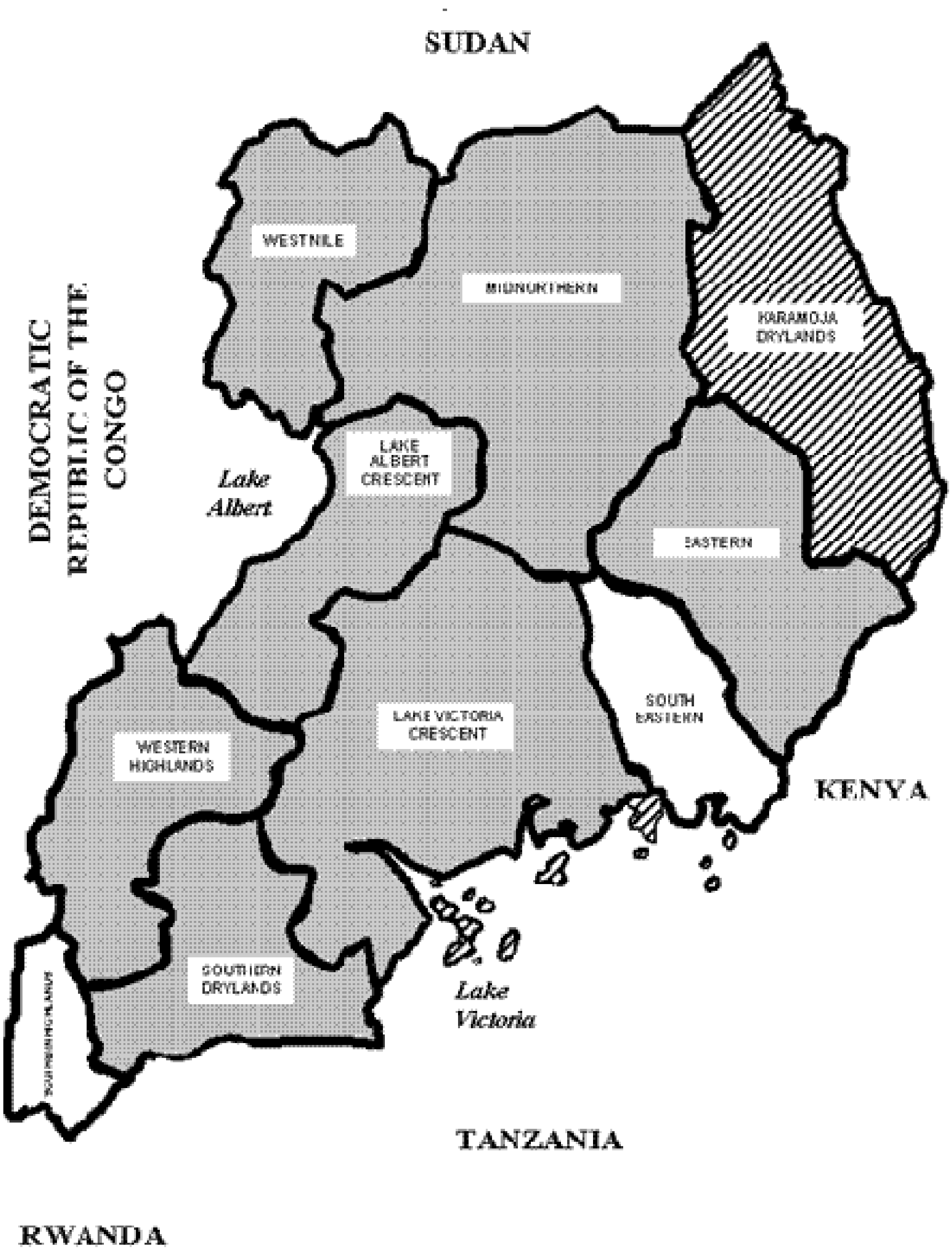

Figure 3.4 Map of Uganda showing those agroecological zones in which samples tested positive for BQCV.

Key: Grey – virus present in zone; white – no positive samples found in this zone; crosshatched – area not sampled in this study.

3.5 Discussion

The many benefits of beekeeping in Uganda have been identified by several authors (Roberts, 1971; Ogaba and Akongo, 2001; Ogaba, 2003; UEPB, 2005; Kajobe 2006). Advantages include a rich variety of indigenous *Apis* populations, the readily availability of cheap local materials necessary for beekeeping, and the fact that apiculture is a low-technology practice, compatible with Ugandan crop production systems. Moreover, honey and other hive products, for example beeswax, can be effectively processed using simple tools. A beekeeper does not need to be a landowner in order to keep bees; hives can be placed on community held land, making apiculture a practical occupation for poorly resourced groups, cooperative programmes, and otherwise marginalized sections of society across Uganda (Ogaba and Akongo, 2001; Ogaba, 2003; Marris, 2006).

At present, Ugandan apiculture is generally practiced on a small scale, providing a vital source of food and income in poor rural communities. However, in order to meet the objectives of a comprehensive development framework for poverty reduction and economic growth, the government has embarked on a reformation programme to increase agricultural outputs from existing subsistence, to full-scale commercial levels of production (MFPED, 2000; Ssewakiryanga, 2005; UEPB, 2005). Beekeeping is recognised as being a very important sector in recent programmes for development, poverty alleviation, conservation and sustainable use of forest resources in Uganda (Ogaba, 2003; Kajobe, 2006), and the apiculture industry, although still in its infancy, offers real potential for income generation and socio-economic development. A considerable market exists for African bee products, not just as foodstuffs; Pharmaceutical and Cosmetic industries also make extensive use of honey and other non-perishable hive products, such as propolis and bees wax (UEPB, 2005). In order to realize all of the above advantages, it is vital that Uganda maintains a robust national bee population. To this end, Uganda's National Agriculture Research Organisation (NARO) has been mandated to undertake, promote and coordinate research in all aspects of apiculture, including bee health. Recent surveys have provided valuable information about the threats posed to beekeeping by predators such as insects, reptiles, primates and other mammals (Roberts, 1971; Kajobe, 2006; Kajobe and Roubik, 2006), but the national incidence of viral pathogens, which are microscopic, and more insidious in their effects, is under-researched and poorly understood (Commonwealth Secretariat, 2002).

This study screened samples of larval, pupal and adult *A. mellifera* collected from a total of 16 districts across the country for the presence of seven types of virus. Of these, only BQCV was found to be present in bee colonies. This is the first molecular detection of BQCV in any East African honey bee stocks. BQCV was initially isolated in the 1970s, from the remains of developing queens found decomposing within blackened cells (Bailey and Woods, 1977). It is now known to be widespread throughout European honey bee colonies (Aubert et al., 2008). BQCV affects all life stages of *A. mellifera*, but is more often detected in adult bees than in brood or pupae (Siede and Büchler, 2003; Tentcheva et al., 2004a); this was also found to be the case in Ugandan samples testing positive for BQCV. Diseased larvae have a pale yellow appearance, and tough saclike skin (Ball and Bailey, 1997). In Australia, BQCV is thought to be the most common cause of death in queen larvae (Anderson, 1993). The annual incidence cycle of BQCV is closely associated with that of a microsporidian gut parasite of bees, *Nosema apis*, and the presence of the virus has been shown to increase the pathogenicity of *N. apis* (Bailey et al., 1981, 1983). Overwintering losses and problems with queen rearing in French bee stocks have been associated with BQCV infection (Faucon et al., 2002), and both *N. apis* and BQCV have been linked with colony losses. The incidence of *N. apis* in Uganda is currently unknown.

Figure 3 illustrates the distribution of BQCV-positive samples across Uganda's agroecological zones. Although data collected in this study suggests that certain areas may be virus free, given that infected zones directly border apparently healthy zones, this is highly unlikely to be the case. Absence of positive samples is more likely to reflect the comparatively small sample sizes, coupled with the possibility that virus is present at low (sub clinical levels). It is the view of these authors that BQCV should be assumed present throughout the country. Regarding the implications of BQCV infection for Ugandan apiculture, with one exception, samples collected for the purposes of this study came from asymptomatic colonies, so on this basis it is clear that the presence of virus within a colony does not necessarily result in overt disease. It has been observed elsewhere that various types of virus can be found in apparently healthy adult bees and pupae (Dall, 1985; Anderson and Gibbs, 1988; Hung et al., 1996a), and only when they occur in colonies co-infested with the parasitic mite *Varroa destructor* will virus-induced mortality follow (Shimanuki et al., 1994; Hung et al., 1996b). This suggests that on some occasions, viruses multiply to lethal levels when activated by feeding mites (Aubert et al., 2008 and references cited therein). *Varroa* has been shown to play several roles in the manifestation and spread of viral diseases: mites have a weakening effect on their hosts, rendering them more susceptible to infection (Yang and Cox-Foster, 2005); dissemination of pathogens is also facilitated when viruses enter through the lesions caused by feeding mites (Ball, 1989). For certain viruses, transmission by *V. destructor* has been proven experimentally (Békési et al., 1999; Nordström et al., 1999; Chen et al., 2004; Tentcheva et al., 2004a), and it has also been suggested that *Varroa* may act as vectors for BQCV (Bailey, 1976). Although some studies (in Hungary and France) have been unable to confirm this assumption (Tentcheva et al., 2004b; Forgách et al., 2008), BQCV has been detected in mites from Thailand (Chantawannakul et al., 2006). In South Africa, BQCV has been linked with notably increased honey bee mortality when associated with *Varroa* (Swart et al., 2001; Davison et al., 2003), and it has been shown that latent BQCV infections can be triggered by injecting bee pupae with insect Ringer solution, further implicating feeding mites in activation of BQCV disease (Shen et al., 2002).

Although there is no available data for the incidence of *Varroa* in Uganda, the mite is believed to be absent from this country (Kajobe, R., *pers. obsev.*), and current evidence suggests that *Varroa* is also absent from the neighbouring countries of Kenya, Tanzania, Sudan and DRC (Griffiths and Bowman, 1981; Kigatiira, 1984, 1988; Matheson, 1993; Ellis and Munn, 2005). The status of *Varroa* in Rwanda is unknown (Ellis and Munn, 2005). However, the mite is prevalent throughout much of the rest of the world, having spread rapidly in recent years from outside its natural range in Asia to all continents except Australia (Webster and Delaplane, 2001 and references cited therein), and *Varroa* has been confirmed as present and spreading in sub-Saharan Africa (Allsop, 1997a, 1997b, 1999), including a recent report in Nigeria (Ukattah, 2008). This means that while the current impact of BQCV in Uganda may be minimal, should *Varroa* mites reach districts where BQCV is endemic, then the combination of the mite and the virus could have a much greater negative effect on beekeeping.

Data presented in the current study clearly demonstrates the presence of at least one virus in Ugandan honey bee stocks. Screening for additional potentially damaging viruses, e.g. *Cloudy wing virus*, and the further characterization of BQCV from Uganda could provide useful information on virus provenance. Similar distribution patterns for DNA-based pathogens in *A. mellifera*, including statutory notifiable organisms like *Melissococcus plutonius* and *Paenibacillus larvae*, would also provide valuable information to ensure the continued health of honey bee stocks. Given the proven relationship that exists between incidence of BQCV and *Nosema* sp. (Bailey et al., 1981, 1983), there is a need for further screening of samples to ascertain whether *N. apis* (or related species *N. ceranae*) is present, especially in those sites where BQCV has been confirmed. Moreover, *A. mellifera* is not the only type of bee utilised in Ugandan apiculture. At least six species of stingless bees are hunted and/or cultured for their honey and brood (Cunningham, 1996; Kajobe, 2006; 2007; Kajobe and Roubik, 2006). The incidence of viral pathogens in these additional bee stocks is yet to be investigated, and their susceptibility to honey bee viruses is entirely unknown.

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