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Exploring the beekeeping potential in the Republic of Benin by examining the melliferous trees, the honeybees and the honey they produce

Felicien Amakpe

Promotor: Prof. Dr. Dirk de Graaf

Co-promotors: Prof. Dr. Ir. Brice Sinsin and Em. Prof. Dr. Frans Jacobs

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Supervisors

Promotor: **Prof. Dr. Dirk de Graaf**

Laboratory of Molecular Entomology and Bee Pathology (L-MEB)

Department of Biochemistry and Microbiology

Faculty of Sciences, Ghent University, Belgium

Co-promotor: **Prof. Dr. Ir. Brice Sinsin**

Laboratory of Applied Ecology (LAE)

Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin

Co-promotor: **Em. Prof. Dr. Frans Jacobs**

Honeybee Valley

Faculty of Sciences, Ghent University, Belgium

Examination committee

Prof. Dr. Bart Braeckman (Ghent University, Belgium, Chairman)

Dr. Lina De Smet (Ghent University, Belgium, Secretary)

Prof. Dr. Jean-Luc Hornick (University of Liège, Belgium)

Prof. Dr. Ir. Patrick Van Damme (Ghent University, Belgium)

Prof. Dr. Ir. Guy Smagghe (Ghent University, Belgium)

Dr. Wim Reybroeck (Technology and Food Science Unit, Institute for Agricultural and Fisheries Research, Belgium)

Dean: Prof. Dr. Herwig Dejonghe

Rector: Prof. Dr. Anne De Paepe

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Author's email address:

famakpem@hotmail.com

To all those who keep or like bees.

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LIST OF ABBREVIATIONS

ABPV	Acute bee paralysis virus
AFB	American foulbrood
ALPV	Aphid lethal paralysis virus
As	Arsenic
B-DTE	Benino-dry tropical ecotype of honeybee
BIC	Bayesian information criterion
BLAST	Basic local alignment search tool
BQCV	Black queen cell virus
B-SE	Benino-Soudanian ecotype of honeybee
B-SGE	Benino-Soudano Guinean ecotype of honeybee
BSRV	Big Sious River virus
CBPV	Chronic bee paralysis virus
CCD	Colony collapse disorder
Cd	Cadmium
CENAD	Cercle Nature et Développement
CI	Cubital index
CIAT	Centre Intégré d'Apiculture Tropicale
CNAC	Comité National d'Agrément et de Contrôle des Produits Phytopharmaceutiques
COI-COII	Cytochrome Oxydase I- Cytochrome Oxydase II
Cr	Chromium
Cu	Copper
DA	Diastase activity
DBH	Diameter at breast height
DL50	Lethal dose that kills 50% of the bees of the colony
DNA	Deoxynucleic acid
DWV	Deformed wing virus
E	East
EC	Electrical conductivity
EFB	European foulbrood

EPILOBEE	Epidemiological surveillance programme on honeybee colony mortality
EU	European Union
EURL	European Union Reference Laboratory for honeybee health
FL	Femur length
FV	Filamentous virus
GC	Gas chromatography
GC-MS/MS	Gas chromatography-mass spectrometry
GDP	Gross Domestic Product
GPS	Global Positioning System
HDI	Human Development Index
HMF	Hydroxymethylfurfural (5-(hydroxymethyl-) furan-2-carbaldehyde)
HPLC	High performance liquid chromatography
IAPV	Israeli acute paralysis virus
IFN	Inventaire Forestier National du Bénin
IFS	International Foundation for Science
IHC	International honey commission
ILVO	Flemish Institute for Agricultural and Fisheries Research
INSAE	Institut National de la Statistique et de l'Analyse Economique du Bénin
KBV	Kashmir bee virus
KTBH	Kenyan top bar hive
LC-MS/MS	Liquid chromatography–mass spectrometry
LSV	Lake Sinai virus
MDG	Millennium Development Goal
ML	Maxium likelihood model
MLPA	Multiplex ligation probe dependant amplification
MP	Maximum parsimony method
MRL	Maximum residue limit
mtDNA	Mitochondrial DNA
BL	Basitarsus length
N	North
NGO	Non-governmental Organization
Ni	Nickel

OTC	Oxytetracycline hydrochloride
PAM	Programme Alimentaire Mondial
Pb	Lead
PBF2	Projet Bois de Feu Phase 2 du Bénin
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
RHPS	Relative honey potential score
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SBV	Sacbrood virus
SHB	Small hive beetle
S-MR	Silvo-melliferous region
SPR	Subtree-Pruning-Regrafting
SWOT	Strengths, weaknesses, opportunities and treats analysis
TL	Tibia length
TTL	Total leg length
tRNA	Transfert RNA
UNDP	United Nation Development Programme
USA	United States of America
WA	West Africa
WASAR	West African Seminar on Apicultural Development
WGS 1984	World Geodesic System of 1984
WL	Wing length
WW	Wing width
Zn	Zinc

SUMMARY

The Republic of Benin is a West African country that has a variety of climatic conditions ranging from sub-humid (Guinean) in the south to semi-arid (Soudanian) in the north. In this country where high pressure is put on the natural resources, beekeeping is found to be one of the best niche activities for sustainable livelihood that, at the same time, have positive impacts on the environment. In order to make the activity a real income source for poverty alleviation, this study focused on the appraisal of the beekeeping potential on a nationwide basis, by analysing the following factors, which determine the managerial, environmental and commercial sustainability of beekeeping in a given ecological area:

- study of the melliferous plants available for the honeybee *Apis mellifera*;
- study of the pathogenic status of the honeybees;
- screening of pesticide residues in honey and honey quality and
- the morphometric and genetic characterization of the native honeybees.

A nationwide study of the melliferous tree distribution and diversity indicated that the country can be divided into six silvo-melliferous regions (S-MRs) with specific beekeeping potential. There were the South S-MR, the Central S-MR, the Central West S-MR, the Central North S-MR, the Middle North S-MR and the Extreme North S-MR. The South S-MR is dominated by industrial plantations that mainly produce pollen and has the poorest honey potential while the Central West S-MR proved to be the best beekeeping area with 46.7% nectar-producing trees, 9.4% pollen-producing trees and 40.6% plants that provide both resources to bees.

The bee health study indicated that, apart from *Varroa destructor* and *Aethina tumida,* there is no major bee disease in the Republic of Benin. In fact, American and European foulbrood, chalk-brood disease, acariosis and nosemosis are absent. However, 13 bee samples (15.5%) were found to be infected by viruses as determined by PCR-based techniques. Acute bee paralysis virus (ABPV; 8.3 % of infected hives) and Black queen cell virus (4.8%) were the most common. From the first we found a strain with an unusual molecular signature of the capsid region, which was taxonomically positioned between ABPV and Israeli acute paralysis virus. We also found Lake Sinai virus (LSV; 3.6%) for the first time in Africa.

A multi-residue control analysis indicated that all the honeys were free from 293 residues including the 64 pesticides in use in the country at the different limits of determination. All honeys also comply with the international standards with the exception of two the honey of two sites that had a high hydroxymethylfurfural (HMF) concentration and a low diastase activity as a consequence of the long storage. Though honey quality parameters and residues are dynamic, this research indicated that the beekeeping environment in the Republic of Benin is very safe. The wholesalers would only have to avoid contamination during processing, packaging and transport to comply with the international standards for honey export.

Based on morphometric data the native honeybees can be divided into three distinct ecotypes. The sequence analysis of the mitochondrial DNA COI-COII regions, indicated 3 new and clearly separated haplotypes. These haplotypes miss the typical P_0 region but they all possess a duplication or triplication of the Q region. Sequence analyses also confirmed that all haplotypes belong to the A lineage and are closely related to the *adansonii*, *scutellata* and *iberiensis* races which represent respectively 66%, 27% and 7% of the honeybee population in the Repubic of Benin.

As a whole, there is a good potential for beekeeping in the country, especially in the central and the northern regions where the activity can efficiently contribute to the different strategies for poverty alleviation in the Republic of Benin.

SAMENVATTING

De Republiek Benin is een West-Afrikaans land met verschillende klimatologische omstandigheden, gaande van het subhumide (Guinees) in het zuiden tot de semi-aride (soudanian) in het noorden. In dit land waar een hoge druk wordt gezet op de natuurlijke hulpbronnen, wordt de bijenteelt gezien als één van de beste niche-activiteiten voor duurzaam levensonderhoud met tegelijkertijd een positieve invloed op de omgeving. Met de betrachting om van deze activiteit een echte inkostenbron te maken voor armoedebestrijding, concentreerde deze studie zich op de beoordeling van het bijenteeltpotentieel van het land door het analyseren van factoren die bepalend zijn voor de leidinggevende, milieu- en commerciële duurzaamheid van de bijenteelt in een bepaald ecologisch gebied:

- de studie van de bijenplanten beschikbaar voor de honingbij *Apis mellifera*;
- de studie van de pathogenen-last van de honingbijen
- screening naar residuen van pesticiden en de kwaliteit van de honing en
- de morfometrische en genetische karakterisering van de inheemse honingbijen.

Een natiewijde studie naar de verspreiding en diversiteit aan bijenbomen maakte het mogelijk om het land te verdelen in zes silvo-mellifere regio's (S-MRs), elk met zijn specifieke bijenteelt-potentieel. Het betrof de Zuidelijke, de Centrale, de Centraal-Westelijke, de Centraal-Noordelijk, de Midden-Noordelijke en de Uiterst-Noordelijke S-MR. De Zuidelijke S-MR wordt gedomineerd door industriële plantages die voornameijk pollen produceren en heeft het laagste bijenteelt-potentieel terwijl de Centraal-Westelijke S-MR het meest geschikt lijkt met 46,7% nectar-producerende bomen, 9,4% pollen-producerende bomen en 40,6% planten die beiden leveren.

De studie van de bijengezondheid gaf aan dat, behalve *Varroa destructor* en *Aethina tumida*, er geen belangrijke bijenziekten voorkomen in de Republiek Benin. Met name, Amerikaans en Europees vuilbroed, kalkbroed, acariose en nosemose komen niet voor. Echter, 13 stalen (15,5%) waren virus-geïnfecteerd zoals bleek uit de PCR-techniek. Het Acute bee paralysis virus (ABPV; 8,3%) en Black queen cell virus (4,8%) waren het meest voorkomend. Van de eerste vonden we een stam met een ongewone moleculaire signatuur van de capside regio, waardoor het virus

taxonomisch gepositioneerd blijkt tussen ABPV en het Israel acute paralysis virus. We hebben ook voor het eerst Lake Sinai bij bijen in Afrika gevonden.

Een multi-residue controle-analyse gaf aan dat alle honingstalen vrij waren van residuen van 293 pesticiden waaronder alle 64 pesticiden die in dit land gebruikt worden, tot op de detectielimiet. Alle honing voldoet aan de internationale normen, met uitzondering van twee sites die een hoge hydroxymethylfurfural (HMF) concentratie en een lage diastase-index hadden als gevolg van de langdurige opslag. Hoewel honingkwaliteitparameters en residuen dynamisch zijn, gaf dit onderzoek aan dat de bijenteelt-omgeving in de Republiek Benin erg veilig is. De groothandelaren zouden enkel moeten pogen te verhinderen dat contaminatie gebeurt tijdens de verwerking, verpakking en transport, om te voldoen aan de internationale normen voor honingexport.

Op basis van de morfometrische gegevens konden de lokale honingbijen onderverdeeld worden in drie gescheiden ecotypes. De sequentie-analyse van de mitochondriaal DNA COI-COII regios, gaf het bestaan van 3 nieuwe en duidelijk te onderscheiden haplotypes aan. Deze haplotypes missen de typische P_0 regio maar bezitten allen een duplikaat of een triplikaat van de Q region. Sequentie-analyse bevestigde dat al de haplotypes behoren tot de A afstamming en nauw verwant zijn met de *adansonii*, *scutellata* en *iberiensis* rassen, welke respectievelijk 66%, 27% en 7% van de bijenpopulaties uitmaken in de Republiek Benin.

Alles samen kan gezegd worden dat er een goed potentieel is voor bijenteelt in dit land, vooral dan in de centrale en noordelijke regio's waar de activiteit kan bijdragen tot verschillende strategiën van armoedebestrijding in de Republiek Benin.

CHAPTER 1

General introduction

A beekeeper with his own hand made protective clothes and smoker in the district of Abomey.

CHAPTER 1

General introduction

1. Beekeeping context in the Republic of Benin

1.1. Honey hunting and traditional beekeeping

The communities in the Republic of Benin have been using honeybees for obtaining sweet commodities since ancient times. The widespread method for this was honey hunting in which bee colonies in termitaria or trees are burned and all found honey is collected. Nowadays, honey hunting has disappeared in many areas of the country as a consequence of the constant destruction of the wild colonies and habitat loss (Amakpe, 2008).

The system of keeping honeybees in traditional hives is common with the Somba and sedentary Fulani tribes in the north. These hives are made of tree logs, clay jars, tree barks, woven sorghum or grass straw, and placed high in the trees (figure 1). In this traditional way of beekeeping, the hives are opened at night during the dry season and the honey and brood combs are removed without totally killing the colonies which are sometimes able to recover from the fire the farmer uses to avoid too many stings.

Figure 1: Traditional hives in the district of Toukountouna (north Benin) made of clay jar (left) and a tree log (right)

1.2. Management system and profit to beekeepers

According to Hussein (2000), beekeeping with designed movable frame hives, smoker and protective clothes at an apiary is very recent in the country and started only in 1972. It was the 3rd West African Seminar on Apicultural Research (WASAR) held in December 1995 in Cotonou that effectively boosted the interests of NGOs and the government in integrating beekeeping in their strategies for poverty alleviation and natural resource management (Botoyiye, 1999).

Despite the great interest in the activity, beekeeping is still limited to small-scale farmers. The hives are also usually abandoned with no care and many apiaries may be left for more than twelve months awaiting the first colonisation. In 2008, when a survey was conducted in order to set up this PhD programme, only 40 active apiaries could be identified in the entire country. That is why in 2010, we tried to establish additional apiaries from the south to the north of the country in order to have at least 120 sites from which honey and bee samples may be collected for the different investigations.

The apiary usually consists of 2 to 5 traditional or Kenyan top bar (KTB) hives with the exception of some rare apiaries located in the districts of Djidja, Ouesse and Parakou where up to 20 active hives can be encountered. Bee colonies are always obtained from natural swarms and no artificial queen rearing or colony introduction is reported. Almost nothing is known on bee diseases, and in consequence, no chemicals are applied on the colonies.

As far as the post-harvest is concerned, honey is the only marketable product from the apiaries and honey extraction is done by hand. Pollen, combs, larvae and propolis are usually thrown away or used for medicinal purpose by some rare beekeepers. The harvested honey is sold at the local market and some to the neighbouring countries (Nigeria, Togo, Niger, and Burkina Faso).

The generated income to the beekeepers is poorly documented. But depending on the level of intensification and the care to the honeybees, it is estimated that a beekeeper can earn, in addition to his main activities, 20 to 50 US dollars (USD) per hive per year (CeRPA, 2006; Amakpe, 2008; Yédomonhan & Akoègninou, 2009) in a country where more than 36% of the population lives below the poverty line of 1.90 USD per day (UNDP, 2014). With this contribution to the household income, beekeeping is then a great opportunity for poverty alleviation but deeper investigations are needed into the country's possibilities to develop sustainable beekeeping.

2. Beekeeping potential components

2.1. Definition

The beekeeping potential is the total of the latent capacities or abilities of a specific ecological area to develop beekeeping as a sustainable income source for the communities. According to Hilmi *et al.* (2012), Nazzi *et al.* (2014) and Famuyide *et al.* (2014), beekeeping potential is

determined by the managerial skills, the prevailing climatic conditions, the pest environment, the bee diversity and the melliferous plant availability which is influenced by the complex of bushfires / grazing / tree harvesting. These elements interact to yield the hive products, the value of which is determined by specific quality measures and the presence of pesticide residues or contaminants. Figure 2 summarises the different factors that determine the beekeeping potential.

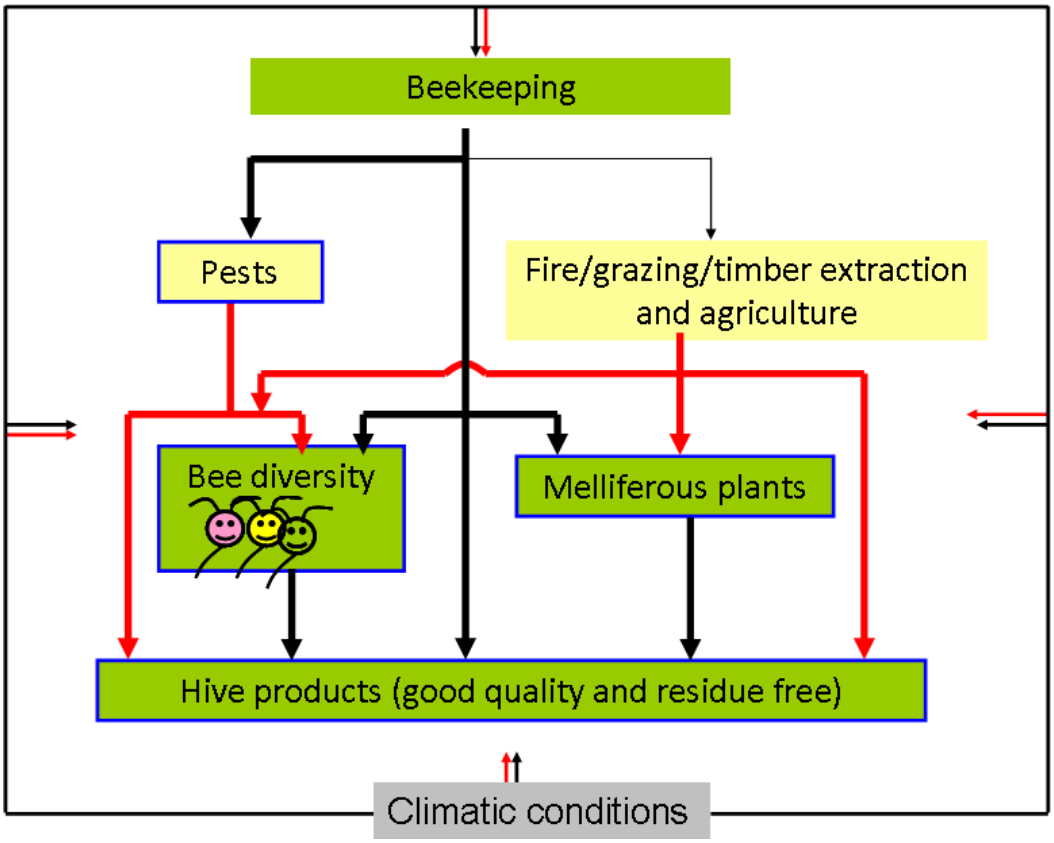

Figure 2: Determinant factors of the beekeeping potential.
Negative components are shaded in yellow, and positive components are shaded in green. Red arrows indicate negative impacts. Black arrows indicate positive or managerial impacts on the targeted factor. The lighter arrow from "beekeeping" toward "fire/grazing/timber extraction/agriculture" indicates the low impact the beekeeper has on these parameters. Climatic conditions may affect the whole system positively or negatively (represented by red and black arrows starting from the same point). The factors with blue border lines are the ones we studied in this PhD programme

2.2. Beekeeping or managerial skills

In a given ecological area, the hive product yields are determined by the managerial skills of the beekeeper. He is the main actor who manages the other factors to obtain the desired hive products. In their model of innovation adoption in agriculture, Feder & Umali (1993) and Abadi Ghadim & Pannell (1999) found that the most important factors affecting adoptions in rural areas

are the agro-climatic environment and the availability of extension services. In fact, the agro-climatic conditions first determine the feasibility of the innovation and reduce the distance between practitioners. The extension services ease or improve the frequency of contact and exchanges between practitioners to disseminate the innovation. In addition to these general factors, Ekumankama *&* Nwankwo (2002), Deressa *et al.* (2009), Jaafar-Furo (2007) indicated that the beekeeping adoption is specifically influenced by the age and gender of the beekeeper and fear of being stung. In this respect, these authors found that the fear of being stung is the main reason why elder people and women are less interested in this activity.

2.3. Melliferous plants

2.3.1. Availability and measurement

The main hive products (pollen, honey and propolis) are obtained from live plants which constitute the core beekeeping factor. The first approach to evaluate the melliferous resource availability is measuring the flowering intensity, which is the total flowering plant density of an area (Hoffman *et al.*, 1990; Al-Ghamdi *et al.*, 2014). But according to Lobreau-Callen *et al.* (1986) and Damblon *&* Lobreau-Callen (1991), the total flowering plant density does not necessarily determine the availability and quality of melliferous resources because not all plants may produce harvestable pollen, nectar or honeydew to bees, and the plant-bee relationship is more complex than food availability. Nectar and pollen harvesting possibility also depends on the bee shape and the tongue length as some plant species may only be pollinated by long-tongued bee species (Borrel, 2005; Danforth *et al.*, 2013). The most reliable parameter for determining the melliferous plants potential of an area is then the effectively visited plants density (melliferous plants) as this includes other parameters such as abundance, relative distance from the colony, sugar content of the nectar, flower colour, etc. (Lobreau-Callen *&* Damblon, 1994; Collevatti *et al.*, 2000; Beil *&* Horn, 2008). In the tropical areas, the melliferous plant density is affected by the complex of fire, grazing and tree harvesting, which determine the global flora as indicated by Biaou (2009).

2.3.2. Fire, tree harvesting and grazing impacts

According to Sinsin (1993), Biaou (2009), Houedanou *et al*. (2015) and Sinsin *et al*. (2013), the dynamic of the plant community in the tropical areas is deeply affected by the synergic influences of grazing, bushfire and wood extraction.

In most tropical areas, bushfire is considered as an ecological factor which cannot be prevented. It is only possible to manage it in order to reduce its influence on the bee population and on the melliferous plants during the dry season as indicated by Attuquayefio & Wuver (2003) and Linsoussi (2011). As far as grazing pressure is concerned, it is a problem of an unsustainable livestock system based on pastoralism in the entire West African (Abiola *et al.,* 2005; Schonegg *et al.,* 2006). It also includes tree pruning during the dry season, which negatively impacts the flowering capacity and the regeneration of melliferous trees (Bufford & Gaoue, 2015). Tree harvesting also includes bark and leaf harvesting (for medicine). But the most damaging tree extraction is the shifting cultivation, which totally converts the landscape into annual crops with no melliferous value (Figure 3).

Figure 3: Fire in agriculture and its impacts on melliferous trees.
At the right in the photo, the farmer has removed the entire vegetation for annual crop. At the left is the destructive bushfire, which is likely to impact the melliferous plants and the honeybees.

The control of transhumance and shifting cultivation lies in a total transformation of the nomadic pastoral system into a sedentary one and into a more sustainable agriculture at the West Africa regional level (Abiola *et al.,* 2005). But these actions are beyond the possibilities of the local beekeepers who can only invest in the colony management to improve hive product yields.

2.4. Bee pests and other threats

2.4.1. Anthropogenic and non-pathogenic impacts

The bee population is threatened worldwide by the loss of habitat and habitat fragmentation, agriculture intensification, pesticides and other polluants which also make them more vulnerable to diseases (Johansen *et al.*, 2006; Williams *et al.*, 2010). In this respect, Atkins & Kellum (1984)

and Sande *et al.* (2011) indicated that more than 70,000 California (USA) honeybee colonies were destroyed during the agricultural boom in the year 1967 by carbaryl use and soil fumigation. Unfortunately, reliable data on the impacts of agricultural development on honeybees are lacking in the Republic of Benin where pesticides are widely used on cotton. The commonly used pesticides in the country and their toxicity to the honeybee are summarised in table 1.

Table 1: Active components of main pesticides used in the Republic of Benin and their toxicity to bees.

Active component	Risk class[1]	LD50[2]	Residue[3]
Aldicarb	I	0.35	1 to 2 days and more
Acetamiprid	I	-	
Carbaryl	I	1.5 to 26.5	2 hours to 12 hours and more
Chlorpyrifos	I	0.11	5 hours to 6 hours and more
Cypermethrin	I	0.015	2 days to 3 days and more
Deltamethrin	I	0.032	2 days
Diazinon	I	0.37	1 to 2 days and more
Dimethoate	I	0.19	2 hours to 3 days and more
Ethion	III	-	Less than 2 hours
Fenitrothion	I	0.20	3 days
Lindane	I	-	More than 2 days
Malathion	I	0.73	2 hours to 5 days and more
Parathion	I	0.18	10 hours to 1 days and more
Permethrin	I	0.16	3 hours to 12 days and more

[1] Risks classes: I = highly toxic to bees, II = moderately toxic to bees, III = relatively non-toxic to bees.
[2] LD_{50} = the lethal dose required to kill 50% of the colonies in micrograms per bee (µg/bee).
[3] Period of the residual toxicity to bee after the product is sprayed.

Data adapted from Delaplane (1999), WHO (2003), Johansen *et al.* (2006) and MAEP (2010).

In savannah areas, recurrent bushfires constitute another great threat to the bees and their habitat. According to Delmas (1991), Scholes *et al.* (1996) and Devineau *et al.* (2010), most of the world's bushfires occur in the African savannah areas, and at least 17% of the African surface is burned annually. This causes the extinction of many bee species either by direct burning or destruction of habitat (Frost, 1984; Flannigan *et al*., 2009; Jurgen *et al*., 2012).

In the Republic of Benin, savannah represents more than 75% of the vegetation and more than 60% of the country total area is burned every year (Akouegninou, 2004; Mouillot *&* Field, 2005). This high fire pressure progressively removes trees from the landscape (Houinato *et al.*, 2001), which are shelters and the main floral resources of bees.

The country also harbours transhumant herders from Nigeria, Burkina Faso, Mali and Niger (figure 4). Because of insufficient grass for their livestock, they also prune many melliferous trees such as *Pterocarpus erinaceus, Khaya senegalensis* and *Afzelia africana* that make up the bulk of

the melliferous resources in the dry season (Bufford & Gaoue, 2015). On the other hand, the honey hunters systematically destroy all the surviving wild colonies they encounter in the dry season and abandon empty or brood combs which are likely to spread diseases.

Figure 4: The landscape after recurrent annual bushfire.
The trails are the footprints of transhumant herders who graze green plants and prune melliferous trees in the dry season.

2.4.2. Small hive beetles (SHB)

In terms of predators, the most important bee enemy in the West African countries is *Aethina tumida*, the small hive beetle (SHB). It is endemic to the tropical area where it can survive and perfectly reproduce in rotten fruit (Lundie, 1940). It creates a lot of stress in the colonies by feeding on brood, pollen and nectar, and can even transmit viral diseases (Eyer *et al.,* 2009). In the United States of America, the pest may be controlled by using pesticides in the hive or in the soil against its larvae as an interruption of their life cycle. But the safest way to control this pest is the use of trapping devices as pesticides may be harmful to bees and disqualify the honey in a residue analysis (Rosenkranz *et al.*, 2010; USA/UADA, 2015).

In West African, the impact of the beetles in the apiaries is so obvious that the small hive beetle and the large hive beetle (*Oplostomus fuligineus*) are the only pests that beekeepers report in the Republic of Benin (Amakpe, 2008). Other bigger predators are lizards, ants, birds and rats. Though they can sometimes create big losses in abandoned apiaries, they may be mechanically controlled in well-managed apiaries and are not of great importance to beekeepers.

2.4.3. Mite diseases

The two most important threats to the worldwide honeybees are *Varroa destructor* and *Acarapis woodi (OIE, 2015;* Heath, 1985; Matheson, 1996; van Dooremalen, 2012; Strauss *et al.*, 2015*)*. According to Anderson *&* Trueman (2000), Martin *et al.* (2012) and Sammataro *et al.* (2013), they are found worldwide with the exception of Australia and some islands in Hawaii due to the isolation of these eco-regions.

A. woodi is an internal parasite of the bee trachea where it feeds on hemolymph. It is known to cause great losses in apiaries especially in the case of massive infestation in hard winter in combination with other pests (Sammataro *et al.*, 2013; Nguyen, 2010). On the other hand, *V. destructor* is responsible for much more damage worldwide. It is associated with viral diseases and infests adult bees (phoretic form) while the reproductive form infests brood (Kuenen *&* Calderone, 1997; Rosenkranz *et al.*, 2010). Feeding on brood and adult's hemolymph, the mite weakens each individually infested bee and continuously stresses the colony, which becomes more vulnerable to viral and microbial diseases because varroa mite is also known to suppress the antimicrobial and immunity system of bees (Navajas *et al.*, 2008; Yang & Cox-Foster, 2005).

As far as the control of the mites is concerned, hard synthetic chemicals with organophosphate, coumaphos, flumethrin and formamidine as active components against varroa are also effective in reducing the incidence of *A. woodi* (Ellis *&* Munn, 2005). But this chemical control practice is associated with a high risk of contaminating the hive products and increasing the resistance of *V. destructor* to pesticides. It is advised to alternate hard chemicals with soft chemical treatments like organic acids and essential oils, mite trapping, drone brood population control and genetic tolerance improvement, which are more sustainable (Aliano *&* Ellis, 2005; Rosenkranz *et al.*, 2010).

2.4.4. Fungal diseases

According to Prest *et al.* (1974) and Gilliam *&* Taber (1991), *Ascosphaera, Nosema* and *Aspergillus* are the three main fungus genera that affect honeybees. In opposition to the other honeybee diseases such as AFb and Varroa, their virulence is very much influenced by the climatic and feeding conditions. In fact, high humidity is known to contribute to the establishment and spread of fungal diseases (Flores *et al.,* 1996; Fries 2010; Higes *et al.,* 2008). The most important fungal bee disease is chalkbrood, which is caused by *Ascosphaera apis* (Anderson *et al.*, 1998; Aronstein,

2010). It is a worldwide distributed brood disease which is normally not considered as a problem in Asian beekeeping. But in Europe and North America, where the climat is temperate, it is known to create a lot of losses (Gilliam *et al.*, 1997; Yakobson *et al.,* 2003; Zaghlou *et al.*, 2005).

In the African countries, it was first reported in Tunisia by Heath (1985) and later in Ethiopia where up to 87% of the colonies were infected in some areas (Gebeya *&* Genet, 2006; Yohannes *et al.,* 2009). Other African countries where chalkbrood was reported are South Africa, Egypt (Strauss *et al.,* 2013; Sanad *&* Mohanny, 2011) and Nigeria (Ajao *&* Babatunde 2013; Akinwande *et al.,* 2013).

Stonebrood caused by *Aspergillus spp*. is a saprophytic soil fungus that creates similar symptoms to the ones of *A. apis*. The difference between the two lies in the fact that chalkbrood mummies are spongious while stonebrood mummies are hard and make a specific sound if the hive is shaken (Shoreit *&* Bagy, 1995; Vojvodic *et al.,* 2011; Foley, 2014). The African countries in which this disease is reported are the Mediterranean countries and Nigeria (Shoreit *&* Bagy, 1995; Hussein, 2000).

Other pathogenic fungi that infect honeybees are *i) Nosema ceranae,* which is originally a parasite of *Apis cerana*, and *ii) Nosema apis,* which is known to infect *Apis mellifera* (Fries; 2002; Paxton *et al.,* 2007; Martin-Hernandez *et al.,* 2012). They parasitize adult honeybees worldwide (Huang *et al.,* 2007; OIE, 2015). Nevertheless, there seems to be a geographic prevalence of the two species. *N. apis* is more prevalent in warmer climates while *N. ceranae* is more prevalent in temperate climates. In Africa, nosemosis is sparsely documented in some countries where the etiological agent has not been determined. It is reported in the northern and eastern parts of Africa, Zimbabwe, South Africa, Senegal and Gambi as “nosemosis” with no distinction between *N. ceranae* and *N. apis* (Muli *et al.*, 2014; Strauss *et al.*, 2013; Fries *et al.*, 1996).
As far as their virulence is concerned, *N. ceranae* is reported to be associated with colony collapse disorder in the USA (Higes *et al.,* 2008; Tapaszti *et al.,* 2009; Tentcheva *et al.,* 2004). In the European countries, *Nosema ceranae* is believed to be responsible for the massive losses of Spanish colonies in 2009 (Higes *et al.,* 2009). But the contribution of *Nosema* to the general winter colony losses in Europe is still controversial. In fact, until now it has not been proven that the pathogen is associated with the winter losses, alone or in combination with varroa or other

stresses from pollution, global climate change and loss of habitat (Runckel *et al.,* 2011; Fernandez *et al.*, 2012; Ravoet *et al.*, 2013).

As far as the fungal disease control is concerned, there is almost no effective chemical control, and the impact of fungal diseases in the apiaries is also small. The most efficient methods are managerial ones, such as high hygiene in the apiary (clean and new comb, ventilation of the hive), selecting and keeping strong colonies and those with a good hygienic behaviour (FAO, 2006; Campano *et al*., 1999).

2.4.5. Bacterial diseases

The most important bacterial honeybee diseases are the American foulbrood (AFB), which is caused by the spore-forming bacterium *Paenibacillus larvae* and the European foulbrood (EFB) caused by the non-spore-forming bacterium *Melissococcus plutonius* (de Graaf *et al.*, 2006; Genersch *et al.,* 2010; Forsgren, 2010). These two brood diseases seem to be limited to the European and the American apiaries (Bailey 1974, Hornitsky *&* Wilson, 1989; Allen *et al.,* 1990). But they have also been reported in South Africa where the beekeeping system is the same as in the European and American apiaries (Swart, 2001; Ostiguy, 2010).

Until now, bacterial diseases have not been reported in the West African countries, where there is a very low managed colony density and where the native *A. mellifera* absconds a lot, reducing the outbreak of bacterial diseases. Moreover, weak colonies or abandoned combs are immediately destroyed by wax moth and small hive beetle invasions, interrupting any outbreak or dispersion of bacterial diseases (Human *et al.,* 2011; Ellis *&* Munn, 2005). Adjare (1990) also explained the absence of bacterial diseases in the West African apiaries by the widespread traditional beekeeping system in which farmers remove the entire honey and brood combs from the hive, cutting down any brood disease in the colony.

Due to the high lethality of the bacterial diseases, conservative measures of isolation and destruction of diseased colonies as well as burning of infected materials are applied. This is particularly recommended for *P. larvae,* the spore of which is very resistant and can stay infective for many years (Roetschi *et al.*, 2008; Forsgren, 2010). For the European foulbrood, managerial techniques such as the “shook swarm” method are effective. It consists in shaking bees out of the infected colony into a new foundation hive and destroying the infected combs. It must be

used without antibiotic combination to avoid bacterial spreading and resistance to antibiotics (Hornitsky *et al*., 1989; Waite *et al.*, 2003). In other countries, oxytetracycline hydrochloride (OTC) or other antibiotics are used for the successful control of both European foulbrood and American foulbrood as indicated by Reybroeck *et al.* (2011), Forsgren (2010), and Miyagi *et al.* (2000). However, in some countries this led to the development of resistance against antibiotics.

2.4.6. Viral diseases

Although viral disease are not OIE regulated, their impact on bee health should not be underestimated. At least 18 pathogenic bee viruses are known, and some of them may be found at a latent stage in the colonies (Tentcheva *et al.,* 2004; FAO, 2006; de Miranda *et al.,* 2010). Despite of this large number, it is the occurrence of the problem of colony collapse disorder (CDD) that really boosted the scientific community to focus greater interests on the contribution of the viral pathogens to beekeeping (Holden, 2006).

With the exception of the filamentous virus (FV), which is a DNA virus, and the chronic bee paralysis virus (CBPV) which has more complex RNA strands, they are all single-stranded positive RNA viruses (De Smet *et al*., 2012; 2008; Evan & Hung, 2000; Blanchard *et al.*, 2007; Olivier *et al.,* 2008). Most of them belong to the Iflavirideae and Dicistrovirideae families (Picornavirale order) with the exception of CBPV and Lake Sinai virus (LSV), which are unclassified (Ravoet *et al.,* 2015a; Daughenbaugh *et al.,* 2015). The phylograph of the main virus species of the two families is presented in figure 5.

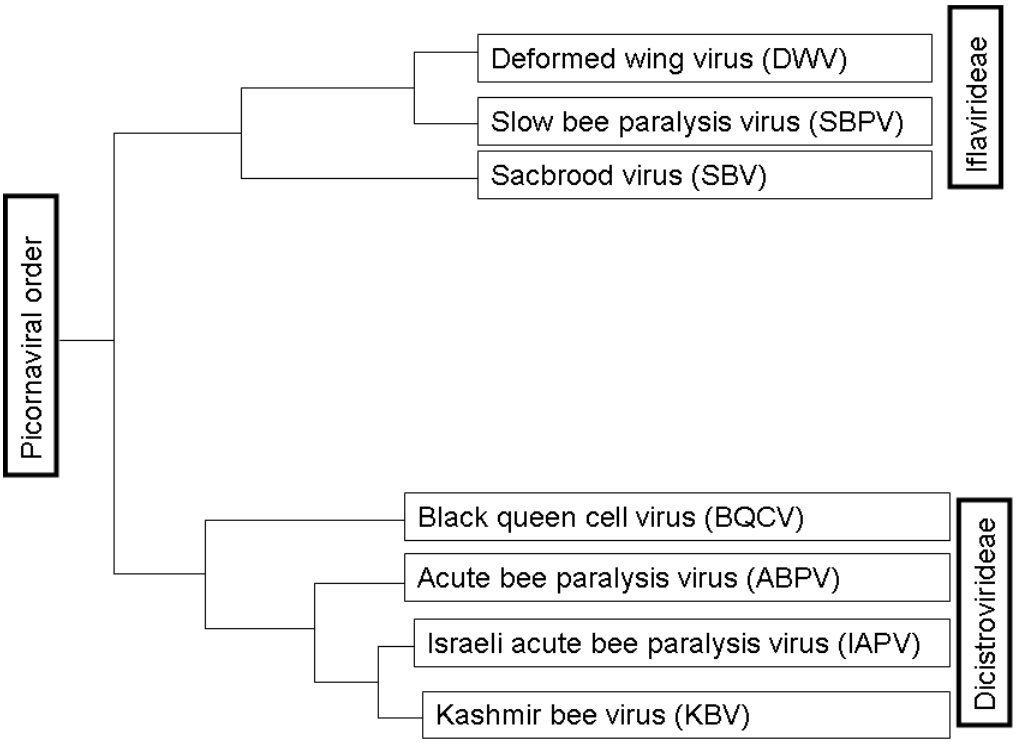

Figure 5: Phylogeny of the main honeybee viruses of Dicistroviridae and Iflaviridae families.
Phylogeny built on the available genomes from GenBank according to Johansson *et al.*, 2013.

The most feared and studied of them are acute bee paralysis virus (ABPV), black queen cell virus (BQCV), deformed wing virus (DWV), kashmir bee virus (KBV), sacbrood virus (SBV) and Israeli acute paralysis virus (IAPV) (Runckel *et al.,* 2011). Viral infections are usually asymptomatic in the colony and require sophisticated methodologies in well-equipped laboratories to be identified accurately (Chen *&* Siede, 2007; De Smet *et al.*, 2012). Nevertheless, this does not apply to some viruses as they have specific symptoms such as wing deformities (DWV), dark shiny hairless bees that cannot fly (CBPV), specific colour of dead larvae (BQCV), which can be recognised by well-informed beekeepers. The technical skills required for detecting bee viruses is the main cause of the limited knowledge of them in developing countries where viral detection is still based on symptomatic appraisal. Table 2 summarises the main viral bee diseases and their impact on the colonies.

In the African countries, there seems to be a geographic distribution or prevalence of viral diseases. In fact, Loucif *et al.* (2013) and Mumoki *et al.* (2014) found that the Mediterranean colonies are mainly infected by deformed wing virus (DWV), which infected 42.1 to 100% of colonies, and acute bee paralysis virus (ABPV), which infected 60% of colonies. In South African beekeeping, Swart *et al.* (2001) and Strauss *et al.* (2013) found a high level of BQCV (more than 62%) followed by IAPV (15%), and they were not associated with varroa. The only bee virus found

in East Africa was the BQCV, which infested more than 35% of the apiaries (Kajobe *et al.,* 2010). Apart from these countries the viral status of the African countries is still unknown as indicated by Allen *&* Ball (1996) and Ellis *&* Munn (2005).

As far as the viral disease treatment is concerned, there is no specific control and some viral symptoms may disappear without any treatment. The most defendable viral disease treatments are vector control (Eyer *et al.*, 2009), especially against varroa, which is the most important viral outbreak factor.

Table 2: Main honeybee viruses and their impacts on the infected colonies.

Virus	**Discovery**	**Distribution**	**Main symptoms and impacts on colonies**
Black queen cell virus (BQCV)	In 1977 from dead queen larvae	Worldwide	Death of queen larvae. Associated with *Nosema apis* for worker infection.
Bee virus X (BVX)	In 1974 during other viral study	-	No visible symptom. Reduces life span of workers
Bee virus Y	In 1980 in UK from dead bees		Worker death. Associated with *Nosema apis*
Chronic bee paralysis virus (CBPV)	Since antiquity	Worldwide	Black shiny hairless bees that tremble before death. High mortality
Deformed wing virus (DWV)	In 1983 from Japanese bees	Worldwide	Deformed wings and shape of the bees at birth. High worker mortality. Associated with *Varroa destructor*.
Filamentous virus (FV)	In the USA (1977)	-	DNA virus with no visible symptom in the colonies
Acute bee paralysis virus (ABPV)	In 1963 during CBPV study	World wide	Weakens colonies. Associated with V*arroa destructor*.
Kashmir bee virus (KBV)	From Kashmir *Apis cerana* in 1974	Europe, Australia	Rapid mortality of individual bee without any symptom. Weakens the colony. Associated with *Varroa destructor*
Israeli acute paralysis virus (IAPV)	In 2002 from dead bees of Israel	North America, Europe, Asia, South Africa	Individual death. Low impact on the colony.
Sacbrood virus (SBV)	In 1917 from dead brood	Worldwide	Saclike appearance of diseased larvae. Weakens colonies. Associated with *Varroa destructor*
Slow bee paralysis virus (SBPV)	In 1974 during BVX study	Very rare	Paralysis of the forelegs before death. Rare on the field. Associated with *Varroa destructor*

2.4.7. Other potential diseases

In addition to these pathogens of great concern to the world beekeeping, other pathogens are the trypanosomatids represented by *Crithidia mellificae* and *Lotmaria passim* (Ravoet *et al.*, 2015b; Schwarz *&* Evans, 2013) and the gregarine pathogens represented by *Apicystis bombis* (Lipa *&* Triggiani, 1996). They are discovered on honeybees and solitary bees (Schwarz *et al.*,

2013), but little interest is given to them and the probability of their lethality to the honeybees has still not been proven.

2.4.8. Colony collapse concerns

According to Cox-Foster *et al.* (2007) and vanEngelsdorp *et al.* (2009), the colony collapse is defined by a mix of criteria which mainly include a rapid loss of adult bees, leaving broods, or few young bees in a hive with a "workerless queen" with enough food. The pest invasions are rather a consequence of the dramatic loss of workers than the cause of the collapse. With this definition, the colony collapse can only be assessed in well-managed and well-monitored colonies, which are very rare in the Republic of Benin. The root cause of the problem is still not clear. But it is attributed to pesticides, pollution and pathogenic burdens represented by Varroa mite infestations, nosemosis, viruses and stresses from environmental factors such as drought, water or food shortage (Neumann & Carreck, 2010; Ellis *et al.*, 2010; vanEngelsdorp *et al.,* 2010).

The impact it has on beekeeping varies from country to country. In the United States, where it was first reported in 2006 as the colony collapse disorder (CCD), it created an estimated loss of managed colonies of 38% to 90% per year from the year 2000 to 2007 according to Cox-Foster *et al.* (2007) and NASS (2007). But from 2012, Spleen *et al.* (2013) reported a decrease in the phenomenon to 22.5% in the entire country. In the European countries, the problem of massive adult bee loss is best described as the winter colony losses, which occur during winter. Fortunately there is also an improvement in the 17 countries of the epidemiological surveillance programme on honeybee colony mortality (EPILOBEE) and the winter colony losses which ranged between 2.4%-15.4% in 2012-2013 decreased to 0.04-11.1% in 2014 (Nguyen *et al.*, 2010; Laurant *et al.,* 2015).

In the African countries, colony collapse was reported in South Africa and the Mediterranean where the phenomenon is getting worse and worse (Pirk, 2014). In the Republic of Benin, which is a tropical country with no winter, the colonies keep brood rearing all the year long. The problem that may more affect the bees in such an ecological area is the colony collapse disorder. In fact, the country hosts all the main causes of the phenomenon which are pesticides, habitat loss, varroasis, SHB and predators. These factors may act in conjunction with the recurrent bushfires, which are likely to kill a lot of workers in dry season (vanEngelsdorp *et al.,* 2013;

Neumann & Carreck, 2010). Then, though there is no reliable literature on this, the colony collapse disorder may be of great concern in Benin and deserves more attention in sustainable beekeeping.

2.5. Honey quality and residues

Nowadays, food quality and pesticide residues are major concerns for consumers and producers worldwide. As far as the honey and other hive products are concerned, people are more and more demanding regarding the honey origin, the absence of adulterant substances, residues, contaminants and false labelling that are likely to harm their health (Bertelli *et al.*, 2010; Consonni *et al.*, 2013). In this respect, honey quality and residues, the two key properties that determine the safe consumption of any food, are also regulated by international standards which all involved actors in beekeeping have to comply with on international markets or for domestic consumption (Hamilton *et al.*, 2003; European commission, 2001; Van der Valk, 2013; WHO & FAO, 1975; England Regulation, 2015).

2.5.1. Honey residues and contaminants

According to WHO & FAO (1975), a pesticide residue is any substance or mixture of substances in food for man or animals resulting from the use of a pesticide. It includes any specified derivatives, such as degradation and conversion products, metabolites, reaction products and impurities, which are considered to be of toxicological significance. They are essentially different from contaminants as they originate from pesticide use while a contaminant is a biological, chemical, physical, or radiological substance which is normally absent in the environment but affects living organisms through air, water, soil and food when they reach a certain concentration in consumed commodities. Both categories of substances are health hazards and are strictly regulated by international standards, and the methodologies for determining their acceptable limits of concentration are well-documented (Hamilton *et al.*, 2003, 2004; Krakowska *et al.*, 2015). In the European countries, Bernal *et al.* (2010) and Johnson *et al.* (2014) found at least 22 insecticides/acaricides, 29 fungicides and 2 herbicides residues in honey while 9 insecticides/acaricides and 8 fungicides residues are common in pollen. The most frequent active components of residues are fluvalinate, chlorfenvinphos, fipronil, malathion, chlorpyriphos, thiacloprid and neonicotinoid.

As far as their origins are concerned, Aghamirlou *et al.* (2015) and Christodoulou *et al.* (2015) found that most residues and contaminants are the consequence of bad beekeeping systems or poor hygiene such as the use of forbidden pesticides and drogues or inappropriate materials used for honey processing and packaging. This is particularly the case for zinc (Zn), which contaminates honey kept or processed in galvanized containers. But according to Abaga *et al.* (2011) and Krakowska *et al.* (2015), some pesticide residues, X-ray and heavy metal contaminants such as lead (Pb), copper (Cu), chromium (Cr), cadmium (Cd) arsenic (As) and nickel (Ni) originate from industry, agriculture and pollution, which are beyond the beekeeper's control.

In the Republic of Benin, no chemical bee pest control is applied and industries are almost absent in beekeeping areas. The residue may only arise from agricultural pesticides especially on cotton, the main crop on which pesticides are intensively used in the country.

2.5.2. Honey quality

Honey quality is a mix of physical, biological and chemical properties that distinguish honey from other sweet commodities. CODEX Alimentarius (1981) defined and characterised honey as "the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature". It is only a plant-originated product made by bees and essentially contains sugar and organic acids, enzymes and particles that are added by the bees and should not contain other artificially added ingredients. The different honey quality parameters include moisture, sugar content, colour, enzyme content which must be between specific limits as indicated in table 3 and table 4.

Table 3: Main honey quality parameters according to the European Honey Legislation Council Directive 2001/110/EC and Directive 2014/63/EU

Quality criteria	**Value**
Diastase activity, in diastase number (Schade scale)	Not less than 8 for blending or all retail honeys in general
	Not less than 3 for honeys with low enzyme content such as *Citrus spp* honey and an HMF content not more than 15 mg/kg
Hydroxymethylfurfural HMF (5-(hydroxymethyl-)furan-2-carbaldehyde)	Not more than 40 mg/kg in general in conjunction with the diastase activity not less than 8
	Not more than 80 mg/kg for tropical declared honeys and blend of these honeys
Moisture	Not more than 20% (except for particular stated honey)
	Not more than 23% for heather honey (*calluna*)
Reducing sugars	Not less than 65g/100g unless stated for particular honey Not less than 60g/100g for honeydew or blends of honeydew honey and blossom honeys
Sugar contents (sum of glucose and fructose)	Not less than 60 g/100g unless stated for the particular honey
	Not less than 40 g/100g for honeydew honey, blends of honeydew honey with blossom honeys
Appearance sucrose content	Not more that 5 g/100g in general (unless stated otherwise)
	Not more that 10 g/100g for honeydew and blends of honeydew and blossom) and other honey
Water insoluble solids content	Not more than 0.1 g/100 g in general
	Not more than 0.5 g/100 g for pressed honey
Electrical conductivity	Not more than 0.8 mS/cm in general (millisiemens per centimetre) More than 0.8 mS/cm for honeydew, blends of honeydew and blossom honeys 0.2-1.8 for *Tilia, Erica, Eucalyptus, Gossipium, Lavandula, Calluna honeys*
Free acid	Not more than 40 milli-equvalents acid per 1000 g in general
	Not more than 80 milli-equivalents acid per 1000 g for baker's honey
Ash	Not more than 1 per 100g for honeydew,
	Not more than 0.6 per 100g for other honey

Table 4: Additional honey quality proposed by the International Honey Commission (IHC, 2015).

Quality criteria	**Value**
Composition and origin	Naturally collected and transformed ingredients by honeybees from live plants
Colour	No artificial colorant added
Heating	Never above 45 °C
Proline content (ripeness and genuine origin index)	At least 180mg/kg in general
Crystallisation	Not influenced by chemical or biochemical treatments
Specific rotation ($[\alpha]^{20}_{D}$): angle of rotation of polarised light at the wavelength of the sodium D line at 20 °C of an aqueous solution of 1 dm depth and containing 1 g/ml of honey	Negative for blossom honey and positive values for honey dew (but may differ from region to region)
Invertase activity: unit of micromoles of substrate destroyed per minute (in Siegenthaler scale)	More than 50 in general More than 20 for honey with low enzyme activity

Adapted from CODEX Alimentarius (1981) and International honey commission (2015).

2.6. Honeybee diversity

The contribution of the bees to the beekeeping potential is determined by their biodiversity, which reinforces the colony's resistance to diseases and other environmental hazards. In this respect, Moritz *et al.* (2010) and Jaffe *et al. (*2009) found that resistance to diseases and hive product yields are higher in ecological areas that have the best diversity of bee populations. This diversity is made up of the different bee races, sub-races, ecotypes, genetic and morphometric diversity available for the beekeepers.

2.6.1. Bee races and evolutionary lineage

In systematics, "bee" is used to refer to a large group of more than 18,191 species categorised into 7 families that make up the super family of the Apoidea (Danforth *et al.*, 2013). Michener (2000) and Hedtke *et al.* (2013) established the bee phylogeny and described the different bee families, which are dominated by the Apideae family, containing 5,700 described species. The other families are the Melitideae (200 described species), the Megachilideae (3,170 described species), the Andrenideae (2,900 described species), the Halictideae (4,300 described species), the Stenotridideae (only 21 described species) and the Colletideae with 2,500 described species (figure 7). In non scientific language, "bee" is used to refer to the bees of the Apideae family, especially *Apis mellifera (L.)*. As far as their lineage is concerned, the honeybees *Apis mellifera* has been divided into five evolutionary lineages. They are "M" lineage (the West Mediterranean and the Western European honeybees); the "C" lineage (North Mediterranean honeybees); the "A" lineage (the African honeybees); the "O" lineage (Oriental) distributed in the Middle East and the "Y" for the Ethiopian subspecies (Ruttner, 1988, Franck *et al.,* 2000, Whitfield *et al.,* 2006; Alburaki *et al.,* 2011).

In the African continent (figure 6), the honeybees derive from the migration and evolution of the European *Apis mellifera,* which has evolved into 12 different races (*adansonii, capensis, intermissa, lamarckii, littorea, major, monticola, scutellata, unicolor, sahariensis, simensis and jementica*) due to the various ways of coping with the different ecological zones of the continent (Rutner, 1992; Meixner *et al.,* 2011; Hedtke *et al.*, 2013).

In the Republic of Benin, Villière (1987) and Ruttner (1992) found that all the honeybees belong to the *Apis mellifera adansonii* race of West Africa. But according to beekeepers, three different

"types" of bees exist in the country: the small and aggressive bees of the forest areas, the big mild bees of open high lands and a third intermediate type (Hounkpe *et al.,* 2006, Amakpe, 2010).

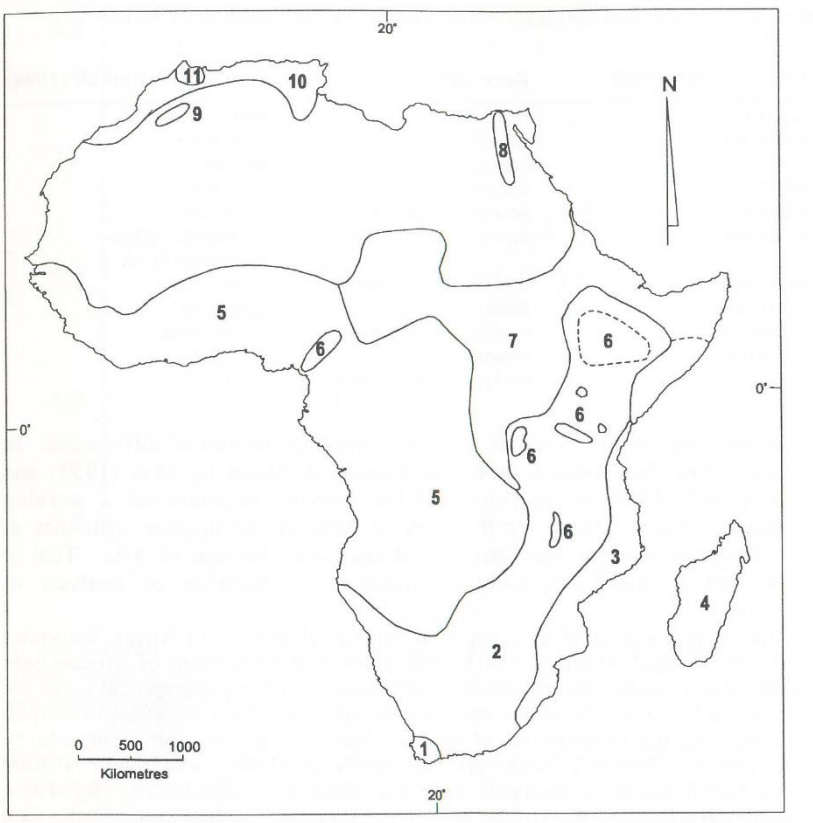

Figure 6: Geographical distribution of the African honeybee races as defined by Ruttner (1988).
1. *capensis,* 2. *scutellata,* 3. *litorea,* 4. *unicolor,* 5. *adansonii,* 6. *monticola,* 7. *jemenitica,* 8. *lamarckii,* 9. *sahariensis,* 10. *intermissa,* 11. *major*.

2.6.2. Biotic factors affecting the honeybee diversity

According to Le Conte *&* Navajas (2008), Louveaux *et al.* (1966), Shimanuki *&* David (2000), the biotic factors that determine the honeybee diversity consist of the energetic relationship between the bees, the melliferous plants and other species which the bees interact with (inter-specific stress) and between different bee colonies (intra-specific stress).

The first component (inter-specific stress) consists of the bee pests and the bee-plant relation. The bee pest stress is determined by the position of the honeybees in the trophic chain of the ecological area (Clauss, 1994; Londt, 1993; FAO, 2006). Under these stresses, Heath (1985) and Matheson (1996) found that the bees develop ethological, morphometric and behavioural strategies (hygiene, mimesis, aggressiveness, resistance/tolerance and even life cycle duration) for combating against or surviving in the same environment with their enemies. In the plant-bee relation, the bees are also engaged with other pollinators (mammals, birds, insects, *etc.*) in sharing the floral load capacity in the most efficient way (Struck, 1994). Submitted to the risk of

an overcollection of their pollen which may lead to a limited seed production, some plants have succeeded in separating their pollen and nectar phenology from the bee active periods or hiding these resources in deep organs. The difficulties in finding or collecting the available floral resources from plants have created the need for special morphological and ethological adaptations in each ecological area.

The inter-specific stress is represented by robbing, which emerges in situation of food shortage in the tropical areas (Micheal *et al.,* 2004). In this robbing process, the weak colonies usually disappear because of starvation or death of their queen, and this leads to the loss of genetic diversity as found by Bell *et al.* (1974) and DeGrandi-Hoffman *et al.* (1998).

2.6.3. Non biotic factors affecting the honeybee diversity

According to Dyer (2002), Parvu *et al*. (2013) and Cooper *&* Schaffer (1985), the bee foraging efficiency, their reproductive capacity and response to stress are influenced by the prevailing climatic factors such as temperature, air humidity, daylight and rainfalls. In fact, although the honeybees are poikilotherms which do not regulate their internal body temperature, they keep the colony temperature between 32°C and 36°C for the optimal brood development and the colony harmony (Heinrich, 1981; Southwick *&* Heldmaier, 1987; Beshers, 2001).

The most important climatic factors which impact the bees' survival outside the hive and their foraging capacity in the tropical areas are the ambient temperature (from 10° to 44°C), drought, and the rain fall (Winston, 1991; Prost, 1987). In this respect, Becher (2010), Cooper *&* Schaffer (1985) and Bishop *&* Armbruster (1999) found that the more humid and cloudy a climate is, the less active the bees are, and this works out in low hive product yields and high disease pressure. These factors also directly impact tree diversity and density which dramatically decrease as the climate becomes drier (Paltineanu *et al.,* 2007; Schmidt *et al.,* 2010; Haln-Hadjali *et al.,* 2006). In the Republic of Benin, drought and temperature are determined by the south-north rainfall gradient, which makes the country drier as the latitude increases (Adjadohoun *et al.,* 1989; Akoegninou, 2004). This gradient is likely to determine the honeybee population's diversity and distribution in the country.

3. Aims and scope

3.1. Objectives

Rural communities of Africa in general and in the Republic of Benin in particular, contribute to a great part of the gross domestic product by small-scale agriculture with limited investment capacity. In such situations, innovation in rural transformation and well-being should focus on niche activities that may be implemented with little investment and provide enough income for livelihood improvement (Chambers *&* Conway, 1992; Ellis, 1998; Van Dillen, 2002). In fact, the introduction of high input projects in rural areas always fails because of the lack of the required financial resources and competences to sustain their implementation.

The Republic of Benin is one of the least developed countries in the world with a Human Development Index (HDI) of 0.476 in 2013. According to INSAE (2014) and UNDP (2014), 23% of the population lives in permanent food insecurity, and more than 39% of the population lives under the poverty line in rural areas. In this situation, the strategies for improving the livelihoods in the country are mainly based on agriculture diversification/ intensification and forest products extraction. As stated by Peterken (1993), these strategies based on forest products and agriculture cannot be sustainable as they negatively impact the natural resources and put the possibility of the future generation to creating its own livelihood at stake.

Developing alternative income sources to the extensive land-based activities is then the most reliable strategy to secure a sustainable livelihood and a balanced natural resources management. But the question of which environmentally smart activity should be promoted in order to ensure a decent living standard for the community remains. As an answer to this legitimately challenging question, Bradbear (2004), Buchmann *&* Nabhan (1995), Klein *et al. (*2006), Roubik, (1995), and Kearns *et al. (*1998) found that beekeeping is the only farming system which needs very little investment, provides enough income in rural areas and contributes, beyond the direct income for the owner, to biodiversity conservation and the global welfare of human beings on earth because of the pollination function the bees perform.

This research program focused then on the development of beekeeping as a sustainable contribution to livelihood improvement in the Republic of Benin. The development of this activity is based on three interconnected components which are the bees, their pests and the floral

resources (Le Conte & Navajas, 2008; Williams *et al.*, 2010; vanEngelsdorp, 2010). The specific objectives of the research, which covered the entire country, are:

- Evaluation of the available melliferous resources;
- Description of the main pests and diseases that may affect the honeybees;
- Evaluation of the quality of the honey and the residues they contain and
- Study of the different honeybee ecotypes and races.

3.2. Thesis lay out

The output of this research is presented in four research chapters. The first presents a spatial analysis of the melliferous resources and determines a categorisation of the country into different melliferous regions. As the country is dominated by savannah lands, and the main floral resources available to bees during the dry season are perennial trees, we focused our investigation on the timber trees which produce pollen or nectar to honeybees. This helped to determine the different silvo-melliferous regions which are areas characterised by a specific set of trees that produce harvestable pollen or nectar and at the same time can be used as timber. This also helps to make a hierarchic classification of the different regions in order to determine the areas with the best floral resources. The second chapter analyses the pathogenic status of the honeybees in the Republic of Benin. The bees are accurately screened for the determination of the most important pathogenic agents from predators to viruses. A spatial analysis of the pests is performed as well as the correlation between multiple pests. Honey is the ultimate product of beekeeping in the country. In the third research chapter we then analysed the honey quality and searched for pesticide residues in order to find out if the hive products are marketable on the international market. The fourth research chapter analyses the different honeybee races and their lineage. The corresponding morpho-ecotypes of those races in the different ecological areas of the country are also analysed as a measure of their biodiversity in sustainable beekeeping.

The general discussion analyses and compares the melliferous resource availability, the health status, the honey quality and the biodiversity of the honeybee of the different regions in order to evaluate the beekeeping potential of the country. From this we proposed a categorisation of the country into practical beekeeping regions.

CHAPTER 2

Melliferous plants suitable for timber production

The landscape in dry season. Trees remain the main floral source after the bushfire has passed.

CHAPTER 2

Melliferous plants suitable for timber production

Personal contribution:
- Methodology design
- Implementation of forest surveys
- Database on the melliferous plants
- Data analysis
- Writing the manuscript

Parts of this chapter are published in:

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1. Abstract

Perennial plants are the main pollen and nectar sources for bees in the tropical areas where most of the annual flora is burned in dry seasons. Therefore, perennial plants constitute the most reliable biomaterials for determining and evaluating the beekeeping regions of the Republic of Benin. A silvo-melliferous region (S-MR) is a geographical area characterised by a particular set of homogenous melliferous plants that can produce timber. Six S-MRs could be identified in the country, i.e. the South, the Central, the Central West, the Central North, the Middle North and the Extreme North region. At the country level, the melliferous plants were dominated by *Vitellaria paradoxa,* which is common in all regions. The most diversified family was the Caesalpiniaceae (12 species) followed by the Combretaceae (10 species), and *Combretum* was the richest genus. The effect of dominance is particularly high in the South region where *Elaeis guineensis* alone represented 72.6% of the tree density. The total melliferous plant density varied from 99.3 plants/ha in the Central region to 178.0 plants/ha in the Central West region. On the basis of nectar and pollen source, the best region for beekeeping is the Central West region while the South region, characterised by an unbalanced distribution of pollen and nectar trees, was the poorest silvo-melliferous region in the Republic of Benin.

2. Introduction

The deep relationship between bees, climate and flora of any ecological area almost makes the bees an ecological factor of the same rank as temperature, rainfall, humidity and sunstroke with which they interact and cope (Shimanuki & David, 2000). The necessity for studying the linkage of the biotic community as a determinant of the ecosystem's dynamics and stability is well-recognised by the scientific community which also agrees on the spatial distribution pattern of living organisms as a key attribute of the ecological factors (Loreau *et al.*, 2001). However, very little is known on the particular function of the melliferous plants and their distribution as a part of the multi-functionality of the ecosystem (DeGrandi-Hoffman *et al*., 1990; Amakpe, 2008).

Beekeeping is a very old activity carried out by humans to ensure their livelihood. In poor countries such as the Republic of Benin, it is one of the rare niche activities that need little

investment, provide a substantial income to local people and have a positive impact on the global environment at the same time.

Apart from the prevailing climatic conditions, the most important factor that determines the hive productivity in any ecological area is the available floral resources from which the bees collect nectar, pollen and propolis (Shimanuki & David, 2000). The study of melliferous plants available to the honeybees then lies at the heart of the decision-making as far as the beekeeping development is concerned.

In dry tropical areas, most of the annual plants are burnt during the dry season by recurrent bushfires (Biaou, 2008). The main nutritional source for bees during the dry season, when the bees are more active, is covered by perennial melliferous plants, which represent more than 40% of the total pollen collection (Dongock *et al.*, 2004). Thus, sustainable beekeeping in these areas is an enterprise which mainly relies on perennial trees. In the Republic of Benin, where the savannah zone reaches the Atlantic Ocean in the south and where more than 87% of the territory lies in the Sudanian zone (Akoègninou, 2004); the dependence of beekeeping on the perennial melliferous trees is even greater. We therefore decided to determine the distribution of these trees at a nationwide level in order to categorize the land based on its potential for beekeeping.

The vegetation, its structure and the dynamics of the different stands from the coastal zone in the south to the dry savannah in the north are well-documented for Benin (Sinsin, 1993; Akoègninou *et al.,* 2006). Moreover, in 2007 the General Directorate of Forest and Natural Resources conducted a countrywide forest survey, and a database on the different timber species is available (IFN, 2010).

At the regional and international levels, we could rely on excellent descriptive works on tropical melliferous plants and the resources they issue to bees (pollen or nectar) (Adjare, 1990; Dongock *et al.*, 2011). Although there is plenty of information on the vegetation in general, the knowledge of the melliferous resources of the country is sparse and limited at local or village levels (Yédomonhan *et al.*, 2012). Some institutions and projects conducted empiric inventories of the plants visited by bees in order to set a beekeeping plan for selected areas, but a nationwide study that categorizes the land based on its potential for the beekeeping sector is still lacking. This

study was aimed to fill this gap in order to improve the living standards of the beekeepers by extending their working field and to evaluate the underestimated honey production function of trees beside the timber function that forests in tropical areas commonly have.

3. Materials and methods

3.1. Study area

The study area is the entire Republic of Benin (112,622 km²) located in the "Dahomey Gap" where the savannah reaches the coast. The climate is sub-tropical from the coast to 7°10'N and evolves progressively to the dry tropical climate in the north, dividing the country in three main climatic areas (Akoègninou *et al.*, 2006; ASECNA, 2012). From the coast to the 7th parallel stands the littoral and sub-littoral climatic area with 900 mm annual rainfall in the west and 1400 mm annual rainfall in the east. The transitional tropical humid zone stands from the 7th to the 9th parallel with 1200 mm to 1300 mm annual rainfall. Above the 9th parallel is the dry tropical climate with one rainy season of 900 mm to 1200 mm annual rainfall.

The vegetation varies from the Guinean forest in the south-east to the semi-desert vegetation in the extreme north. The diversity of the vegetation in the different ecological area leads to 10 phyto-geographic districts, each with a specific floristic composition (Adomou, 2005).

3.2. Sampling and plots

The forest surveys were conducted from April 2010 to July 2011 on systematic fixed area plots established all over the country at a regular distance of 15 km. The first point from which the other points were generated was located in the south-eastern extremity of the country as close as possible to the south-western Nigerian border. The Universal Transverse Mercator (UTM/WGS1984) of the 31 northern meridian (zone 31N) geographical coordinates of this first point are X = 465,000 east and Y = 705,000 north.

The geographical coordinates of the other points were automatically generated by the software "grille d'échantillonnage 1.5" of 22/08/02 developed by the Forestry Economy and Management

Unit of the Faculty of Agronomy Sciences of Gembloux, Belgium. Based on the shape of the country and its total area, a theoretic number of 507 plots were determined. However, 25 plots located at peaks of mountains and water plans were not reachable. Thus, the surveys were conducted on 482 fixed area circular plots with a radius of 18 m (1017.3 m² each) distributed all over the country (figure 7). The centre of each plot was determined by its UTM/WGS1984 zone 31N geographical coordinates. The navigation for finding the different plot centres was performed using a GPS (Garmin) with 0.7 m precision.

3.3. Surveyed population and data collection

The surveyed population consisted of any perennial plant that had a minimum Diameter at Breast Height (DBH) of 10 cm (DBH≥10 cm) with the exception of climbing plants and bamboos. With regard to palm trees, only those that had their stem free of leaves at a minimum height of 1.3 m were counted, and their diameters were not measured.

In this chapter, a silvo-melliferous plant is any live plant that is known to produce nectar or pollen for the honeybees (melliferous plants) and with a minimum DBH of 10 cm (timber). Different publications dealt already with the melliferous plants identification in tropical areas and in Benin (Gadbin, 1980; Comlan *et al.*, 2009; Dongock *et al.*, 2008, 2011; Adjare, 1990; Yédomonhan *et al.*, 2012). We used these sources for determining whether the surveyed perennial plants were melliferous plants or not. Depending on the two main foods a plant can produce for the bees, the melliferous plants were classified as follows: pollen source (P) for the plants that mainly produce pollen; nectar sources (N) for the plants that mainly produce nectar; nectar and pollen sources (NP) for plants that produce the two foods for the bees.

In this survey, dead plants were not taken into account as they have no nutritional value for the honeybees. The accurate determination of the plant species was performed by using the botanical works of Ern (1988) and Akoègninou *et al.* (2006) and when required, by comparing the collected plant with the specimen available at the national herbarium of the Republic of Benin. The different data collected at each plot were: [1] general information on the plot such as the administrative location, the geographic coordinates, etc., [2] the number of stems per species and [3] the DBH of each living tree or stem with a diameter of more than 10 cm.

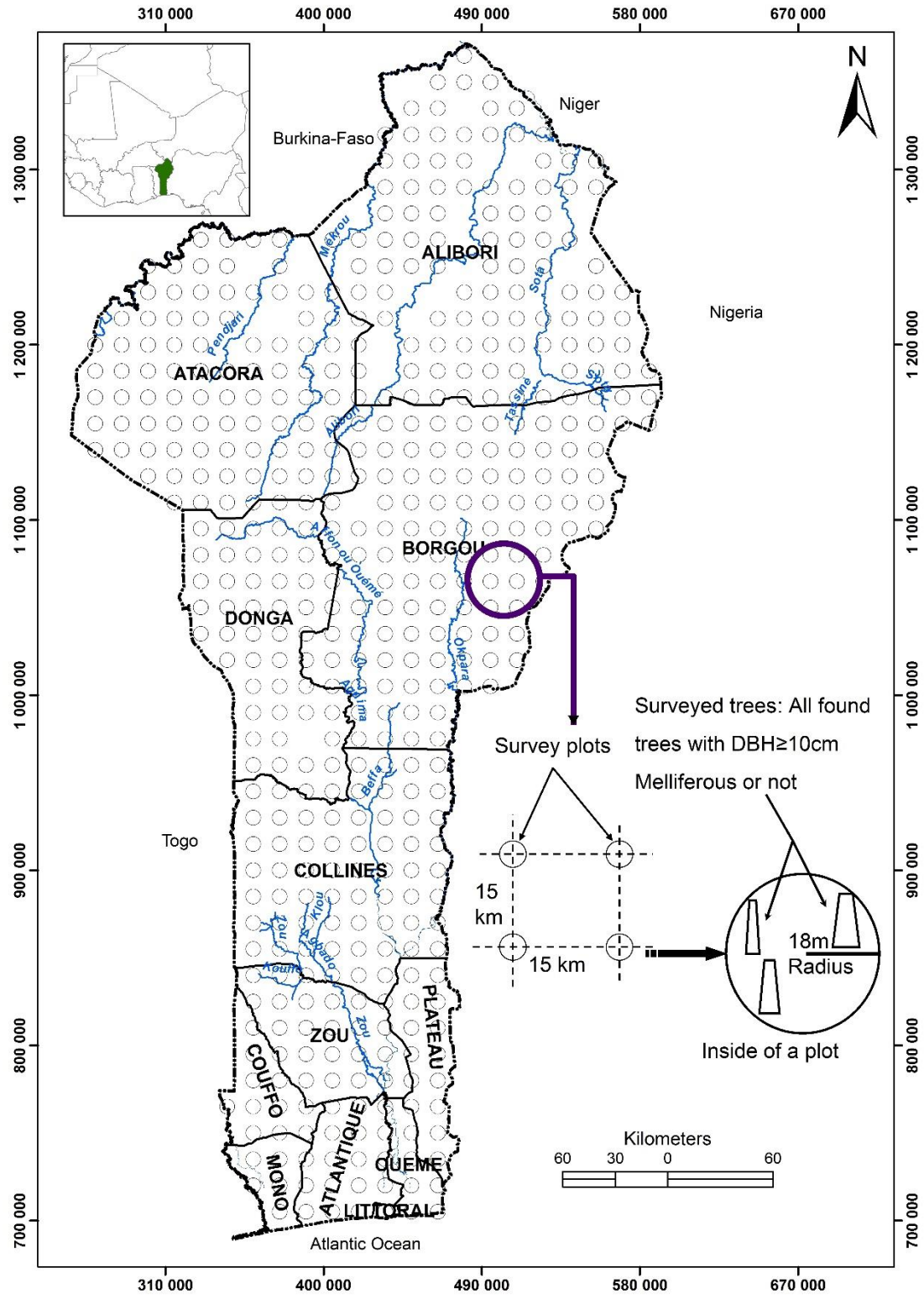

Figure 7: Distribution of the survey plots in the Republic of Benin.
The international border is marked by a black dotted line; departmental borders by black lines; perennial water by a blue line and survey plots by grey circles. In the left corner is a map of West Africa with the Republic of Benin marked in green.

3.4. Data processing

Statistical analysis was conducted with the GraphPAD PRISM® version 6.01. First, a descriptive statistic analysis was performed on the tree density, the species frequency and the basal area for each individual plot. The classification of the plots and their distribution in the gradient axis consisted in grouping the survey plots in homogenate clusters based on the melliferous plant species composition and the abundance of each species per plot.

Empty plots located on roads, tilled lands, houses, rocky areas and in water plans were excluded from the classification analysis. The plots that had no melliferous plants were also excluded from the analyses, which were finally performed on 248 plots that bore at least one melliferous plant. After the melliferous regions were determined, the empty plots which had been excluded from the classification analysis were integrated into their respective region for the determination of the different dendrometric parameters of each melliferous region.

The classification and ordination of the plots in clusters was performed by the Bray-Curtis similarity using the nearest neighbour cluster method (Cottam & Curtis, 1956; Bray & Curtis, 1957). In order to reduce the influence of species which were too much abundant in some plots, we performed a Log_{10} transformation of the row abundance values per plot before the Bray-Curtis similarity value BC_{jk} (equation 1) was calculated (Jongman *et al.*, 1995; Legendre & Gallagher, 2001). The BC_{jk} value was calculated per pair of plots in a matrix of 248 plots x 248 plots and served in automatically comparing the Euclidean distance between each pair of plots.

$$(1) BC_{jk} = \frac{2\sum_{i=1}^{p} \min(N_{ij}, N_{ik})}{\sum_{i=1}^{p} (N_{ij} + N_{ik})}$$

In the equation (1) N_{ij} is the Log_{10} of the abundance of the species i in the plot j; N_{ik} is the Log_{10} of the abundance of the same species i in the plot k; min is the minimum of the two values, and the sum of the numerator and denominators is for all the species encountered in the two plots.

The initial clusters identified by the ordination graph were projected in the shape file of the country by ArcGis 9.02 to visualise their geographical distribution. The geographical area of concentration of each cluster in the country map feature was used as a background for cutting a polygon that represented a particular silvo-melliferous region, of which we determined the area with the "Xtool Pro 4.0" functions of the ArcGIS 9.02.

The spectrum of the melliferous plants for each region was characterized by the diametric structure and by the relative total plant importance (IP%) of melliferous plants in order to identify the leading melliferous plants and the relative contribution of each species to the entire melliferous potentiality of the region. The IP% of a determined melliferous plant was calculated by the following equation (2) according to Cottam and Curtis (1956):

$$(2) IP\% = \frac{n_s}{N_T} + \frac{n_p}{N_p} + \frac{\sum_{i=1}^{n} G_i}{G}$$

In the equation (2) n_s is the number of stems of the species in the region; N_T is the total number of stems present in the region; n_p is the number of plots that bear the species; N_P is the total number of species in the region; G is the total basal area of all the found species in the region; $G_i = (\pi/4)(DBH)^2$ is the tree individual basal area of the species and DBH was the measured diameter at breast height by a tape, graduated in diameter-equivalence of the tree circumference.

Other parameters we used for characterizing the different S-MRs were: (i) the tree basal area per ha of the region which equals the total of the individual tree area divided by the total surface of the survey plots of the region; (ii) the tree density per ha which is the total number of tree stems encountered in a region divided by the total surface of the survey plots of the region; (iii) the species diversity which is the number of different species encountered in the region (also determined for the different genera and families), (iv) the exclusive silvo-melliferous species which is a tree species only found in a particular region, and (v) the total nectar density per region which is the sum of the nectar producing plant density (N) and the nectar and pollen producing (NP) density (DeGrandi-Hoffman *et al.,* 1990 and Al-Ghamdi *et al.*, 2014).

The total nectar density also helped to establish the relative honey potential score (RHPS) for each region. This is the total nectar tree density divided by the lowest value which was obtained in the south region.

4. Results

4.1. Climatic gradient and silvo-melliferous regions

There was a strong correlation between the values of the ecological gradient and the ecological distance of Bray-Curtis matrix. The correlation value is 0.7, and the significance level for the test of zero correlation was very low ($P<0.01$). The plot distribution pattern in the Euclidean distances and the ordination indicated three main clusters which were projected in the country shape file. One was below the ordination value 100, another between 100 and 180 and the third above the value 180 (figures 8 and 9). These plot clusters were distributed in the different parts of the country as follows.

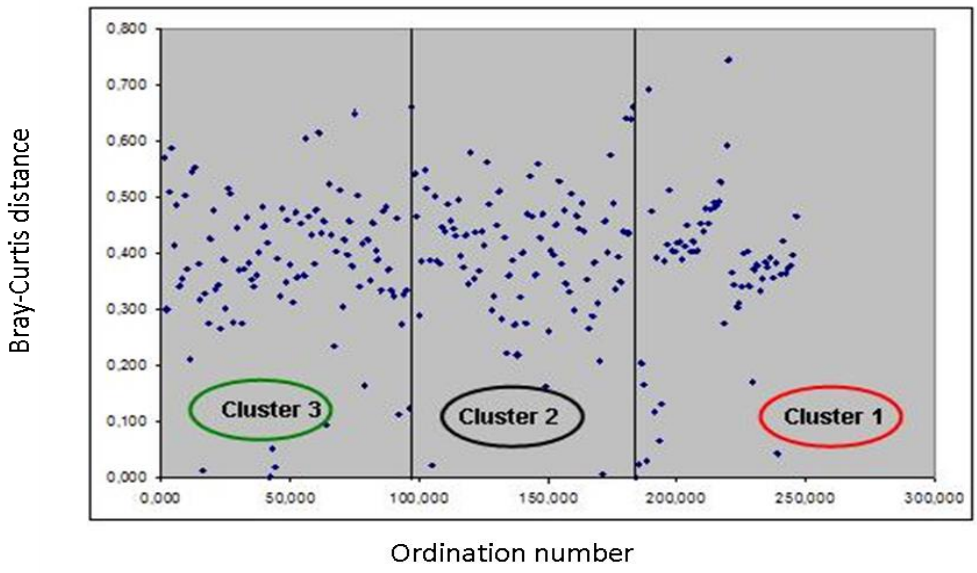

Figure 8: Distribution of the plots in the Euclidean axes.
The plots of cluster 1 are presented as triangles in the shape file of the country. The plots of the cluster 2 are presented as dots, and the plots of the cluster 3 are presented as stars in the country shape file.

The first plot cluster (triangles in figure 9) was exclusively concentrated in the south of the country under the latitude of Aplahoué (department of Couffo) and Covè (department of Zou) in the Sudano-Guinean climate area. It was dominated by industrial plantations of *Elaeis guineensis, Gmelina arborea, Tectona grandis* and *Acacia auriculiformis*.

The second cluster was made of plots (dots in figure 9) that were absent in the south and the central-west which benefit from a better annual rainfall. They were almost absent in the band of Bembèrèkè-Sinende to Segbana, known as the *Vitellaria paradoxa* (butter tree) region. Apart from this particular area, they were spread all over the transitional climatic region of the country in a mixture of some scattered plots from the first and the third clusters.

The third plot cluster (stars in figure 9) was divided into two pure ecotypes located in two distinct geographical regions in the south and the north of the second cluster. One was located in the central-west humid climatic region of the country (region of Bassila) with an annual rainfall of 1300 mm. The second geographical type of this cluster covered a region that extends from the north-east of the department of Borgou to the central-west border of the department of Atacora with the Republic of Burkina Faso.

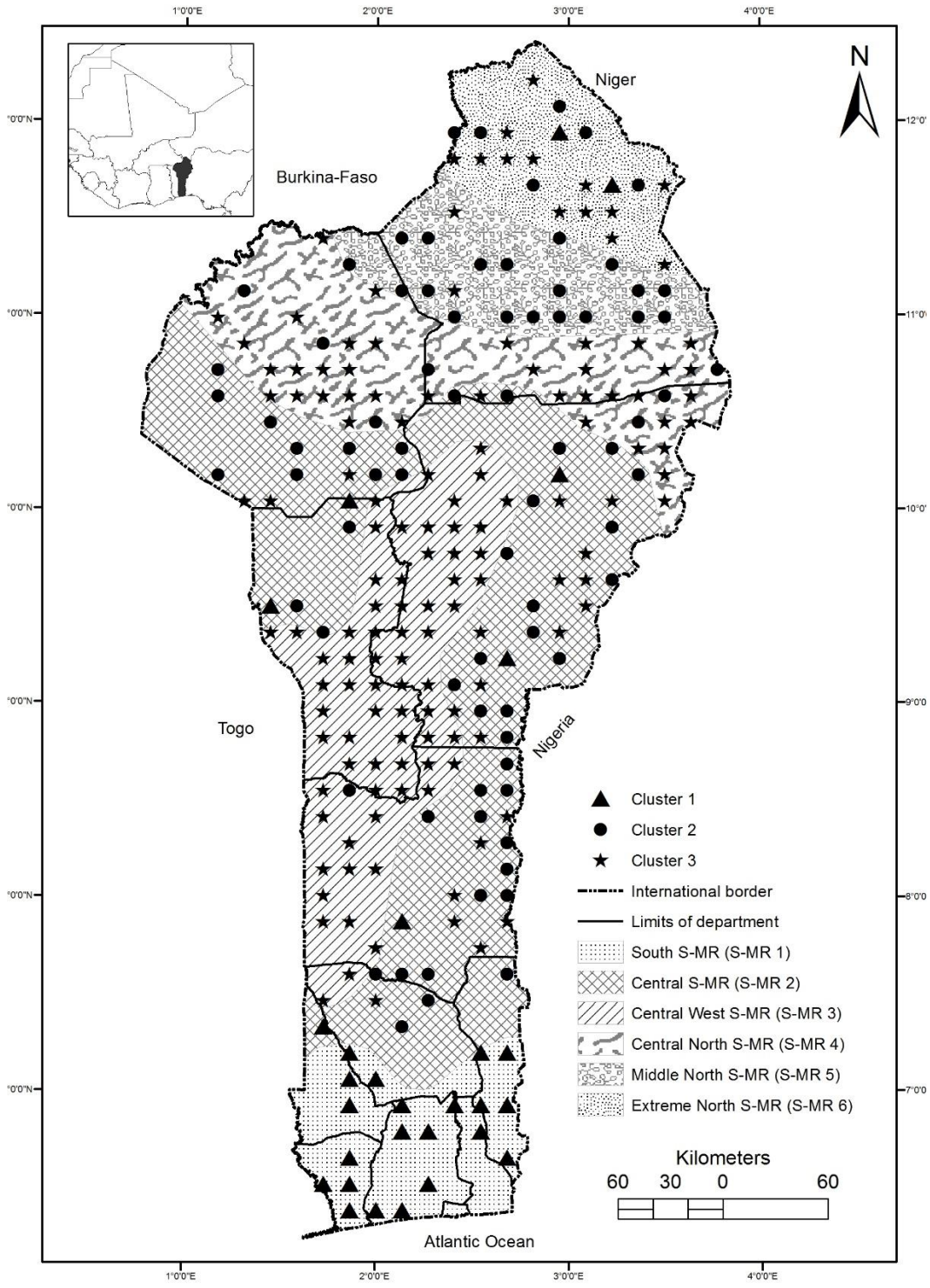

Figure 9: Location of the 3 main plot clusters.

The triangles (▲) are the plots that constituted the first cluster from the ordination analysis. Their concentration corresponded to the silvo-melliferous region (S-MR) of the South. The dots (●) are the plots of the second cluster. Their concentration in the north corresponded to the Middle North S-MR. The stars (*) constituted the third cluster, the concentration of which made up the Central West S-MR and the Central North S-MR. The mixture of triangles, dots and stars corresponded to the Central S-MR in the centre and the Extreme North S-MR in the extreme north of the country.

4.2. Spectrum of the silvo-melliferous regions

The geographic distribution of the plot clusters indicates that the Republic of Benin can be divided in six S-MRs with different beekeeping potential (Figure 9). The dendrogram generated from the Euclidean distance pair comparison (figure 10) indicated the segregation between the different regions (R=0.7 and P<0.01).

The first region is the South Silvo-melliferous Region (S-MR 1), which covered 1,102,906.23 ha and was totally located in the south. It bore six exclusive melliferous trees (*Acacia auriculiformis, Ceiba pentandra, Eucalyptus* sp*., Gmelina arborea, Newbouldia laevis* and *Phoenix reclinata)*. It was dominated by *Elaeis guineensis, Gmelina areborea, Cola* sp. and *Tectona grandis,* which represented 196% of the total tree importance. The melliferous tree density was 140.4 trees/ha represented by 24 species distributed across 20 genera and 14 families sharing 299.1% of the total tree importance. This total importance was dominated by pollen producing trees (211.0%) while the share of nectar producing trees was only 31.8%. In this region, the food production is unbalanced, and the bulk of the melliferous plants density was made up of pollen producing trees (mainly *Elaeis guineensis* and *Tectona grandis*) that represented 87.7% of the melliferous density. The nectar producing plants represented 5.3% and the plants that produced nectar and pollen for bees represented 6.7% of the plant density. This region had the lowest total nectar density

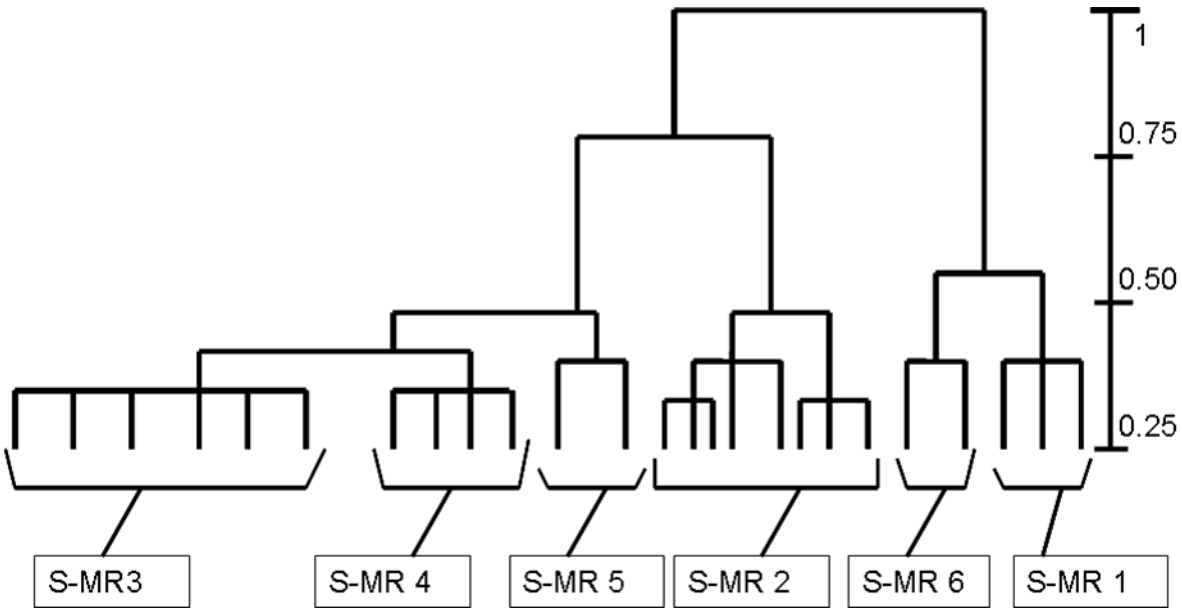

Figure 10: Dendrogram of the position of clusters that made up each Silvo-melliferous Region (S-MR: silvo-melliferous region).

(16.9/ha) and the relative honey potential score was 1. The richest genus was the *Acacia* with 3 species, and the Arecaceae family was the most diversified with 3 genera and 3 species. The average diameter of the melliferous trees in this region was 18.6 cm (+/- 7.5 cm) and varied from 10 to 34 cm. There was one non-melliferous plant (*Ficus* sp.) of the Moraceae family that represented 0.3% of the total tree density.

The second region is the Central Silvo-Melliferous region (S-MR 2) of 4,175,094.9 ha. It is an elongated area that covers the north of the department of Couffo, the department of Plateau, the department of Zou, the east of Collines, the east of Borgou, the north-west of Donga and the west of the department of Atacora. It bore 68 melliferous tree species of 26 families and 59 genera, which represented 203.2% of the total tree importance (141.0% of nectar plants, 113.5% of plants that produces both and 39.1% of pollen plants). The total density of melliferous plants was 99.3 plants/ha. This total melliferous density is made up of 40.6% of nectar producing plants, 14.5% of pollen producing plants and 41.5% of nectar and pollen plants. The total nectar density of this region was 84.3/ha, and the corresponding relative honey potential score is 5. The most important melliferous plants were *Vitellaria paradoxa, Anogeissus leiocarpa, Pterocarpus erinaceus* and *Danielia oliveri,* which mainly produce nectar. The Caesalpiniaceae was the most diversified family with 10 species distributed in 8 genera. The richest genus in this region was the *Combretum* that bore 4 species. The exclusive species were *Adansonia digitata, Blighia sapida, Borassus aethiopum, Dalbergia* sp*., Daniellia* sp*., Feretia* sp*., Phyllanthus amarus, Ximenia americana.* The average diameter of the melliferous trees was 20.3 cm (+/-16.8 cm) and varied from 10 to 135 cm. The non-melliferous plants species were found in the families of Meliaceae, Mimosaceae, Moraceae, Sapindaceae and Simaroubaceae, which represent 3.4% of the total tree density.

The third region is the Central West Silvo-Melliferous Region (S-MR 3) of 2,162,444,24 ha. It was an area starting from the north of the district of Djidja (department of Zou) and covered the west of the department of Collines, the west of Borgou and the east of the department of Donga. In this region, the species were evenly distributed, and there is no dominance effect although *Vitellaria paradoxa* is the most important plant with 14.9% of the total melliferous density. The exclusive silvo-melliferous species were *Albizia* sp*., Chrysobalanus icaco, Dialium guineense,*

Garcinia ovalifolia, Morelia senegalensis, Parkia bicolour, Pterocarpus santalinoides and *Uvaria* sp. The total melliferous density was 178.0 plants/ha made up of 46.7% nectar sources, 9.4% pollen producing plants and 40.6% plants that issue both feed for the honeybees. The total nectar density was 160/ha corresponding to a relative honey potential score of 10. The diversity of the melliferous plants was distributed between 60 species of 53 genera and 21 families that shared 293.8% of the total tree importance. The most diversified family was the Caesalpiniaceae that bore 9 genera and 10 species. The *Combretum* genus was the richest genus with 4 species. The melliferous trees had an average diameter of 19.8 cm (+/- 8.7 cm), which varied from 10 to 57.5 cm. The non-melliferous plants were found in the families of Anacardiaceae, Anonaceae, Caesalpinianceae, Asteraceae, Euphorbiaceae, Fabaceae and Moraceae which represented 3% of the total tree density.

The fourth region is the Central North Silvo-Melliferous Region (S-MR 4). It covered 1,966,018.80 ha in the Central part of the north of the country. It is a geographic ecotype of the third region which covered the north-east of the department of Borgou, the south of the department of Alibori and centre of the department of Atacora. The only exclusive species of this region was *Albizia zigia*. The most important species were *Anogeissus leiocarpa*, *Burkea africana*, *Crossopteryx febrifuga* and *Detarium microcarpum,* representing 85.0% of the total importance. The total melliferous tree density was 157.0 plants/ha and consisted of 27.9% of nectar plants, 10.3% of pollen plants and 57.4% of plants that issue both (nectar and pollen) to bees. The diversity was made up of 53 species belonging to 20 families and 44 genera which represented 291.8% of the total tree importance. The total nectar density of the region was 140.0/ha, and the relative honey potential score was 8. The most diversified family remained the Caesalpiniaceae that bore 7 genera and 9 species. The *Combretum* was also the richest genus with 4 species. The melliferous trees had an average diameter of 17.9 cm (+/- 7.0 cm), which varied from 10 to 38 cm. The non-melliferous plant species are found in the families of Caesalpiniaceae, Asteraceae, Fabaceae, Moraceae and Ulmaceae representing 4.2% of the total tree density of the area.

The fifth region is the Middle North Silvo-Melliferous Region (S-MR 5), which covered 1,084,913.1 ha. It was dominated by *Vitellaria paradoxa* and *Anogeissus leiocarpa,* which represented 22.0% of the total melliferous tree density. No exclusive species were found in this region, which is a geographic subdivision of the second (the Central Silvo-Melliferous) region with

which it shared all the species. The total melliferous density was 108.12/ha and consisted of 31.9% nectar producing trees, 15.2% pollen producing trees and 48.6% that produces nectar and pollen for the bees. The most important species were *Vitellaria* paradoxa*, Isoberlinia doka, Terminalia* sp. and *Anogeissus leiocarpa,* which represent 95% of the total importance. There were 42 species of 18 families, and the most diversified family was the Caesalpiniaceae that had 7 genera and 8 species which represented 288.0% of the total tree importance. The total nectar density was 90.9/ha corresponding to a relative honey potential score of 5. *Combretum* was also the richest genus with 4 species. The average diameter of the melliferous trees was 18.7 cm (+/- 9.4 cm) and varied from 10 to 63.7 cm. Three non-melliferous plants were found in this region and belonged to the Moraceae, Sapotaceae and Caesalpiniaceae families representing 4.1% of the plant density.

The sixth region is the Extreme North Silvo-Melliferous Region (S-MR 6). It covered 1,044,625.1 ha in the sub-saharian climate type and bore two exclusive species (*Balanites aegyptiaca* and *Stereospermum* sp.). There were 29 melliferous species in this region, made up of 14 families and 23 genera, which represented 292.0% of the total melliferous tree importance. This melliferous importance consisted of 77.3% of nectar sources, 43.2% of pollen sources and 171.4% of plants that produce both resources for the bees. The most diversified family was the Caesalpiniaceae with 5 genera and 6 species. The *combretum* was the richest genus with 5 species. *Vitellaria paradoxa, Anogeissus leiocarpa*, *Terminalia* sp. and *Detarium microcarpum* were the most important species with 111% of the total importance. The total melliferous tree density was 101.4/ha made up of 22.1% of nectar producing trees, 15.2% of pollen producing trees and 59.0% of pollen and nectar producing trees. The total nectar density of the region was 85.3/ha, and the relative honey potential score was 5. The average diameter of the melliferous trees was 19.4 cm (+/- 11.0 cm) varying from 10 cm to 66 cm. The non-melliferous plant species encountered in this region belonged to the Anacardiaceae, Anonaceae, Caesalpiniaceae and the Fabaceae families, which make up 3.9% of the total tree density.

The dendrometric characteristics of the 20 most important silvo-melliferous trees of each region are summarised in table 5.

Table 5: Dendrometric parameters of the twenty most important melliferous species per silvo-melliferous region (S-MR)

N: nectar producing trees; NP: nectar and pollen producing trees; P: pollen producing trees; RBA, relative basal area; D%: relative density; F%, relative frequency; IP%, total importance.

Species	Food type	Silvo-melliferous regions (SM-R)																							
		South S-MR				Central S-MR				Central West S-MR				Central North S-MR				Middle North S-MR				Extreme North S-MR			
		D%	RBA	F%	IP%	D%	RBA	F%	IP%	D%	RBA	F%	IP%	D%	RBA	F%	IP%	D%	RBA	F%	IP%	D%	RBA	F%	IP%
Acacia auriculiformis	P	0.3	0.7	3.3	4.3																				
Acacia polyacantha	P	1.7	2.4	3.3	7.4																	1	1.1	2	4.1
Acacia sp.	P	3.9	7.7	1.7	13.3													1.5	0.8	2.3	4.6	9.8	5.1	5.2	20.1
Afzelia africana	N									1.2	3.1	1.7	6	1.2	3.2	<0.1	4.4					1.2	3.2	2	6.4
Anacardium occidentale	N					3.1	1.6	2.3	7.0	7.1	3.6	1.7	12.4												
Anogeissus sp.	NP																	7.8	4.4	2.3	14.5				
Anogeissus leiocarpa	NP					8.2	9.8	5.1	23.1	2.2	5	2.2	9.4	5.8	6.2	<0.1	12.0					8.1	10	6.5	24.6
Azadirachta indica	N	1.3	2.5	6.7	10.5																				
Bombax costatum	NP																	2.4	6.7	4	13.1				
Bridelia ferruginea	P					1.9	0.6	0.4	2.9																
Burkea africana	N					3.3	3	2.8	9.1	4.8	5.5	6	16.3	4.1	5.3	<0.1	9.4	1.9	2.1	1.7	5.7	1.7	1.7	2.6	6
Ceiba pentandra	NP	0.1	1.3	1.7	3.1																				
Cola sp.	P	2.2	23.5	1.7	27.4																				
Combretum collinum	NP																	2.9	1.4	3.5	7.8	3.4	1.5	3.9	8.8
Combretum glutinosum	NP																	6.1	2.6	5.8	14.5	5.9	2.6	9.2	17.7
Combretum micranthum	NP																					1	0.3	1.3	2.6
Combretum nigricans	NP																	2.4	1.5	1.7	5.6	3.2	1.1	3.9	8.2
Combretum sp.	NP																	4.4	2.3	4.6	11.3	3.9	2	5.2	11.1
Crossopteryx febrifuga	NP					1.8	0.8	1.8	4.4	2.8	1.9	3.1	7.8	12.8	8.3	<0.1	21.1	6.3	2.2	5.8	14.3	7.1	3.9	7.8	18.8
Daniellia oliveri	N					3.3	7.3	4.8	15.4	2.4	5.7	3.8	11.9	1.1	4.4	<0.1	5.5								
Detarium microcarpum	NP					1.9	0.9	1	3.8	1.6	0.6	1.9	4.1	11	5.2	<0.1	16.2	5.3	2	3.5	10.8	9.6	5.1	5.9	20.6
Diospyros mespiliformis	N	0.1	0.5	1.7	2.3									1.1	1.7	<0.1	2.8								
Elaeis guineensis	P	72.6	0	43.3	115.9	2.4	0	0.1	2.5																
Entada sp.	P													3.5	2.1	<0.1	5.6								

Melliferous plants suitable for timber production

Eucalyptus sp.	N	3.2	7	1.7	11.9																			
Gmelina arborea	NP	3.6	23.1	1.7	28.4																			
Grewia sp.	P	0.1	0.3	1.7	2.1																			
Isoberlinia doka	N					3.9	5.3	3.2	12.4	7.4	10.7	3.3	21.4					5.3	10.4	5.2	20.9	1.7	7.2	2.6	11.5
Isoberlina tomentosa	N									3	5	1.6	9.6					2.4	5.6	0.6	8.6				
Lannea sp.	P	0.4	0.7	1.7	2.8									2.9	3.7	<0.1	6.6	2.2	5.7	4.6	12.5	3.7	5.4	7.2	16.3
Lannea other sp.	P					3.5	3.2	2.8	9.5	3.1	3.1	6.4	12.6												
Lonchocarpus sericeus	NP	1.8	4.3	5	11.1																			
Maranthes polyandra	NP									4.3	2.1	3.3	9.7	2.1	1.6	<0.1	3.7							
Mitragyna inermis	NP	0.6	1.4	1.7	3.7									6.3	4	0	10.3	2.7	2.6	0.6	5.9	5.4	10	1.3	16.7
Monotes kerstingii	NP					1.8	1	2.1	4.9	4	2.3	2.1	8.4	1.6	0.9	<0.1	2.5								
Nauclea latifolia	NP																	1.9	0.6	1.7	4.2				
Parinari curatellifolia	NP					1.9	1.5	1.3	4.7	3.8	2.2	4.7	10.7												
Parkia biglobosa	NP	0.1	0.8	1.7	2.6	2.2	3.9	4.8	10.9					1	3.6	<0.1	4.6								
Pericopsis laxiflora	NP					1.8	1	0.7	3.5					1.6	1.1	<0.1	2.7								
Piliostigma sp.	NP					3	1.1	1.4	5.5	2.3	1	3.5	6.8	2	0.9	<0.1	2.9					2.7	2.7	3.3	8.7
Prosopis africana	N					1.7	1.5	2.11	5.31	1.64	2.9	2.9	7.44	1.4	2.1	<0.1	3.5	1.4	1.7	2.8	5.9	1.2	0.9	2.6	4.7
Pseudocedrella kotschyi	NP									1.1	1.1	2.4	4.6												
Pteleopsis suberosa	NP													1.1	0.8	<0.1	1.9								
Pterocarpus erinaceus	N					4.3	5.4	5.8	15.5	2.8	5.3	3.8	11.9	2.8	7.1	<0.1	9.9	1.7	4.2	4	9.9				
Spondias mombin	N	0.1	0.8	1.7	2.6																				
Sterculia setigera	P	1.4	7.3	1.7	10.4													1.7	3.7	2.9	8.3				
Strychnos spinosa	N																					2.2	0.8	1.3	4.3
Tectona grandis	P	4.8	12.5	6.7	24	2	0.9	1	3.9																
Terminalia sp	NP					6.5	4.5	2.3	13.3	8.2	4.8	7.1	20.1	5.5	3.8	<0.1	9.3	5.8	3.4	6.3	15.5	7.1	9.3	6.5	22.9
Trichilia prieureana	NP	0.1	1.5	1.7	3.3																				
Uapaca togoensis	P									1.9	0.9	1.7	4.5												
Vitellaria paradoxa	N	0.6	0.8	3.3	4.7	13.6	15.4	12.4	41.4	14.9	13.2	10.9	39	13.8	16	0.1	29.9	14.3	15.2	10.3	39.8	11.8	14.2	9.2	35.2

5. Discussion

From the total of 2807 floral species distributed in 1129 genera and 185 families found in Benin (Akoègninou *et al*., 2006), the first national forest survey found 186 timber trees (IFN, 2010). Our study identified 90 perennial melliferous plants, distributed in 29 families and 73 genera. At the country level, this represented a tiny part of 3.2% of the total floral diversity. But when taking into account the timber trees only, the melliferous plants found in this study represented 48.4% of the timber tree diversity. Though the nectar silvo-melliferous plant density in the country is low (40.0 plants/ha), the tree distribution is well-balanced to make pollen and nectar available for the bees. In fact, our investigation found that there are almost as much pollen producing trees as nectar producing trees all over the country (29.1% of nectar trees, 25.4% of pollen trees and 42.3% of trees that produce both resources for the bees) with only 3.2% of non-melliferous trees. Beekeeping is then an activity which can, at first sight, thrive in the entire country.

The diversity of the melliferous species appeared to be higher than found by Dongock and colleagues (Dongock *et al*., 2004) in Cameroon, which had 78 melliferous species of 33 families. In Benin the most important found families were Caesalpiniaceae (13.3%) and the Mimosaceae (11.1%) whereas Asteraceae and Solanaceae dominated the melliferous families in Cameroon (Dongock *et al*., 2004), and the Combretaceae were the dominant family in Senegal (Ricciardelli & Compagnucci, 1991).

The availability and the balance of pollen and nectar from which the bees make the hive products and the distribution of the melliferous trees over the country was also determined by the south-north ecological gradient as Adomou (2005) and Sinsin *et al.* (2004) found in the global flora of the country. In that system, the Central West and the Central North regions located in greener areas also had the highest melliferous tree density, which was also well-balanced in pollen and nectar source. But our findings proved that the availability and diversity of melliferous plants and the beekeeping potentiality of a region did not necessarily correlate with the global prevailing floral diversity. In fact, the Central Region (located in the centre of the country with the highest tree diversity) had less melliferous trees per hectare than the Extreme and Middle North regions located in more difficult climate conditions with poorer global floral diversity. In the same way, the south, which benefits from a better rainfall, had the best global floral biodiversity of the country (Adjadohoun *et al.*, 1989; Akoègninou *et al*., 2006). Unfortunately, this was the poorest

apicultural region because the perennial melliferous flora was simplified and reduced to industrial plantations of *Elaeis guineensis, Tectona grandis* and *Acacia* trees, which mainly produce pollen (Yédomonhan *et al.*, 2012). In addition to the poorer nectar production, the South Region is very cloudy and has high air humidity all year long. This meant that, as indicated by Louveaux *et al.* (1966) and Klein *et al.* (2007), the honeybees will be less active, and this region is less suitable for beekeeping than the other melliferous regions.

The total relative importance of the different species showed that *Vitellaria paradoxa* was the most important species in the entire melliferous region except the south where *Elaeis guinensis* dominated the melliferous flora. This dominance of *Vitellaria paradoxa,* which is endemic to the country (Jurgens *et al.*, 2012), is due to its high economic value. In fact, the species is usually conserved by peasants during the slash and burn cropping system (Glèlè Kakaï, 2011), and the landscape sometimes evolves into "pure *Vitellaria paradoxa* plantations" in most S-MRs. The honey flow periods in the country will then be very influenced by the blossoming dynamics of this species.

The exclusive species that produce nectar could give their specific signature to the honey that would be produced in the region. In that, we found that the south region's honey will be characterised by *Gmelina arborea*, *Eucalyptus spp*. and *Newbouldia lae*vis. The honey from the central regions will be characterized by nectar from *Borassus aethipium*, *Ximenia americana*, *Danielia sp., Blighia sapida, Phyllantus sp. and Feretia sp*.; the honey from the Central West regions will be characterized by *Garcinia ovalifolia*, *Dialium guinense*, *Peterocarpus santalinoides* and *Chrysobalanus icaco*; the honey from the extreme north regions will be characterized by nectar from *Balanites aegyptiaca* and *Stereospermum sp*. The Middle North and the Central North had no exclusive tree that produces nectar, and our findings suggested that the honey from these two areas could have no particularity that could distinguish them from other surrounding regions.

The diversity of the melliferous trees per regions indicated that the Central region, which had the lowest melliferous density, was the most diversified area with 68 species of 26 families. It is followed by the Central West region and the Central and Middle North regions that respectively bore 60 species 53 and 42 melliferous tree species. This diversity of the melliferous trees was the main reason why these three areas had the best balance in nectar and pollen sources. Though

the Extreme North region was less diversified compared to the four first regions, it is far better than the South region with 29 melliferous species and a better balance in nectar and pollen sources. In addition to that, it is located in better climatic conditions and we classified this region as the fifth S-MR of the country.

The dry season is the period when the bees are most active (Adjare, 1990), and this period ecologically coincides with the blossoming of more than 70% of the tropical trees (Arbonier 2002, Akoègninou 2006). With this positive ecological synchronization of the active period and the plant blossoming, the annual pollen or nectar production of a region will mainly be determined by the size of the trees that it bears. But this research proved that all the regions were dominated by small sized trees of less than 40 cm DBH as the country is located in a savannah area from the north to the south. The best parameter for characterizing the different regions was then the melliferous tree relative density (pollen and nectar sources) as indicated in figure 11.

By integrating all these parameters and the relative honey potential score of the different regions, we suggest the following classification of the different regions of the country as far as their floral potentiality is concerned. The first beekeeping area of the country is the Central-West

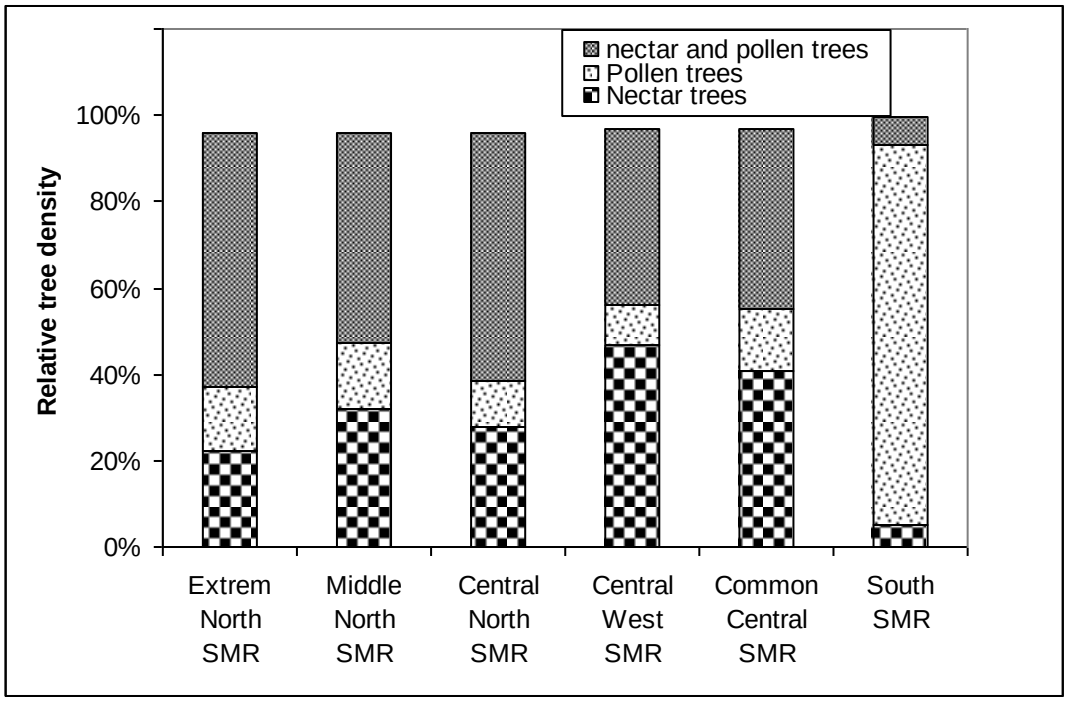

Figure 11: Distribution of nectar and pollen production per silvo-melliferous region (S-MR).
The South S-MR had too many pollen trees and very few nectar sources; the other regions had a mixture of pollen and nectar producing trees for bees.

region, also known as the Bassila honey belt in the country which can produce 10 times more honey than the south. It is followed by the Central North Region which can produce 8 times more honey than the south. These regions were characterized by a good balance and diversity of nectar and pollen producing trees. The Middle North Region, the Extreme North Region and the Central Region are similar as far their relative honey potential score are concerned and can each produce 5 times more honey than the south. But the Middle North region is even better than the Extreme North, which is also better than the Central region despite the higher diversity in melliferous tree of the Central Region. The marginal region in beekeeping is the South region, which, in addition to having a poor melliferous flora, is too humid and cloudy all year long, and the bee will be less active.

This classification is really conform to what is commonly adopted in the field works of different actors involved in the beekeeping sector in Benin, and to the findings of Adjinakou (2000) and Kokoye (1991) who suggest that real beekeeping in Benin starts from the latitude of Abomey (north limit of South S-MR) to the north, and that the area of Bassila (corresponding to the Central West S-MR) is the best beekeeping region.

6. Conclusions

The density of the melliferous resources and their quality indicated that the south is the poorest region while the best melliferous region of the country is located in the Centre West. Even if the silvo-melliferous conditions of the other regions are fair, they benefit from good climatic conditions for the bees, and a beekeeping enterprise can thrive well in these areas.

The research was limited to the perennial plants that issue the bulk of pollen and nectar to bees in the tropical areas in the dry season. But the bees do not only rely on the perennial trees or timber trees of more than 10 cm diameter at breast height, and many annual plants produce nectars and pollen. The hive product yields are also determined by the colony management and the honeybee diversity, which are analysed in the next chapters.

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Melliferous plants suitable for timber production

CHAPTER 3

Pathogenic status of the honeybees in the Republic of Benin

Worker honeybees chasing small hive beetles on the top bars of a Kenyan top bar hive.

CHAPTER 3

Pathogenic status of the honeybees in the Republic of Benin

Personal contribution: Bee and honey samples collection
Pest identification in the fields and at lab
Data analysis
Writing the manuscript

Parts of this chapter are published in:

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1. Abstract

Samples of bees and honeys were collected from apiaries in the entire Republic of Benin (West Africa). Visual inspection of hives, broods and adult bees revealed high *Varroa destructor* and *Aethina tumida* infestations. A pathogen screen based on bacterial/fungal cultures and tissue microscopy revealed the absence of American and European foulbrood, chalkbrood disease, acariosis and nosemosis. However, for the 84 sites for which a PCR based viral screening method was validated, 13 bee samples (15.4%) were found to be infected by acute bee paralysis virus (ABPV; 8.3%), Black queen cell virus (BQCV; 4.7%) and Lake Sinai virus (LSV; 3.5%).

2. Introduction

Beekeeping is a human activity that has been carried out since Ancient Egyptian time. Today it represents an important economic activity in many parts of the world because of the honey that the bees (*Apis mellifera*) produce, while the economic value of the pollination services is many times higher. In fact, more than 60% of the 1330 cultivated crop species depend on bee pollination, which is also known to improve crop quality (Jacobs *et al.,* 2006; Klein *et al.,* 2007; Roubik, 1995; Kearns *et al*., 1998; Hopping *et al*., 1993). The contribution of honeybees to the Gross Domestic Products is estimated to be over $15 billion per year in the United States and £200 million per year in the United Kingdom (Morse & Calderone, 2000).

In poor countries such as the Republic of Benin, beekeeping is one of those rare activities that need little investment, but nevertheless provide substantial incomes to local people. Developing this activity could be one of the most efficient strategies for combating poverty in these areas. But in addition to the weak management system that limits hive product yields, beekeeping in developing countries is negatively impacted by numerous pests, which also determine the possibility of the country to export hive products (OIE, 2009). It then becomes urgent and compulsory to document the bee health status in order to make sure the country fits the international market standards for more profitable beekeeping in the Republic of Benin.

The health status of the African honeybees is far less characterized than that of their European and North American counterparts. In the last comprehensive review of the world bee health, information from Sub-Saharan African countries was mostly lacking or incomplete and was mainly based on predators such as ants, mice and the widespread small hive beetle *Aethina tumida* (Ellis, 2004). Since then, the situation has hardly improved. New data on the prevalence of the bee lice *Braula coeca* in East African Ethiopia became available (Gidey *et al.,* 2012). A screen for the most common bee viruses in Uganda revealed the presence of Black queen cell virus (BQCV) for the first time. The pathogen load of South African stationary and migratory *Apis mellifera scutellata* apiaries were compared (Straus *et al*., 2013). And recently a comprehensive nationwide survey was conducted in East African Kenya in order to assess the presence of parasites, viruses and pesticide contaminants (Muli *et al*., 2014). However, data on the pathogen load of most West African countries, including the Republic of Benin, is still missing. In order to fill that gap, we conducted a nationwide pathogen screen focussing on bacterial, fungal and viral bee pathogens and other parasites and predators in the Republic of Benin.

3. Materials and methods

3.1. Study area

The honeybee samples were collected from established apiaries in the entire Republic of Benin. In the country, beekeeping is mainly based on natural swarms from the neighbourhood of the beekeeper, and no bee colony introduction has been reported till now. The most common hive in use is the Kenyan top bar hive (KTBH), and very few people use frame hives. The average number of hives per beekeeper is 5 hives with a maximum of 20 hives per beekeeper in a few apiaries located in the departments of Borgou, Donga and Zou (Amakpe, 2008). The studied colonies were obtained from trained beekeepers who received hives in 2010 from a supporting local NGO "*Cercle Nature et Développement*" (CENAD-NGO) in the south and the centre of the country and from the "*Centre Intégré d'Apiculture Tropicale*" (CIAT-NGO) in the north. The hives were baited and allowed to be colonized freely by natural swarms in order to make sure that the bees really belonged to the surroundings of the apiaries.

3.2. Sampling

Samples were collected from 101 apiaries established all over the country at an average distance of 50 km (figure 12). At the time of sample collection, all the hives bore colonies of minimum 6 months old. The colonies also had at least one full mature honeycomb, enough caped larval combs and at least 8 combs in total. The collection period ran from February 2012 to December 2012.

At each apiary, approximately 100 bees were collected from the inner parts of one hive with an adapted hand aspirator, and then immediately put in 90% alcohol. At the same time, a piece of mature honeycomb was cut and put in a 0.25 l jar, which was immediately sealed. Also a piece of worker brood and drone brood were taken for immediate examination of mites and other brood diseases. The collected bees and honey were kept at the local ambient room temperature (25-32°C) until they were brought to the laboratory of Molecular Entomology and Bee Pathology in Ghent (Belgium) in February 2013. From then on, they were stored at 5°C for one month before the start of the analyses.

3.3. Testing for bacterial pathogens

Honey samples were tested for the presence of the etiological agents of American foulbrood (*Paenibacillus larvae*) and European foulbrood (*Melissococcus plutonius*) by cultivation. For the American foulbrood 5 g of honey was diluted with an equal amount of PBS, heated at 50°C, vigorously shaken and then centrifuged at 3000 rpm for 30 min, after which the supernatant was discarded (de Graaf *et al.,* 2006). The pelleted sediment was re-suspended in 150 µl of PBS, heat treated (80°C for 10 min) in order to kill the vegetative stages of contaminating bacteria and plated out on MYPGP-agar with 0.1% of two antibiotics (pipemidic acid and nalidixic acid). After at least 4 days of incubation at 37°C under aerobic atmosphere, the plates were inspected for bacterial colonies and compared with a positive plate obtained from the pathogen stock of the Laboratory of Molecular Entomology and Bee Pathology. Suspicious colonies were further characterized by the catalase test and Gram-staining.

Pathogenic status of the honeybees

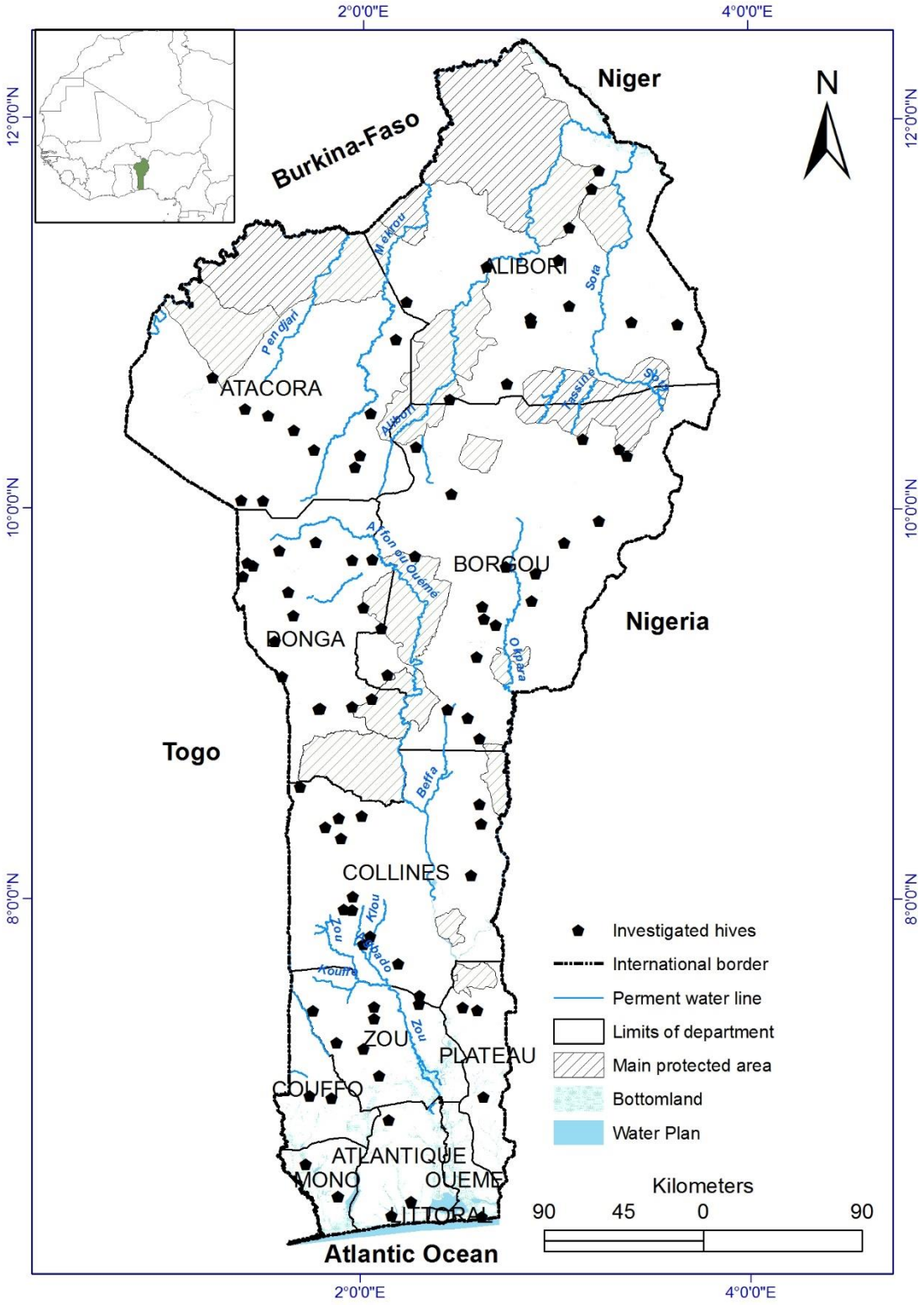

Figure 12: Locations of the investigated colonies in the Republic of Benin.

The test for European foulbrood was mainly performed as mentioned above, but we omitted the heat treatment steps, and the sediment-PBS mixture was plated out directly on an appropriate medium (Bailey solid medium) according to Bailey & Collins (1982) and incubated

under anaerobic conditions at 37°C for at least 4 days and then compared with a positive control plate. Again suspicious colonies were further characterized by Gram-staining.

3.4. Testing for fungal pathogens

Honey samples were also tested for the spores of *Ascosphaera apis*, the etiological agent of chalkbrood disease. Similarly as foulbrood, the honey samples were diluted in PBS, centrifuged, and the sediment-PBS mixture was plated out (without heat treatment) on an appropriate medium (solid Sabouraud medium). Incubation was done at 28°C for 72 hours and suspicious fungal growth was further examined microscopically and compared with a positive control sample incubated in the same conditions as the investigated samples.

3.5. Parasitic examination

At the apiary, beehives were examined for the presence of *Varroa destructor*, including both the phoretic and reproductive mites. The phoretic mite determination was done by the alcohol wash on 50 workers and drones while 50 drone and worker brood cells were open on the field for the screening of the reproductive mite and of other brood diseases. The presence of the beetles *Aethina tumida* (small hive beetle) and *Oplostomus fuligineus* (large hive beetle) was also recorded and was based on the finding of their eggs, larvae or adults in the inspected hives.

In the laboratory, adult bees were examined for the presence of the tracheal mite (*Acarapis woodi*) and *Nosema* spores. With regard to the tracheal mite, the thoraces of 10 bees per sample were dissected, and the exposed tracheas were examined microscopically for the presence of mites (Denmark *et al.,* 2000; Fakhimzadeh, 2001). As far as the *Nosema* spores are concerned, the whole abdomina of 10 bees per sample were homogenized by mechanical beating in PBS at maximum speed, and twenty microliters of the obtained suspension was examined under microscope at a magnitude of 400x.

3.6. Initial screening for viruses with the BeeDoctor

As the bee samples were kept in alcohol for 4 to 12 months, the viral disease inspection methods were validated with a positive sample from the pathogen stock of the Laboratory of Molecular Entomology and Bee Pathology (Belgium) which had been kept in alcohol for 12 months at non-controlled room temperature.

The tissue homogenization and RNA isolation were preceded by three wash steps of 10 bees per sample in PBS at 5°C under continuous agitation in an end-over-end rotator for 72 and 24 (twice) hours respectively. The washed bees were transferred to 4 ml of PBS and grounded by mechanic agitation in a TissueLyser (Precellys) for 10 min at maximum speed in the presence of 100 µl of zirconia beads and 2 metal stainless beads (De Smet *et al.,* 2012), and thereafter stored at -80°C until further use. Once they were thawed, we performed two centrifugation steps and each time transferred the supernatants to a clean vial, and RNA was extracted from 140 µl of supernatants with the QiaAmp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions.

BeeDoctor, a MLPA-based method for simultaneous detection of multiple bee viruses was used in its mode to detect 10 honeybee viruses or virus complexes (De Smet *et al*., 2012): chronic bee paralysis virus (CBPV), deformed wing virus (DWV) complex, acute bee paralysis virus (ABPV) complex, black queen cell virus (BQCV), slow bee paralysis virus (SBPV), sacbrood virus (SBV), Israeli acute paralysis virus (IAPV), aphid lethal paralysis virus (ALPV) strain brookings, Big Sioux River virus (BSRV) and Lake Sinai virus (LSV) complex (Ravoet *et al.,* 2013). All probes were synthesized by Integrated DNA Technologies (Leuven, Belgium). The tests were done with 1 µl starting material (RNA solution). Reagents typical for the MLPA reactions were purchased from MRC-Holland. Finally, amplicon lengths were determined by 4% high-resolution agarose gel electrophoresis. In order to validate putative negative results, samples were re-analyzed by MLPA in a setting with the reference gene β-actin as recommended by De Smet *et al.* (2012). This step offers the opportunity to detect virus strains with minor deviations when compared to the reference sequences, and we subsequently confirmed viral presence by RT-PCR followed by amplicon sequencing.

3.7. Reverse transcriptase-PCR and sequencing

The confirmation of virus presence was done by reverse transcriptase-PCR (RT-PCR) followed by sequencing. First, RNA (5 µl) was reverse transcribed using the RevertAid^{TM} First Strand cDNA Synthesis Kit (Thermo Scientific) with random hexamer primers according to the manufacturer's instructions. Subsequent PCR reactions were done in a mixture of 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1.25 U Hotstar Taq DNA polymerase (Qiagen), 1 µl cDNA product and the appropriate primer set (2 µM of each primer). Primer sequences can be found in Ravoet *et al.,* 2014). Positive samples for the ABPV complex were re-analysed with primers covering parts of the capsid gene specific for ABPV, IAPV and Kashmir bee virus (KBV) designed by Singh *et al.* (2010). Positive and negative controls were always included. All PCR products were electrophoresed in 1.4% agarose gels, stained with ethidium bromide and visualized under UV light. The IAPV amplicon was cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen). Amplicons were sequenced on an ABI 3130XL platform using virus-specific primers or M13 primers. These sequences were analyzed using Geneious R7. The deviating ABPV sequences were deposited to GenBank as KP025950-KP025952.

3.8. Phylogenetic analysis

Similar amino acid sequences were downloaded in Geneious R7 using a blast search. Sequences with more than 40% pair wise identity were MUSCLE aligned and trimmed. The best fitting maximum likelihood (ML) model was selected using the Bayesian information criterion (BIC), as implemented in MEGA6. Phylogenetic trees were inferred via ML using PhyML 3.0 (Guindon *et al*., 2010) with the Jones-Taylor-Thornton amino acid substitution model with a discrete gamma distribution (JTT+G) and approximate likelihood ratio test non-parametric branch support based on a Shimodaira-Hasegawa-like (aLRT SH-like) procedure.

4. Results

The outcome of the pathogen screen is summarized in Table 6 and figure 13 and presents the distribution of the bee viruses in the country. We were unable to detect any of the bacterial and fungal pathogens, nor the parasites *Nosema* spp. or *A. woodi*.

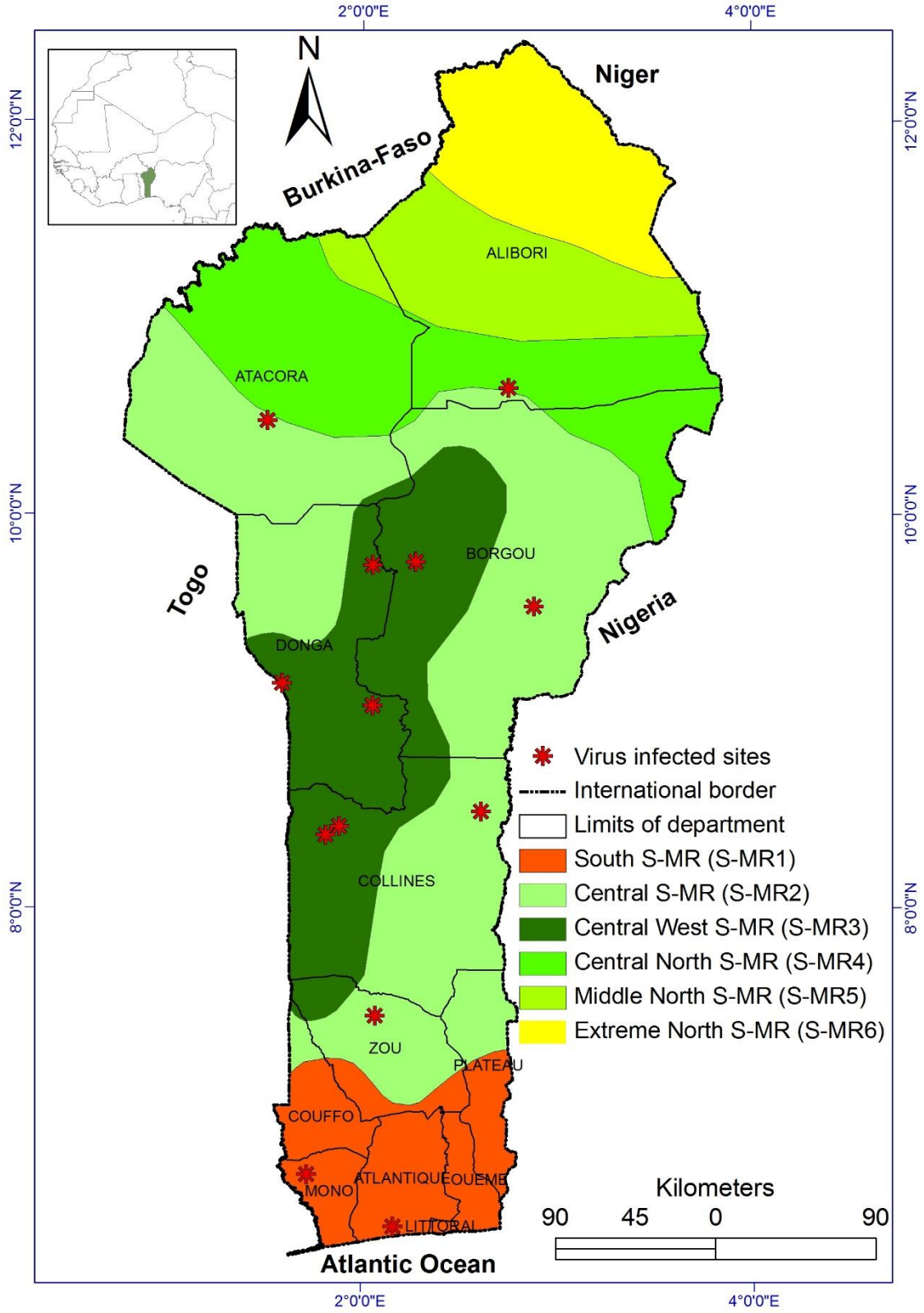

Figure 13: Distribution of viral diseases in the Republic of Benin.
S-MR = silvo-melliferous region; see chapter 2.

The search for viruses yielded a small number of positive cases. The initial screening with the BeeDoctor produced 17 suspicious results, 14 of which could be confirmed by the subsequent RT-PCR analyses. Only the faint bands for CBPV (sample 76), LSV (sample 95) and SBPV (sample 99) remained unconfirmed. From the seven samples that were positive in the BeeDoctor for ABPV complex, 6 could be assigned to ABPV and one to IAPV in the confirmative RT-PCR. But when the sequencing data of the latter became available we found that it clustered more to the ABPV clad than to the IAPV clad as indicated on the phylogram (Figure 13). We therefore considered it an unusual ABPV strain with an intermediate molecular signature. Besides, for the very first time we found LSV in samples from the African continent (samples 32, 67 and 77) and four samples that were positive for BQCV (samples 11, 32, 45 and 78). Here again, RT-PCR amplicons were additionally subjected to sequencing in order to confirm the virus status of the samples. Taking into account the number of validated negative and positive results (84 samples), our study revealed a prevalence of 8.3%, 3.5% and 4.7% for ABPV, LSV and BQCV, respectively and one sample was positive for BQCV and LSV.

With regard to the mite diseases, approximately half of the examined beehives were infested with *Varroa destructor* (48 hives). But surprisingly, of the 13 hives that were virus-positive only 6 were found to be infested with *Varroa*-mites. The small hive beetle was almost omnipresent with 90 infested hives. The large hive beetle was much less prevalent with only 13 infested hives. The varroa mite and beetles infestations of the investigated hives are presented in annex 1.

Table 6: Pathogens found in the nationwide health screen of Benin.

Only the outcomes of samples that were positive for at least one pathogen are given. 0 = not found; 1 = found; Bac: bacteria; Fun: fungi; Vir: virus; AFB: American foulbrood; EFB: European foulbrood; Asc: *Ascosphaera apis*; Aca: *Acarapis woodi*; Nos: *Nosema* spp.; ABPV: acute bee paralysis virus; ALPV: aphid lethal paralysis virus; BQCV: black queen cell virus; BSRV: Big Sioux River virus; CBPV: chronic bee paralysis virus; DWV: deformed wing virus; IAPV: Israeli acute paralysis virus; LSV: Lake Sinai virus; * = virus with an unusual molecular signature.

Sample codes	**Location (district)**	**Bac**	**Bac**	**Fun**	**Par**	**Par**	**Vir**	**Vir**	**Vir**	**Vir**	**Vir**	**Vir**	**Vir**	**Vir**	**Total**
		AFB	**EFB**	**Asc**	**Aca**	**Nos**	**ABPV**	**ALPV**	**BQCV**	**BSRV**	**CBPV**	**DWV**	**IAPV**	**LSV**	
11	Dipika (Gogounou)	0	0	0	0	0	0	0	1	0	0	0	0	0	1
15	Kouarfa (Toukountouna)	0	0	0	0	0	1	0	0	0	0	0	0	0	1
32	Pahou (Ouidah)	0	0	0	0	0	0	0	1	0	0	0	0	1	2
42	Soonon (N'dali)	0	0	0	0	0	1	0	0	0	0	0	0	0	1
45	Sounoumon (N'dali)	0	0	0	0	0	0	0	1	0	0	0	0	0	1
59	Kokoro (Ouèssè)	0	0	0	0	0	1	0	0	0	0	0	0	0	1
66	Koko (Bantè)	0	0	0	0	0	1	0	0	0	0	0	0	0	1
67	Bantè centre (Bantè)	0	0	0	0	0	0	0	0	0	0	0	0	1	1
76	Gosso (Djougou)	0	0	0	0	0	1	0	0	0	0	0	0	0	1
77	Wannou (Bassila)	0	0	0	0	0	0	0	0	0	0	0	0	1	1
78	Tchetou (Bassila)	0	0	0	0	0	0	0	1	0	0	0	0	0	1
85	Lokossa centre (Lokossa)	0	0	0	0	0	1	0	0	0	0	0	0	0	1
100	Kasehlo (Djidja)	0	0	0	0	0	1*	0	0	0	0	0	0	0	1
Total		0	0	0	0	0	7	0	4	0	0	0	0	3	14

5. Discussion

5.1. Bacteria, fungi, mites and beetles

The failure to find the etiological agents of American and European foulbrood was not unexpected. Indeed, although both diseases have a broad distribution, they are underrepresented in Sub-Saharan Africa, especially in the western part. Clinical outbreaks of American foulbrood occur in South Africa (Human *et al.,* 2011), but the limited information from the western part of the continent only talks about contaminated honey samples (Brodsgaard & Hansen, 2003). Human and colleagues (Human *et al.,* 2011) describe that absence of clinical AFB cases in Sub-Saharan Africa can partially be explained by the fact that the African honeybee, *Apis mellifera scutellata*, abscond more frequently from their hive when disturbed. Moreover, weak colonies and absconded hives are, according to Sanford (2003), rapidly invaded by SHB and wax moths that totally destroy the broods and combs and thus stop further spread of the foulbrood diseases. The absence of chalkbrood disease is also in line with expectations as this disease has only been reported in one country in Sub-Saharan Africa, i.e. South Africa, which has almost the same apicultural practice as the Western and Asian countries (Ellis & Munn, 2005). There are some older (Fries *et al.,* 2002) and recent (Muli *et al.,* 2014) reports on the occurrence of nosemosis in Sub-Saharan Africa, though they are not numerous from the west. The closest country where *Nosema spp*. was found is Niger (Matheson, 1996).

The purpose of the present study was to identify putative threats to the beekeeping practice in the different beekeeping regions of the Republic of Benin, and based on what we found; honeybee diseases do not represent a major threat to the beekeeping practice in this country. In fact, the very low hives density and the widespread management system in which the colonies are renewed each year reduce the cross infection. In addition to this situation, the native *Apis mellifera* subspecies is known to be very defensive and hygienic. It also absconds a lot, and the abandoned combs or broods are destroyed by SHB and wax moth invasion as explained earlier (Adjare, 1990; Sanford, 2003), reducing the probability of establishment or outbreak of diseases.

Unfortunately, the *Varroa*-mite and the small hive beetle were highly rooted in the apiaries of this country as in the entire tropical area (Paraïso *et al.,* 2011; Lundie, 1940). But as found by

Strauss *et al.* (2013) this apparently has no impact on the occurrence of bee viruses as in our study bee viruses were even more frequently found in apiaries where the *Varroa*-mite was lacking.

5.2. Virus concern in the Republic of Benin

With only 13 virus infected apiaries found nationwide, corresponding to a prevalence of 15.4%, the viral load of bee colonies in Benin is still very low when compared to the European, American and Asian situations where up to 80% of the colonies were virus positive (Choe *et al.*, 2012; De Smet *et al.,* 2012; Tentcheva *et al.*, 2004). Again, we believe that this is due to (with regards to the fungal, microbial and parasitic mites diseases) the very low apiary density and a widespread beekeeping system based on natural swarms and in which the beekeepers remove all the broods and combs from the hive during the harvesting periods, interrupting the life cycle of many diseases.

With respect to the virus species, our data confirms earlier reports that BQCV and ABPV are the most commonly found viruses in the African continent (Swart *et al.,* 2001; Kajobe *et al.,* 2010; Straus *et al.,* 2013; Muli *et al.,* 2014). The dominance of BQCV was found in the European apiaries as well (Antunez *et al.,* 2006). However, the finding of 3 samples that were positive for LSV was remarkable. It concerns a newly found bee virus, and its discovery was so far only limited to the USA and Europe (Runckel *et al.,* 2011; Ravoet *et al.,* 2013; Granberg *et al.,* 2013). It seems to be a polymorphic viral species that also occurs in solitary bees (Ravoet *et al.,* 2014). The finding of an ABPV with an unusual molecular signature of the capsid region is in line with the expectations, as different closely related bee viruses including ABPV, KBV and IAPV are now considered part of a complex within the family of Dicistrovirideae (de Miranda *et al.,* 2010). Nevertheless, the taxonomic position of the found ABPV strain as intermediate between ABPV and IAPV could be evolutionary of importance, seeing the isolation of the apiaries from Benin (figure 14). Previously, a deviating ABPV strain (Genbank: AAK15543) was also detected in Hungary. Later on, it appeared to be related to a virus isolated from the ant *Formica exsecta* (Johansson *et al.,* 2013). This might indicate that unusual viruses from the ABPV complex are present in Hymenopteran insects, but are rarely detected.

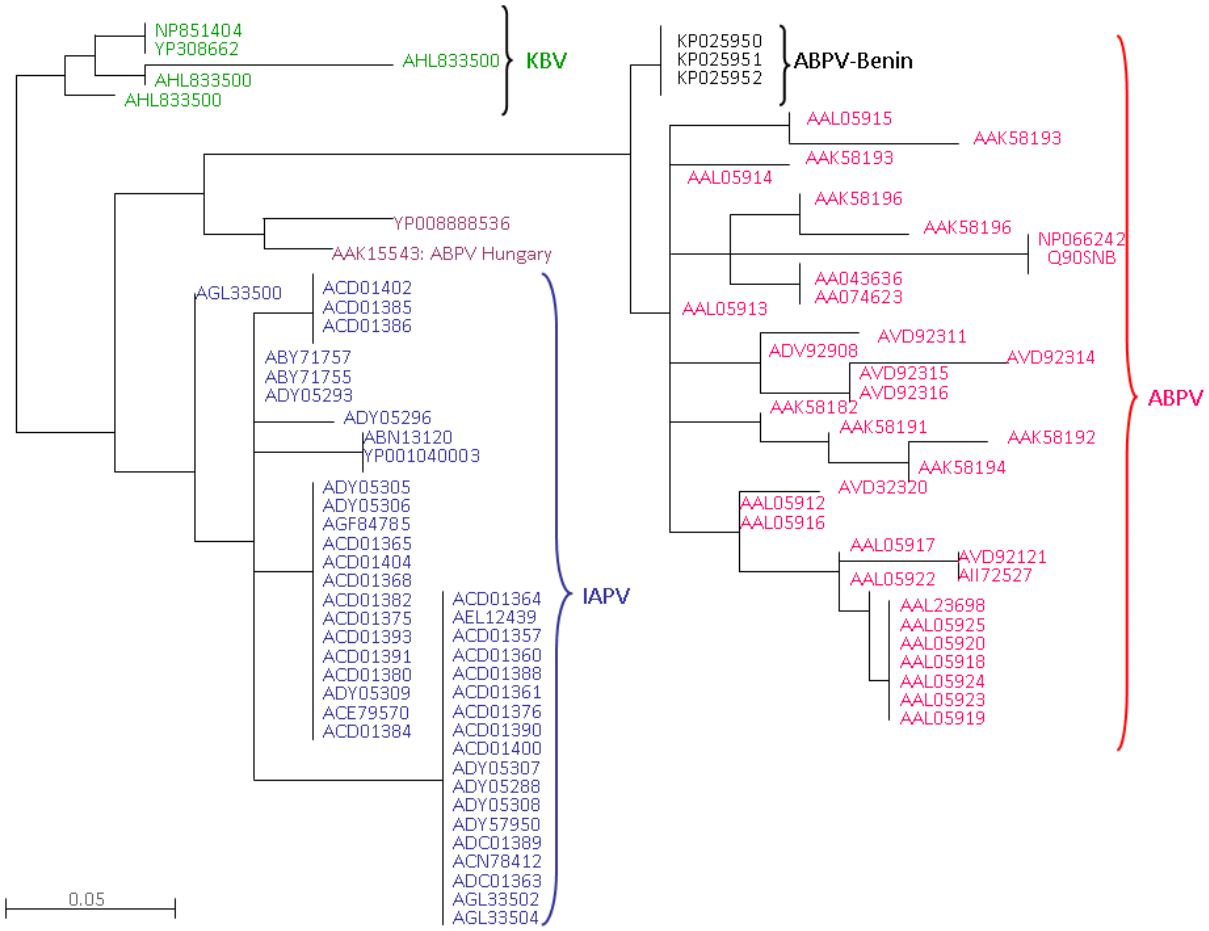

Figure 14: Phylogram of different isolates from the ABPV complex, as inferred from the partial capsid protein. ABPV strains are shown in red, KBV strains in green and IAPV strains in blue. The unusual strain from Hungary and *Formica exsecta* virus 1 are visualised in purple. The ABPV strains described in this paper, which are part of the ABPV cluster, are shown in black. Nodes supported by aLRT values >80% are exposed.

6. Conclusion

The present study did not reveal any major threats or diseases listed by the International Office of Epizooty. Thus, there is a great market opportunity for the beekeeping development in the strategies of poverty alleviation in the Republic of Benin. Though the viral infection level is still very low, it provided new data on the spread of recently discovered bee viruses in this unexplored western part of the African continent.

ACKNOWLEDGEMENTS

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Annex 1: *Varroa*-mites and pest species found in the nationwide health screen in Benin.
0 = not found; 1 = found

Sample code	*Varroa* (on workers)	*Varroa* (on drones)	Small hive beetle	Large hive beetle
1	1	1	1	0
2	0	0	1	1
3	0	0	0	0
4	1	1	1	1
5	1	1	1	1
6	1	1	0	1
7	0	0	1	0
8	1	1	1	1
9	0	0	1	0
10	0	0	0	0
11	0	0	1	0
12	0	0	1	1
13	1	0	1	0
14	1	1	0	0
15	0	0	1	0
16	1	1	1	0
17	0	0	1	0
18	1	1	1	0
19	1	1	1	0
20	0	0	1	0
21	0	0	1	0
22	1	1	1	0
23	0	0	1	0
24	1	1	1	0
25	1	0	1	0
26	1	1	1	0
27	0	0	0	0
28	0	0	1	0
29	0	0	1	0
30	0	0	1	0
31	0	0	1	0
32	0	0	1	0
33	1	0	1	0
34	1	1	1	0
35	1	1	1	0
36	1	1	1	0
37	1	1	1	0
38	0	1	1	0
39	0	1	1	0
40	0	0	1	0
41	1	1	1	0
42	1	1	1	0
43	1	1	1	0
44	1	1	1	0
45	1	1	0	0
46	0	0	1	0
47	0	0	1	0
48	0	0	0	0
49	0	0	1	0
50	0	0	1	0
51	1	1	0	0
52	0	0	1	0

53	0	0	1	0
54	0	0	1	0
55	0	0	1	0
56	0	1	1	0
57	0	1	1	0
58	0	0	1	0
59	0	1	1	0
60	0	0	1	0
61	0	1	1	1
62	0	0	0	0
63	1	0	1	0
64	1	0	1	0
65	0	0	0	0
66	0	0	1	0
67	0	0	1	0
68	1	1	1	0
69	1	1	1	0
70	0	0	1	0
71	0	0	1	0
72	0	0	1	0
73	1	1	1	0
74	0	0	1	0
75	0	0	1	0
76	0	0	1	0
77	1	1	1	0
78	0	0	1	0
79	0	1	1	0
80	1	1	1	0
81	1	0	1	0
82	0	0	1	1
83	0	0	1	0
84	0	0	1	0
85	1	1	1	1
86	1	1	1	0
87	0	0	1	0
88	0	0	1	0
89	0	0	1	0
90	0	0	1	0
91	0	0	1	0
92	0	0	1	0
93	0	0	1	0
94	0	0	1	0
95	1	1	1	0
96	1	1	1	0
97	1	1	1	1
98	0	0	0	1
99	1	1	1	1
100	1	1	1	1
101	1	1	1	0

CHAPTER 4

Pesticide residues in honey and honey quality in the Republic of Benin

Honey extraction materials of a local beekeeper in the district of Djidja.

CHAPTER 4

Pesticide residues in honey and honey quality in the Republic of Benin

Personal contribution: Methodology design
Honey samples collection
Data analysis
Writing the manuscript

Parts of this chapter are published in:

Amakpe F., De Smet L., Brunain M., Ravoet J., Jacobs F.J., Reybroeck W., Sinsin B., de Graaf D.C. (2016) Discovery of Lake Sinai virus and an unusual strain of acute bee paralysis virus in West African apiaries. *Apidologie* **47:** 35-47.

1. Abstract

Eight honey samples were collected from apiaries in regions of Benin where pesticides are intensively used on cotton crop. The samples were analysed for evaluating the honey quality and potential pesticide residues. The different honey quality parameters were determined by methods belonging to the list of harmonized methods of the International Honey Commission, The pesticide residues were determined by liquid and gas chromatography-mass spectrometry (LC-MS/MS & GC-MS/MS). This research indicated that all analysed honeys were free from the 293 targeted pesticide residues, and most samples complied with the European honey standards except for the honey of two samples with a too high HMF content, two samples with a too low diastase activity and one sample with a borderline result for the moisture content.

2. Introduction

One of the most important challenges of the beekeeping enterprise in a country is the acceptability of the hive products on the international market (OIE, 2009). In fact, food residue monitoring and quality control strategies are mostly applied on hive products which are disqualified for human consumption when they contain a residue above the defined maximum residue limit or level (MRL) (Regulation 37/2010; Regulation 396/2005) or when a quality parameter is out of the regulated ranges (Directive 2014/63/EU). For the European Commission, a country cannot be accepted for exporting hive products if its national quality and residue control plan is not operational and if the analytical methods are not sensitive and reliable to assure that the honey complies with the European standards (Council Directive 96/23/EC; Commission Decision 2004/432/EC; Commission Decision 2005/34/EC). As far as the pesticide residues in particular are concerned, the exporting country should clearly demonstrate and sustain that it is able to determine the residues at the required regulatory level especially the residues of prohibited substances such as carbamates, pyrethroids, organochlorine and organophosphorous compounds (Council Directive 96/23/EC). The knowledge about the presence of pesticide residues and the honey quality status in a country is as important as the other beekeeping factors. In fact, residues and quality are the final parameters which determine if the hive products can be consumed safely.

In the European, American and Asian countries, the origins of pesticide residues in honey are well-documented. According to Rosenkranz (2010), Reybroeck *et al.* (2012), Bogdanov (2006), honey pesticide residues originate from agricultural use of pesticides or from honeybee pest control. For the wholesalers, the residues may also come from bad post harvest systems such as contaminated containers which may alter the status of a product which was delivered residue free from the hive or by the small-scale beekeepers (Aghamirlou *et al.,* 2015 and Christodoulou *et al.*, 2015). In the Republic of Benin, where no chemical control is performed on the colony, the main potential contamination sources remain the crop protection uses, particularly from cotton crop on which pesticides are intensively used.

As far as the honey quality is concerned, the quality parameters vary in function of the geographic areas, the floral origins, the type of honey and the processing conditions (Bertelli *et al.*, 2010; Consonni *et al.*, 2013). They are also regulated by international standards (Directive 2014/63/EU; Van der Valk 2013; WHO & FAO, 1975; England Regulation, 2015) and help to distinguish genuine honeys from adulterated ones, to prevent false labelling and to ensure a healthy product for the consumer.

In opposition to the European countries where the pesticides residues and honey quality analyses are routinely monitored and well-documented (Hamilton *et al.*, 2004; Krakowska *et al.*, 2015; Bernal *et al.*, 2010 and Johnson *et al.*, 2014), almost nothing is known on the honeys of the Republic of Benin, which in consequence is not on the list of the accepted countries for exporting honey to the European Community market (Commission Implementing Decision (EU) 2015/1338). This research is aimed at filling this gap by evaluating specific honey quality parameters and monitoring potential pesticide residues in honey directly collected from the areas of the country which are most exposed to pesticides.

3. Methodology

3.1. Investigated sites

Cotton is the main annual cash crop in the Republic of Benin on which pesticides in the categories of herbicides, fungicides, insecticides and acaricides are used. From the period of 2005 to 2015,

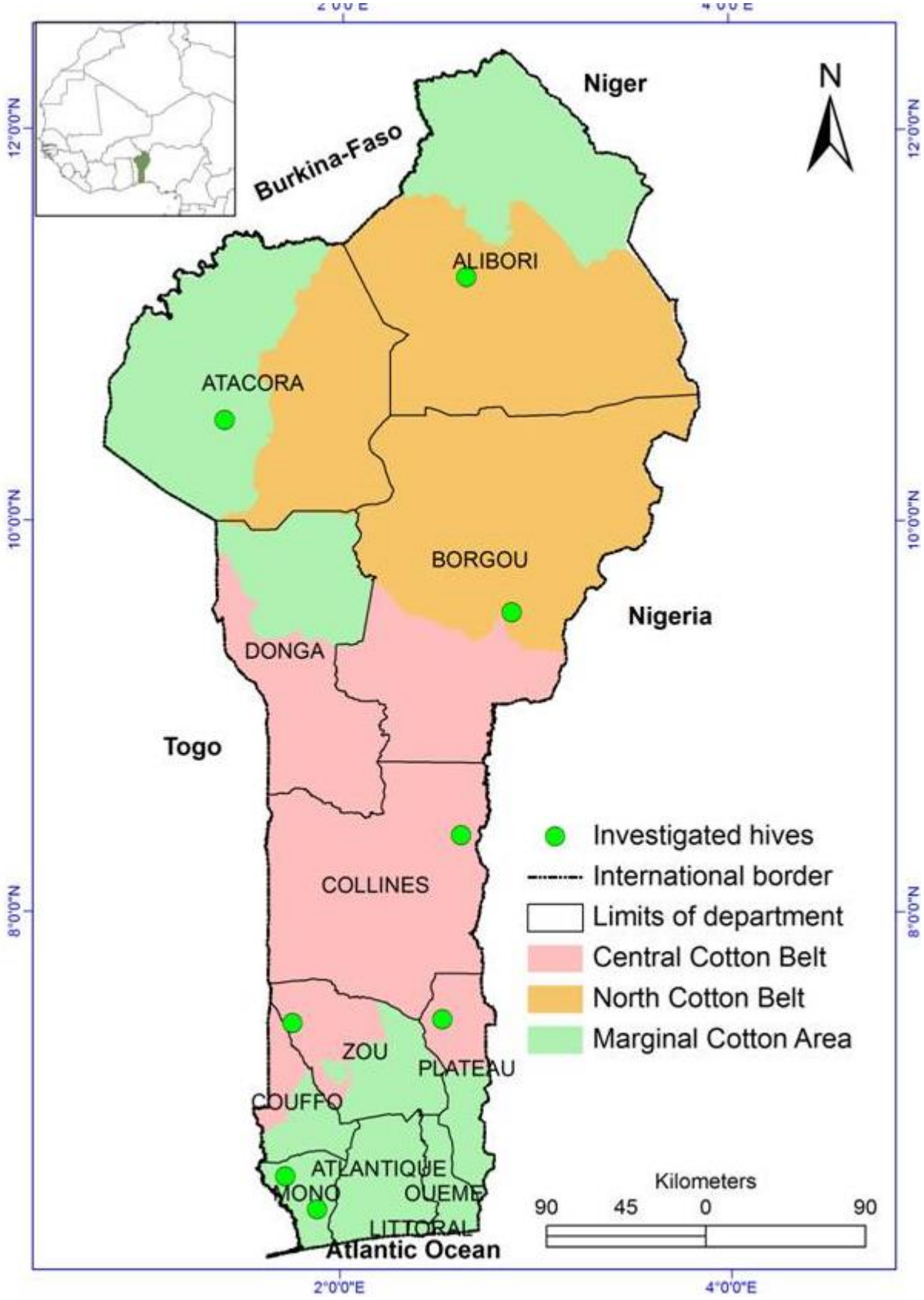

Figure 15: Location of the investigated apiaries for pesticide residue and honey quality analyses. The different cotton regions are obtained from MAEP (2001).

the “Comité National d’Agrément et de Contrôle des Produits Phytopharmaceutiques” (CNAC) authorised 64 pesticides containing up to 30 different active substances for use in the country (MAEP, 2010). We assumed that these pesticides may be found in honey samples from the different agro-regions where cotton is intensively cultivated. Eight honey samples from the two main regions of pesticide use were retained for multi-residue and honey quality analyses (MAEP, 2001):

- Three apiaries were selected from the south cotton belt region in the districts of Djidja, Ouessè and Kétou;
- Two apiaries were selected in the cotton belt of the north of the country in the districts of Banikoara and N’dali and
- Three apiaries were selected in the marginal cotton region in the districts of Toukountouna (in the north), Comè and Lokossa in the South (Figure 15).

The honeys were directly collected from an old honey comb of a hive at each apiary from April to May 2012 (honey flow period). The collected honey combs were directly put in jars of 0.25 l which were kept at ambient temperature till they were brought to the Flemish Institute for Agricultural and Fisheries Research (ILVO) for honey quality analysis and to Intertek Food Services GmbH (Bremen, Germany) for the pesticide residue analysis in February 2013.

3.2. Honey quality analysis

Honey is essentially made of sugars (fructose and glucose), organic acids, enzymes, and solid particles added by the honeybees. The colour and physico-chemical properties depend on the plant origins and the storage conditions (Reybroeck, 2014), and the different quality criteria are strictly regulated by international standards as indicated in table 3 and table 4 (Directive 2014/63/EU; Council Directive 2001/110/EC). The International Honey Commission has described harmonized methods suitable for assessing the honey quality (Bogdanov *et al.,* 2006; IHC, 2015). In accordance to these methods, five honey quality parameters were determined from the collected honeys at the Belgian Institute for Agricultural and Fisheries Research (ILVO):

- The moisture was determined by refractometry by means of an Abbé refractometer;

- The electrical conductivity (EC) was determined with an electrical conductivity cell and an electric conductivity meter;
- The specific rotation was measured in a clear, filtered aqueous honey solution by means of a polarimeter;
- The diastase activity (DA) was photocolorimetrically determined by a harmonized method with Phadebas tablets consisting of an insoluble blue dyed cross-linked type of starch as substrate. The substrate is hydrolysed by the enzyme, yielding blue water-soluble fragments, determined photometrically at 620 nm. The absorbance of the solution is directly proportional to the diastase activity of the sample.
- The HMF was determined based on a measurement of UV absorbance of HMF at 284 nm in a spectrophotometer. This harmonized method is based on the original work of White (IHC, 2015).

3.3. Pesticide residue analysis

The samples were tested on the presence of 293 carbamates, pyrethroids, organochlorine and organophosphorous compounds at Intertek Food Services GmbH (Bremen, Germany). Liquid chromatography-mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS) were used for searching for the different pesticides residues in the honey samples as indicated by Abaga *et al.* (2011) and Bogdanov (2006). The targeted pesticides of each method are summarised in table 7. The limit of quantification (LOQ) for most pesticides was 10 µg/kg with the exception of fenthion, iprovalicarb, pirimicarb, phosphamidon, oxydemeton-methyl (demeton-S-methyl sulfoxide) for which the LOQ was 20 µg/kg. For the LC-MS/MS analysis, 1 mL of the filtered extract was diluted to 5 mL in methanol and filtered through a 0.22 µm syringe filter. The GC-MS/MS analyses were performed on a gas chromatograph (GC), and ultra high purity helium (99.999%) was used as the carrier gas for separating the different targeted pesticides.

Table 7: Targeted pesticides from the two residue analysis methods.

Pesticides that can be identified by LC-MS/MS	**Pesticides that can be identified by GC-MS/MS**	**Pesticides that can be identified by both methods**
Abamectin; Acetamiprid; Aldicarb; Aldicarb sulfone; Aldicarb sulfoxide; Amitraz; Azoxystrobin; Benalaxyl; Bitertanol; Boscalid; Bromacil; Bromuconazole; Bupirimate; Buprofezin; Cadusafos; Carbaryl; Carbendazim; Carbofuran; Carbofuran (3-Hydroxy-); Chloroxuron; Clofentezine; Clomazone; Clothianidin; Cymiazole; Cyproconazole; Cyprodinil; Demeton-S-methyl; Demeton-S-methyl; Diethofencarb; Diethyltoluamid (DEET); Difenoconazole; Diflubenzuron; Dimethoate; Dimethomorph; Dimoxystrobin; Diniconazole; Diphenylamine; Disulfoton; Disulfoton-PS-sulfone; Disulfoton-PS-sulfoxide; Ditalimfos; Diuron; Dodine; EPN; Epoxiconazole; Ethiofencarb; Ethoprophos; Ethoxyquin; Famoxadone; Fenamiphos; Fenarimol; Fenazaquin; Fenbuconazole; Fenhexamid; Fenoxycarb; Fenpropimorph; Fenpyroximate; Fenthion; Fenthion-oxon; Fenthion-PO-sulfone; Fenthion-PS-sulfone; Fenthion-sulfoxide; Fluazifop-P-butyl; Fluazinam; Fludioxonil; Flufenoxuron; Fluquinconazole; Flusilazole; Fonofos; Hexaconazole; Hexythiazox; Imazalil; Imidacloprid; Indoxacarb; Iprovalicarb; Isofenphos; Isofenphos-methyl; Isoproturon; Kresoxim-methyl; Linuron; Lufenuron; Malaoxon; Malathion;	Aclonifen; Acrinathin; Alachlor; Aldrin; Benfluralin; Bifenthrin; Binapacryl; Bromophos (-methyl); Bromophos-ethyl; Bromopropylate; Captan; Carbophenothion; Chlordane (alpha-,cis-); Chlordane (Oxy); Chlordane (gamma-, trans-); Chlorfenapyr; Chlorfenson; Chlormephos; Chlorobenzilate; Chloroneb; Chloropropylate; Chlorothalonil; Chlorpropham; Chlorpyrifos (-ethyl); Chlorpyrifos-methyl; Chlorthal-dimethyl; Chlorthion; Chlorthiophos; Chlozolinate; Coumaphos; Cyanofenphos; Cyanophos; Cyfluthrin; Cyhalothrin (lambda-); Cypermethrin; DDD (o,p-); DDD (p,p-); DDE (o,p-); DDE (p,p-); DDT (o,p-); DDT (p,p-); Deltamethrin; Diazinon; Dichlobenil; Dichlofenthion; Dichlofluanid; Dicloran; Dicofol; Dieldrin; Endosulfan (alpha-); Endosulfan (beta-); Endosulfan-sulfat; Endrin; Esfenvalerate; Ethion; Etofenprox; Etridiazole; Etrimfos; Famphur; Fenchlorphos; Fenitrothion; Fenpropathrin; Fenson; Fensulfothion; Fenvalerate; Fluchloralin; Flucythrinate; Fluvalinate,; Folpet; Formothion; Halfenprox; HCH (alpha-); HCH (beta-); HCH (delta-); Heptachlor;	Acephate; Azinphos-ethyl; Azinphos-methyl; Chlorfenvinphos; Dichlorvos; Fipronil; Methamidophos

Mecarbam; Mepanipyrim; Mepronil; Metalaxyl; Metamitron; Metazachlor; Methiocarb; Methiocarb; Methiocarb; Methomyl; Methoxyfenozide; Metobromuron; Metolcarb; Metribuzin; Monolinuron; Myclobutanil; Nitenpyram; Nuarimol; Omethoate; Oxadixyl; Oxamyl; Oxydemeton-methyl; Penconazole; Pencycuron; Pirimicarb; Pirimicarb (Desmethyl-); Prochloraz; Propamocarb; Propargite; Propiconazole; Propoxur; Propyzamide; Pymetrozine; Pyraclostrobin; Pyridaben; Pyridaphenthion; Pyrifenox; Pyrimethanil; Pyriproxyfen; Quinoxyfen; Rotenone; Spinosad; Spirodiclofen; Spiromesifen; Spiroxamine; Tebuconazole; Tebufenozide; Tebufenpyrad; Teflubenzuron; Terbutylazine; Tetraconazole; Thiabendazole; Thiacloprid; Thiametoxam; Thiodicarb; Thiophanate-methyl; Triadimefon; Triadimenol; Trichlorfon; Trifloxystrobin; Triflumizole; Triforine	Heptachlor epoxide (cis-, exo-); Heptachlor epoxide (trans-, endo-;); Heptenophos; Hexachlorobenzene (HCB); Hexaflumuron; Iodofenphos; Iprobenfos; Iprodione; Isazofos; Isocarbofos; Isodrin; Isoxathion; Leptophos; Lindane (gamma-HCH); Methacrifos; Methidathion; Methoxychlor; Mevinphos; Mirex; Monocrotophos; Nitrapyrin; Nitrofen; o-Phenylphenol; Paraoxon-ethyl; Paraoxon-methyl; Parathion-ethyl; Parathion-methyl; Pendimethalin; Pentachloroaniline; Pentachloroanisole; Permethrin; Phenthoate; Phorate; Phorate-sulfone; Phosalone; Phosmet; Phosphamidon; Piperonyl butoxide; Pirimiphos-ethyl; Pirimiphos-methyl; Procymidone; Profenofos; Profluralin; Propetamphos; Prothiophos; Pyrazophos; Quinalphos; Quintozene; S 421 (octachlorodipropyl ether); Sulfotep; Sulprofos; Tecnazene; Tefluthrin; Terbufos; Tetrachlorvinphos; Tetramethrin; Tetradifon; Tetrasul; Thionazin; Tolclofos-methyl; Tolylfluanid; Triallate; Triazophos; Trichloronat; Trifluralin; Vinclozolin	

4. Results and discussion

Table 8 summarises the output of the different quality and residue analyses. With respect to the honey quality, the HMF of two samples (sample 14: 93.5 mg/kg; sample 85: 231.6 mg/kg) exceeded the European standard of 80 mg/kg for honeys from tropical regions. The diastase activity of two samples (sample 80: 6.1 U/g; sample 88: 2.7 U/g) was also below the standard of 8 U/g. Finally, the moisture content in sample 93 was 20.2 g/100 g, exceeding the general standard of 20g/100g. All other results were in line with the European honey quality legislation (Directive 2014/63/EU).

The high HMF and the low diastase activity in two honey samples could be explained by the long storage at room temperature (average of 32°C) between sampling and analysis. But the low diastase activity could also be caused by the fact that it concerns honeys with a low enzyme activity due to the botanical origin of the nectar. The high moisture content in one sample may be explained by the fact that the honeys were directly collected from the hive for research purposes without necessarily taking into account their ripeness at the moment of sample collection.

As far as the residues are concerned, all honey samples were free from residues of 293 different carbamates, organochlorine and organophosphorous pesticides. This means that the honey samples correspond to the Regulation (EC) 396/2005, as well as Regulation (EC) 470/2009 in conjunction with Commission Regulation (EU) 37/2010 on pesticide residues from apicultural use. These results could be explained by the fact that initial concentrations of pesticides in nectar are very low (Schur & Wallner, 2000), and that most of the pesticides used today are unstable and disintegrate quickly after use (Bogdanov, 2006). Furthermore, in the Republic of Benin, pesticides are mainly used on annual crops, and honeybees collect nectar from trees during the dry season when the usage of pesticides is limited, and the contamination probability from the foraging activity of the bees is very low. Finally, pesticides show more affinity to beeswax compared to honey (Ravoet *et al*., 2015d). According to Bogdanov (2006), pesticide contaminants in the honey could also originate from substances used for the bee pest control. The absence of residues in the honeys which were directly collected from the apiaries can then be the

consequence of the organic apiculture in the country where no beekeeper was found to use any pesticide or chemical in the colonies.

Table 8: Honey quality and pesticide residue analyses.
Underlined values were not in correspondence to the European honey quality legislation (Directive 2014/63/EU of the European Parliament and of the Council); HMF = hydroxylmethylfurfural.

Sample code, location	Cotton region	Moisture content (g/100 g)	Electrical conductivity (µs/cm)	Specific rotation	HMF-content (mg/kg)	Diastase activity (U/g)	Pesticides
9, Banikoara	North cotton belt	19.7	597	-12.5	23.9	9.7	Not found
14, Toukountouna	Poor cotton area (north)	17.0	1290	-10.0	93.5	10.4	Not found
98, Djidja	Central cotton belt	17.2	703	-10.5	36.5	9.7	Not found
60, Ouessè	Central cotton belt	19.7	609	-11.0	60.5	8.2	Not found
80, N'dali	Poor cotton belt (north)	17.9	656	-11.5	64.5	6.1	Not found
85, Lokossa	Poor cotton belt (south)	18.8	1178	-9.3	231.6	10.0	Not found
88, Comè	Poor cotton belt (south)	18.9	543	-12.0	76.2	2.7	Not found
93, Kétou	Central cotton belt	20.2	391	-11.5	17.7	10.2	Not found

5. Conclusion

This research indicated that honeys directly collected from the apiaries in the Republic of Benin are free of residues, and most of them comply with the international honey quality standards except for a sample with a borderline result for moisture; two samples with a too high HMF concentration and two samples with a too low diastase activity, most probably due to the long period between sampling and analysis. This is a great opportunity for the beekeeping enterprise in a country where no chemical is used in the colony and where there is a temporal separation between the agricultural pesticide use and the honey flow period. But these two important parameters are very dynamic, and the residue risk is still present especially from the centre to the north of the country where pesticides are widely used on cotton crop. The quality and residue status of the final blend-honey from local beekeepers for export are also determined by the collection and storage conditions, which may yield a final product of poorer quality.

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CHAPTER 5

The morphometric and genetic diversity of the native honeybee *Apis mellifera* in the Republic of Benin

Open air nest of *Apis mellifera* in *Anacardium accidentale* tree (district of Djidja).

CHAPTER 5

The morphometric and genetic diversity of the native honeybee *Apis mellifera* in the Republic of Benin

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Personal contribution:	Methodology design
	Bee samples collection
	Morphometric measures
	Data analysis
	Writing the manuscript

1. Abstract

Morphometric characteristics combined with genetic markers are powerful tools used for determining honeybee subspecies. Bees samples collected from 94 established apiaries distributed over the entire Republic of Benin were analyzed using 7 morphometric parameters and the COI-COII regions of mitochondrial DNA. Based on the morphometric data the native honeybees could be divided into three distinct ecotypes. The sequence analysis of the mitochondrial DNA COI-COII regions, indicated 3 new and clearly separated haplotypes. These haplotypes miss the typical P_0 region but they all possess a duplication or triplication of the Q region. Sequence analyses also confirmed that all haplotypes belonged to the A lineage and are closely related to the *adansonii*, *scutellata* and *iberiensis* races which represents respectively 66%, 27% and 7% of the honeybee population in the Repubic of Benin.

2. Introduction

According to Schmidt *et al.* (2010), Moritz *et al*. (2010) and Jaffe *et al*. (2009), beekeeping sustainability depends on the diversity in the honeybee populations as this reinforces their resistance to diseases and improves hive product yields. It is then important to evaluate such diversity in order to improve the apicultural performance in the Republic of Benin. At the African bio-geographic level, 11 races are commonly described (Ruttner, 1988). But other races could exist as well because all the adaptation factors of the continent have not yet been elucidated as demonstrated by Meixner *et al*. (2011). In the Republic of Benin, the honeybees have been classified in the *adansonii* race that dominates the West African region by Villière (1987) based on morphometric measurements. But since then, the studies of the honeybees have hardly evolved and are limited to key body parts measurements in isolate villages located in the centre and the north of the country (Hounkpe *et al.,* 2007; Amakpe, 2010; Paraïso *et al*., 2011).

Morphological characteristics are still considered very important methods in the classification of honeybees (Meixner *et al*., 2011; Farshineh *et al*., 2007; Fresnaye, 1981) and more than 40 parameters can be used. Among them, the wing length, the wing width, the cubital veins, the femur length, the basitarsus and the tibia lengths of workers are the easiest and non-biased quantitative parameters for the morphometric analyses (Francoy *et al.,* 2008; Farshineh *et al.*, 2007; Sheppardm & Smith, 2000; Marghitas *et al.,* 2008). On the other hand, this approach is not

well suited to characterize honeybees at subspecies level and to analyze phylogenetic relationships, because the morphometric parameters can be sensitive to environmental selection pressures, starvation and pathological status (Franck *et al.,* 2000; Miguel *et al.,* 2011; Ahmed *et al.,* 2012). A more accurate and reliable method is the use of the mtDNA COI-COII intergenic region which is the specific genetic marker for the genus *Apis* (Cornuet & Garnery, 1991; Garnery *et al.,* 1993; Bodur *et al.,* 2007). In fact, variations in the sequence of this region can be used for differentiating among the five honeybee lineages and to discriminate among *A. mellifera* subspecies (Garney *et al.*, 1992; Franck *et al.,* 2000b; Sheppard & Smith, 2000).

The objective of this research is to determine the morphometric and genetic variations of feral honeybee colonies in the Republic of Benin using morphometry and DNA sequencing analysis. This will help to determine *i*) the lineage of *Apis mellifera* in the country, *ii*) determine which races are present and *iii*) to analyse the different morphometric diversity of the honeybee.

3. Methodology

3.1. Sample collection

The study covered the entire Republic of Benin from the south to the north. Beekeeping in the country is mainly performed by small-scale peasants using traditional hives. All the colonies are obtained from natural swarms, which are directly captured by hive baiting. The studied bees were collected from 94 established apiaries distributed all over the country at an average distance of 40 km ranging from 5 km to 80 km (Figure 12). Each apiary consisted of 5 to 15 colonies of at least 6 months old obtained from natural swarms. For the bee collection, some 100 young bees were collected from the inside of one randomly chosen hive per apiary. The sampled bees were immediately put in 70% ethanol until the different morphometric measurements and genetic analyses occurred.

3.2. Morphometric study

3.2.1. Morphometric measurements

The morphometric measurements were performed on 20 bees selected at random from the 100 bees of each colony. The right fore wing and the right hind leg of each bee were accurately cut.

They were afterwards mounted and fixed on slides by being covered with a thin transparent adhesive plastic.

The bee parts were scanned using a micro numeric microscope camera CCMEX-1300 at a total 60x magnification as described in Rostecki (2007) and Quezada-Euan *et al*. (2007). The measurements were performed on the scanned digital images using the software image focus V3.0. The classical morphological traits used in this analysis included:

- On the right fore wing: the wing length (WL), the wing width (WW), the cubical veins "a" and "b";
- On the right hind leg: the femur length (FL), the tibia length (TL), and the basitarsus length (BL).
- In addition to these 7 measures, two parameters were calculated according to Marghitas *et al. (*2008) and Cornuet *et al. (*1975).
    - The total leg length per bee: TTL = FL+TL+BL
    - The cubital index per bee: CI = b/a

A good repeatability of the measuring methods was obtained by using exactly the same measuring tools (Microscopes, photo adapter, slides and adhesive cover), the same operator who performed the operation during the same period of the day as recommended by the British Standards Institution (1975), Bland & Altman (1986) and Ogbuehi & Osuagwu (2012). The repeatability was first tested and accepted ($r\% < 0.01$) by performing twice, the different measurements on two groups of ten bees from one colony.

3.2.2. Morpho-ecotype distinction

The morphometric data analysis was performed with R 3.0.3 software. The colony sample means and standard deviation were first calculated and were used as representative estimates for each colony.

The entire morphometric parameters were first submitted to a stepwise canonical discriminant analysis in order to identify the body parts that add the highest predictive power to the discriminant functions. The classification of the colonies into ecotypes was performed by the

cluster analysis based on the Euclidian distance and the Wards method (Bray *&* Curtis, 1957; Milligan, 1980; Shrestha & Kazama, 2007; Bhat *et al.*, 2014). This served to identify homogeneous colony clusters (groups) which were assimilated to the different honeybee morpho-ecotypes.

The colonies that made up each group of the discriminant analysis were given a specific symbology in the feature class after they were projected by their geographic coordinates in the country "shape file" by ArcGIS 9.02®. The distribution of the clusters plots in the country map was visually inspected and the concentration area of each symbology was assimilated to the corresponding ecotype region.

The south-north, west-east and topographic morphometric gradient analyses were performed by a multiple regression between the environmental parameters (Longitude, latitude, and elevation) and the body parts that best discriminate the colony groups (Meixner, 2011; Alattal *et al*., 2014; Ruttner, 1988).

3.3. Genetic study

3.3.1. DNA extraction

The same samples used in the morphometric analysis were also used in the genetic characterization. As the bees were preserved in ethanol, they were rinsed in PBS for 72 hours before the DNA extraction started. DNA extraction was performed like described by Garnery *et al.* (1991). Briefly, DNA was extracted by grinding one bee per colony in 0.1M TrisHCl (pH 8.0), 10 mM EDTA, 100 mMNaCl, 0.1% SDS, 50 mM DTT and 0.25 mg/ml proteinase K. Extracts were incubated for 4h at 37°C, then centrifuged for 5 min at 500G. Deproteination was performed with two phenol-chloroform extractions. DNA was precipitated with ethanol overnight at -20°C, pelleted (30 min at 18 000G), rinsed with 70% ethanol and after drying resuspended in 1ml of TE (10mMTris, 1mM EDTA, pH8).

3.3.2. PCR amplification and sequencing

The mtDNA region including the $tRNA^{leu}$ gene, the COI-COII intergenic region and the 5' end of the COII subunit gene was PCR-amplified using gene-specific primers (E2) 5'-GGCAGAATAAGTGCATTG-3' and (H2) 5'-CAATATCATTGATGACC-3' (Garnery *et al*., 1993). The 25

µl PCR reaction contained 2.5 µl 10x buffer, 2mM $MgCl_2$, 25 pM of primers E2 and H2, 25 nM of each dNTP and 1.25 units of HotStarTaq DNA Plus polymerase (Qiagen) and 1 µl of DNA extract as template. The temperature cycling protocol was set as follows: 2 min at 95°C; denaturation, 45 s at 95°C; annealing, 45 s at 54°C; extension, 1 min at 72°C, 35 cycles; final holding, 5 min at 72°C. A fraction of the PCR product was run on a 1% agarose gel as quality control and for total size determination. PCR products were sequenced in both directions by GATC biotech using a ABI 3730xl DNA genetic analyser (Applied Biosystem). Sequences were assembled using Vector NTI software. They were BLASTed and compared with the sequences available in the GenBank database (NCBI) and an *in silico Dra*I restriction analysis was performed using the Vector NTI software. The resulting restriction fragments were used to determine the different haplotypes (Garnery *et al.,* 1998).

3.3.3. Phylogenetic analyses

The analyses involved 70 nucleotide sequences and different reference sequences including all evolutionary lineages. Evolutionary analyses were conducted in MEGA7 (Kumar, 2016). DNA sequences were aligned using Muscle in the MEGA7 software. A phylogenetic tree was constructed using the Maximum Parsimony method (MP) on the alignment (Chouhan & Pardasani, 2008). Due to the large size variation in the COI-COII region of *A. mellifera* the MP analysis was unrooted, the gaps were treated as missing characters, and no outgroup taxa was used. Parsimony bootstrap analysis included 1000 replicates (Felsenstein, 1985) using the branch and bound algorithm. Branches corresponding to partitions that reproduced in less than 53% bootstrap replicates were collapsed. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 2 in which the initial trees were obtained by the random addition of sequences.

4. Results

4.1. Ecological gradient and honeybee morpho-ecotypes

As indicated in table 9, the stepwise discriminant analysis of the morphometric characteristics revealed that the total leg length, the wing length and the cubital vein “b” were the main morphometric measures that really discriminated the honeybee populations in the Republic of Benin ($P<0.001$ for the wing and the total leg length and $P<0.05$ for the Cubital vein “b”). As far

as the cubital index which is the most used morphometric parameter for distinguishing the honeybee population is concerned, the difference of the means of the cubital index of the different colonies was not significant (P=0.75), suggesting that all the bees could be classified in the same population.

Table 9: Stepwise discriminant analysis matrix of the morphometric parameters.

Morphometric characteristics	F statistics	P value	Wilks'lambda
Total leg length	116.03	<0.001	0.28
Wing length	15.85	<0.001	0.21
Cubital vein "b"	3.56	0.033	0.19
Wing width	0.18	0.833	-
Cubital vein "a"	0.28	0.757	-
Femur length	0.79	0.456	-
Tibia length	0.93	0.398	-
Basitarsus length	0.60	0.551	-
Cubital index	0.28	0.758	

The assessment of the effect of the geographic parameters (latitude, longitude and altitude) revealed that the bee total leg length and the wing length were highly determined by the latitude which respectively explained 29% (P<0.001) and 37% (P<0.001) of the total difference in the bee leg and wing lengths. In fact, the higher in latitude, the longer the bee legs and wings were, indicating a positive south-north gradient in the bee size. On the other hand, the hive longitudes did not influence the morphometric parameters (R=0.1 and P=0.1). This means that there is no east-west ecological gradient determinant in the bee parts. The different morphometric characteristics are neither correlated with the amplitude in the elevation (14 m in the south to 405 m in the north-west) of the inspected apiaries (R=0.25 and P>0.10).

The discriminant analysis of the morphometric parameters indicated that the honeybees belonged to three distinct morphometric groups (figure 16). The canonical function 1 explained 98.6% of the total variation and is determined by the total leg length and wing length. The canonical function 2 explained 1.4% of the total variation and is influenced by the cubital vein "b". The projection of the different groups of colonies in the shape file of the country indicated that they were distributed in three particular regions that determined the honeybee ecotypes of the Republic of Benin (figure 17).

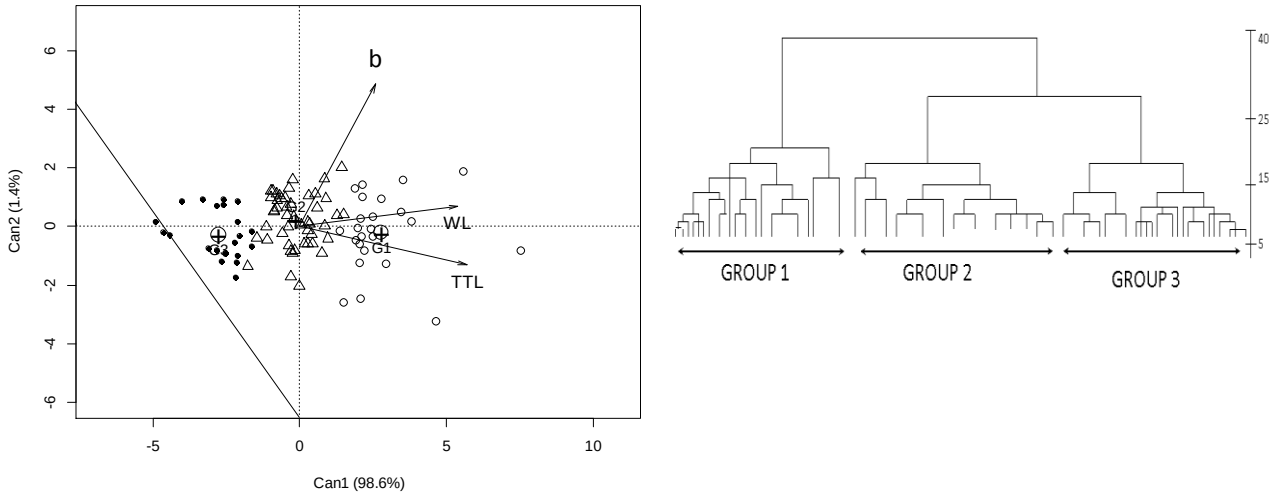

Figure 16: Distribution of colonies in the canonical axes (left) and the corresponding dendrogram (right).

Group 1 represented by blue dots in figure 17 corresponded to the Benino-dry-Tropical ecotype of Bees. Group 3 represented by red dots in figure 17 corresponded to the Benino-Soudano-Guinean ecotype. Group 2 represented by green squares in figure 17 are mixed with some colonies of the group 3 in the centre and corresponded to the Benino-Soudanian ecotype of bees. TTL: total leg length. b: cubital vein "b". Can: canonical axe.

The first group (G1) was made of colonies which were entirely located in the dry tropical climate area in the north beyond the 10th parallel and were characterised by high values of total leg length and wing length. They constitute the first morpho-ecotype, referred as the Benino-dry Tropical-ecotype (B-DTE) which covered a distribution area of 5,008,637 ha (44% of the country area) in the dry savannah. The hives that made up the third group (G3) were located in the south of the country, from the coast to the 7th parallel in the Soudano-Guinean climate. They are the Benino-soudano-guinean ecotype (B-SGE) which distribution area covered 849,30 ha (8% of the country area). They had the shortest legs and wings and are located in the south where the climate is Soudano-Guinean, and the vegetation is originally the Guineo-Congolian forest. Some rare colonies of this can be seen in relic forests in the central west. Between these two geographically opposite ecotypes, the colonies of the second group (G2) were scattered and mixed with colonies of G3 in the central part of the country. They have intermediate leg and wing length but some higher values of the Cubital vein "b". They made up the Benino-soudanian ecotype (B-SE) from the north of the region of Abomey (7th parallel) to the latitude of Bembereke (10th parallel). The ecological distribution of this type covered a large area of 5,404,259 ha (48% of the country area) in the Soudanian climatic zone with the woodlands as dominant vegetation. The morphometric parameters of each morpho-ecotype is summarised in table 10

The morpho-genetic diversity of the honeybees

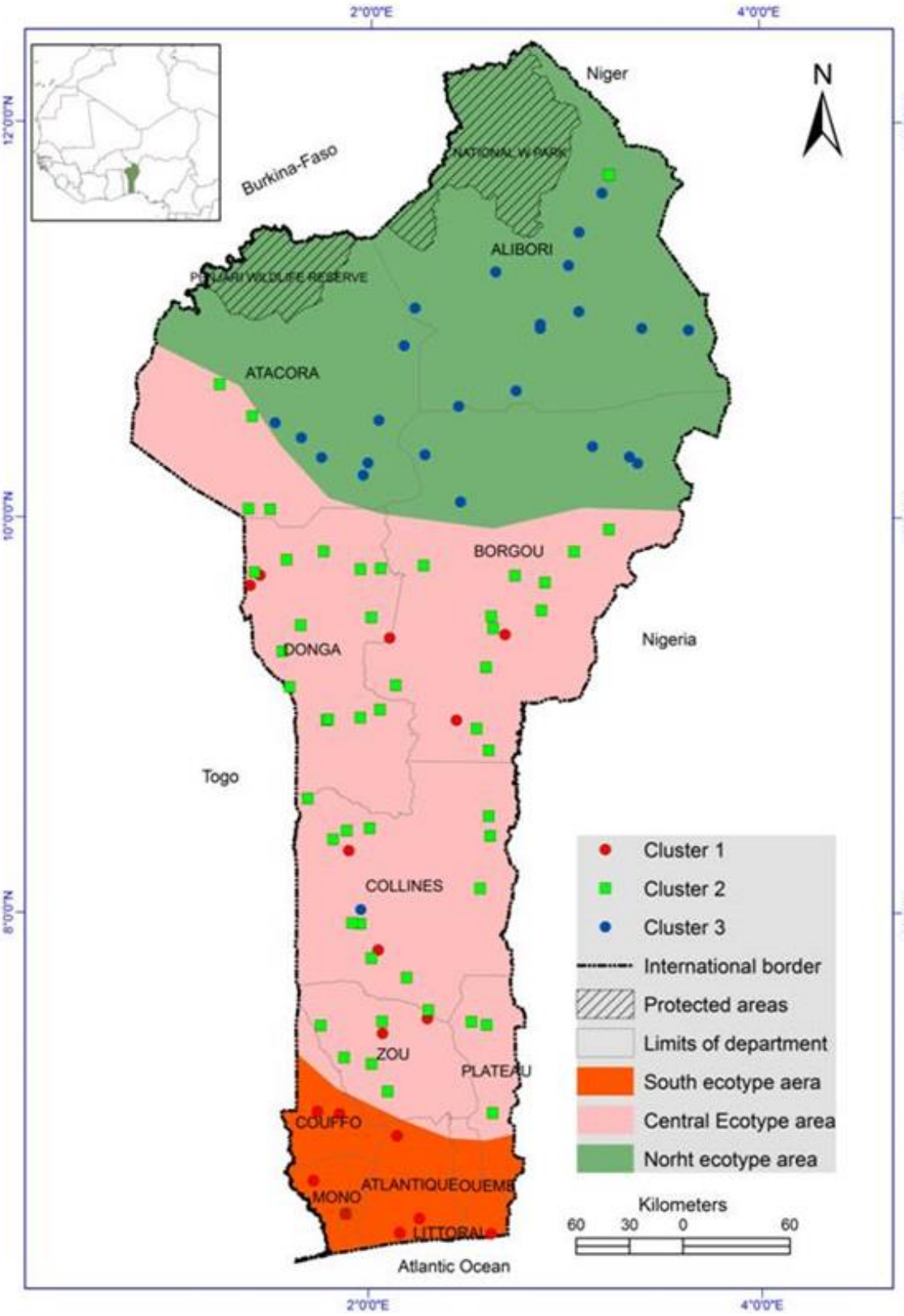

Figure 17: Spatial analysis of the bee ecotypes.

Table 10: Morphometric characteristics of the different bee ecotypes of Benin.
B-SGE Benino-soudano guinean ecotype. B-SE: Benino-soudanian ecotype
B-DTE: Benino-dry tropical ecotype
The morphometric parameters are in mm

Ecotype	Covered area		Morphometric parameter							
	Km²	%		Wing length	Wing width	Femur length	Tibia length	Basitarsus	Cubital index	Total leg length
B-DTE	8.493	8	Average	8.7	3.1	2.6	3.1	2.2	2.0	7.9
			STDV	0.1	0.1	0.1	0.1	0.1	0.3	0.2
B-SE	54.043	48	Average	8.5	3.0	2.5	3.0	2.2	2.1	7.7
			STDV	0.1	0.1	0.1	0.1	0.1	0.4	0.2
B-SGE	50.086	44	Average	8.2	2.9	2.4	2.9	2.1	2.1	7.4
			STDV	0.2	0.1	0.1	0.1	0.1	0.4	0.2
Benin	112.622	100	Average	8.5	3.0	2.5	2.3	2.2	2.1	7.7
			STDV	0.2	0.1	0.1	0.1	0.1	0.4	0.3

4.2. Honeybee haplotypes

Of the 70 *A. mellifera* samples for which the DNA sequencing analysis worked, 12 samples were found to have three new haplotypes (Axx, Axy, Axy'). All other sequences showed a characteristic *Dra*I restriction pattern that is typical for the A1, A4, A13 and A28 haplotype. The alignment of the new haplotypes with known sequences from the A lineage showed that they miss the P_0 sequence which was until now a typical pattern for the A lineage. The difference between the new haplotypes is the repetition of the Q sequences. Three samples possess two copies of the Q region while the nine other samples possess three Q region copies. One sample of the last group has an additional *Dra*I site. Missing the P_0 or P_1 element was until now a characteristic of the C lineage. However, the new haplotypes could not be mistaken for the C lineage because individuals of the C lineage have only one copy of the Q region.

Based on the *Dra*I restriction pattern 15 different haplotypes could be identified (Table 11). On the other hand, when considering the sequence variations (single nucleotide polymorphism, SNP), 22 haplotypes were distinguished. Three haplotypes are identical to previously described haplotypes (Acc. Ef033649.1; ef033650.1; gu326335.1). The other haplotypes were new.

Due to the limited amount of analyzed sequences it is difficult to study the distribution of the different haplotypes between the sampling regions. However, the newly identified haplotypes belong all to the *adansonii* race and were only detected in the north and central part of the country.

Table 11: Haplotypes based on fragment length and SNP of mt COI-COII intergenic region.

***Dra*I haplotype**	**Location**	**Fragment length**	**N**	**Haplotype percentage**	**# SNP in *Dra*I haplotype**
A1	C	47 108 483	26	37,14	
	N		1	1,43	4
	S		4	5,71	
A1 VARIANT 1	C	47 108 482	1	1,43	
	N		0	0	
	S		0	0	
A1 VARIANT 2	C	47 110 483	3	4,29	
	N		0	0	
	S		0	0	
A1 VARIANT 3	C	47 107 483	0	0	
	N		0	0	
	S		1	1,43	
A4	C	47 108 192 483	3	4,29	
	N		3	4,29	2
	S		1	1,43	
A4 VARIANT 1	C	47 108 191 483	2	2,86	
	N		0	0	
	S		0	0	
A4 VARIANT 2	C	47 108 193 483	1	1,43	
	N		0	0	
	S		0	0	
A4 VARIANT 3	C	47 108 192 486	2	2,86	
	N		4	5,71	3
	S		0	0	
A4 VARIANT 4	C	47 107 192 483	0	0	
	N		1	1,43	
	S		0	0	
A4 VARIANT 5	C	47 107 192 488	0	0	
	N		1	1,43	
	S		0	0	
A28	C	50 109 192 191 487	3	4,29	
	N		0	0	
	S		0	0	
A13	C	47 302 483	0	0	
	N		1	1,43	
	S		0	0	
QQ	C	47 40 191 484	3	4,29	
	N		0	0	
	S		0	0	
QQQ	C	47 40 192 192 483	2	2,86	
	N		6	8,57	
	S		0	0	
QQQ'	C	47 40 192 192 63 420	1	1,43	
	N		0	0	
	S		0	0	

The phylogenetic tree is used for grouping members of the same lineage together in the same branch. All analysed samples clustered together in the A lineage and are, based on the clustering, closely related to the *adansonii* (66%), *scutellata* (27%) and *iberiensis* (7%) races. Figure 18 shows the phylogenetic tree of all analysed samples together with reference strains belonging to the C, O, Y, M and A lineage. The newly identified haplotype is also clustering in the A lineage although it misses the typical P_0 region which is characteristic for the C lineage. The C lineage is missing the P_0 region but has only one copy of the Q region while the new haplotype possesses two or more copies from the Q region. The ecotypes determined based on morphometric data are grouping together although some exceptions are present.

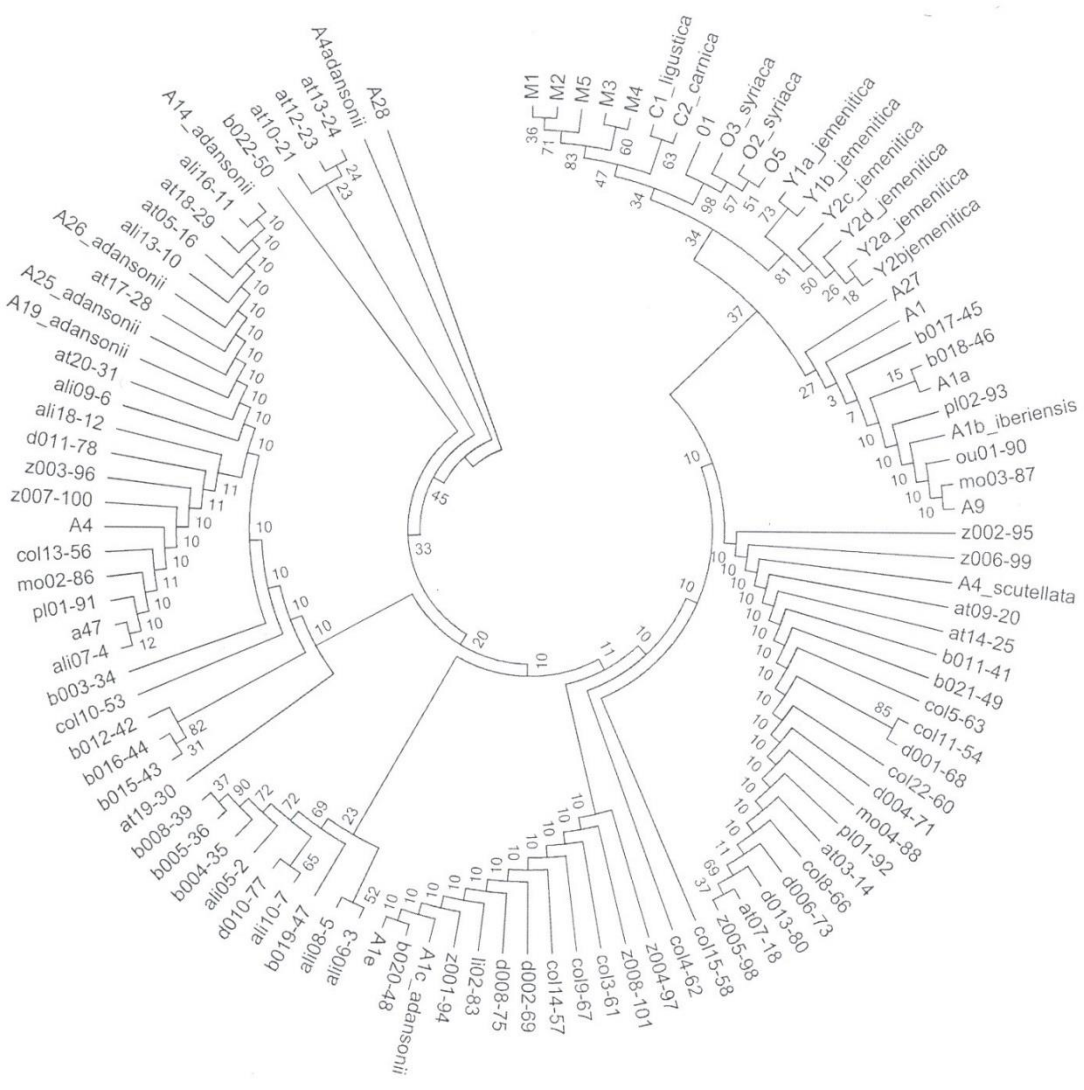

Figure 18: Maximum parsimony phylogenetic tree of all analysed samples with other *A. mellifera* haplotypes which represent all five lineages.

5. Discussion

5.1. Morphometric diversity in the honeybees

We found that the high amplitude of the wing length (7 mm-8.5 mm), the total leg length (6 mm - 7 mm) and of the cubital index (1.04-3.7) is in the same range as found by Amakpe (2010) and Paraizo *et al.* (2011) in the Republic of Benin for bees located in the north and the centre. According to Romet (2008) and Franck *et al. (*2000), the high amplitude in the Cubital index and other morphometric parameters is characteristic of the honeybees from the Democratic Republic of Congo to Senegal, which are all classified as the *adansonii* race (Ruttner, 1988). The distribution of the bees in the cubital index classes (figure 20) indicated that all three bee ecotypes of the Republic of Benin belong to the same bee population. But the 2,05±0.39 value of the Cubital index in the country (Amakpe, 2010; Paraïzo *et al.*, 2011; Hounkpe *et al.*, 2007) is far below the cubital index in Cameroon and the whole of Africa (Cornuet *et al.,* 1975; Romet, 2008; Ruttner, 1988). Based on the cubital index, our findings and the previous researches in the country suggested that the bees of the Republic of Benin may be considered as a mixture of different races or that the cubital index alone could not be used for distinguishing the honeybee populations in the country.

Though the three morpho-ecotypes had the same cubital index distribution shape, they are totally different as far as their body sizes are concerned. In fact, the smaller bees of the B-SGE are separated from the bigger bees of the B-DT by the intermediate size bees of the B-SE (figure 19). The highest diversity in the morphometric parameters (number of classes) was obtained in the central part. As stated by Louveaux *et al.* (1966), Borrel (2005), Danforth *et al.* (2013), Williams *et al. (*2010) and Danforth *et al. (*2006), this higher morphometric heterogeneity is the consequence of the interaction of the bee genetic diversity and the survival strategies which lead to anatomic and physiologic adaptations in order to cope with recurrent annual stresses such as food shortage, predators, diseases, bushfires and climatic hazards.

In regards to the ecological gradient impact on the bees, there is no significant west-east gradient on the bee parts as most biological parameters that do not change too much in the east-west direction in absence of important topographic barriers (Borcard *et al.,* 1992; McKinney, 1997). We also found that the elevation was neither correlated to the bee sizes in the Republic of Benin. These may partially be explained by the fact that the country has very low topographic amplitude

and the east-west distance is too small to lead to measurable diversities in the size of the African honeybee (Farshineh *et al.*, 2007; Romet, 2008).

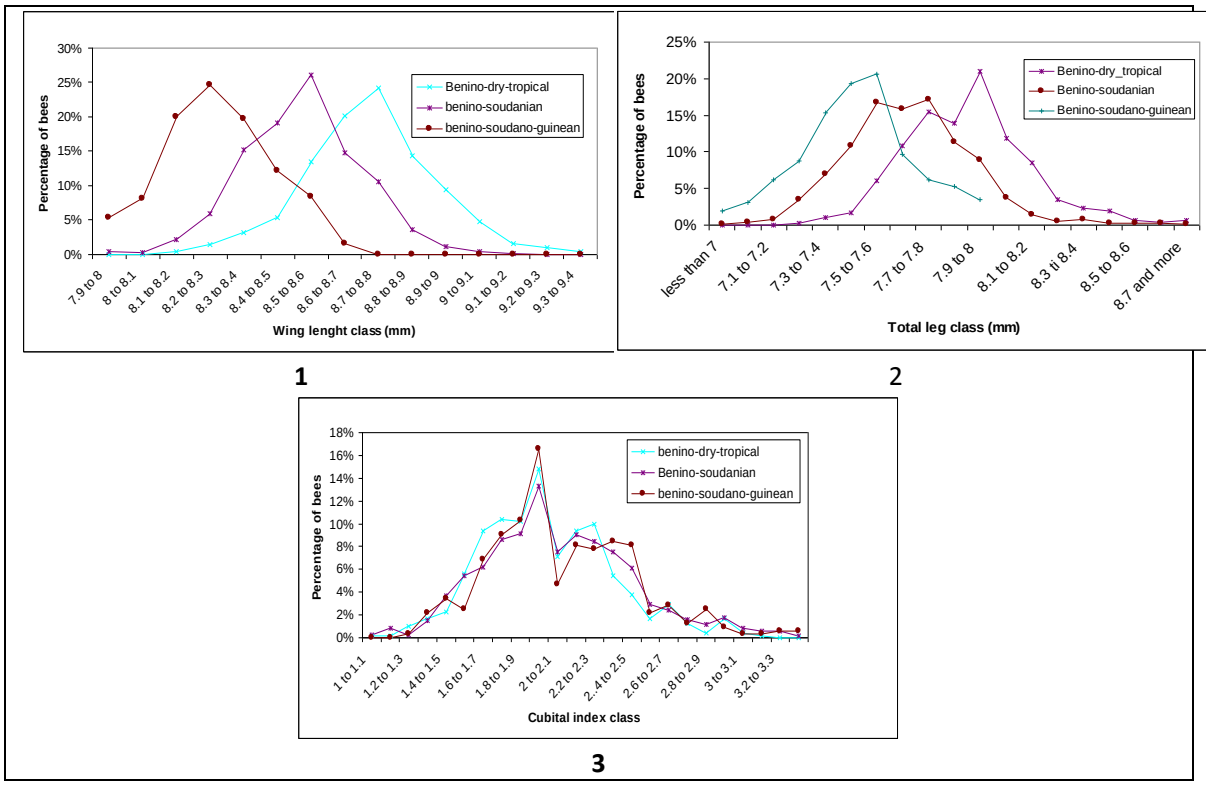

Figure 19: Morphometric structure of the different bee ecotypes.
1. Wing length; 2. Total leg length and 3. Cubical index.

5.2. Genetic diversity in the honeybees

All analyzed honeybees from the Republic of Benin belong to the A lineage. This is in correspondence with Franck *et al.* (2001) and Wallberg *et al.* (2014) who determined that all honeybees in that part of Africa, including *adansonii*, *scutellata* and *capensis* races belong to the mitotypes of lineage A. Our finding also confirms that honeybees from north-western Africa and from tropical and southern Africa highly diverge when mitochondrial and microsatellite data are considered (Franck *et al.,* 2001). In the same line, this research proved that *adansonii* is the widespread bee race in West Africa where it represents more than 66% of the bee population in the Republic of Benin. But the finding of *iberiensis* race (7% of the bee population) is not in line of expectation as this European honeybee is normally distributed in the Iberian Peninsula and originally belong to the M lineage (Rutter *et al.,* 1978; Franck *et al.,* 1998; Garnery *et al.,* 1995). The A linage of the *iberiensis* in this research, supports that it is not originated from a queen introduction from European beekeepers. It is then an African native *iberiensis* race that had not

been discovered earlier because of the low population density. Till now, the distribution area of the *scutellata* race is the southern Africa (Ruttner *et al.,* 1978) and it was also not expected that this could be found in the Republic of Benin. But for more than 27% of the honey bee population is it really a common bee race in West Africa which may come from the high migration capacity of the African honeybee and a probable queen introduction from the southern part of Africa.

All haplotypes have been fully characterized by sequencing, which explains the high number of different haplotypes/variants based on the *Dra*I restriction pattern. Restriction fragments of many variants differ only in 1 nucleotide which would not be recognized by RFLP typing and can in fact be grouped under the SNP variations. These results indicate that using the *Dra*I restriction pattern is not sufficient to explore the variation among samples and that sequencing is necessary for the complete exploration of variation like suggested previously (Cornuet & Garnery, 1991; Magnus *et al.*, 2011). Furthermore, we found three novel mtDNA haplotypes which were grouped in the A lineage based on the phylogenetic study. The identification of new haplotypes increases as found by Garnery (1995) the diversity within the A evolutionary lineage where some samples showed a duplication and a triplication of the Q segment. But in opposition to former studies (Alburaki *et al.,* 2011; Garnery*, et al.,* 1995) the A lineage of the country is missing the P_0 segment in the intergenic region, which is characteristic for the C lineage. These results suggest that may be a new lineage of the *Apis mellifera* is present in West Africa.

A comparison of the genetic methods and the morphometry indicated that there is good correspondence between the morpho-ecotypes and the races distribution areas in the Republic of Benin (figure 20). In fact, the B-DTE is made up by a pure population of *adansonii* in the north of the country, the B-SE is a mix of *scutellata, adansonii* and *iberiensis* races in the central part while the B-SGE ecotype is mainly made up of the *iberiensis* and the *adansonii* races in the southern part of the country. The uniformity in the morphometry of the honeybee in each ecological area regardless their race can then be considered as the results of specific natural selection forces which shape the phenotype of the bee that had highly diversified genetic origin (Ruttner, 1988; Sheppard *et al.,* 1991; Oldroyd *et al.,* 1994).

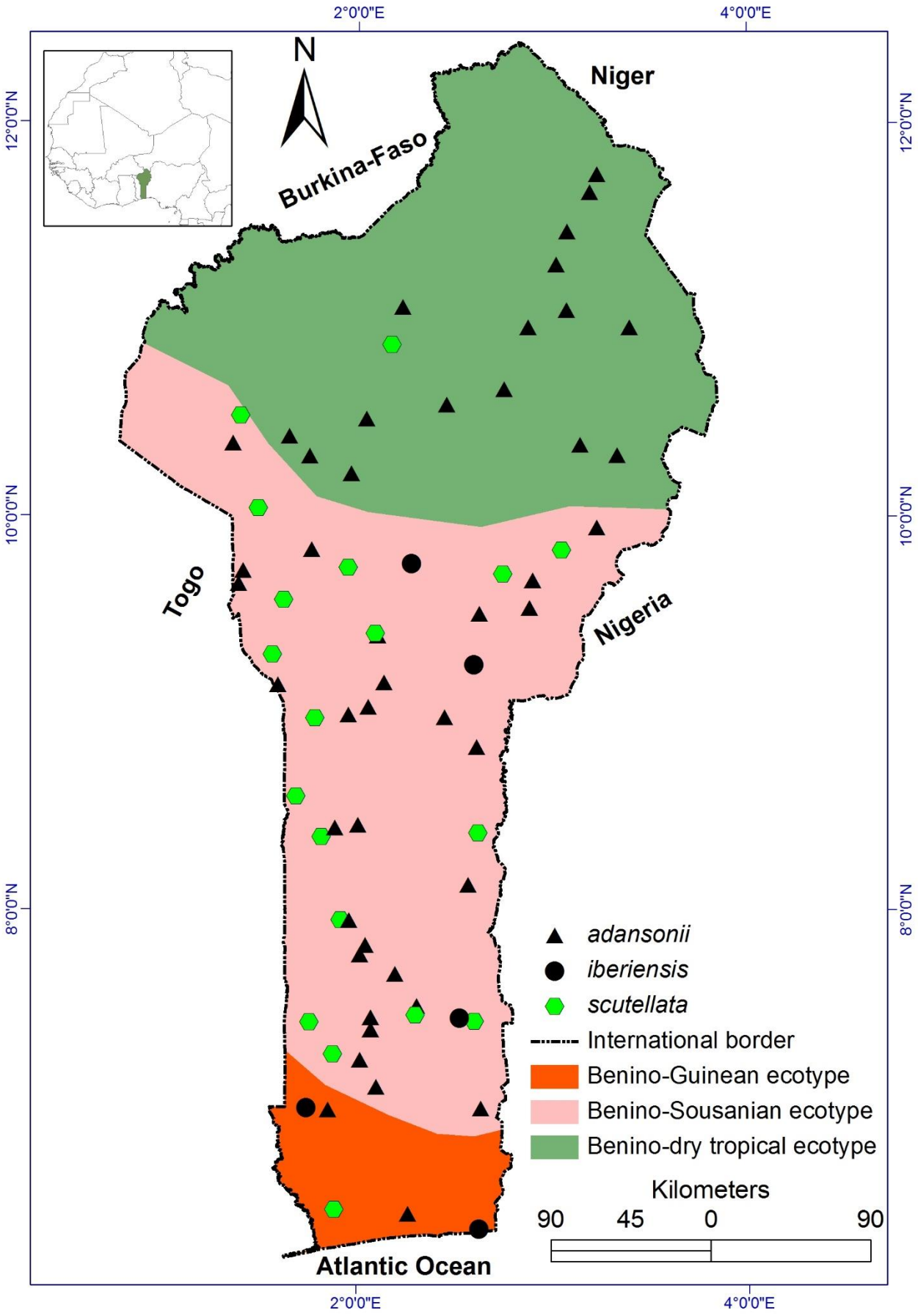

Figure 20: Morphometric and genetic map of the honeybees in the Republic of Benin.

The bigger size of the bees in the north may partially be explained by, as stated by Adger *et al.* (2005) and Le Conte & Navajas (2008), the fact that the honeybees always adjust their adaptive behaviour to the prevailing ecological conditions and stay more active and fly longer distances in open areas compared to closed forests making the forest ecotypes smaller than those of open lands (McKinney, 1997; Henle, *et al* 2004; Becher, 2010). This also may be explained by the *adansonii* race that determines the mean of the morphometric parameter per area. Being bigger than the other race, the mean of each morphometric parameter in an ecological area may be influenced by the percentage of the *adansonii* race in the bee population of the considered area.

5.3. Honeybee diversity and implication for beekeeping potential

Beekeeping in the Republic of Benin is based on three races in opposition to the common belief that *adansonii* is the only one race available in West Africa (Ruttner, 1988; Paraïso, 2011). The southern and the central parts of the country can be considered as a hybridization area of the three races and have the highest genetic diversity in the bee population. On the other hand, beekeeping in the north of the country is only based on a morphometrically uniform population of the *adansonii* race. With this lower genetic diversity in the north, the bee in that part could be more vulnerable to disease than the population of the south and centre. But as found elsewhere (Amakpe *et al.* 2015), the north has the lowest bee pest pressure where viral disease is also absent, this may be due to the high hygienic and defensive behaviour of the *adansonii* race which can contribute to a higher resistance to diseases (Thomas *et al.,* 2004; Williams *et al.,* 2010; Moritz *et al.*, 2010; Jaffe *et al.,* 2009).

6. Conclusion

Beekeeping in the Republic of Benin relies on three main morphometric and genetic bee populations distributed in the different ecological areas. The north is made up of the pure population of *adansonii* race which is the B-DTE. The south and the centre of the country are dominated by the B-SGE and B-SE complex where *adansonii*, *sctutellata* and *iberiensis* races coexist. The research results indicated that morphometry can perfectly be used to determine the ecological diversity. But as the morphometric parameters are influenced by the prevailing environmental conditions, only genetic methods could be used for determining the bee races and their evolutionary lineage.

Acknowledgements

This research is made possible by a funding from the International Foundation for Science (IFS) that we really thank. We also thank the NGO Cercle Nature et Developpement (CENAD-NGO) and the Centre Intégré d'Apiculture Tropical (CIAT-NGO) who helped in training the beekeepers and establishing the apiaries all over the country.

The morpho-genetic diversity of the honeybees

CHAPTER 6

Discussion and conclusion

Beekeepers of the village Kaodji, district of Covè during a training section.

CHAPTER 6

Discussion and conclusion

1. Discussion

1.1. Melliferous trees in the Republic of Benin

Beekeeping in the Republic of Benin is mainly based on perennial trees which are also globally well balanced in nectar and pollen plants with the exception of the south where the flora is dominated by pollen producing trees of industrial plantations of *Tectona grandis* and *Gmelina arborea*. But, the climatic conditions may negatively impact the melliferous plants availability and require specific management skills from the beekeepers. In fact, during the rainy season where the bees are less active (Cooper *&* Schaffer, 1985; Bishop *&* Armbruster, 1999), the melliferous resources are too much diluted or washed by heavy rainfalls. This creates a dearth period where artificial feeding is needed in order to keep the colonies strong. But due to lack of appropriate knowledge, beekeeping in the country is considered as a scavenging activity which benefits by no care from the beekeepers and many colonies are lost in the rainy seasons.

In the dry season where the bees are more active, many melliferous plants are destroyed both by bushfires and by being pruned by herders as fodder (Adjare, 1990; Bufford & Gaoue, 2015). This leads to limited blossom availability to the bees when compared to the summer periods in the Western apiculture (Hepburn & Radolff, 1998). As it is almost impossible to prevent bushfires in the country, it is necessary to establish a global fire management system to reduce the fire impact on the melliferous trees in dry season (Attuquayefio & Wuver 2003; Linsoussi, 2011). Tree pruning is also a West African problem which cannot be controlled by local beekeepers. However, the melliferous flora can be improved by targeting the reforestation programs on melliferous trees such as *Pterocarpus erinaceus*, *Khaya senegalensis* and *Afzelia Africana* which are pruned nationwide in the dry season.

1.2. Pathogenic environment

The bee pathosphere of a certain region is made up of all the pathogens (fungi, bacteria, viruses, protozoa and arthropods) which are known to disease the honeybee. In consideration to this, the honeybee pathosphere of the Republic of Benin seems to contain only *Varroa* and viral diseases.

In this pathosphere, *Varroa* is nationwide distributed while viral diseases are concentrated in the hybridization areas of *adansonii, scutellata* and *iberiensis* races in south and the centre. The

northern part, which is dominated by adansonii race, is free of viral disease although the absence of honeybee viruses is not common in areas of high *Varroa* and SHB infestation (Navajas *et al.*, 2008; Eyer *et al.,* 2009).

The small hive beetle (*Aethina tumida*), the big hive beetle (*Oplostomus fuligineus)*, wax moths (*Galleria Mollonella*) and other predators, which are widespread in the country, are not considered as part of the bee pathosphere as they are not real hosted organisms and have no immune influence on the honeybee (Ravoet, 2015c; Fries *et al*., 1996; Anderson & Morgan, 2007; Runckel *et al.,* 2011; Schwarz *et al*., 2015). They are also the main reason of brood disease scarcity as they destroy weak colonies and the remaining combs of abandoned hives (Strauss *et al*., 2015; Mumoki *et al*., 2014). But *A. tumida* is an OIE regulated pest which, in association with wax moth depreciates honey combs during storage (Bradbear, 2010; Hilmi *et al*., 2012). The restrictive definition of the bee pathosphere in Benin could then be extended to a particular pest environment which is made up of *Varroa*, viruses, beetles and predators. All these pests require appropriate control by the beekeepers in order to have safe and marketable hive products.

1.3. Honey quality and residues

The absence of pesticide residues and contaminant in honey nationwide is a great opportunity for the beekeeping potential as this is the prerequisite for exporting hive products (Bradbear, 2010; CODEX Alimentarius, 2001). As far as the honey quality is concerned, most analysed honeys also comply with the international standards. Though these important conditions are met on collected honeys from the apiaries, the quality and residue parameters are dynamic and are assessed on the blend of honeys ready for export. In the cotton crop areas, there is still a high residue risk as the consequence of intensive and uncontrolled pesticide use. The honey in these areas is likely to be depreciated by pesticide residues from water pollution, and residual products on melliferous plants or from contaminated containers (Bogdanov, 2006; Christodoulou, 2015).

In order to maintain residue free and of good quality the hive products, the beekeepers must be well-trained on international quality, on residues standards and on the post harvest system. For the residues in particular, more commitment should be obtained from the central government and the civil society for setting internal regulations which will prevent abuse of chemicals. But till now, practical actions to integrate beekeeping in the different strategies for agricultural

development are lacking from the government. In fact, most agricultural development strategies in the country are targeted at industrial crops dominated by cotton on which pesticides are intensively used (INSAE, 2015).

1.4. Honeybee morphometric and genetic diversity

In the Republic of Benin, the honeybee genetic diversity is made up of three bee races (*adansonii, scutellata and iberiensis*). These races are adapted to the different climatic condition of the country as three morpho-ecotypes. The central and the southern parts bear a mixture of the three bee ecotypes and races and have the highest biodiversity potential available for improving the apicultural performance such as resistance to disease, aggressiveness and hive product yields. The northern part is characterised by one particular morpho-ecotype of the adansonii race. In regards to the high homogeneity of the bee populations in the north, the bees in this area may be more vulnerable to the environmental hazards and diseases (Hepburn & Radolff, 1998). But this research indicated that the adansonii race (B-DE) is free of virus while there is no difference in the SHB and *Varroa* prevalence between the different honey bee races and the corresponding ecotypes.

1.5. Beekeeping potential in the Republic of Benin

In regards to the melliferous plant availability, the pest status, the residues risk and the honeybee diversity, the regions where beekeeping can best be promoted stands from the north of the district of Abomey (7th parallel) to the north of the district of Bembèrèkè (11th parallel). This area covers the west, the central north and the central silvo-melliferous regions which have the best melliferous flora. The problems of these regions lie in the potential risks of pesticide residues, bushfire and tree pruning by herders in the dry season. In addition to these factors which are likely to negatively impact the melliferous plants and depreciate the honey quality, these regions also have the highest viral prevalence. As the potential impacts of bee viruses are not known in the country, the pest environment in these areas is probably of greater concerns than the one of the north where viral disease is absent. The south of the country (from the coast to the 7th parallel) is characterized by a poor melliferous flora in a too humid and cloudy climate. Beekeeping in this area will stay less profitable to the beekeeper even if the managerial skills are

improved to the same standards to the one the other regions. Figure 21 summarizes the geographic analysis of the beekeeping potential in the Republic of Benin.

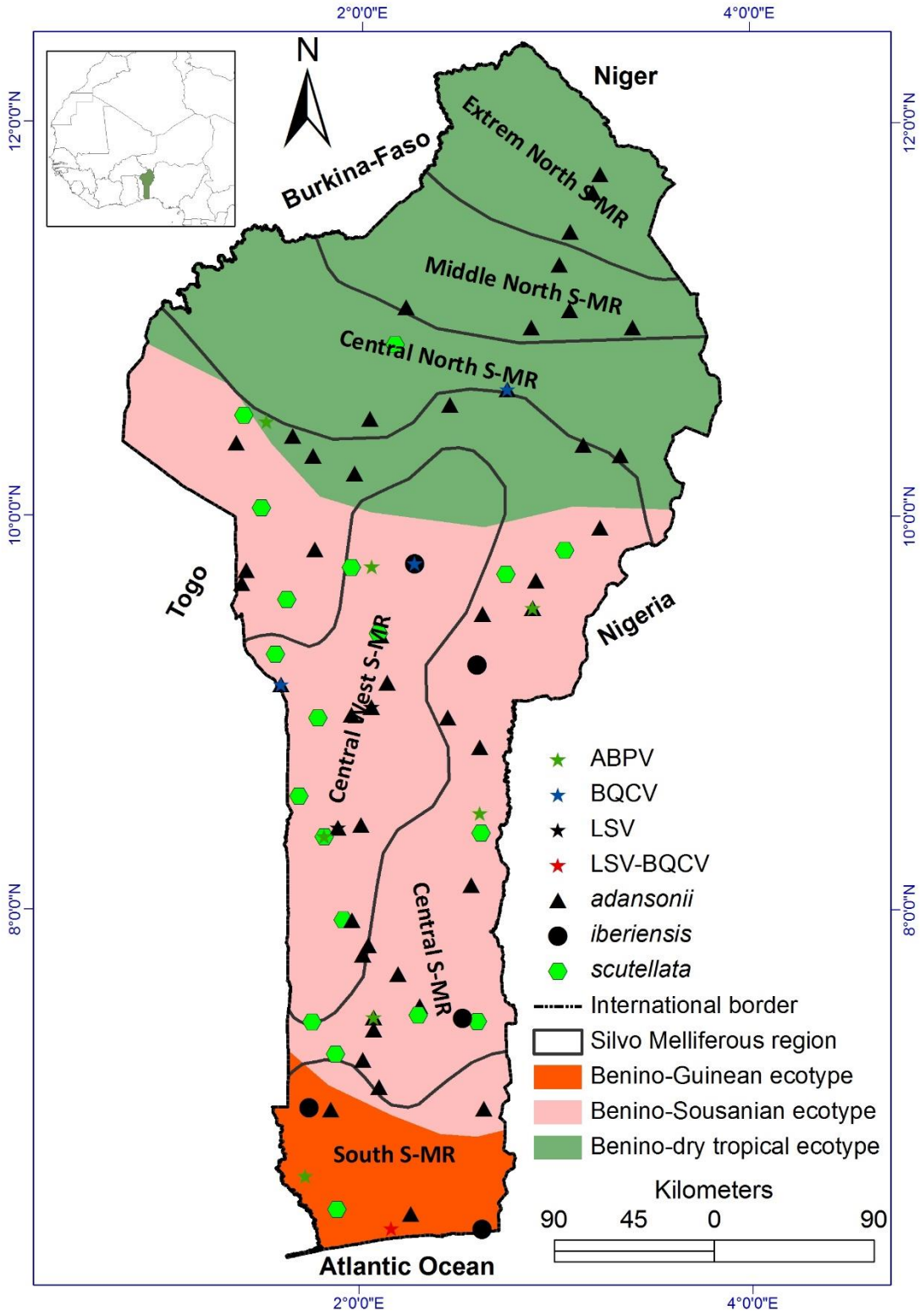

Figure 21: Geographic analysis of the beekeeping potential in the Republic of Benin.
S-MR = silvo-melliferous region; see chapter 2.

Table 12: Strengths, weaknesses, opportunities and threats analysis of the beekeeping regions.

Region	Strengths	Weaknesses	Opportunities	Threats
South Region	Very low pesticide residue risk; bear three bee races	Too much air humidity; very poor in melliferous trees, poor beekeeping skills; high *Varroa* and SHB pressure.	Available market for hive product (high population density); low bushfire risk	Honeybee disappearance from population growth
Central Region	High diversity of the bee ecotypes and races (three races and three ecotypes); good beekeeping skills.	High *Varroa* and SHB pressure	Available market for hive products	Tree pruning by herders in dry season; bushfire; pesticide residues risk
Central west Region	Good beekeeping skills; good melliferous plant availability; two bee races (*adansonii* and *scutellata)*	High *Varroa* and SHB pressure; viral prevalence	Available market for hive products; traditional beekeeping habits	Tree pruning by herders in dry season; bushfire; pesticide residues risk
Central North Region	Very low viral prevalence; good Beekeeping skills	High *Varroa* and SHB pressure; only one bee ecotype and one race (*adansonii*)	Available market for hive products; traditional beekeeping habit	Pesticide residues risk; bushfires; tree pruning by herders in dry season
Middle North Region	No viral disease; low pesticide residue risk	Low melliferous tree density; high *Varroa* and SHB pressure; only one bee ecotype and one race (*adansonii*)	Traditional beekeeping habits; available market for hive products	Area submitted to desertification; pesticide residues risk; bushfire
Extreme North Region	No viral disease; low pesticide residue risk	Low melliferous plant density; only one bee ecotype and one bee race (*adansonii*)	Available market for hive products; traditional beekeeping habits	Area submitted to desertification; tree pruning by herders in dry season

2. Conclusion and recommendations

Beekeeping in the Republic of Benin is based on three races which are distributed in three morpho-ecotypes by the adaptation to the environmental factors of the different ecological areas. A comparison of the different regions indicated that the best beekeeping regions stand from the north of the district of Abomey (7° North) to the north of Bembèrèkè (11° North). These regions bear the highest genetic and morphometric diversity in the honeybee population. They also have the best melliferous plants density which is well-balanced in nectar and pollen sources. This research found that there is no pesticide residue in the honeys which are of good quality at the apiary. The country is also free of the main OIE pests, such as American foul brood, the European foul brood, and nosemosis. *Varroa* and small hive beetle are the most important pests

that the beekeepers can control by adequate integrated pest management to reduce residue contamination risks.

As a whole, beekeeping can really be used as a sustainable income source in the different strategies of poverty alleviation in the Republic of Benin. But all these factors which make up the beekeeping potential of the country are dynamic, and are influenced by the climatic conditions, the managerial skills, the socio-economic and politic conditions. The potential of each region may be more valued for the community wellbeing by implementing the following actions.

- In the Republic of Benin, shifting cultivation is the widespread cropping system, especially from the centre to the north. In these areas, there is a real threat of shortage of melliferous resources as a consequence of the conversion of the entire flora into plantations or annual crops of limited melliferous value (Al-Ghamdi, 2014). It is then very important that the different agricultural extension programmes focus on improving the melliferous flora by saving an acceptable number of melliferous trees per ha, encouraging reforestation and replacing the shifting cultivation by a sedentary agriculture in which farmers will not open new land every year.

- The central parts of the country (central-west, central-north, middle-north and the central regions) have been considered the best beekeeping areas for a long time (MAEP, 2001), and many extension services and institutions have concentrated their interventions on these areas in order to have an immediately measurable impact. In this process, the south and the extreme north have been downgraded to secondary zones and the south is still far behind. Having less contact with managed apiaries, most beekeepers in these two areas are innovators of poor managerial skill. Beekeeping development then needs a well-designed extension programme that will strengthen the technical capacities of beekeepers all over the country. In order to get the best benefit from the beekeeping potentials of each region, deep appraisals of the involved actors and the socio-economic environment are also necessary to analyse the specific conditions required for a real participation of the community;

- Beekeeping in the country is basically considered a niche activity performed by isolated peasants with no formal organisation. The development of this activity needs a stronger

involvement from the government and the civil society for adding value to the hive products and establishing an effective beekeeping line. This implies i) setting regulations related to choosing and applying pesticides that protect the bees, ii) changing the shifting cultivation and transhumance to a sedentary agriculture and husbandry and iii) setting an operational residue control and pest management plan as the prerequisite for the acceptance of the hive products on the international markets. The government and the civil society implication is also necessary in the establishment of a fire management plan and in targeting melliferous trees in the different reforestation programmes in order to improve the melliferous tree density.

3. Future perspectives

Four main remarks about the future arise from the outcomes of this study.

- The research is limited to perennial plants which make up the bulk of the pollen and nectar sources in the tropical areas. But many other plants are visited by the bees in the country. These plants are made up of weeds, shrubs and trees of less than 10 cm of diameter, climbing plants and annual crop which also produce nectar and pollen for the honeybees. The bees also collect honeydew and specific researches could also target on the honeydew plants. Evaluating the contribution of all these plants will help determine the real melliferous plant potential of the country.

- One of the most important constraints to beekeeping in the country is the natural swarms on which it relies. Artificial queen rearing should then be introduced in order to take more advantage of the beekeeping potential of each region. But the success of artificial queen rearing depends on many factors such as the bee ethology, the pest impacts and the climate which also determine the establishment of the artificial nucleus. Thorough research is then needed on the artificial queen rearing in the country in order to establish a beekeeping system which no longer depends on the unreliable natural swarm.

- Despite of the heavy *Varroa* and SHB infestation in the entire country, viral disease prevalence is very low. This research also found Lake Sinai virus in African apiaries and a new ABPV strain for the first time. More investigations need then to be done on the low viral prevalence in a country characterized by a high *Varroa* infestation. The new ABPV

strain also needs more investigations and the intrusion of the Lake Sinai virus in the Republic of Benin must be elucidated.

- The P_0 segment is normally present in the A lineage of honeybee and absent in the C lineage. But this research found that the A lineage to which all the honeybees of the Republic of Benin belong misses this segment. In the same line, we also found that the *iberiensis* race in this area of West Africa belongs to the A lineage and is not originated from a recent queen introduction from the Mediterranean or European beekeepers. In-depth genetic analyses on the honeybees of the country are then needed in order to elucidate the particularity of the A lineage and the origin of the *iberiensis* race in the West African honeybee populations.

REFERENCE LIST

Abadi Ghadim A. K. & Pannel D. J. 1999 A conceptual framework of adoption of an agricultural innovation. Agricultural Economics **21:** 145-154

Abaga N. O. Z., Alibert P., Dousset S., Savadogo P. W., Savadogo M., Sedogo M. 2011 Insecticide residues in cotton soils of Burkina Faso and effects of insecticides on fluctuating asymmetry in honey bees (*Apis mellifera* Linnaeus). Chemosphere, **83:** 585–592

Abiola F. A., Teko-Agbo A., Biaou C., Niang M. 2005 Impacts socio-économiques et zoosanitaires de la transhumance. Conf OIE 89-103

Adger N. W., Arnell N. W., Tompkins E. L. 2005 Successful adaptation to climate change across scales. Global Environ Chang, **15***:* 77-86.

Adjanohoun E. J., Adjakidjè V., Ahyi M. R. A., Ake Assi L., Akoègninou A., d'Almeida J. 1989 Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. Agence de Coopération Culturelle et techniques, Paris.

Adjare S. 1990 Beekeeping in Africa. FAO Agricultural Service Buletin 68/6, Rome, 80p

Adjinakou A. R. S. 2000 Etude de quelques facteurs influençant l'apiculture au sud du Bénin. Mémoire DIT/CPU/UNB/Cotonou-Bénin.

Adomou A. C. 2005 Vegetation patterns and environment gradients in Benin: Implication for biogegraphy and conservation. Ph.D Thesis; Wageningen University, Wageningen.

Adomou A. C., Sinsin B., Akoègninou A. & Van der Maesen L.J.G. 2007. Les patrons de végétation et gradients environnementaux au Bénin: Implications pour la biogéographie et la conservation. Notes of the Laboratory of Applied Ecology, **1 :** 1-17.

Agence de la Sécurité et du Contrôle de la Navigation Aérienne (ASECNA) 2012. Fiche météorologique du Benin. Agence Régionale de Bohicon

Aghamirlou H. M., Khadem M., Rahmani A., Sadeghian M., Mahvi A. H., Akbarzadeh A., Nazmara S. 2015 Heavy metals determination in honey samples using inductively coupled plasma-optical emission spectrometry Journal of Environmental Health Science & Engineering 13:39 DOI 10.1186/s40201-015-0189-8

Ahmed A., Mohammad A., Yehya A., a Nuru A. 2012 Morphometric diversity of indigenous Honeybees, *Apis mellifera* (Linnaeus, 1758), in Saudi Arabia (Insecta: Apidae) Zoology in the Middle East, **57**: 97-103.

Ajao A., & Babatunde S., 2013. Isolation and identification of microorganisms in comb and body parts of wild and domesticated honey bees of two ecozones of Nigeria. Erudite Journal of Microbiology and Biodiversity, **2**: 8–15.

Akinwande K., Badeja M., Ogbogu S. 2013 Challenges associated with the honey bee (*Apis mellifera adansoni*) colonies establishment in south western Nigeria. African Journal of Food, Agriculture, Nutrition and Development. **13**: 7467–7484.

Akoegninou A. 2004 Recherches botaniques et écologiques sur les forêts actuelles du Bénin. PhD Thesis; Université de Cocody-Abidjan, Côte d'Ivoire.

Akoegninou A., Van der Burg W. J., Van der Maesen L. J. G., Adjakidjè V. J. P. E., Sinsin B. Yédomonhan H. 2006 Flore analytique du Bénin, Backuys Publishers, Leiden, the Netherlands.

Alattal Y., Alsharhi M., Alghamdi A., Alfaify S., Migdadi H., Ansari M. 2014 Characterization of the native honey bee subspecies in Saudi Arabia using the mtDNA COI-COII intergenic region and morphometric characteristics. Bulletin of Insectology. **67 (1)**: 31-37

Alburaki M., Moulin S., Egout H., Alburaki A., Garnery L. 2011 Mitochondrial structure of Eastern honeybee populations from Syria, Lebanon and Iraq. Apidologie. **42** : 628–641 DOI: 10.1007/s13592-011-0062-4

Al-Ghamdi A., Adgaba N., Getachew A., Tadesse Y. 2014 New approach for determination of an optimum honeybee colony's carrying capacity based on productivity and nectar secretion potential of bee forage species. Saudi Journal of Biological Sciences. http://dx.doi.org/10.1016/j.sjbs.2014.09.020

Aliano N.P., Ellis M.D., 2005. A strategy for using powdered sugar to reduce Varroa populations in honey bee colonies. J. Apicult. Res. **44 (2):** 54–57.

Allen M. F., Ball, B. V., Underwood B. A., 1990 An isolate of *Melissococcus pluton* from *Apis laboriosa*. J. Invertebr. Pathol. **55**: 439–440.

Allen M., Ball B. 1996 The incidence and world distribution of the honey bee viruses. Bee World. **77**: 141-162.

Amakpe F. 2008 Beekeeping in the strategies of biodiversity conservation, desertification control and poverty alleviation in Benin: A monograph of the honey bees in the district of Djidja. University of Liège - Faculty of Agronomy Sciences of Gembloux. Gembloux, Belgium.

Amakpe F., 2010, The Biodiversity of the Honey Bees (*Apis Mellifera Adansonii*) in the District of Djidja, Republic of Benin. Int J Environ Cult Econ Soc Sustain. Common Ground Publishing LLC. **6(6):** 90-104

Amakpe F., De Smet L., Brunain M., Ravoet J., Jacobs F. J., Reybroeck W., Sinsin B., de Graaf D. C. 2015 Discovery of Lake Sinai virus and an unusual strain of acute bee paralysis virus in West African apiaries. Apidologie INRA, DIB and Springer-Verlag France. DOI: 10.1007/s13592-015-0372-z

Amakpe F., J., Zuber ., Jacobs F J. 2008 Role of Beekeeping in the household food security and livelihoods: Implication for the sector policy development in Ethiopia; Gents Africa Platform (GAP) 2nd Symposium 22nd December 2008, University of Ghent.

Anderson D. L., & Morgan M. J. 2007 Genetic and morphological variation of bee-parasitic tropilaelaps mite (Acari: Laelapidae): New and redefined species. Exp. Apl Acarol. **43**: 1-24

Anderson D. L., Gibbs A. J., Gibson N; L. 1998 Identification and phylogeny of spore-cyst fungi (*Ascophaera spp*) using ribosomal DNA sequences. Mycol Res. **102 (5):** 541-547

Anderson D. L., Trueman J. W. H. 2000 *Varroa jacobsonii* (Acari: Varroides) is more than one species. Exp Appl Acarol. **24**: 165-189

Antunez K., D'Alessandro B., Corbella E., Ramallo G., Zunino P. 2006 Honeybee viruses in Uruguay. J. Invertebr. Pathol. **93**: 67–70.

Arbonier A. 2002 Arbres, arbustes et lianes des zones sèhes de l'Afrique de l'Ouest. CIRAD, Deuxième Edition Muséum National d'Histoire Naturelle

Aronstein K. A., Murray K. D., 2010 Chalbrood disease in honey bee. Journal of Invertebrate Pathology. **103**: 520-529

Atkins E. L., Kellum D. 1984 Microencapsulated pesticides - visual microscopical detection of capsules - quantification of residue in honey and pollen. American Bee Journal. **124 (11):** 800-804

Attuquayefio D. K. & Wuver A. M. 2003 A study of bushfires in a Ghanaian costal wetland I. Impact on small mammals. African Journal of Applied Ecology, Vol 4

Bailey L., 1974. An unusual type of *Streptococcus pluton* from the Eastern Hive bee. J. Invertebr. Pathol. **23**: 246–247.

Bailey L., Collins M.D. 1982 Reclassification of '*Streptococcus pluton*' (White) in a new genus *Melissococcus* as *Melissococcus pluton* nom. rev. - comb. nov. J. Appl. Bacteriol. **53:** 215-217.

Baker A. C. and Schroeder D. C. 2008 The use of RNA-dependent RNA polymerase for the taxonomic assignment of Picorna-like viruses (order Picornavirales) infecting *Apis mellifera* L. populations. Vorology Journal 5:10 doi 10.1186/1743-422x-5-10

Ball B. V. 1983 The association of Varroa jacobsoni with virus diseases of honeybees. In *Varroa jacobsoni* Oud. affecting honey bees: Present status and needs Edited by: Cavalloro R. Rotterdam: Commission of the European Communities: 21-23

Becher M. 2010 The influence of developmental temperatures on division of labour in honeybee colonies. Martin-Luther-Universitat Halle-Wittenberg. Thesis

Beil M., Horn H. and Schwabe A. 2008 "Analysis of pollen loads in a wild bee community (Hymenoptera: Apidae) a method for elucidating habitat use and foraging distances," Apidologie. **39**: 456-467.

Bell W. J., Breed M. D., Richards K. W., Michener CD. 1974 Social, stimulatory and motivational factors involved in intraspecific nest defense of a primitive lyeusocial halictine bee. J. Comp. Physiol. **93**: 173–81

Bennett M. 1972 Field control of strawberry fruit rot (*Botrystis cinerea* Fr) by fungicides (Li). J. Hort. Sci. and Biotech. **47**, 321-&.

Bernal J., Garrido-Bailon E., Del Nozal M. J., Gonzalez-Porto A. V., Martín-Hernandez R., Diego J. C., Jiménez J. J., Bernal J. L., Higes M. 2010 Overview of Pesticide Residues in Stored Pollen and Their Potential Effect on Bee Colony (*Apis mellifera*) Losses in Spain Journal of Economic Entomology. **103(6)**:1964-1971. 2010 doi: http://dx.doi.org/10.1603/EC10235

Bertelli D., Lolli M., Papotti G., Bortolotti L., Serra G., Plessi M. 2010 Detection of honey adulteration by sugar syrups using one-dimensional and two dimensional high-resolution nuclear magnetic resonance. Journal of Agricultural and Food Chemistry. **58**: 8495e8501.

Beshers S. N, Huang Z. Y, Oono Y., Robinson G. E. 2001 Social inhibition and the regulation of temporal polyethism in honey bees. J Theor Biol. **213**: 461–479

Bhat S. A., Meraj G., Yaseen S., Pandit A. K. 2014 Statistical Assessment of Water Quality Parameters for Pollution Source Identification in Sukhnag Stream: An Inflow Stream of Lake Wular (Ramsar Site), Kashmir Himalaya. Journal of Ecosystems. ID 898054 http://dx.doi.org/10.1155/2014/898054

Biaou S. S. H. 2009 Tree recruitment in West African dry woodlands: The interactive effects of climate, soil, fire and grazing. PhD Thesis, Wageningen University, Wageningen.

Bilu A. Dag A. Elad Y and Shafir S. 2004 Honey bee dispersal of Biocontrol Agents. An evaluation of dispersing devices. Biocontrol Sci. Techn. **14**: 607-617.

Bischoff I., Feltgen K., Breckner D. 2003 Foraging Strategy and Pollen Preferences of *Andrena vaga* (Panzer) and *Colletes cunicularius* (L.) (Hymenoptera: Apidae). Journal of Hymenoptera Research. **12 (2)** : 220-237

Bishop J. A. & Armbruster W. S. 1999 Thermoregulatory abilities of Alaskan bees: effects of size, phylogeny and ecology. Functional Ecology. **13**: 711-724

Blanchard P., Ribiere M., Celle, O., Lallemand P., Schurr F., Olivier V., Iscache A.L., Faucon J.P., 2007. Evaluation of a real-time two-step RT-PCR assay for quantitation of Chronic bee paralysis virus (CBPV) genome in experimentally-infected bee tissues and in life stages of a symptomatic colony. J. Virol. Methods. **141** : 7–13

Bland JM, Altman DG. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, i, 307-310.

Bodur, C., Kence, M., Kence, A. 2007 Genetic structure of honeybee, *Apis mellifera* L. (Hymenoptera: Apidae) populations of Turkey inferred from microsatellite analysis. J. Apic. Res. **46**: 50–56

Bogdanov S. 2006. Contaminants of bee products. Apidologie. **37**: 1-18.

Borcard D., Legendre P. & Drapeau P., 1992 Partialling out the spatial component of ecological variation. Ecology. **73**: 1045–1055.

Borrell B. J. 2005 Biotropica Long Tongues and Loose Niches: Evolution of Euglossine Bees and Their Nectar Flowers. Biotropica. **37 (4):** 664-669

Botoyiye S.E. 1999 36th Congress of Apiculture. Apimondia, Vancouver, Abstracts, 233°p.

Bradbear N. 2004 Beekeeping and Sustainable Livelihoods. Agricultural Support Systems Division. Food and Agriculture Organization of the United Nations Rome, 143°p.

Bradbear N. 2010 Le rôle des abeilles dans le développement rural Manuel sur la récolte, la transformation et la commercialisation des produits et services dérivés des abeilles. Produits forestiers non ligneux 19. FAO Rome. 248p

Bray J. R. & Curtis J. T. 1957 An ordination of the upland forest communities of southern Wisconsi. Ecological Monographs. **27**: 325-349

British Standards Institution 1975 Precision of test methods 1: Guide for the determination and reproducibility for a standard test method (BS 597, Part 1). London: BSI (1975).

Brocklesby, M. A. & Fisher, E. 2003 Community Development in sustainable livelihood approaches-an introduction. Community Development Journal. **38** : 185-198.

Brødsgaard C., Hansen H. 2003 Ondart bipest en verdensomspaedende sygdom. Tidsskrift Biavl. **137(7)**: 198-201.

Buchmann S. L. and Nabhan G. P. 1995 The forgotten pollinators. Island Press, 282°p.

Bufford J. L. & Gaoue O. G., 2015 Defoliation by pastoralists affects savanna tree seedling dynamics by limiting the facilitative role of canopy cover Ecological Applications. Ecological Society of America. **25(5):** 1319-1329

Cambers R. and Conway, G., 1992 'Sustainable rural livelihoods: practical concepts for the 21st century', IDS Discussion Paper 296, Brighton: IDS

Campano F., Flores J.M., Puerta F., Ruiz J.A., Ruz J.M. 1999 Fungal diseases of the honeybee (*Apis mellifera* L.) in Colin M.E. (ed.), Ball B.V. (ed.), Kilani M. (ed.) Bee disease diagnosis 61-68

Cardinal S, Straka J, Danforth B. N. 2010 Comprehensive phylogeny of apid bees reveals the evolutionary origins and antiquity of cleptoparasitism. Proc. Natl. Acad. Sci. USA. **107**:16207-11

CeRPA (Centre Régional pour la Promotion Agricole) Atacora/Donga. 2006. Rapport annuel d'activité. Atacora, Bénin. pp. 5-13

Chen Y. P., Siede R (2007) Honey bee viruses. Adv Virus Res. **70**: 33-80.

Choe S.E., Nguyen L.T.K., Noh J.H., Koh H.B., Hwa J.Y., Kweon C.H., Kang S.W. 2012 Prevalence and distribution of six bee viruses in Korean *Apis cerana* populations. J. Invert. Pathol. **109**: 330–333.

Chouhan U., Pardasani K., 2008. A maximum parsimony model to reconstruct phylogenetic network in honey bee evolution.-International Journal of Biological and Medical Sciences. **3**: 220-224.

Christodoulou D. L., Kanari P., Kourouzidou O., Constantinou M., Hadjiloizou P., Kika K., Constantinou P. 2015 Pesticide residues analysis in honey using ethyl acetate extraction method: validation and pilot survey in real samples, International Journal of Environmental Analytical Chemistry, **95(10):** 894-910, DOI: 10.1080/03067319.2015.1070408

Clauss 1994 Cleptoparasitic drone flies. Proceeding of the fifth International Conference on apiculture in tropical climates. Trinidad and Tobago, PP 113-115

Codex Alimentarius Commission. 1981. Codex Standard for Honey. Codex Alimentarius. **12**:1-8.

Collevatti R. G., Schoereder J. H. and Campos L.A.O. 2000 "Foraging Behavior of Bee Pollinators on the Tropical Weed *Triumfetta semitriloba*: Flight Distance and Directionality," Revista Brasileira de Biologia. **60 (1):** 29-37

Comlan M.K., Thérèse M.E., Atsu K.G., Komlan B., Koffi A. 2009 Inventaire des taxons polliniques des miels de la zone guinéenne du Togo : Cas des zones ecofloristiques IV et V. European Scientific Journal, **8** : 1857-7881.

Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Off. J. Eur. Union 2010 L15: 1-72.

Consonni R., Cagliani L.R., Cogliati C. 2013 Geographical discrimination of honeys by saccharides analysis Food Control. **32**: 543-548

Cooper P. D., & Schaffer W. M. 1985 Temperature regulation of honey bees (*Apis mellifera*) foraging in the Sonoran desert. J. exp. Biol. **114 :** 1-15

Cornuet J. M. & Garnery, L. 1991 Mitochondrial DNA variability in honeybees and its phylogenetic implications. Apidologie. **22:** 627-642

Cornuet J. M., Fresnaye J., Tassecourt L. 1975 Discrimination et classification de populations d'abeilles à partir de caractères biométriques, Apidologie. **6 (2) :** 145-187

Cottam G. & Curtis J.T. 1956. The use of distance measurements in phytosociological sampling. Ecology. **37:** 451-460.

Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing directives 85/358/EEC and 86/469/EEC and decisions 89/187/EEC and 91/664/EEC. Off J Eur Communities 1996 L125:10–32

Council of the European Union (2001) Council Directive 2001/110/EC of 20 December 2001 relating to honey

Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities L10/47

Cox-Foster D. L., Conlan S., Holmes E. C., Palacios G., Evans J. D.,. Moran N. A., Quan, P-L., Briese T., Hornig M., Geiser D. M., Martinson V., vanEngelsdorp D., Kalkstein A. L., Drysdale A., Hui J., Zhai, J., Cui L.,. Hutchison S. K., Simons J. F., Egholm M., Pettis J. S., Lipkin W. I. 2007 A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder. Science. **318:** 283 DOI: 10.1126/science.1146498

Damlon F. & Lobreay-Callen D. 1991 Bee foraging in north and West Africa. Acta Horticultura **288**: 121-126

Danforth B N., Sipes S, Fang J, Brady SG. 2006. The history of early bee diversification based on five genes plus morphology. Proc. Natl. Acad. Sci. USA. **103:**15118–23

Danforth B. N., Cardinal S., Praz C., Almeida E. A. B., and Michez D. 2013. The Impact of Molecular Data on Our Understanding of Bee Phylogeny and Evolution. Annu. Rev. Entomol. **58**: 57–78

Daughenbaugh K. F, Martin M., Brutscher L. M., Cavigli I., Garcia E. Lavin M., Flenniken M. 2015 Honey Bee Infecting Lake Sinai Viruses. Viruses. **7(6):** 3285-3309; doi:10.3390/v7062772

de Graaf D.C., Alippi A.M., Brown M., Evans J.D., Feldlaufer M., Gregorc A., Hornitzk M., Pernal S.F., Schuch D.M.T., Titera D., Tomkies V., Ritter W. 2006 Diagnosis of AFB in honey bees: A synthesis and proposed analytical protocol. J. Invert. Pathol. **92**: 585-590.

de Miranda J.R., Cordoni G., Budge G. 2010 The Acute Bee Paralysis Virus-Kashmir Bee Virus-Israeli Acute Paralysis Virus complex. J. Invert. Pathol. **103 (1) :** S30–S47.

de Miranda J.R., Drebot M., Tyler S., Shen M., Cameron C. E., Stoltz D. B., Camazine S. M. 2004 Complete nucleotide sequence of Kashmir bee virus and comparison with acute bee paralysis virus. J Gen Virol. **85:** 2263-2270.

De Smet L., Ravoet J., de Miranda J.R., Wenseleers T., Mueller M.Y., Moritz R.F.A., de Graaf D.C. 2012 BeeDoctor, a versatile MLPA-based diagnostic tool for screening bee viruses. PLoS ONE. **7(10):** e47953. doi: 10.1371/journal.pone.0047953

DeGandi-Hoffman G., Terry I., Morales F., Weinhold C. 1990 Influence of blossom density, bloom phenology, honey bee foraging activity and blossom quality on apple tree set. Southwestern Entomologist VOl 15 N°3

DeGrandi-Hoffman G., Collins A.M., Martin J.H., Schmidt J.O., Spangler H.G. 1998. Nest defence behaviour in colonies from crosses between Africanized and European honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae). J. Insect Behav. **11**:37–45

Delaplane, K.S. 1999 The Small Hive Beetle (*Aethina tumida*), Bugwood Publication, Entomology and Forest Resources Information Digital Working Group. **98**: 0111.

Delmas, R.J., Loudjani, P., Podaire, A. et Menaut, J-C. 1991. Biomass burning in Africa: an assessment of annually burnt biomass. In: J.S. Levine (ed.) Global Biomass Burning: Atmospheric, Climatic and Biospheric Implications, pp. 126-133. MIT Press, Cambridge, Mass.

Denmark H.A., Cromroy H.L., Sanford M.T. 2000 Honey bee tracheal mite, *Acarapis woodi* (Rennie) (Arachnida: *Acari: Tarsonemidae*). Entomology and Nematology Department, Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Deressa T. T., Hassan M., R., Ringler C., Alemu T., Yusuf M. 2009 Determinants of farmers' choice of adaptation methods to climate change in the Nile Basin of Ethiopia. doi:10.1016/j.gloenvcha.2009.01.002

Devineau J-L., Fournier An., Nignan S. 2010 Savanna fire regimes assessment with MODIS fire data: Their relationship to land cover and plant species distribution in western Burkina Faso (West Africa). Journal of Arid Environments. **74**: 1092-1101

Directive 2014/63/EU of the European Parliament and of the Council of 15 May 2014 amending Council Directive 2001/110/EC relating to honey. Off. J. Eur. Union. **L164**: 1-5.

Dongock N. D., Tchoumboue J., Youmbi E., Zapfack L., Mapongmentsem P., Tchuenguem F. F. N. 2011 Predominant melliferous plants of the western Sudano Guinean zone of Cameroon. African Journal of Environmental Science and Technology. **5(6):** 443-447.

Dongock N.D., Tchoumboue J., Youmbi E., Zapfack L., Mapongmentsem P., Tchuenguem F. F. N. 2004. Inventaire et identification des plantes mellifères de la zone soudano-guinéene d'altitude de l'ouest Cameroun. Tropicultura: **22(3)** : 139-145

Dongock N.D., Tchoumboue J., Youmbi E., Zapfack L., Mapongmentsem P., Tchuenguem F. F. N. 2008. Caractéristiques polliniques des plantes mellifères de la zone soudano-guinéenne d'altitude de l'ouest Cameroun. Tropicultura. **26(3):** 150-154.

Dyer F. D. 2002 The Biology of the Dance Language. Annu. Rev. Entomol. **47**: 917-49

Ekumankama O. O. & Nwankwo G. Radio Farm Brodcasts: A study of Adoption of Agricultural Innovation in the Umuahla Zone, Abia State. Journal of Agricultural Extension. **6:** 17-24

Ellis F. 2000 The determinants of rural livelihoods diversification in developing countries. Journal of Agricultural Economics. **51 (2):** 289-302

Ellis J D; Evans J D; Pettis J S. 2010. Colony losses, managed colony population decline and Colony Collapse Disorder in the United States. Journal of Apicultural Research. **49(1):** 134-136. DOI: 10.3896/IBRA.1.49.1.30

Ellis J.D. 2004 The ecology and control of small hive beetles (*Aethina tumid*a Murray). PhD-dissertation, Rhodes University.

Ellis J.D., Munn P.A. 2005 The world health status of honey bees. Bee World. **86**: 88-101.

Ellis, F. 1998 'Survey article: Household strategies and rural livelihood diversification'. The Journal of Development Studies. **35 (1):** 1-38.

England regulations 2015: The Honey (England) Regulations 2015 No. 1348

Ern H. 1988 Flora and vegetation of the Dahomey Gap. A contribution to the plant geography of West tropical Africa. Annals of the Missouri Botanical Garden. **25** : 520-571.

Evans J.D. and Hung A. C. 2000 Molecular phylogenetics and the classification of honey bee viruses Archives of Virology (Arch Virol). **145**: 2015-2026

Eyer M., Chen Y. P., Schäfer M. Ol., Pettis J, Neumann P. 2009 Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses. Apidologie. **40 (4):** 419-428

Fakhimzadeh K. 2001 Detection of major mite pests of *Apis mellifera* and development of non-chemical control of varroasis. PhD-dissertation, Department of Applied Biology University of Helsinki, Finland.

Famuyide, O.O., Adebayo, O., Owese, T., Azeez, F.A., Arabomen, O., Olugbire, O.O, Ojo, D. 2014 Economic Contributions of Honey Production as a Means of Livelihood Strategy in Oyo State. International Journal of Science and Technology. **3 (1):** 7-11

FAO 2006 Honey bee diseases and pests: a practical guide. Agricultural and Food Engineering technical report 4. www.fao.org/3/a-a0849e.pdf accessed on 08/08/2015

FAO, 1996. The state of food and agriculture. Food Security, some macroeconomic dimensions. FAO Agriculture serie N° 029 Food and Agriculture Organisation of the United Nations, Rome, 352P.

Farshineh M.B; Gencer, H.V; Firatli, C., Bahreini R. 2007 Morphometric characterization of Iranian (*Apis mellifera meda*), Central Anatolian (*Apis mellifera anatoliaca*) and Caucasian (*Apis mellifera caucasica*) honeybee populations. J. Apicult. Res. Bee World. **46:** 225-231.

Felsenstein J., 1985 Confidence limits on phylogenies: An approach using the bootstrap.- Evolution. **39**: 783-791.

Ferder G. & Umali D. L. 1993 The Adoption of Agricultural Innovations. A review. Technological Forecasting and Social Change. **43**: 215-239

Fernandez JM, Puerta F, Cousinou M, Dios-Palomares R, Campano F, Redondo L. 2012 Asymptomatic presence of Nosema spp. in Spanish commercial apiaries. J Invertebr Pathol. **111**:106–110.

Flannigan M. D., Krawchuk M. A., de Groot W. J., Wotton B. M., Gowman L. M. 2009 Implications of changing climate for global wildland fire. International Journal of Wildland Fire. **18**: 483–507

Flores J.M., Ruiz J.A., Ruz J.M., Puerta F., Bustos M., Padilla F. & Campano F. 1996. Effect of temperature and humidity of sealed brood on chalkbrood development under controlled conditions. Apidologie. **27**: 185–192.

Foley K., Fazio G., Jensen AB., Hughes W.O.H: 2014 The distribution of *Aspergillus spp*. opportunistic parasites in hives and their pathogenicity to honey bees. Vet Microbiol. **169**: 203-210.

Forsgren E. 2010 European foulbrood in honey bees. Journal of Invertebrate pathology. **103**: 55-59

Franck P., Garnery L., Loiseau A., Oldroyd B. P., Hep- Burn H. R., Solignac M., Cornu J. M., 2001.- Genetic diversity of the honeybee in Africa: microsatellite and mito- chondrial data.- Heredity. **86**: 420-430.

Franck P., Garnery L., Solignac M., Cornuet J-M. 1998 The origin of West European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data. Evolution. **52(4):** 1119-1134

Franck P., Garnery L., Solognac M., Cornuet J.M. 2000 Molecular confirmation of a fourth lineage in honeybee from the Near East. Apidologie. **31** : 167-180.

Franck, P., Garnery, L. Cekebrabi, G., Soignac M., Cornuet J.M. 2000b Hybrid origins of honeybees from Italy (*Apis mellifera ligustica*) and Sicily (*A.m. sicula*) Molecular Ecology. **9:** 907-921

Francoy, T.M., Wittmann, D., Drauschke, M., Müller, S., Steinhage, V. 2008 Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. Apidologie. **39:** 488–494

Fresnaye, J. 1981 Biométrie de l'abeille. INRA 2éme édition, 54°p.

Fries I. 2002 African honey bees (*Apis mellifera scutellata*) and nosema (*Nosema apis*) infections. In Jones, R. eds. Bees without frontiers, Sixth European Bee Conference, Cardiff, UK, 1-5 July 50-55

Fries I., 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). Journal of Invertebrate Pathology. **103:** S73–S79.

Fries I., Feng F., Da silva A., Slemenda S.B., Pieniazek N.J. 1996. *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphologycal and molecular characterization of a Microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). Eur. J. Protistol. **32**: 356–365.

Frost, P.G.H. 1984. The responses and survival of organisms in fire-prone environments. In: P. de V. Booysen et N.M. Tainton (eds), Ecological Effects of Fire in South African Ecosystems. Springer-Verlag, Berlin pp. 273-309..

Gadbin C., 1980. Les plantes utilisées par les abeilles au Tchad méridional. Apidologie. **10(2)** : 217-254.

Garnery L., Cornuet J. M., Solignac M. 1992 Evoluationary history of the honeybee *Apis mellifera* inferred from mitochondrial DNA analysis. Molecular Ecology. **1**: 145-154

Garnery, L., Franck, P., Baudry E., Vautrin, D. Cornuet, J.M. Solignac, M. 1998 Genetic diversity of the west European honey bee (*Apis mellifera mellifera* and *A.m. iberica*) II. Microsatellite loci. Genetics Selection Evolution. **30**: 49-74

Garnery, L., H. Mosshine, B. P. Oldroyd, And J.-M. Cornuet. 1995. Mitochondrial DNA variation in Moroccan and Spanish honey bee populations. Mol. Ecol. **4**:465-471.

Garnery, L., Solignac, M. mCelebrano, G. Cornuet J.M. 1993 A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure of *Apis mellifera* L. Experientia. **49**: 1016-1021

Gebeya, R. & Genet, S. 2006. Chalk brood in Ethiopia. Bees For Development Journal 78: Online at: http:// planbee.org.uk/uploads/BfDJ78%20EthiopiaChalk brood061.pdf.

Genersch, E., Evans, J.D. and Fries, I. 2010. Honey bee disease overview. J. Invertebr. Pathol. **103**: 2-4.

Gidey A., Mulugeta S., Fromsa A. 2012 Prevalence of bee lice Braula coeca (*Diptera*: *Braulidae*) and other perceived constraints to honey bee production in Wukro Woreda, Tigray Region Ethiopia. Global Vet. **8**: 631-635.

Gilliam M. & Taber S. 1991. Diseases, pests, and normal microflora of honeybees, *Apis mellifera*, from feral colonies. Journal of Invertebrate Pathology. **58**: 286–289.

Gilliam M., Lorenz B. J., Wenner A. M., Thorp R. W. 1997 Occurrence and distribution of *Ascosphaera apis* in North America: chalkbrood in feral honey bee colonies that had been in isolation on Santa Cruz Island, California for over 110 years1. Apidologie, Springer Verlag (Germany). **28 (6):** 329-338.

Glèlè Kakaï R., Akpona T.J.D., Assogbadjo A.E., Gaoué O.G., Chakeredz S., Gnanglè P.C. 2011 Ecological adaptation of the shea butter tree (*Vitellaria paradoxa* C.F. Gaertn.) along climatic gradient in Benin, West Africa, African Journal of Ecology. **49:** 440-449.

Goulson D. 1999 Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. Perspectives in Plant Ecology, Evolution and Systematics. **2 (2):** 185-209

Granberg F., Vicente-Rubiano M., Rubio-Guerri C., Karlsson O.E., Kukielka D., Belak S., Sanchez-Vizcaino, J.M. 2013 Metagenomic detection of viral pathogens in spanish honeybees: co-infection by aphid lethal paralysis, Israel acute paralysis and lake sinai viruses. PLoS One 8(2): e57459. doi: 10.1371/journal.pone.0057459

Guindon S., Dufayard J.F., lefort V., Anisimova M., Hordijk W., Gascuel, O. 2010 New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0 Syst. Biol. **59**: 307-321.

Hahn-Hadjali K., Schmidt M. & Thiombiano, A. 2006 Phytodiversity dynamics in pastured and protected West African savannas. In: S. A. Ghazanfar & H. J. Beentje (eds.), Taxonomy and ecology of African Plants: their conservation and sustainable use. Proceedings of the 17th AETFAT Congress Addis Abeba, pp. 351–359. Royal Botanic Gardens, Kew.

Hamilton D. J., Ambrus A., Dieterle R. M., Felsot A. S., Harris C. A., Holland P. T., Katayama A., Kurihara N., Linders J., Unsworth J., Wong S. S. 2003 Regulatory Limits For Pesticide Residues In Water (IUPAC Technical Report) Pure Appl. Chem. **75 (8):** 1123-1155.

Hamilton D., Ambrus A., Dieterle R., Felsot A., Harris C., Petersen B., Racke K., Wong S-S, Gonzalez R., Tanaka K., Earl M., Roberts G., Bhula R. 2004 Pesticide residues in food-acute dietary. Pest Manag Sci 60:311-339 doi 10.1002/ps.865

Heath L. A. F. 1985 "Occurrence and Distribution of Chalk Brood Disease of Honeybees," Bee World. **66:** 9-16.

Hedtke S. M., Patiny S. and Danforth B. N., 2013 The bee tree of life: a supermatrix approach to apoid phylogeny and biogeography. BMC Evolutionary Biology. **13**:138

Heinrich B. 1981 The mechanisms and energetics of honeybee swarm temperature regulation. J Exp Biol. **91**: 25-55

Henle K., Davies K. F., Kleyer M., Margules C.R., Settele J. 2004 Predictors of species sensitivity to fragmentation. Biodiversity and Conservation. **13**: 207–251.

Hepburn H. R. Radolff S.E. 1998 Honeybees of Africa. Berlin; Heidelbert, New York, Baecelona, Budapest, Hong Kong, London, Milan, Paris, Satan, Clara, Singapour, Tokyo, Springler, ISBN 3-540-64221-8 DBN 95.287264.1

Hepburn H.R., Jacot Guillarmod A. 1991 The Cape honeybee and the fynbos biome. South African Journal of Science. **87**:70-73

Higes M., Martin-Hermandez R., Garrido-Bailon E., Gonzalez-Porto A. V., Garcia-Palencia P., Meana A., Nozal M. J. D., Mayo R., Bernal J. L. 2009 Honey bee colony collapse due to *Nosema ceranae* in professional apiaries. Environmental Microbiology Reports. **1**: 110-113.

Higes M., Martin-Hernandez R., Botias C., Bailon E.G., Gonzales-Porto A., Barrios L., Del Nozal M.J., Palencia P.G., Meana A. 2008 How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environment. Microbiol. **10**: 2659–2669

Hilmi M., Bradbear N., Mejia D. 2012 Beekeeping and sustainable livelihoods. Diversification booklet number 1. Second edition Rural Infrastructure and Agro-Industries Division FAO, Rome. 83p

Holden C. 2006 Ecology, Report warns of looming pollination crisis in North America. Science. **314**: 393

Hopping M.E., Martyn J.A.K., Hacking N.J.A. 1993 Comparison of growth and yield of kiwifruit on different vine support structures. New. Zeal. J. Crop. Hort. 295-301.

Hornitzky A.Z., Wilson S. 1989. A system for the diagnosis of the major bacterial brood diseases. J. Apicult. Res. **28: 191**–195.

Hornitzky M.A.Z., Smith L.A. 1999. Sensitivity of Australian *Melissococcus pluton* isolates to oxytetracycline hydrochloride. Austr. J. Exp. Agricult. **39:** 881–883.

Houehanou T.D., Romain L. Glele Kakaı R.L., Assogbadjo A.E., Kindomihou V., Houinato M., Wittig R., Sinsin A. B. 2015 Change in the woody floristic composition, diversity and structure from protected to unprotected savannahs in Pendjari Biosphere Reserve (Benin, West Africa). African Journal of Ecology. **51:** 358-365

Houinato M., Sinsin B. Lejoly J. 2001 Impact des feux de brousse sur la dynamique des communautés végétales dans la forêt de Bassila (Bénin), Acta Botanica Gallica **148(3)** : 237-251, DOI: 10.1080/12538078.2001.10515891

Hounkpe N. U. H., Mensah G. A., Koutinhouin B., Pomalegni S. C. B., Goergen G., 2007 Typisation des abeilles melliferes dans le Nord Bénin. Bul. Rec. Agr. Bénin. **58**: 56-59

Huang W. F., Jiang J. H., Chen Y. W., Wang C.H, 2007 *A nosema cerana* isolate from the honey bee (*Apis mellifera*, Hymenoptera: Apidae). Plos Patholg 6, e10011160.

Huitchinson J., Dalziel J. M. 1972 Flora of West Tropical Africa 1953-1972 HMSO, London UK

Human H., Pirk C.W.W., Crewe R.M., Dietemann V. 2011 The honeybee disease American foulbrood: An African perspective. Afr. Entomol. **19(3):** 551–557

Hussein M.H. 2000. Beekeeping in Africa: I. North, East, North-East and West African countries. Apiacta **1**: 32–48.

IHC 2009 Harmonised methods of the International Honey Commission. URL http://www.bee-hexagon.net/en/network.htm

IHC 2015 International honey Commission 2009 Harmonised methods URL http://www.bee-hexagon.net/en/network.htm

INSAE 2014. Quatrième recensement général de la population. Que retenir des effectifs de populations de 2013. Direction des Etudes Démographiques

INSAE 2015 Les Echanges Extérieurs du Bénin en 2014. Grands traits du Commerce Extérieur au Bénin. Institut National de la Statistique et de l'Analyse Economique, Mars 2015

Inventaire Forestier National (IFN) 2010: Rapport final de l'Inventaires Forestiers National au Benin. Méthodologies, résultats et annexes. Projet Bois de Feu Phase 2, Direction Générale des Forêts et Ressources Naturelles.

Jaafar-Furo M. R. 2007 Appraising the perception of farming communities towards adoption of apiculture as a viable source of income in Adamawa state, Nigeria. Apicta. **42**: 1-15

Jacobs F.J., Simoens C., de Graaf D.C., Deckers J. 2006 Scope for non-wood forest products generation from rehabilitation areas: focus on beekeeping. J. Drylands. **1:** 171-185.

Jaffe R., Dietemann V., Allsopp M. H., Costa C., Crewe R. M., Dall'olio R., De La Rua P., El-Niweiri M. A. A., Fries I., Kezic N., Meusel M. S., Paxton R. J., Shaibi T., Stolle E., Moritz R; F. A. 2009 Estimating the density of honeybee colonies across their natural range to fill the gap in pollinator decline censuses. Conservation Biology DOI: 10.1111/j.1523-1739.2009.01331.x

Jaffe R., Dietemann V., Crewe R. M. Moritz R. F. A. 2009 Temporal variation in the genetic structure of a drone congregation area: an insight into the population dynamics of wild African honeybees (*Apis mellifera scutellata*). Molecular Ecology. **18**: 1511-1522

Johansen E., Riedl H., Brewer L., Barbour J. 2006 How to reduce bee poisoning from pesticides. A Pacific Extension publication, Oregon State University, University of Idaho, Washington University, 28°p

Johansson H, Dhaygude K, Lindström S, Helantera H, Sundström L, Trontti K 2013 A Metatranscriptomic Approach to the Identification of Microbiota Associated with the Ant *Formica exsecta*. PLoS ONE 8(11): e79777. doi:10.1371/journal.pone.0079777

Johnson P. Huxdorff C., Simon G. Santillo D. 2014 The bees' burden. An analysis of the pesticide residues in comb pollen (beebread) and trapped pollen from honey bees (*Apis mellifera*) in 12 European countries. Greenpeace Research Laboratories technical report 03/2014. 56p

Jongman R.H., ter Braak C. J. L. F., Van Tongeren, O. F. R. 1995 Data analysis in community and landscape ecology. Cambridge University Press, Cambridge.

Jürgens N., Schmiedel U., Haarmeyer D. H., Dengler J., Finckh M., Goetze D. 2012. The BIOTA Biodiversity Observatories in Africa: a standardized framework for large-scale environmental monitoring. Environmental Monitoring and Assessment. **184**: 655–678.

Kajobe R., Marris G., Budge G., Laurenson L., Cordoni G., Jones B., Wilkins S., Cuthbertson A.G.S., Brown M.A. 2010 First molecular detection of a viral pathogen in Ugandan honey bees. J. Invert. Pathol. **104**: 153-156.

Kearns C. A., Inouye D. W. and Waser N. 1998 Endangered mutualisms: the Conservation of plant-pollinator interactions. Annu. Rev. Ecol. Syst. **29**: 83-112

Keasar T, Shmida A, Motro U, 1996. Innate movement rules in foraging bees: flight distances are affected by recent rewards and are correlated with choice of flower type. Behav Ecol Sociobiol. **39**:381–388.

Klein A.M., Vaissière B.E., Cane J.H., Dewenter S. I., Cunningham S. A., Kremen C. 2007. Importance of pollinator in changing landscapes for world crops. Proceedings of the Royal Society B. **274**: 303-313.

Klein A.M., Vaissière, B.E., Cane, J.H., Dewenter, S.I., Cunningham, S.A., Kremen, C., Tscharntke, T. 2007 Importance of pollinator in changing landscapes for world crops. Proc. Roy. Soc. B. **274**: 303-313.

Knutsson P. 2006 The Sustainable Livelihoods Approach: A Framework for Knowledge Integration Assessment. Human Ecology Review. **13** No. 1

Kokoye S.J. 1991 Guide pratique d'initiation à l'apiculture tropicale. Service production animale du CARDER-Atacora, Ministère du Développement Rural, Benin.

Kovac H., Stabentheiner A. Schmaranzer S. 2010 Thermoregulation of water foraging honeybees-Balancing of endothermic activity with radiative heat gain and functional requirements Journal of Insect Physiology. **56**: 1834–1845

Krakowska A., Muszynska B., Reczynski W., Opoka W., Turski W 2015 Trace metal analyses in honey samples from selected countries. A potential use in bio-monitoring, International Journal of Environmental Analytical Chemistry. **95(9):** 855-866, DOI: 10.1080/03067319.2015.1055475

Kuenen L.P.S., Calderone N.W., 1997 Transfers of Varroa mites from newly emerged bees: preferences for age- and function-specific adult bees. J. Insect Behav. **10**: 213–228

Kumar S., Stecher G., Tamura K. 2016 MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger data sets. Mol Biol Evol.

Laurant M., Hnedrix P., Bibiere-Chabert M., Chauzat M-P. 2015 A pan-European epidemiological study on honeybee colony losses 2012-2014. European Union Reference Laboratory for honeybee health (EURL)

Le Conte Y., & Navajas M. 2008 Climate change: Impact on honeybee populations and diseases. Rev. Sci. tech. off. Int. Epiz. **27(2)** : 499-510.

Legendre P. & Gallagher E. D. 2001 Ecologically meaningful transformations for ordination of species data. Oecologia. **129**: 271-280.

Linsoussi A. C. 2011 Bio regionalization of remote sensing based spatial decision support system for bush fire management in Benin. Thesis University of Bonn,

Lipa J. J. Triggiani O. 1996 *Apicystis gennov* and *Apicystis bombi* (Liu, Macfarlane & Pengelly) Comb nov (Protozoa: Neogregarinida), a cosmopolitan parasite of *Bombus* and *Apis* (Hymenoptera: Apidae) Apidology. **27**: 29-34

Lobreau-Callen D, Damblon F, 1994. Spectre pollinique des miels de l'abeille *Apis mellifera* L. (Hymenoptera, Apidae) et zone de végétation en Afrique occidentale et méditerranéenne. Grana. **33** : 245-53.

Lobreau-Callen D, Darchen R, Le Thomas A. 1986. Apport de la palynologie à la connaissance des relations abeilles / plantes en savanes arborées du Togo et du Bénin. Apidologie. **17(4):** 279-306.

Londt J.G.H. 1993 Afrotropical robber fly (Diptera: Asilidae) predation of honey bees, *Apis mellifera* Linnaeus (Hymenoptera Apidae). African Entomology. **1**: 167-173

Loreau M., Naeem S., Inchausti P., Bengtsson J., Grime, J. P., Hector A. *et al*. 2001 Biodiversity and ecosystem functioning current knowledge and future challenges. Science. **294** : 804-808.

Loucif-Ayad, W., Chefrour, A., Algharibeh, M., Haddad, N. 2013 First detection of deformed wing virus of honeybees in Algeria. Phytoparasitica. **41**: 445-447

Louveaux J., Albisetti M., Delangue M. & Theurkauff J. 1966 Les modalités de l'adaptation des abeilles (*Apis mellifica L*.) au milieu naturel. Ann. Abeille. **9 (4)** : 323-350.

MAEP 2001 Etude sur les conditions de vie des ménages ruraux (ecvr2) Edition 1999-2000. Profil de la pauvreté rurale et caractéristiques socio-economique des ménages ruraux Projet BEN/96/001.

MAEP 2010: Liste officielle des pesticides autorisés en Républic du Benin par le Comité National d'Agrément et de Contrôle des Produits Phytopharmaceutiques (CNAC). Document de travail, CARDER Mono-Couffo

Magnus R. M., Tripodi A. D., Szalanski A. L., 2011 Mitochondrial DNA diversity of honey bees, *Apis mellifera L*. (Hymenoptera: Apidae) from queen breeders in the United States.- Journal of Apicultural Science**. 55:** 37-46.

Marceau J, Boily R, Perron JM, 1990. The relationship between hive productivity and honey bee flight activity. J Apic Res. **29**:28-34.

Marghitas L. A. l., Paniti-Teleky O., Dezmirean D., Margaoan R., Bojan C., Coroian C., Laslo L., Moise A. 2008 Morphometric differences between honeybees (*Apis mellifera carpatica*) populations from transylvanian area. Lucraristiin Ńifice Zootehnie si Biotehnologii. **41 (2):** Timisoara

Martin S. J. Highfield A. C. Brettel L. Villalobos E. M. Budge G. E. Powel M., Nikaido S. Schroeder 2012 Global honey bee viral landscape altered by a parasitic mite. Science. **336**: 1304-1306

Martin-Hernandez, R; Meana, A; Garcia-Palencia, P; Marin, P; Botias, C; Garrido-Bailon, E; Barrios, L; Higes, M 2009 Effect of temperature on the biotic potential of honey bee microsporidia. Applied and Environmental Microbiology. **75**: 2554-2557.

Matheson A. 1996 World bee health update. Bee World. **77**: 45-51.

Mayack C. & Naug D. 2009 Energetic stress in the honeybee *Apis mellifera* from Nosema *ceranae* infection Journal of Invertebrate Pathology. **100**: 185–188

McKinney M. L. 1997 Extinction vulnerability and selectivity: Combining ecological and paleontological views. Annual Review of Ecology and Systematics. **28**: 495-516..

Meixner M. D., Leta M. A., Koeniger N., Fuchs S. 2011 The honeybees of Ethiopia represent a new subspecies of *Apis mellifera-Apis mellifera simensis* n. ssp. Apidologie. **42**: 425-437

Michael D. B., Ernesto GN., and Greg J. H. 2004 Defensive behaviour of honey bees: Organization, Genetics, and Comparisons with Other Bees. Annu. Rev. Entomol. **49**: 271-98doi:10.1146/annurev.ento.49.061802.123155

Michener C.D. The Bee of the world. The Johns Hopkins University Press, Baltimore, 2000, 913°p.

Miguel, I., Baylac M., Iriondo M., Manzano C., Garnery L., Estonba A. 2011: Both geometric morphometric and microsatellite data consistently support the differentiation of the *Apis mellifera* M evolutionary branch. Apidologie. **42:** 150-161

Milligan G. W. 1980. An Examination of the Effect of Six Types of Error Perturbation on Fifteen Clustering Algorithms. Psychometrika. **45**:325-342.

Miyagi, T., Peng, C.Y.S., Chuang, R.Y., Mussen, E.C., Spivak, M.S., Doi, R.H., 2000. Verification of oxytetracycline-resistant American foulbrood pathogen *Paenibacillus larvae* in the United States. J. Invertebr. Pathol. **75**: 95–96.

Moritz R.F.A, de Miranda J., Fries I., Le Conte Y., Neumann P., Paxton R.J. 2010 Research strategies to improve honeybee health in Europe. Apidologie. **41**: 227–242

Morse R., Calderone N.W. 2000 The value of honey bees as pollinators of US Crops in 2000. Bee Cult. **128**: 1-15.

Mouillot F, Field C.B 2005 Fire history and the global carbon budget: a 1◦× 1◦ fire history reconstruction for the 20th century. Global Change Biology. **11**: 398–420. doi:10.1111/J.1365-2486.2005.00920.X

Muli E., Patch H., Frazier M., Frazier J., Torto B., Baumgarten T., Kilonzo J., Kimani J.N., Mumoki F., Masiga D., Tumlinson J., Grozinger C. 2014 Evaluation of the distribution and impacts of parasites, pathogens and pesticides on honey bee (*Apis mellifera*) populations in East Africa. PLoS ONE. **9(4):** e94459. Doi: 10.1371/journal.pone.0094459

Mumoki F.N., Fombong A., Muli E., Muigai A.W.T., Masiga D. 2014 An inventory of documented diseases of African honeybees African Entomology. **22(3):**473-487. DOI: http://dx.doi.org/10.4001/003.022.0313

NASS 2007 National Agricultural Statistics Service 2007, Honey. www.nass.usda.gov

Navajas M., Migeon A., Alaux C., Martin-Magniette M. L., Robinson G. E., Evans J. D., Cros-Arteil S., Crauser D., Le Conte Y. 2008 Differential gene expression of the honey bee *Apis mellifera* associated with Varroa destructor infection BMLC Genomic. **9:** 301 doi:10.1186/1471-2164-9-301

Nazzi F., Annoscia D., Caprio E., Di Prisco G., Pennacchio F. 2014 Honeybee immunity and colony losses. Entomologia volume. **2 (203):** 80-86

Neumann P., Carreck N. 2010 Honey bee colony losses Journal of Apicultural Research IBRA **49(1):** 1-6 DOI 10.3896/IBRA.1.49.1.01

Nguyen B.K., Mignon J., Laget D., de Graaf D. C., Jacobs F. J vanEngelsdorp D., Brostaux Y., Saegerman C., Haubruge E.2010 Honey bee colony losses in Belgium during the 2008-9 winter. Journal of Apicultural Research and Bee World. **49(4):** 337-339 DOI: 10.3896/IBRA.1.49.4.07

Ogbuehi K. C., Osuagwu U. L. 2012 Repeatability and interobserver reproducibility of Artemis-2 high-frequency ultrasound in determination of human corneal thickness. Clinical ophtamology. **(6)** 761–769

OIE 2015 Code sanitaire pour les animaux terrestres 2015. Chapitre 9, Apidea. Office International des Epizooties. http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/

OIE. Terrestrial Animal Health Code (2009) Office International des Epizooties, URL http://www.oie.int/eng/normes/Mcode.html

Oldroyd, B. P., D. Rowe, J.-M. Cornuet, T. E. Rinderer, And R. H. Crozier. 1994. Racial admixture of *Apis mellifera* in Tasmania, Australia: similarities and differences with natural hybrid zones in Europe. Heredity. **74:**315-325.

Olivier V., Massou I., Celle O., Blanchard P., Schurr F., Ribière M., Gauthier M. 2008 *In situ* hybridization assays for localization of the chronic bee paralysis virus in the honey bee (*Apis mellifera*) brain. Journal of Virological Methods. **153**: 232–237

Ostiguy S., 2010 Sustainable beekeeping: managed pollinator CAP coordinated agricultural project: a national research and extension initiative to reverse pollinator decline. Am. Bee J. **150**: 149–152

Paltineanu C., Mihailescu I. F., Seceleanu I., Dragota C., Vasencius F. 2007 Using aridity indices to describe some climate and soil features in Eastern Europe: a Romanian case study. Theoritical and Applied Climatology. **90**: 263-274

PAM, 2014 Analyse globale de la vulnérabilité et de la sécurité alimentaire (AGVSA). Republic du Benin. Programme Alimentaire Mondial. 146p

Paraïso A., Cornelissen B., Viniwanou, N. 2011 Varroa destructor infestation of honey bee (*Apis mellifera adansonii*) colonies in Benin. J. Apicult. Res. **50(4):** 321-322.

Paraïso A., Viniwanou N., Akossou A. Y. J., Mensah G. A. & Abiola W., 2011 Caractérisation morphométrique de l'abeille *Apis mellifera adansonii* au Nord-Est du Bénin. Int. J. Biol. Chem. Sci. **5(1):** 331-344

Parvu M., Andronie I. C., Simion V. E., a Amfim A. 2013 Studies Concerning the Impact of Climate Changes on Honeybees Behaviour. Animal Science and Biotechnologies. **46 (2):** 282-284

Paxton R .J. 2010 Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*)? Journal of Apicultural Research. **49(1):** 80-84 DOI 10.3896/IBRA.1.49.1.11

Paxton R. J., Klee J., Korpela S., Fries I. 2007 *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie. **38**:558-565

Peterken G., 1996. Woodlands conservation and management. 2nd edition 374°p.

Pirk C. W. W., Human H., Crewe R. M., vanEngelsdorp D. 2014 A survey of managed honey bee colony losses in the Republic of South Africa - 2009 to 2011 Journal of Apicultural Research. **53(1):** 35-42 IBRA DOI 10.3896/IBRA.1.53.1.03

Prest D.B., Gilliam M., Taber S. Mills J.P. 1974 Fungi associated with discolored honey bee, *Apis mellifera*, larvae and pupae. Journal of Invertebrate Pathology. **24:** 253–255.

Prost J. 1987 Apiculture : connaître l'abeille, conduire le rucher. Edition Lavoisier. Technique et documentation, 6ème édition revu et complétée, 570°p

Quezada-Euan, J. J. G., Paxton R. J., Palmer K. A., Itza, W. J. M., Tay, W. T., Oldroyd, B. P. 2007 Morphological and molecular characters reveal differentiation in a neotropical social bee, *Melipona beecheii* (Apideda : Meliponini). ©INRA/DIB-AGIB/EDP Sciences, Apidologie. **38**: 247-258.

Rabajante J.F., Figueroa R.B. Jr., Jacildo A.J. 2009 “Modeling the area restrict searching strategy of stingless bees, *Trigona biroi*, as a quasi-random walk process,” Journal of Nature Studies. **8 (2):** 15-21.

Ravoet J. De Smet L., Wenseleers T., de Graaf D. C. 2015a Genome sequence heterogeneity of Lake Sinai Virus found in honeybees and Orf1/RdRP-based polymorphisms in a single host. Virus Research. **201** : 67–72

Ravoet J. 2015c Exploring the involvement of honey bee pathogens in colony collapses from epidemiological data, pathogen genotyping and host immune response. PhD thesis, Ghent University

Ravoet J., Reybroeck W., de Graaf, D.C. 2015b Pesticides for apicultural and/or agricultural application found in Belgian honey bee wax combs. Bull. Environ. Contam. Toxicol. **94:** 543-548 DOI 10.1007/s00128-015-1511-y.

Ravoet J., De Smet L., Meeus I., Smagghe G., Wenseleers T., de Graaf D.C. 2014 Widespread occurrence of honey bee pathogens in solitary bees. J. Invert. Pathol. **122**: 55-58.

Ravoet J., Maharramov J., Meeus I., De Smet L., Wenseleers T., Smagghe G., de Graaf D.C. 2013 Comprehensive bee pathogen screening in Belgium reveals *Crithidia mellificae* as a new contributory factor to winter mortality. PLoS ONE **8(8):** e72443. doi: 10.1371/journal.pone. 0072443

Ravoet J., Schwarz R. S., Descamps T., Yanez O., Tozkar C. O., Martin-Hernandez R., Bartolome C., De Smet L., Higes M., Wenseleers T., Schmid-Hempel R., Neumann P., Kadowaki T., Evans J. D., de Graaf D. C. 2015b Differential diagnosis of the honey bee trypanosomatids *Crithidia mellificae* and *Lotmaria passim*. Journal of Invertebrate Pathology. **130:** 21-27

Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food of plant and animal and amending Council Directive 91/414/EEC. Off. J. Eur. Union **L70:** 1-16.

Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Off. J. Eur. Union **L152:** 11-22.

Reybroeck W. 2014 Quality Control of Honey and Bee Products. In Beekeeping for Poverty Alleviation and Livelihood Security Springer Science+Business Media Dordrecht. R.K. Gupta *et al*. (eds.) DOI 10.1007/978-94-017-9199-1_18

Reybroeck W., Daeseleire E., De Brabander H. F. Herman L. 2012 Antimicrobials in beekeeping. Vet Microbiol. **158:**1-11 DOI 1016/j.vetmic.2012.01.012

Ricciardelli D. G. & Compagnucci R. 1991 Apicultura e spettro pollinico di alcuni mieli del Senegal. Apicoltura. **7:** 33-49.

Roetschi A., Berthoud H., Kuhn R., Imdorf A., 2008. Infection rate based on quantitative real-time PCR of *Melissococcus plutonius* the causal agent of European foulbrood, in honeybee colonies before and after apiary sanitation. Apidologie. **39**: 362–371.

Romet A.2008 *Apis mellifera adansonia* in the uplands of West Cameroon. Bee for Dev. J. N°59)

Rosenkranz P., Aumeier P., Ziegelmann B. 2010 Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology. **103**: 96–119

Rostecki P., Samborski J., Prabucki J. 2007 Chuda-Mickiewicz B. A comparison of various hardware for the Measurement of the cubical index. J. Apicult. Sci. **51**: 1-5.

Roubik D. W. 1995 Pollination of cultivated plants in the tropics. Food and Agriculture Organization of the United Nations, Rome, Italy. Bull. 118°p.

Rozenkranz P., Aumeier P., Ziegelmann B. 2010 Biology and control of *Varroa destructor*. J. Invertebre Pathol. **103**: S96-S119 DOI 1016/j.jp.2009.07.016

Runckel C., Flenniken M.L., Engel J.C., Ruby J.G., Ganem D., Andino R., De Risi J.L. 2011 Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*. PLoS One. **6(6):** e20656 doi: 10.1371/journal.pone.0020656

Ruttner F 1988 Biogeography and taxonomy of honeybees. Springer, Berlin, xii+284 pp

Ruttner, E, L. Tassencourt, And J. Louveaux. 1978. Biomet-rical-statistical analysis of the geographic variability of *Apis mellifera* L. Apidologie. **9:**363-381.

Sammataro D., de Guzman L., George S., Ochoa R., Otis G. 2013 Standard methods for tracheal mite research Journal of Apicultural Research. **52 (4):** DOI 10.3896/IBRA.1.52.4.20

Sanad R., Mohanny K. 2011. Survey and management of the chalk brood fungal disease infecting honeybee colonies by natural agents at Qena Governorate, Upper Egypt. Egyptian Journal of Biological Pest Control. **21**: 251–256.

Sande D. Mullen J., Wetzstein M., Houston J. 2011 Environmental Impacts from Pesticide Use: A Case Study of Soil Fumigation in Florida Tomato Production. Int. J. Environ. Res. Public Health. **8 :** 4649-4661; doi:10.3390/ijerph8124649

Sanford M.T. 2003 Diseases and pests of the honey bee. CIR766, Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Schmidt M., Agonyissa D., Ouedraogo A., Hahn-Hadjali K., Thiombiano A., Koulibaly A., Goetze D., Zizka G. 2010 Changes in plant species composition following a climatic gradient in West Africa. In: X. van der Burgt, J. van der Maesen& J.-M. Onana (eds), Systematics and Conservation of African Plants, pp. 823-828. Royal Botanic Gardens, Kew.

Scholes, R. J., Kendall, J., Justice, C. O. 1996 The quantity of biomass burned in southern Africa. Journal of Geophysical Research 101 (D19): **23:** 667-23,676.

Schonegg G., Martel P., Sano B., Noufou S. 2006 Les conflits liés à la transhumance transfrontalière entre le Niger, le Burkina Faso et le Benin. DED, Division Développement rural, Gestion des eaux et ressources naturelles

Schur A., Wallner K. 2000. Bewertung des individuellen Wirkstoffeintrages von Sammelbienen nach Pestizidapplikation in blühende Kulturen. Apidologie. **31:** 615-616.

Schwarz R. S., Schwarz Q. H., Evans J. D. 2015 Hologenome theory and the honey bee pathosphere Insect sciences http://dx.doi.org/10.1016/j.cois.2015.04.006

Schwarz R. S., Evans J. D. 2013 Single and mixed-species trypanosome and microsporidia infections elicit distinct, ephemeral cellular and humoral immune responses in honey bees. Dev Comp Immunol. **40**: 300–310.

Sheppard, W. S., T. E. Rinderer, J. A. Mazzoli, J. A. Stelzer, And H. Shimanuki. 1991. Gene flow between African and European-derived honeybee populations in Argentina. Nature. **349**: 782-784.

Sheppardm W., Smith D. 2000 Identification of African-derived bees in the Americas : a survey of methods. Annals of the Entomological Society of America. **93**: 159-176

Shimanuki H. & David K. A. 2000 Diagnosis of Honey Bee Diseases. United States Department of Agriculture Agricultural Research Service. Agriculture Handbook Number 690.

Shoreit, M.N., Bagy, H.M., 1995. Mycoflora associated with stonebrood disease in honey bee colonies in Egypt. Microbiol. Res. **150:** 207–211.

Shrestha S. & Kazama K. 2007 Assessment of surface water quality using multivariate statistical techniques: A case study of the Fuji river basin, Japan. Environmental Modelling & Software **22:** 464-475

Singh R., Levitt A.L., Rajotte E.G., Holmes E.C., Ostiguy N., Vanengelsdorp D., Lipkin W.I., Depamphilis C.W., Toth A.L., Cox-Foster D.L. 2010 RNA viruses in hymenopteran pollinators: evidence of inter-Taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. PLoS One 5, e14357.

Sinsin B. & Kampmann D. 2011 Atlas de Biodiversité en Afrique de l'Ouest : Tome I, Bénin. Frankfurt, Allemagne, 526p.

Sinsin B. 1993 Phytosociologie, écologie, valeur pastorale, production et capacité de charge des pâturages du périmètre Nikki-Kalalé au Nord-Bénin. Ph.D Thesis; Fac. Sc., Lab. Bot., Syst. & Phytosoc., Univ. Lib. Bruxelles, Brussels.

Sinsin B., Eyog M.O., Assogbadjo A.E., Gaoué O.G. Sinadouwirou T. 2004 Dendrometric characteristics as indicators of pressure of Afzelia africana Sm. dynamic changes in trees found in different climate zones of Benin. Biodiversity and Conservation. **13(8):** 1555-1570.

Sinsin F., Glele Kaka R., Orthmann B., Sinsin B. 2013 Tree-ring: a suitable implement for spatial and temporal fire distribution analysis in savannah woodland and dry forest. J. For. Res. DOI 10.1007/s11676-015-0047-7

Solesbury W. 2003 Sustainable Livelihoods: A case study of the evolution of DFID Policy. ODI Working Paper 217. London

Southwick EE, Heldmaier G. 1987 Temperature control in honey-bee colonies. Bioscience. **37**:395-399

Spleen, A. M; Lengerich, E. J; Rennich, K; Caron, D; Rose, R; Pettis, J. S; Henson, M; Wilkes, JT; Wilson, M; Stitzinger, J; Lee, K; Andree, M; Snyder, R; vanEngelsdorp, D. 2013 A national survey of managed honey bee 2011-12 winter colony losses in the United States: results from the Bee Informed Partnership. Journal of Apicultural Research. **52(2):** 44-53. DOI:10.3896/IBRA.1.52.2.07

Stabentheiner A., Kovac H., Hetz S. K., Käfer H., Stabentheiner G. 2012 Assessing honeybee and wasp thermoregulation and energetics-New insights by combination of flow-through respirometry with infrared thermography. Thermochimica Acta. **534**: 77-86

Strauss U., Human H., Gauthier L., Crewe R.M., Dietemann V., Pirk, C.W.W. 2013 Seasonal prevalence of pathogens and parasites in the savanah honeybee (*Apis mellifera scutellata*). J. Invert. Pathol. **114**: 45-52.

Strauss U., Pirk C W. W., Crewe R.M., Human H., Dietemann V. 2015 Impact of Varroa destructor on honeybee (*Apis mellifera scutellata*) colony development in South Africa Exp Appl Acarol **65**:89-106 DOI 10.1007/s10493-014-9842-7

Struck 1994 Flowers and their insect visitors in the arid winter rainfall region of southern Africa: observations on permanent plots. Insect visitation behaviour. Journal of Arid Environments. **28**: 51-74

Swart D.J., Johannsmeier M.R., Tribe, G.D., Kryger, P. 2001 Diseases and pests of honeybees. In: Johannsmeier, M.F. (Ed.), Beekeeping in South Africa. Plant Protection Handbook No. 14. Agricultural Research Council, Pretoria, pp. 198–222.

Tapaszti Z., Forgach P., Kovago C., Bekesi L., Bakonyi T., Rusvai M. 2009 First detection and dominance of *Nosema ceranae* in Hungarian honeybee colonies. Acta Veterinaria Hungarica. **57**: 383–388.

Tentcheva D., Gauthier L., Zappulla N., Dainat B., Cousserans F., Colin M.E., Bergoin M. 2004 Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. Appl. Environ. Microbiol. **70**: 7185-7191.

Thomas C.D., Cameron A., Green R. E., Bakkenes M., Beaumont, L. J., Collingham Y.E., Erasmus B.F.N., Ferreira de Siqueira M., Grainger A., Hannah L., Hughes L., Huntley B., van Jaarsveld A.S., Midley G. F., Miles L., Ortega-Huerta M. A., Peterson A. T., Phillips O.,L., Williams S. E. 2004 Extinction risk from climate change. Nature. **427:** 145-148.

UNDP 2014: Human Development Report 2014. Sustaining Human Progress: Reducing vulnerability and building resilience UNDP

USA/UADA 2015 Managing small hive beetles. USA University of Arkanas Division of Agriculture. Agriculture and Natural Resources FSA FSA7075-PD-6-10N. http://www.uaex.edu

Van der Valk H. & Koomen I. 2013 Aspects determining the risk of pesticides to wild bees: Risk profiles for focal crops on three continents Food and Agriculture Organisation of the United Nation FAO Rome. 82p

Van Dillen, S. 2002. Book review: Rural livelihoods and diversity in developing countries. Journal of Development Economics. **70 (1):** 248-252.

Van Dooremalen C., Gerritsen L., Cornelissen B., van der Steen J.J.M., van Langevelde F 2012 Winter Survival of Individual Honey Bees and Honey Bee Colonies Depends on Level of *Varroa destructor* Infestation. PLoS ONE. **7(4):** e36285. doi:10.1371/journal.pone.0036285

vanEngelsdorp D., Evans J D; Saegerman C., Mullin C., Haubruge E., Nguyen B K., Frazier M., Frazier J., Cox-Foster D., Chen Y., Underwood R., Tarpy D R., Pettis J. S. 2009 Colony Collapse Disorder: a descriptive study. PLoS ONE. **4 (8):** e6481. DOI: 10.1371/journal.pone.0006481

vanEngelsdorp D., Speybroeck N., Evans J. D., Nguyen B. K., Mullin C., Frazier M., Frazier J., Cox-Foster D., Chen Y., Tarpy D. R., Haubruge E., Pettis J. S., Saegerman C. 2010 Weighing risk factors associated with bee colony collapse disorder by classification and regression tree analysis. J. Econ. Entomol. **103(5):** 1517-1523 DOI: 10.1603/EC09429.

Villières, B. 1987 L'apiculture en régions tropicales et équatoriales de l'Ouest. B. Tech. Apicole. **14:** 193-220

Vojvodic S., Rehan S. M., Anderson K. E. 2013 Microbial Gut Diversity of Africanized and European Honey Bee Larval Instars. PLoS ONE. **8(8):** e72106. doi:10.1371/journal.pone.0072106

Waite R., Brown M., Thompson H., Bew M., 2003. Controlling European foulbrood with the shook swarm method and oxytetracycline in the UK. Apidologie. **34:** 569–575.

Wallberg A., Han F., Wellhagen G., Dahle B., Kawata M., Haddad N., Simões Z. L.P., Allsopp M. H., Kandemir I., De la Rua P., Pirk C. W., Webster M. T. 2014 A worldwide survey of genome sequence variation provides insight into the evolutionary history of the honeybee Apis mellifera. Nature Genetics. **46 (10):** 1081-1088. doi:10.1038/ng.3077

Whitfield, C.W., Behura, S. K., Berlocher, S.H., Clark, A. G., Johnston, J. S., Sheppard, W. S., Smith, D. R., Suarez, A. V., Weaver, D., Tsutsui, N. D. 2006 Thrice out of Africa: ancient and recent expansions of the honey bee, Apis mellifera. Science. **314:** 642–5

WHO 2003 World Health Organisation specifications and evaluations for public health pesticides. report per pesticide World Health Organization Geneva

WHO/FAO 1975 Pesticide residues in food. Report of the 1974 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 97; WHO Technical Report Series, No. 574

William N M. W., Crone E. E., Roulston T. H., Minckley R. L., Packer L., Potts S. G. 2010 Ecological and life-history traits predict bee species responses to environmental disturbances. Biological Conservation. **143:** 2280–2291

Winston, 1991 M L The Biology of the Honey Bee. London England Harvard University Press. Cambridge Massachusetts. Ast edit 1991, 281°p.

Woodards S. H., Lozier J. D., Goulson D., Williams P. H., Strange J. P., JHA S. 2015 Molecular tools and bumble bees: revealing hidden details of ecology and evolution in a model system. Molecular Ecology. **24:** 2916–2936 doi: 10.1111/mec.13198

Woods W. A., Heinrich B., Stevenson R. D. 2005 Honeybee flight metabolic rate: does it depend upon air temperature? The Journal of Experimental Biology. **208**: 1161-1173

World bank 2015. world Bank data at http://data.worldbank.org/country/benin

Yacobson B.A., Elad D., Rosenthal K., Kmer I., Slovecky I., Efrat H. 1991. A recent chalkbrood outbreak in Israel: attempts at therapeutic intervention. Am. Bee J. **131**: 786.

Yang X. L., Cox-Foster D. L. 2005 Impact of an ectoparasite on the immunity and pathology of an invertebrate: Evidence for host immunosuppression and viral amplification. P Nati Acad Sci USA. **102**: 7470-7475

Yédomonhan H & Akoègninou A. 2009 La production du miel à Manigri (Commune de Bassila) au Bénin: enjeu et importance socio-économique. Int. J. Biol. Chem. Sci. **3(1):** 125-134

Yédomonhan H., Houenon G.J., Akoègninou A., Adomou A.C., Tossou G. M., Van der Maesen L. J. G. 2012 The woody flora and its importance for honey production in the Sudano-Guinean zone in Benin. International Journal of Science and Advanced Technology. **2(3):** 64-74.

Yohannes A., Bezabeh A., Yaekob B., Begna D., Shiferaw Y., Kebede Y., Kebede N. 2009 Ecological distribution of honeybee chalkbrood disease (*Ascosphaera Apis*) in Ethiopia Ecological distribution of honeybee chalkbrood disease (*Ascosphaera Apis*) in Ethiopia. Ethiopian Journal of Animal Production. **9(1):** 177-191

Zaghloul, O.A., Mourad, A.K., El Kady, M.B., Nemat, F.M., Morsy, M.E., 2005. Assessment of losses in honey yield due to the chalkbrood disease, with reference to the determination of its economic injury levels in Egypt. Commun. Agricult. Appl. Biol. Sci. **70 (4):** 703-714.

CURRICULUM VITAE

PERSONAL DETAILS

Name	Felicien AMAKPE
Date of birth	31/12/1971
Nationality	Beninese
E-mail	famakpem@hotmail.com

EDUCATION

2011-2016 Ph.D candidate, Laboratory of Molecular Entomology and Bee Pathology (L-MEB). Department of Biochemistry and Microbiology. Faculty of Sciences, Ghent University, Belgium

- Ph.D thesis: Exploring the potential of beekeeping in the Republic of Benin by examining the melliferous tress, the honeybees and the honey they produce.
- Promotor: Prof. Dr. Dirk de Graaf; co-promotors: Prof. Dr. Ir. Brice Sinsin and Em. Prof. Dr. Frans Jacobs

2007-2008 Master complémentaire en gestion des ressources animales et végétales en milieux tropicaux. University of Liège, Gembloux Agro-Bio Tech, Belgium).

- Master thesis: Beekeeping in the strategies of Biodiversity conservation, desertification control and poverty alleviation in Benin: a monograph of the honey bees and their pests in the district of Djidja.
- Promotors: Em. Prof Dr. Frans Jacobs and Prof Dr. Jean-Luc Hornick

1997-1999: Master in Forestry, water and rural engineering. Faculty of Agronomy Sciences (National University of Benin) and Faculty of Forestry (University of Ibadan, Nigeria)

- Master thesis: Contribution to a sustainable management of Trois Rivières forest reserve: structure and dynamics of the main plant communities and study of surrounding population needs.
- Promotors: Dr. Ir. Nestor Sokpon (Benin) and Em. Prof. Dr. Baba Adeyoju (Nigeria)

1993-1997 Bachelor in Science (BSc), General Agronomy (National University of Benin, Faculty of Agronomy Sciences).

SCIENTIFIC OUTPUT

A1 peer reviewed publications

1. Amakpe F., De Smet L., Brunain M., Ravoet J., Jacobs F. J., Reybroeck W., Sinsin B., de Graaf D. C. 2015 Discovery of Lake Sinai virus and an unusual strain of Acute bee paralysis virus in West African apiaries. *Apidologie* **47:** 35-47.

A2 peer reviewed publications

1. Amakpe F., Akouehou G. S., de Graaf D. C., Sinsin B. 2015 Determination of the silvo-melliferous regions of Benin: a nationwide categorisation of the land based on melliferous plants suitable for timber production. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* **116 (2):** 143–156.

2. Amakpe F. 2010 The biodiversity of the honey bee (*Apis Mellifera adansonii*) in the district of Djidja, Republic of Benin. *International Journal of Environmental, Cultural, Economic and Social Sustainability* **6 (6):** 89-104.

B2 Book chapter

1. Amakpe F. & Bartlett R. 2008 The Global book of Transport: Benin Social roads, Schorrell Analysis, Version 1.01.

2. Amakpe F. Gbebada D. Adimou E. 2007 Elaboration du plan foncier urbain de la commune de Djidja : dimensionnement des infrastructures sociocommunautaires et voiries urbaines. CENAD/Mairie de Djidja (Benin) et Marie de Tintigny (Belgique).

3. Amakpe F. 2006 Plan stratégique de la Gestion intégrée participative des déchets dans la commune de Bohicon. CENAD/CISV/Union Européenne.

PARTICIPATION AT CONFERENCES

1. Amakpe F., Akouehou G. S., de Graaf D. C., Sinsin B. 2016 Déterminaition des régions sylvo mellifère en république du Bénin. Séminaire national sur la cartographie des occupations du sol et des changements climatiques au Bénin. Cotonou le 28 Juin 2016. Projet GEOFORAFRI (www.geoforafri.org)

2. Amakpe F., Ezin A. M., Hornick J-C., Jacobs F. J., de graaf D. C., Sinsin B. 2013: Abeilles mellifères et besoins en eau. Séminaire de formation sur « Gestion des ressources en eau souterraine dans le cadre de la gire, dans le contexte de bassin versant ». 09 au 13 Décembre 2013 Porto-Novo, Benin.

3. Amakpe F., J., Zuber, Jacobs F J. 2008 Role of beekeeping in the household food security and livelihoods: Implication for the sector policy development in Ethiopia; Gents Africa Platform (GAP) 2nd Symposium 22nd December 2008, University of Ghent.

4. Amakpe F. 2004 Alliances efficaces: Quels partenariats pour réussir les projets de développement et de conservation au sein de l'organisation. Stage International sur la gestion des projets pour la sécurité alimentaire. Faculté des Sciences Agronomiques de Gembloux. Du 9 avril au 10 juillet 2004.

PARTICIPATION AT WORKSHOPS AND COURSES (2006-2014)

1 Elaboration des plans d'aménagement et gestion durable des forêts en République du Bénin (Université d'Abomey Calavi et Direction Générale des Forêts et Ressources Naturelles du Bénin)
2 Beekeeping for poverty alleviation
3 Course on Data base management and biostatistics, (University of Liège, Belgium)
4 Cartographie appliquée et techniques d'inventaire de la faune en milieu tropical (Gembloux Agro-Bio Tech, Belgium)
5 Ressources cynégétiques, écotourisme et aménagement des chasses en milieux tropicaux (University of Liège)
6 Analyse diagnostique et amélioration des systèmes de production agricoles en régions tropicales (University of Liège and Gembloux Agro-Bio Tech, Belgium)

7 Conservation de la biodiversité et aménagement des parcs en milieux tropicaux (University of Liège, Gembloux Agro-Bio Tech, Belgium)
8 Méthodologie de l'information et de la recherche scientifique (University of Liège, Gembloux Agro-Bio Tech, Belgium) ;
9 Aspects anthropologique de la conservation et du développement durable (University of Liège, Gembloux Agro-Bio Tech, Belgium)
10 Gestion des cycles de projet et économie des productions (University of Liège, Belgium)
11 Course on Advanced Academic English: Conference skills-English Proficiency for Presentations. English Lab, Ghent University 1st to 30 April 2006

Master project

1. Aspects énergétiques de l'alimentation des abeilles mellifères en milieux tropicaux (2007-2008).
2. Bee pathology
3. Epidemiologie et maladies des abeilles en République du Bénin (2007-2008)
4. Elevage des abeilles dans la commune de Djidja (2007-2008).
5. Apiculture dans les stratégies de la conservation de la biodiversité au Bénin (2007-2008).
6. Cartographie et dynamique des ressources forestières en république du Bénin (1997-1999).