# Tree selection by the stingless bee *Melipona beecheii,* Chalatenango, El Salvador



Anouschka Hof 2004 AV2004-25



# Tree selection by the stingless bee *Melipona beecheii,* Chalatenango, El Salvador.

Anouschka Hof 790509-348-070

April 2004

F500-758 Afstudeervak Bosecologie, 24 st. AV2004-25 Wageningen University, Forest ecology and forest management group.

Supervisors

Prof. Dr. F. Bongers Forest ecology and forest management group Wageningen University

Ir. M.A.A.H. Sandker Proyecto de manejo de abejas y del bosque (PROMABOS) Utrecht University

The contents of this report are for internal use only. Reproduction is not allowed, unless prior permission has been obtained from one of the supervisors.

# Table of contents

Sum	nmary		4	
1.	Introd	duction	6	
2.	Meth	Methods		
	2.1.	Study site	9	
	2.2.	Tree selection	9	
	2.3.	Distance to the hive	12	
	2.4.	Tree size and density	12	
	2.5.	Flowering	13	
	2.6.	Pollen and nectar samples	14	
	2.7.	Statistical analyses	15	
3.	Resu	Results		
	3.1	Tree distance, size and density	18	
	3.2	Tree selection	20	
	3.3	Sugar percentages	24	
	3.4	Climatic influences	25	
4.	Discu	28		
	4.1	Tree selection	28	
	4.2	Sugar percentages	31	
	4.3	Climatic influences	32	
5.	Conc	lusions	33	
Ackı	nowledge	ements	35	
Refe	erences		36	

# Summary

Floral preference of the bee *Melipona beecheii* strongly depends on the composition of the vegetation, which is geographically very variable. Little is known about the floral preference of *M. beecheii* in El Salvador. This information is of greatest importance for the reforestation component of the PROMABOS project. PROMABOS will plant major bee food trees on farmers' land with the aim of augmenting the survival of *M. beecheii* colonies and increasing their honey production. For a good understanding of the tree selection by *M. beecheii* for the reforestation component of PROMABOS, the preference of *M. beecheii* for the selected tree species has been studied. This has been done by means of both analyses of the pollen removed from the bees, and an investigation of the impact of the tree density and of their average distance to the hives per tree species, on the tree selection of *M. beecheii*.

Fieldwork has been done from November 2003 till March 2004 in the area of Northwest El Salvador (Central America), department of Chalatenango. The fieldwork area was defined as a circle around three hives of *M. beecheii* with a radius of 500 meter. The PROMABOS project selected 20 tree species to plant at land owned by farmers. Not all these selected tree species flowered during the fieldwork period, or were present in the area. For these reasons the research was restricted to ten species, namely: Anacardium occidentale, Byrsonima crassifolia, Cordia alliodora, Gliricidia sepium, Inga paterno, Liquidambar styraciflua, Persea americana, Psidium guajava, Spondias purpurea, and Vernonia patens. The tree density has been estimated using the stand density index (SDI) (Reineke 1933 cited in Woodall et al. 2003). The number of individuals per tree species were counted and the diameters at breast height were measured. The average distance of the tree species to the hives has been measured using a Global Positioning System (GPS). Furthermore, the timing and duration of flowering of the tree species selected by PROMABOS was recorded. In total 391 samples of pollen were collected from *M. beecheii* individuals returning to the hives. 409 Bees were caught to obtain nectar samples for the measurement of the sugar percentage. The pollen these bees accidentally collected on their body were sampled as well. The fuchsine method has been used to prepare the pollen samples for analyses.

From the studied tree species, *Cordia alliodora* and *Vernonia patens* were nectar sources highly favoured by *M. beecheii*. This in spite of a low sugar concentration of the nectar, especially for *Cordia alliodora*, compared to the mode and expected optimum. Both species have whitish

flowers and mass flowering characteristics, which might be favourable for M. beecheii. Therefore these species are recommended for the reforestation component of PROMABOS. Liquidambar styraciflua was both a pollen and a nectar source for M. beecheii. The green colour of the flowers and the lack of mass flowering characteristics possibly form a disadvantage for the selection by *M. beecheii*. The selection was not high, when considering the fact that the trees were situated in the near vicinity of the hives and that they were present at a high stand density index. If used for reforestation, it is recommended to plant this species close to the hives in a high density and situated closely together. Pollen of *Gliricidia sepium* were not found in the analyses. More experiments based upon spotting or catching of *M. beecheii* on this tree species, and the impact of competition by Apis mellifera need to take place, before conclusions can be drawn about the importance of Gliricidia sepium as a nectar source for M. beecheii. Because the members of the subfamily of the Papilionaceae have stamens that are regularly enclosed; visiting bees rarely collect pollen on their body. Inga paterno and Byrsonima crassifolia were hardly or not selected and therefore not recommended for the reforestation component of the project. Persea Americana, Psidium guajava, Spondias purpurea and Anacardium occidentale did not bloom during the fieldwork period. Data about the density and the SDI are present for these species, but further research when the species have their blooming period needs to take place to be able to draw conclusions about their favourability for M. beecheii. Species of the Compositae and Leguminosae family, Myriocarpa sp. (family Urticaceae), Solanum sp. (family Solanaceae) and Syzygium jambos L. (family Myrtaceae), Tibouchina longifolia (family Melastomataceae) were important pollen and nectar sources for M. beecheii. Eugenia sp. (family Myrtaceae) was an important pollen source for M. beecheii.

# 1. Introduction

Non-stinging or "stingless" bees (*Apidae, Meliponinae*) are native to the tropical Americas. Approximately twenty different species occur in El Salvador of which *M. beecheii* is the preferred species for husbandry. Because of their diversity, their great abundance and because of their co-evolution with the local vegetation, stingless bees are important for pollination of various trees in tropical ecosystems. In particular the species of the genus *Melipona* are important for the pollination and conservation of indigenous trees and plants, because of their specific "buzz pollination" behaviour (PROMABOS 2004). The bee is using the indirect flight muscles to cause a vibration, which gives an access to the pollen grains. Therefore, plant species with an inaccessible anther can be pollinated which otherwise could not (Gracie, 1993).

Floral visitation of *M. beecheii* depends on the composition of the vegetation, which is geographically very variable. This bee species is more selective in its choice for food plants than the honeybee or species from the stingless bee species of the Trigona genus (PROMABOS 2004). Little is known about the floral preference of *M. beecheii* in El Salvador, while this information is of greatest importance for the reforestation component of the PROMABOS project (Proyecto de manejo de abejas y del bosque). This project engages in the management and conservation of forest by local beekeepers through the development of stingless beekeeping in the surrounding forest area of the Montecristo nature reserve in the departments of Chalatenango and Santa Ana in El Salvador. PROMABOS will plant major bee food trees on farmers' land with the aim of augmenting the survival of M. beecheii colonies and increasing their honey production (PROMABOS 2004). The program selected 20 trees for the reforestation component which are known to be important pollen or nectar sources for the honey bee Apis mellifera, but the importance for *M. beecheii* is largely unknown. Therefore, the source selection of *M. beecheii* for their collection of pollen and nectar was being studied, to discover if the trees selected by PROMABOS are favourable to *M. beecheii*. This collection intensity depends on the availability of the selected tree species and its importance for *M. beecheii*.

Stingless bee species select pollen in the surrounding 120 to 853 meters of their hive (depending on the species and experiment) (Nieuwstadt & Ruano Iraheta 1996). When the bees fly farther than their foraging range, the amount of pollen they collect is not enough to compare for their loss of energy. Although the difference in flight range between the four stingless bee species used by Nieuwstadt & Ruano Iraheta (1996) and *M. beecheii* might be substantial, the

distance of the trees to the hive is important for the choice of the bees. Other important factors of tree selection by bees are variables closely related to forest disturbance and resource abundance, such as the density and abundance of trees, air temperature and flowering intensity of trees and shrubs (Hsiang Liow et al. 2001). Research by Percival (1947 cited by Coffey et al. 1997) demonstrated that honeybees in general collected pollen from the most abundant species. Furthermore, research by Hsiang Liow et al. (2001) that took place in tropical lowland forests of Southeast Asia, showed that more stingless bees (Trigona spp.) were found where trees were larger and ambient conditions more constant but flowering intensities lower. The question arises whether *M. beecheii* shows behaviour comparable to honeybees and *Trigona* species and collects pollen tree density or size dependent. Flowering of the trees is defined by the timing and duration. Plants in the tropics have a big variation in these variables (Janzen 1967 and Bawa 1983 cited in Bawa et al. 2003), they can flower each time a year for a few days or for as long as the whole year (Gentry 1974a and Opler et al. 1980 cited in Bawa et al. 2003). The duration of flowering is an important factor for the collection of pollen by bees. Data about the timing and the duration of flowering should be collected for the tree species selected by PROMABOS. Also, differences in flower characteristics such as shape, size and colour are important for the selection by bees, because specific flower characteristics have a higher attraction for bees then others (a.o. McCall et al. 1992, Nagamitsu et al. 1999, Kearns et al. 1993). Furthermore, the sugar concentration of the nectar in the flowers has an impact on the choice by bees. Bees tend to select flowers with a sugar concentration between 35-65% (a.o. Roubik 1995, Biesmeijer et al. 1999a/b/c). Concluding; the choice of bees for specific tree species is a function of many different aspects. This research took place in an existing forest; therefore dealing with all these aspects was difficult. The variables that were considered to be of great importance and were easily possible to measure were taken into account.

For a good understanding of the tree selection by *M. beecheii* for the reforestation component of the PROMABOS project and because there is no data available for this region, the purpose of this research was to study the preference of *M. beecheii* for the selected tree species. First, an investigation of the presence of the twenty tree species selected by PROMABOS in the surrounding 500 meters of the hives has been made. As well the timing and duration of flowering of these trees has been noted. Second, pollen has been removed from *M. beecheii* individuals returning to their hive, and sugar concentration of the nectar collected by *M. beecheii* individuals has been measured. Last, the pollen samples have been analysed to investigate the presence of pollen of the tree species selected by PROMABOS, within the pollen samples

collected from returning *M. beecheii* individuals. Considering the low number of tree species selected by PROMABOS that were selected by *M. beecheii* during the fieldwork period (4) the characteristics of the flowers such as colour and shape, have not been taken into account. Though, the possible effect of these aspects on the choice of the bee for a specific tree species must not be underestimated.

The following research questions have been addressed:

- Which tree species, among the selected species, are favourable to *M. beecheii* for the collection of pollen and of nectar?
- Do the distance of the tree species to the hives and/ or the density and size of the tree species exert a significant influence on the tree selection by *M. beecheii*?
- Does the sugar percentage of the nectar of the selected exert a significant influence on the tree selection by *M. beecheii*?

# 2. Methods

# 2.1. Study site

Fieldwork has been done in the area of Northwest El Salvador (Central America), department of Chalatenango. This is the area surrounding the town of La Palma. The selected area was situated in the surroundings of the village of Caballeros (UTM-coordinates: N14 18'26.9" W089 08'18.2"), at an elevation of 1432 m asl. (Diccionario 1985, personal data Sandker 2004). Palynological analysis took place at the technical facilities of the PROMABOS project that were located in the capital San Salvador (Experimental station of the Ministry of Agriculture and the University of El Salvador).

Chalatenango is a mountainous area at the border with Honduras. This is one of the very few forested areas left in El Salvador. The area is volcanic and consists of an acid soil (Ministerio de medio ambiente y recursos naturales 2000a). The vegetation consists of a pine-oak (*Pinus-Quercus*) complex with cipres (*Cupressus lusitanica*) and liquidambar (*Liquidambar styraciflua*) as other dominant species. Other important families are *Asteraceae, Clethraceae, Lamiaceae, Leguminosae* (*Caesalpiniaceae, Mimosaceae & Papillionaceae*), *Melastomataceae, Solanaceae* and *Verbenaceae* (Smeets 2002). The area has a yearly rainfall of about 2256 mm, but there is a dry period from November till April with only 6 to 68 mm rainfall per month. The average air temperature in La Palma is 21° with a minimum of about 16° and a maximum of about 27° (Ministerio de medio ambiente y recursos naturales 2000b).

# 2.2. Tree selection

The PROMABOS project selected twenty trees to plant at land owned by beekeepers, with the aim of augmenting the survival of *M. beecheii* colonies and increasing their honey production. Because reforestation takes place on the private area of beekeepers, an objective of PROMABOS is reforest with economically productive tree species. This means that the selected trees were, next to being known important food sources for bees, timber producing, fruit producing or ornamental trees. Furthermore, an objective is that the tree species aught to be indigenous to the region, because the project aims at managing the natural forest without disturbing the natural composition of the forest (PROMABOS 2004, Sandker 2003). The twenty selected tree species, whether or not these species were recorded to be visited by *M. beecheii*, and additional uses attractive to farmers are shown in table 1.

Scientific name	Family	Visited by <i>M.</i> beecheii	Additional use attractive to farmer
Acacia angustissima	Fabaceae	No	Forage, fruit tree, medicinal use
Anacardium occidentale	Anacardiaceae	Yes <sup>1</sup>	Fruit tree, ornamental tree <sup>1</sup>
Andira inermis	Fabaceae- papilionidae	Yes <sup>1, 2</sup>	Good wood quality, ornamental tree, shade tree <sup>1</sup>
Bixa orellana	Bixaceae	Yes <sup>1</sup>	Medicinal use, ornamental tree <sup>1</sup>
Bursera simaruba	Burseraceae	Yes <sup>1</sup>	Medicinal use <sup>1</sup>
Byrsonima crassifolia	Malpighiaceae	No	Fruit tree, medicinal use <sup>5</sup>
Cassia grandis	Fabaceae-caesalpinioideae	Yes <sup>1</sup>	Medicinal use, ornamental tree, shade tree <sup>1</sup>
Cedrela odorata	Meliaceae	Yes <sup>1</sup>	Good wood quality <sup>1</sup>
Cochlospermum vitifolium	Cochlospermaceae	Yes <sup>1</sup>	Good wood quality, medicinal use, colorization of clothes <sup>1</sup>
Cordia alliodora	Boraginaceae	Yes <sup>3, 4</sup>	Good wood quality <sup>1</sup>
Croton reflexifolius	Euphorbiaceae	No	Edible leaves, medicinal use <sup>5</sup>
Gliricidia sepium	Fabaceae- papilionidae	No	Edible flowers, medicinal use, shade tree <sup>1</sup>
Inga paterno	Fabaceae-mimosaceae	yes <sup>1</sup>	Fruit tree <sup>1</sup>
Liquidambar styraciflua	Hamamelidaceae	Yes <sup>3, 4</sup>	Good wood quality <sup>6</sup>
Persea americana	Lauraceae	No	Fruit tree <sup>6</sup>
Pithecelobium dulce	Fabaceae-mimosaceae	No	Fruit tree, good wood quality, shade tree <sup>5</sup>
Psidium guajava	Myrtaceae	Yes <sup>1</sup>	Fruit tree <sup>1</sup>
Spondias purpurea	Anacardiaceae	No	Fruit tree <sup>5</sup>
Tabebuia rosea	Bignoniaceae	No	Good wood quality, medicinal use, ornamental tree <sup>1</sup>
Vernonia patens	Asteraceae	Yes <sup>4</sup>	Good wood quality <sup>6</sup>

1: Arce *et al.*, 2001. 2: Frankie *et al.*, 1997. 3: Unpublished results PROMABOS, 2003. 4: Personal observations during pollen analyses 2004. 5: Banco Agricola de El Salvador 2003. 6: According to farmers in the region of La Palma, Chalatenango, El Salvador, 2003.

**Table 1**: The twenty tree species selected for the reforestation component of the PROMABOS project. The family names, visits by *M. beecheii* according to literature, and additional uses attractive to farmers.

Not all the selected tree species were expected to flower during the fieldwork period, or were present in the area. For these reasons the research was, at start of the fieldwork, restricted to ten species, namely: *Anacardium occidentale, Byrsonima crassifolia, Cordia alliodora, Gliricidia sepium, Inga paterno, Liquidambar styraciflua, Persea americana, Psidium guajava, Spondias purpurea,* and *Vernonia patens.* 

After the fieldwork period, still not all these selected tree species had their blooming period, while other species had not finished yet. Six species, namely: *Byrsonima crassifolia, Cordia alliodora, Gliricidia sepium, Inga paterno, Liquidambar styraciflua* and *Vernonia patens*, were in bloom during the fieldwork period. The other species were left out of some of the analyses, because it was not possible for *M. beecheii* to harvest the pollen or nectar of these tree species due to lack of flowers. The list of species with their blooming period is given in table 2. From the species that were not out of bloom at the end of the fieldwork period, the number of trees that did not bloom was counted to take this into account with the further analyses. Table 3 gives information about the mass flowering of the tree species.

Species	Start blooming period	End blooming period	% out of bloom*
Anacardium occidentale	Unknown	Half October	100%
Byrsonima crassifolia	Half February	March	95%
Cordia alliodora	Beginning January	End of February	100%
Gliricidia sepium	Half January	Beginning March	100%
Inga paterno	Half February	March	100%
Liquidambar styraciflua	Beginning February	March	95%
Persea americana	Not during fieldwork period	Not during fieldwork period	0%
Psidium guajava	Not during fieldwork period	Not during fieldwork period	0%
Spondias purpurea	Not during fieldwork period	Not during fieldwork period	0%
Vernonia patens	Half February	March	90%

\*% of the trees that were out of bloom at the end of the fieldwork period

**Table 2**: Information about the blooming period of the tree species selected by PROMABOS and present in the fieldwork area, Caballeros, Chalatenango, El Salvador, 2004, and the percentage of trees per tree species that was out of bloom at the end of the fieldwork period.

Scientific name	Sugar% nectar	Mass flowering
Anacardium occidentale	No data	No <sup>(5)</sup>
Byrsonima crassifolia	Oil <sup>(1)</sup>	No <sup>(5)</sup>
Cordia allidora	15-5% <sup>(2)</sup> / <b>33%</b> *	Yes (5)
Gliricidia sepium	3.3-36% <sup>(3)</sup>	Yes (5)
Inga paterno	No data	No <sup>(5)</sup>
Liquidambar styraciflua	33%*	No <sup>(5)</sup>
Persea americana	44-49% <sup>(2)</sup>	Yes (5)
Psidium guajava	28% <sup>(2)</sup>	No <sup>(5)</sup>
Spondias purpurea	No data	No <sup>(5)</sup>
Tabebuia rosea	39% <sup>(3)</sup>	Yes (5)
Vernonia patens	47.6 <sup>(4)</sup> , <b>50%</b> *	Yes (5)

<sup>1</sup>Vinson *et al.* 1997, <sup>2</sup>Crane *et al.* 1984, <sup>3</sup>Arce *et al.* 2001, <sup>4</sup> Biesmeijer *et al.* 1999c, <sup>5</sup> personal communication Sandker (2004), \*own data 2004

**Table 3**: Sugar percentages of the nectar of the flowers of the tree species selected by PROMABOS, and their mass flowering characteristics.

## 2.3. Distance to the hives

Not many bee species have been studied for their flight range, but obvious correlations exist between flightrange and size. Bees around 10 mm big, such as *M. beecheii* (own observations, 2004), have a flight range estimated at 800 meters (Wille, 1983). But, more than 75% of the forage activity occurs within 40% of the maximum foraging distance (Nieuwstadt & Ruano Iraheta. 1996). This, combining with the time schedule and the difficulties traversing the area, lead to a fieldwork area defined as a circle around the hives with a radius of 500 meter. To be able to acquire a representative collection of pollen and nectar samples at the midpoint of the circle, three hives of *M. beecheii* located closely to each other (about one meter apart) have been used. These hives have been considered as one assemblage of *M. beecheii* individuals. Furthermore, using this tactic, a reasonable amount of pollen and nectar samples for the statistical analyses could be obtained, and the hives could be disturbed as less as possible. The distance of the trees to the hives has been measured using a Global Positioning System (GPS). The Garmin etrex 12-channel GPS device has been used to take the UTM-coordinates. The hives were the starting point and the GPS coordinates were the references for the distance. The surrounding area of the hives has been traversed by foot in the company of a local guide. Each time when a selected tree species was encountered, a GPS point has been taken.

#### 2.4. Tree size and density

When traversing the area surrounding the hives, the number of individuals of the ten selected tree species has been recorded and their size at breast height has been measured. Trees with a diameter below two centimeters were neglected because these trees were considered to be too young to offer a substantial reward for harvesting bees. When one of the selected tree species was grouped together on a small spot of the terrain, the number of individuals of this species was counted and the average diameter size was taken. Because of difficulties getting near *Liquidambar styraciflua* caused by steepness of the terrain and density of the forest, the mean diameter was taken based upon 25% of the total amount of trees, with a minimum of 25 individuals. The trees were grouped into little, mediocre and large diameter, in this way three averages were obtained. The diameter of each individual of *Liquidambar styraciflua* was estimated and classified in one of these three groups.

Tree density has been estimated using the stand density index (SDI) (Reineke 1933 cited in Woodall *et al.* 2003, Zeide 2004). This index, based on the relationship between mean tree size

and number of trees per unit area in a forest stand, was developed for even-aged stands but has been applied in uneven-aged stands. Reineke's stand density index for a stand is the number of trees per hectare as if the ASD (diameter of a tree with average basal area) were 25 centimeters (Daniel *et al.* 1997). The diameter class summation method was used to apply SDI in uneven-aged stands, where SDI values were calculated for each diameter class and then summed for an estimate of total stand density (Stage 1968 and Long 1995 cited in Woodall *et al.* 2003). SDI is defined as the equivalent number of trees per hectare with a specified diameter of 25 cm. The summation method calculates SDI for each diameter class and then sums for a total stand value (Woodall *et al.* 2003). Classes have been defined of the different diameter sizes by the program SPSS (SPSS-inc), because not all the diameters of the individual trees were measured. The data were categorized based on percentile groups, with each group containing approximately the same number of cases. The summed SDI was calculated for each of the selected tree species. The following formula (Woodall *et al.* 2003) was used:

# $SDI = \sum TPH_i (DBH_i/25)^{1.6}$

In which:

- DBH<sub>i</sub>: the midpoint of the *i*-th diameter class (cm)
- TPH<sub>i</sub>: the number of trees per hectare in the *i*-th diameter class.
- i: the different diameter class.
- 1.6: the constant found by Reineke, valid for all species and locations.

# 2.5. Flowering

The timing and duration of flowering of the tree species selected by PROMABOS was recorded. In November the trees had been localised. The area has been traversed each week until March, because this was the season that the selected trees were expected to flower. Each time when the area was traversed, it was recorded whether the selected tree species were flowering or not and it was recorded whether all of the trees per selected tree species had flowered, this because the number of flowering trees was an important factor in the analyses. Flowering characteristics such as shape, colour and size are as well important for the selection by bees. However, the number of selected tree species was too low to verify statistically the influences of these characteristics on the selection by *M. beecheii*.

# 2.6. Pollen and nectar samples

Pollen collections took place at each Tuesday during ten weeks in December until March. Seven collections of 15 minutes at intervals of 90 minutes, took place starting at 7:00 o'clock and ending at 16:15 o'clock. This is the time that *M. beecheii* is harvesting. Pollen samples were obtained from several bees when entering the hive for each collection. The entrances of the hives were closed for 15 minutes to be able to catch bees that returned with pollen samples attached to their hind legs. Not all the bees, but at least four individuals (maximal eight) arriving with pollen were caught per interval per three hives to disturb the colonies as less as possible. The aim was to collect at least 30 samples of pollen each day, 10 per hive. In total 391 samples of pollen were collected. The samples were removed, saved in Eppendorf tubes and stored in the refrigerator until further analyses. The date, collection time and colour of the pollen were noted. Also the pollen that bees accidentally collect on their body when visiting a flower for the harvest of nectar have been collected, because part of the pollen removal occurs passive by bees collecting nectar (Thorp 2000). This occurred under the same circumstances, only the pollen was not removed from the hind legs but, with use of glycerine, from the body. The aim was also to collect at least 30 samples of pollen from the bees collecting nectar each day, 10 per hive. In total 409 samples were collected.

The sugar concentration of the nectar of flowers might have an impact on the selection of trees by *M. beecheii* (a.o. Engel *et al.* 1980, Biesmeijer *et al.* 1999b). Therefore, when collecting the pollen from the bodies of the bees harvesting for nectar, the nectar was also collected. This was done by squeezing the stomach of the bee mildly, in this way, the bee spits up the nectar that is stored in the honey stomach. A capillary was used to collect the nectar when excreted. The sugar percentage of the nectar samples has been measured using an Atago Hand Refractometer. The aim was also to measure at least 30 nectar samples each day, 10 per hive. In total 409 samples of nectar were collected.

Analyses of the samples took place in the research facilities of PROMABOS located in San Salvador at the Experimental station of the Ministry of Agriculture and at the University of El Salvador. The fuchsine method has been used to prepare the pollen samples for analyses. The pollen samples together with a little drop of glycerine were heated, fuchsine was added and a top-glass was used to protect the slide. The slides were analysed using a Nikon eclipse E400 microscope. The relative occurrence of the different pollen within the collections has been categorized using four categories; incidental pollen (less than 3%), frequent pollen (3-20%),

abundant pollen (20-50%), dominant pollen (over 50%) (Engel et al. 1980, Sommeijer 1983). A drawback of the method is that no traces of pollen collections will be found if the pollen is destroyed during the preparing of the slides, and the number of pollen types that can be identified depends on the completeness of the slide, because only a small amount of the total pollen sample can be used. Furthermore, percentages calculated from counts of pollen types in mixed samples of bee collections, for example the pollen samples collected from the bees harvesting nectar on different tree species, are not reliable estimates of the relative importance of each pollen source. The weight and volume of the pollen is also important (Silveira 1991). The volume of the different pollen was visually estimated and taken into account during the analyses, a large pollen species was estimated at a higher percentage than a small pollen species. When a pollen of one tree species was present in a concentration higher then 50% in a pollen sample that was taken of the body from a bee collecting nectar from different tree species, the sugar percentage of the nectar collected by this same bee was assumed to resemble the sugar percentage of this specific tree species. The sugar percentages of species lacking corresponding pollen and nectar samples, have been, when possible, obtained from literature (table 3).

Climatic data such as air temperature, humidity and wind velocities have been obtained from the meteorological station in La Palma (SNET 2004), because these factors can have an impact on the flight range of bees and the sugar percentage of the nectar resulting in a possibly different selection (Kearns *et al.* 1993, Corbet *et al.* 1979, Baker *et al.* 1983 cited in Roubik 1995). Differences in climate might form a certification for unexpected selection.

## 2.7. Statistical analyses

• Which tree species, among the selected species, are favourable to *M. beecheii* for the collection of pollen and of nectar?

The selection of trees by *M. beecheii* has been calculated using the method used by Manly *et al.* (1993). The analysis is based on differences in numbers of available tree species and numbers of used tree species, given that the selecting organism has unrestricted access to the entire distribution of available units. First, a Chi-square test has been used to determine whether there was significant selection among the available selected tree species. When there was significant selection ratios have been used to distinguish preference or avoidance of a

specific tree species. The selection probability quantifies the extent to which a tree species was selected. This has been calculated using the following formula;

$$W_i = o_i / \pi_i$$

$$o_i = u_i / u_+$$

With:

- W<sub>i</sub>: The selection probability for tree species i.
- o<sub>i</sub>: The proportion of selections of tree species i within the total number of available tree species.
- $\pi_i$ : The proportion of tree species i within the total number of available tree species.
- u<sub>i</sub>: The number of selections of tree species i.
- u<sub>+</sub>: The total number of selections of all the tree species.

The standardized selection ratio B<sub>i</sub> gives the estimated probability that a randomly selected tree species will be species i, if all species are equally frequent in the total amount of available tree species (Manly *et al.*, 1972 cited in Manly *et al.*, 1993). This method estimates the strength of the selection.

The standardized selection ratio B<sub>i</sub>:

$$\mathsf{B}_{i} = \mathsf{W}_{i} / (\sum_{i=1}^{1} \mathsf{W}_{j})$$

• Do the distance of the tree to the hives and/ or the stand density index of the tree species have a significant impact on the tree selection by *M. beecheii*?

ANOVA has been used to test whether the distances and the trunk sizes differed significantly from each other per tree species. The mean distance and the mean trunk size of a tree species to the hives have been used, because it was not known which specific tree, within a species, the

bee had used for the harvest. The relation between the mean distance and the mean trunk size to the hives and the selection by *M. beecheii* was calculated using a regression analyses. As well the relation between the stand density index of the selected tree species and the selection by *M. beecheii* was calculated using a regression analyses.

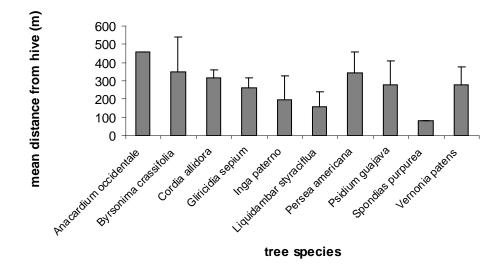
• Does the sugar percentage of the nectar of the selected trees have a significant impact on the tree selection by *M. beecheii*?

The non-parametric Mann-Whitney U test has been used to test whether the means of the sugar percentages of the nectar differed significantly per tree species. A regression analyses has been used to study the impact of the air temperature and the relative humidity on the sugar percentage of the nectar.

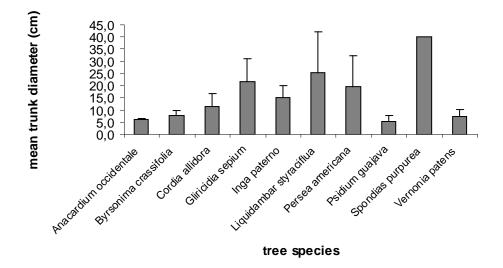
# 3. Results

#### 3.1 Tree distance, size and density

All the ten tree species were found in the fieldwork area. In total 710 trees were present representing these ten species. The distance to the hives varied significantly per tree species (ANOVA: p: 0.000, df: 8) from 81 meters (N = 1, St. dev = 0 m.) to 458 meters (N = 2, St. dev = 0) (Fig. 1). The trunk diameter varied significantly per tree species (ANOVA: p: 0.000, df: 8) from 5 (N = 177, St. dev. = 2.5) to 40 centimeters (N = 1, St. dev = 0 m.) (Fig. 2). The SDI varied from 0.003 to 2.35 (table 4). Often the different tree species were scattered around the fieldwork area, but for example about 95% of *Vernonia patens* was grouped closely together in one site. Also about 90% of the *Cordia alliodora* was situated closely together (Map 1).



**Figure 1**: The average distance of the selected tree species to the hives in the surrounding area of 500 meters, Caballeros, Chalatenango, El Salvador, 2004. (*Anacardium occidentale*: N = 2, St. dev = 0, *Byrsonima crassifolia*: N = 7, St. dev = 194, *Cordia allidora*: N = 18, St.dev. = 42, *Gliricidia sepium*: N = 6, St.dev. = 53, *Inga paterno*: N = 25, St. dev. = 128, *Liquidambar styraciflua*: N =43, St. dev. = 82, *Persea americana*: N = 11, St. dev. = 119, *Psidium guajava*: N = 177, St. dev. = 132, *Spondias purpurea*: N = 1, St. dev. = 0, *Vernonia paters*: N = 32, St. dev. = 98)

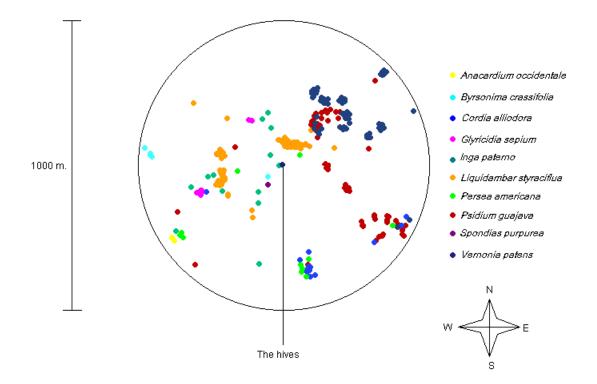


**Figure 2**: The average trunk diameter per tree species, selected by PROMABOS, in the surrounding area of 500 meters, Caballeros, Chalatenango, El Salvador, 2004. (*Anacardium occidentale*: N = 2, St. dev = 0.5, *Byrsonima crassifolia*: N = 7, St. dev = 1.9, *Cordia allidora*: N = 18, St.dev. = 5.4, *Gliricidia sepium*: N = 6, St.dev. = 9.3, *Inga paterno*: N = 25, St. dev. = 5.2, *Liquidambar styraciflua*: N = 43, St. dev. = 16.8, *Persea americana*: N = 11, St. dev. = 12.4, *Psidium guajava*: N = 177, St. dev. = 2.5, *Spondias purpurea*: N = 1, St. dev. = 0, *Vernonia patens*: N = 32, St. dev. = 2.6)

Tree species	Stand Density Index*
	0.00
Persea americana	0,08
Psidium guajava	0,49
Spondias purpurea	0,03
Cordia alliodora	0,10
Liquidambar styraciflua	2,35
Gliricidia sepium	0,06
Anacardium occidentale	0,00
Byrsonima crassifolia	0,01
Inga paterno	0,14
Vernonia patens	0,19
Total	3,12

\*: Number of trees with a basal area with a diameter of 25cm. per hectare.

**Table 4**: The stand density index (number of trees with a basal area with a diameter of 25 centimeters per hectare) for the tree species selected by PROMABOS in the fieldwork area, Caballeros, Chalatenango, El Salvador, 2004.

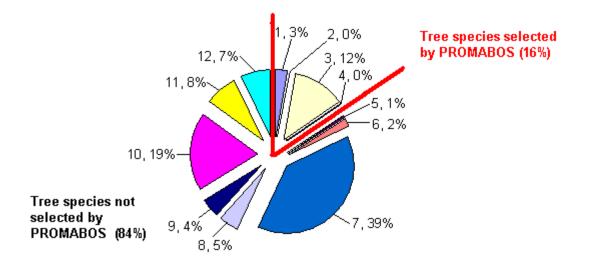


**Map 1:** The locations of the selected tree species, within a circle with a radius of 500 meters with the hives as midpoint. Caballeros, Chalatenango, El Salvador.

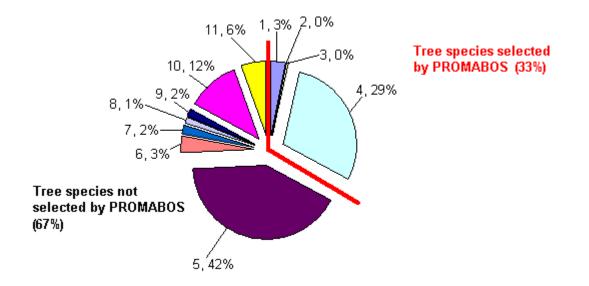
# 3.2 Tree Selection

Next to the tree species selected by PROMABOS, many other tree and shrub species were visited by *M. beecheii* for the collection of pollen and or nectar. Figure 3 and 4 give the frequencies of the visits on these species and the species selected by PROMABOS, shown in percentages of the total amount of collections. Because these other species were not selected by PROMABOS for the reforestation component of the project, the number of individuals per tree or shrub species had not been determined. These frequently visited species or families are: *Compositae*, *Eugenia sp.* (family *Myrtaceae*), *Leguminosae*, *Myriocarpa sp.* (family *Urticaceae*), shrubs of the *Solanum sp.* (family *Solanaceae*), the tree *Syzygium jambos L.* (family *Myrtaceae*), and the shrub *Tibouchina longifolia* (family *Melastomataceae*). The tree species

selected by PROMABOS were dominant (>50%) in 24% of the total amount of pollen samples collected, they were dominant in 16% of the samples from the collection of pollen (fig. 3), and in 33% of the pollen samples taken from the bees harvesting for nectar (fig. 4). The other frequently selected species were dominant in 70% of the total amount of samples, they were dominant in 77% of the samples from the collection of pollen, and in 62% of the pollen samples taken from the bees harvesting for nectar.



**Figure 3**: The frequencies of pollen collection by *M. beecheii* of the tree species selected by PROMABOS and other shrub and tree species most frequently visited by *M. beecheii* for pollen samples, shown in percentages of the total amount of samples (N= 391), Caballeros, Chalatenango, El Salvador, 2004. 1: *Cordia alliodora*, 2: *Inga paterno*, 3: *Liquidambar styraciflua*, 4: *Vernonia patens*, 5: *Compositae*, 6: *Solanum sp.*, 7: *Tibouchina longifolia*, 8: *Syzyguim jambos L*, 9: *Euginia sp.*, 10: *Leguminosae*, 11: *Myriocarpa sp.* 



**Figure 4** The frequencies of accidental pollen collection by *M. beecheii*, harvesting for nectar samples, of the tree species selected by PROMABOS and other shrub and tree species most frequently visited by *M. beecheii* for nectar samples, shown in percentages of the total amount of samples (N= 409), Caballeros, Chalatenango, El Salvador, 2004. 1: *Cordia alliodora*, 2: *Inga paterno*, 3: *Liquidambar styraciflua*, 4: *Vernonia patens*, 5: *Compositae*, 6: *Solanum sp.*, 7: *Tibouchina longifolia*, 8: *Syzyguim jambos L*, 9: *Leguminosae*, 10: *Myriocarpa sp.* 

Some of the tree species selected by PROMABOS, *M. beecheii* did not harvest on. But per visited tree species, the selection differed significantly (Chi-square p: 0.000, df:3). There was a high selection for *Vernonia patens* and for *Cordia alliodora* (table 5). The standardized selection ratios show a selection of respectively 0.55 and 0.40. *Liquidambar styraciflua* and *Inga paterno* were selected with a low rate (standardized selection ratio: 0.03 and 0.01). Because *M. beecheii* selected only four of the tree species selected by PROMABOS, and the prerequisites for the regression analyses were not met, a possible relation between the selection of the tree species and the average distance of these tree species to the hives and the stand density index of these tree species by *M. beecheii* and the sugar percentage of the nectar of these tree species could not be established due to the same reasons. Correlations between these variables did not exist. Table 6 shows the stand density index, the average distance to the hives and the standardized selection ratio of the tree species that were selected by PROMABOS ánd visited by *M. beecheii*.

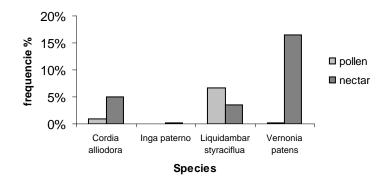
Species	Selection probability	Standardized selection ratio
Cordia alliodora	5.11	0.40
Inga paterno	0.15	0.01
Liquidambar styraciflua	0.35	0.03
Vernonia patens	6.96	0.55

**Table 5**: The extent to which a tree species was selected shown by the selection probability, and the estimated probability that a randomly selected tree species will be species i, if all species are equally frequent in the total amount of available tree species, shown by the standardized selection ratio (Manly *et al.* 1993) for the selected tree species by PROMABOS, visited by *M. beecheii* in the period of December 2003 till March 2004, Caballeros, Chalatenango, El Salvador.

species	SDI	Average distance	Standardized selection ratio
Cordia allidora	0.10	370	0.40
Inga paterna	0.14	197	0.01
Liquidambar styraciflua	2.35	158	0.03
Vernonia patens	0.19	280	0.55

**Table 6**: The stand density index, the average distance of the selected tree species to the hives and the standardized selection ratio of these tree species selected by PROMABOS that were visited by *Melipona beecheii*, in the period of December 2003 till March 2004, Caballeros, Chalatenango, El Salvador.

The frequency with which *M. beecheii* harvested for nectar differed significant from the frequency of harvesting for pollen; considering the tree species *Cordia alliodora* and *Vernonia patens*, (Chi-Square: p: 0.002, df: 1, p: 0.000, df: 1) (fig 5). In the majority of times they harvested nectar from these tree species. *Liquidambar styraciflua* was visited for both the collection of nectar (34.6%) and pollen (65.4%). The pollen of *Liquidambar styraciflua* were significantly (Chi-Square p: 0.005, df: 1) more collected than the nectar. *Inga paterno* was only visited twice, both for nectar.



**Figure 5**: The frequencies of visits by *M. beecheii* for pollen and nectar samples of *Cordia alliodora, Inga paterno, Liquidambar styraciflua and Vernonia patens* in the period of December 2003 till March 2004, Caballeros, Chalatenango, El Salvador.

# 3.3 Sugar percentages

*M. beecheii* collected nectar with a sugar percentage that varied from 15.6% till 67.2%. The average sugar concentration of the collected nectar from the honey stomach of the bees was, from a total off 409 samples, 48.9%. When divided into classes (10-20%, 20-30% etc.), the range from 50-60% was significantly selected the most with 132 times (Chi square p:0.000, df:5) (table 7).

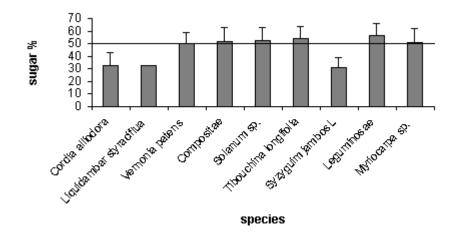
Sugar %	Frequency of selection
10-20%	5
20-30%	28
30-40%	68
40-50%	83
50-60%	132
60-70%	93

**Table 7**: Frequency table of the selected sugar percentage classes in the nectar samples taken from the collections

 by *M. beecheii* (N = 409) in the period of December 2003 till March 2004, Caballeros, Chalatenango, El Salvador.

Sugar concentrations of the nectar of the different tree species that have been selected by PROMABOS for the reforestation, and that have been visited by *M. beecheii* for nectar, have been obtained from literature (table 3). Sugar percentages of the nectar of *Cordia alliodora*, *Vernonia patens* and *Liquidambar styraciflua* have been established also from this research. The sugar concentration in the nectar collected from *Cordia alliodora* and *Vernonia patens* were

significantly different (Mann-Whitney U, p:0.000). The pollen samples that consisted of more then 50% of *Cordia alliodora* pollen corresponded with nectar samples with a mean sugar concentration of 32.6% (N: 15, St. dev: 12). For *Vernonia patens* the corresponding nectar samples had an average sugar concentration of 49.8% (N: 115, St. dev: 10). Just one pollen sample with a corresponding nectar sample consisted of more then 50% *Liquidambar styraciflua* pollen. This sample had a concentration of 32.8% sugar. The frequently visited species other then those selected by PROMABOS produced nectar with an average higher sugar concentration around 55% varying from 51.2% to 60.1%, except for *Syzyguim jambos L* with an average sugar percentage of 31,4%. Figure 6 shows the average sugar percentage of the nectar per species.

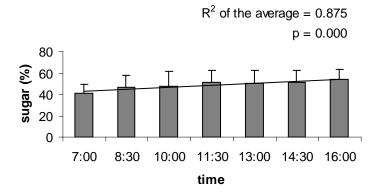


**Figure 6**: The mean sugar percentages in the nectar samples, taken from *M. beecheii* individuals in the period of December 2003 till March 2004, Caballeros, Chalatenango, El Salvador, of which the corresponding pollen slides contained more then 50% pollen of a specific tree species. The selected tree species and the most visited species other then the trees selected by PROMABOS are shown. (*Cordia alliodora* N: 15, St. dev: 12, *Liquidambar styraciflua* N: 1, St. dev: 0, *Vernonia patens* N: 115, St. dev: 10, *Compositae* N: 134, St. dev: 11, *Solanum sp.* N: 18, St. dev: 10, *Tibouchina longifolia* N: 21, St. dev: 10, *Syzyguim jambos L* N: 6, St. dev: 7, *Leguminosae N:* 8, St. dev: 10, *Myriocarpa sp.* N: 36, St. dev: 11)

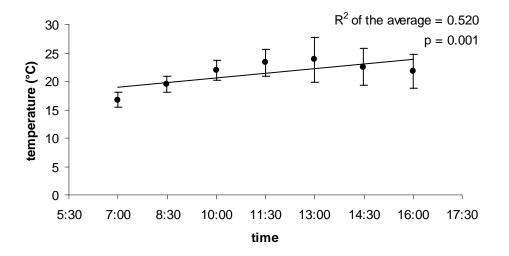
#### 3.4 climatic influences

Both the sugar percentage of the nectar samples (N = 391) (fig. 7) and the air temperature (N = 6 per time interval) (fig 8.) increased significantly during the day (regression analyses p: 0.000, df:1,  $R^2$ : 0.875, p: 0.000, df: 1,  $R^2$ : 0.520). Sugar percentage of the nectar significantly increased with increasing air temperature (regression analyses p: 0.000, df: 1,  $R^2$ : 0.388) (fig.

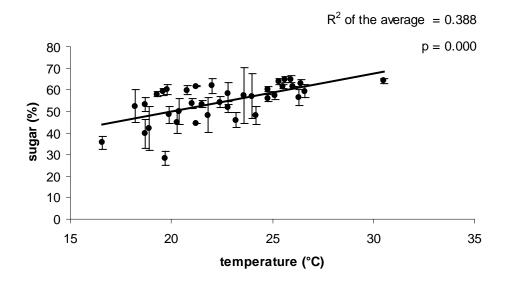
9), and decreased with increasing relative humidity (regression analyses p: 0.009, df: 1,  $R^2$ : 0.432) (fig. 10).



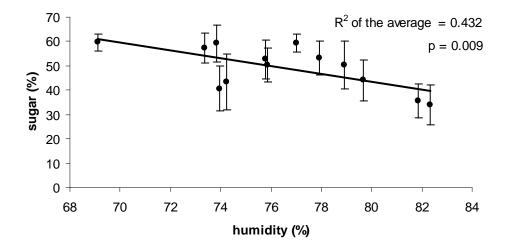
**Figure 7**: Average sugar percentages and the regression line in the nectar samples taken from *M. beecheii* individuals in the period of December 2003 till March 2004, Caballeros, Chalatenango, El Salvador, during the day. (7:00 N: 22, St. dev: 9, 8:30 N: 79, St. dev: 11, 10:00 N: 84, St. dev: 14, 11:30 N: 67, St. dev: 12, 13:00 N: 67, St. dev: 12, 14:30 N: 51, St. dev: 11, 16:00 N: 39, St. dev: 10)



**Figure 8**: Average temperature and the regression line during the day (N = 6), Caballeros, Chalatenango, El Salvador. The temperatures are taken on each day when samples have been taken from *M. beecheii* individuals during the period of January 2004 till March 2004.



**Figure 9**: Relation between the day temperature on the sampling days (temperatures have been measured at each time interval (7) during 6 sampling days, N = 37) in the period of January till March 2004, and the mean sugar concentration of the nectar samples (N = 409) taken from *M. beecheii* individuals Caballeros, Chalatenango, El Salvador. Furthermore, the regression line is shown.



**Figure 10**: Relation between the average humidity on the sampling days (SNET-Servicio Nacional de Estudios Territoriales 2004) (N = 13), and the mean sugar concentration of the nectar samples (N = 409) taken from *M. beecheii* individuals Caballeros, Chalatenango, El Salvador. Furthermore, the regression line is shown.

# **4** Discussion

#### 4.1 Tree selection

Some of the trees selected by PROMABOS were not visited by *M. beecheii* from December until March. Persea americana, Psidium guajava, and Spondias purpurea did not bloom in the fieldwork period and Anacardium occidentale was in the end of the blooming period when the research started. Therefore no conclusions can be drawn for these tree species. Byrsonima crassifolia and Gliricidia sepium were not selected by M. beecheii, based on the nectar and pollen analyses. These tree species were selected by PROMABOS based upon literature about Apis mellifera or other stingless bee species such as Trigona. In literature (Arce et al. 2001) it is stated that Melipona fasciata also visited Byrsonima crassifolia. But, feeding resources for M. beecheii may be different. Furthermore *M. beecheii* may have been suffering severe competition from Apis mellifera. The reason that Byrsonima crassifolia has not been selected by M. beecheii, might be explained, for the selection of nectar, by the fact that the tree species does not produce nectar but oil (Vinson et al. 1997). Experiments with Byrsonima crassifolia closer to the hives and at a higher SDI might give a selection probability, but this is not expected, because of the presence of oil instead of nectar and the lack of pollen collections. For this reason, it is advised to leave this species out of the reforestation component of the PROMABOS project. The members of the subfamily of the Papilionaceae (Gliricidia sepium) have stamens that are regularly enclosed. Therefore, visiting bees rarely collect pollen on their body (Espina et al. 1983). This might give an explanation for the lack of pollen of Gliricidia sepium in the samples, if *M. beecheii* only selects this species for the nectar. Experiments with the spotting or catching of M. beecheii on the trees, and competition of Apis mellifera must make clear while *Gliricidia sepium* is suitable for the reforestation component.

Liquidambar styraciflua was present at the highest stand density index (SDI) and situated at the lowest average distance to the hives, and was for this reason selected with a relatively high frequency for both pollen and nectar. However, considering the high SDI and the short distance, the selection probability was expected to be higher. This lower value might be explained by the flowering. The flowers were not abundant and of a non-conspicuous green colour. Flower colour often influences the selection by bees in general, they do not tend to select for green flowers in a high rate (McCall *et al.* 1992). The frequency with which *Vernonia patens* was selected for nectar was higher then that of *Liquidambar styraciflua*, in spite of a longer average distance to the hive and a lower SDI. Furthermore, *Vernonia patens* was not fully out of bloom yet, therefore

an even stronger selection was expected. It could be concluded that Vernonia patens was more favourable for *M. beecheii* then Liquidambar styraciflua, which was also shown by the different selection probabilities. Vernonia patens though, was densely present at one particular part of the terrain, while Liquidambar styraciflua was scattered throughout the area. A group of trees of one species situated closely together might form an extra attraction for bees because there is a relative high award at a small area, and therefore the relative energy loss will be lower if bees need to fly less far for their harvest (Nieuwstadt & Ruano Iraheta 1996). As well, the mass-flowering characteristics and the white/pink flowers of Vernonia patens could form a reason for the higher selection (McCall et al. 1992). Although based upon one nectar sample, the sugar percentage of the nectar of Liquidambar styraciflua was relatively low compared to the average sugar concentration of the nectar of Vernonia patens, what might also form an explanation for the higher selection for Vernonia patens. Cordia alliodora was selected for nectar with a slightly lower frequency then Vernonia patens, but it must be noted that it was situated at the longest average distance from the hive, and had the lowest SDI. The selection ratio showed that Cordia alliodora was highly selected. Also this species has white flowers and mass-flowering characteristics, what might attract bees strongly because the reward is high, the relative energy loss is low, and the flowers are attractive (McCall et al. 1992). On the other hand, the average sugar concentration was low considering the expected optimum for M. beecheii. This last aspect is therefore of a smaller importance for *M. beecheii* then the SDI, distance and flowering characteristics of Cordia alliodora in this area. Inga paterno was selected only twice and therefore, in combination with the average distance and the SDI, the selection probability was low. Consequently, Inga paterno was not strongly recommended for the reforestation component. Cordia alliodora and Vernonia patens were considered a good choice and Liquidambar styraciflua an optional choice for the reforestation component. Planting this latter species in the near vicinity of the hives in a high density and situated closely together might increase the selection probability.

The analyses showed that *Compositae*, *Leguminosae*, *Eugenia sp., Myriocarpa sp., Solanum sp., Syzygium jambos L*, and *Tibouchina longifolia* were amongst the frequent visited species other then the ones selected by PROMABOS. They composed of 70% of the total harvest by *M. beecheii*. Though, it must be noted that data about the density and SDI of these species are not available. The *Compositae* and *Leguminosae* family comprises a fair amount of species, of which several are floral sources for bees (a.o. Ramalho *et al.* 1989, Nagamitsu *et al.* 1999). There have not been any reports on the importance of *Eugenia* and *Myriocarpa* species for

bees. Research by Engel *et al.* (1980) and a summary of studies by Ramalho *et al.* (1989) have shown that *Solanum sp.* are a pollen and nectar source for at least four *Melipona* species (*M. rufiventris p., M. interrupta i., M. favosa, M.compressip.*) and for at least two *Trigona* species (*T. recursa, T. fulviventris g.*). Research by Landaverde *et al.* (2003) showed that *M. beecheii* as well selected species of the *Solanaceae* family. Also *Syzygium jambos L* is a known important source for bees and *M. beecheii* is a known visitor (Arce *et al.* 2001, Ramalho *et al.*, 1989, Landaverde *et al.*, 2003). Though, this species is not very suitable for reforestation, since it is abundant in the wild vegetation and has no valuable commercial uses for farmers (personal communication Sandker 2004). Furthermore, reports as well state that *Tibouchina longifolia* is selected by *Trigona* species (Ramalho *et al.* 1989). Alas, all these species do not meet all the prerequisites of the reforestation program of PROMABOS.

Though these species do not meet the prerequisites, the question rises what is more important for the project; reforestation with the aim of increasing the income of beekeepers, or reforestation with the aim of increasing the pollen and nectar sources for M. beecheii. Species selected by PROMABOS only account for 24% of the harvest in the season from December until March. This means that more then three quarters of the harvest of nectar and pollen during these months comes from other sources. A species like Tibouchina longifolia is a floral source (39%! of the pollen harvest in the months of December until March) for *M. beecheii* and above all reported to be cultivated and native to El Salvador (Starr et al. 2003). But, this species is a shrub and not cultivated by the beekeepers in Caballeros (Pers. Comm Sandker 2004). As well Syzygium jambos L is a floral source for M. beecheii and can be used for the edible fruits, for medicinal use, as a fodder and for the good quality of the wood etcetera (ARCBC 2004). Nevertheless, the beekeepers in Caballeros consider this species as a weed (Pers. Comm. Sandker 2004). Furthermore the tree is not native to El Salvador. The question rises what is more preferable, reforestation with trees that are native to the country and being utilized by and economically valuable for the beekeepers of Caballeros, or species that are favorable to M. beecheii and yet present. This depends on the importance of the prerequisites of the reforestation component, and of the development and improvement of the Meliponiculture. A suggestion is to reforest a part of the area with trees that meet the prerequisites and a part of the area or understorey with trees/ shrubs that are known to be highly favorable for *M. beecheii* such as Tibouchina longifolia.

There are, mentioned before, many aspects that influence the selection of a food source by bees. The density of the trees, the situation in the field (scattered throughout the area or not), colour and shape of flowers, mass flowering characteristics, sugar percentages of the nectar, competition by other bee species, climatic influences, the presence of natural enemies, and such all affect the selection of food sources. This research took place in an existing forest; therefore dealing with all these aspects was difficult. The variables that were considered to be of great importance and were easily possible to measure were taken into account. To decrease the external influences 'cafeteria' experiments are an option (a. o. Şahin et al. 2003). In these, the bees would be given the opportunity to select their own diet under known conditions. A set SDI for the species, all at an equal distance without the presence of other known food sources would ease the research. Alas, these experiments are time and money consuming.

# 4.2 Sugar percentage

*M. beecheii* collected nectar with a sugar percentage that varied from 15.6% till 67.2%. The average sugar concentration of the collected nectar was 48.9%. These values are in accordance with values found in literature. Research by Roubik (1995) found a range of 19-67% sugar for *M. beecheii*, with a mean of 48%. The range from 50-60% was significantly selected the most. This is explainable because this range is nearest to the optimum sugar percentage for *M. beecheii* found in literature, about 60% (Roubik 1995), and because higher sugar percentages of the nectar were not abundant in the flowers selected by *M. beecheii*.

The pollen samples that consisted of more then 50% of *Cordia alliodora* pollen corresponded with nectar samples with a mean sugar concentration of 32.6%, for *Vernonia patens* 49.8%. The frequently visited species, other then those selected by PROMABOS, produced nectar with an average higher sugar concentration around 55% varying from 51.2% to 60.1%, except for *Syzyguim jambos L* with an average sugar percentage of 31,4%. Data indicate that bees forage for optimal sugar concentration and *M. beecheii* is being called a high sugar specialist (Roubik 1995). Earlier it is stated that the highest selected class was 50 to 60%, what may be near the optimum (Roubik 1995). Therefore *Cordia alliodora, Liquidambar styraciflua* and *Syzyguim jambos L* were mainly selected for pollen; but it can be assumed that especially *Cordia alliodora* was selected for the harvest of nectar for other reasons then the high sugar concentration. The SDI in combination with the mass flowering properties and the location in the field might explain the selection for *Cordia alliodora*. Furthermore, both *Liquidambar styraciflua* and *Syzyguim* 

*jambos L* are not mass flowering trees but are present in a high density in the area (own data and Pers. Com. Sandker 2004).

# 4.3 Climatic influences

Sugar percentage of the nectar significantly increased with increasing air temperature and decreased with increasing relative humidity. Air temperature and relative humidity both can have an impact on the sugar concentration of the nectar of the flower: this affect can differ per flower what might have an impact on the tree selection as well (Kearns *et al.* 1993).

# 5. Conclusions

*Cordia alliodora* and *Vernonia patens* were nectar sources highly favoured by *M. beecheii*. This in spite of a low sugar concentration of the nectar, especially for *Cordia alliodora*, compared to the mode and expected optimum. Both species have whitish flowers and mass flowering characteristics, which might be favourable for *M. beecheii*. Therefore these species are recommended for the reforestation component of PROMABOS.

*Liquidambar styraciflua* is both a pollen and a nectar source for *M. beecheii*. The green colour of the flowers and the lack of mass flowering characteristics possibly form a disadvantage for the selection by *M. beecheii*. The selection was not high, when considering the fact that the trees were situated in the near vicinity of the hives and that they were present in a high stand density. If used for reforestation, it is recommended to plant this species close to the hives in a high density and situated closely together.

Pollen of *Gliricidia sepium* were not found in the analyses. Because the members of the subfamily of the *Papilionaceae* have stamens that are regularly enclosed, visiting bees rarely collect pollen on their body. Therefore, more experiments based upon spotting or catching of *M. beecheii* on this tree species, and the impact of competition by *Apis mellifera* need to take place, before conclusions can be drawn about the importance of *Gliricidia sepium* as a nectar source for *M. beecheii*.

*Inga paterno* and *Byrsonima crassifolia* were hardly or not selected and therefore not recommended for the reforestation component of the project.

*Persea Americana, Psidium guajava, Spondias purpurea* and *Anacardium occidentale* did not bloom during the fieldwork period. Data about the density and the SDI are present for these species, but further research when the species have their blooming period needs to take place to be able to draw conclusions about their favourability for *M. beecheii*.

Species of the Compositae and Leguminosae family, Myriocarpa sp. (family Urticaceae), Solanum sp. (family Solanaceae) and Syzygium jambos L. (family Myrtaceae), Tibouchina longifolia (family Melastomataceae) were important pollen and nectar sources for M. beecheii. Eugenia sp. (family Myrtaceae) was an important pollen source for M. beecheii. Many other species were selected (84% pollen, 67% nectar). Maybe discussions should take place about revising the prerequisites or the objectives of the project.

*M. beecheii* mostly selected nectar with a sugar percentage between 50 and 60%.

The sugar percentage of the nectar increases with an increasing temperature, and decreases with an increasing humidity.

It is recommended for the reforestation program of PROMABOS to plant the trees of one species closely together, and preferably in a high density. This might increase the selection probability considerably.

# Acknowledgements

This research has been made possible thanks to the help of several people who I wish to thank. First of all I would like to thank Sara Schaafsma with whom I spend my days in the field, together we gathered the necessary pollen and nectar samples and analysed the slides. Furthermore I would like to thank my supervisor in Wageningen Prof. Dr. Frans Bongers. I would like to thank all the people from the PROMABOS project in El Salvador, especially Dr. Harriet de Jong and Ir. Marieke Sandker for their help and guidance. Dr. Rinus Sommeijer allowed me to conduct this study at the PROMABOS project. I also had great help from the field guide Jose Miguel Reyna. Last but not least I would like to thank the friends I met in El Salvador for just being there! I had a wonderful time with all of them.

# References

**ARCBC 2004.** ASEAN Regional Centre for Biodiversity Conservation (ARCBC). Website: http://www.arcbc.org/arcbcweb/ASEAN\_Precious\_plants/fruits/Syzygium\_jambos.htm

Arce, H.G., Sánchez, L.A., Slaa, J., Sánchez-Vindas, P.E., Ortiz M., A., Van Veen, J.W., Sommeijer, M.J., 2001. Árboles melíferos nativos de Mesoamérica. Centro de investigaciones apicolas tropicales.

**Banco Agrícola de El Salvador, 2003.** El Salvador Bajo su sombro. Árboles de neustra rincón mágico. Fomento cultural.

**Bawa, K.S., Kang, H., Grayum, M. 2003**. Relationships among time, frequency, and duration of flowering in tropical rain forest trees. American Journal of Botany 90, 6, 877-887.

**Biesmeijer, J.C., Born, M., Lukacs, S., Sommeijer, M.J., 1999a**. The response of the stingless bee *M. beecheii* to experimental pollen stress, worker loss and different levels of information input. Journal of apicultural research 38(1-2): 33-41.

**Biesmeijer, J.C., Ermers, M.C.W., 1999b.** Social foraging in stingless bees: how colonies of *Melipona fasciata* choose among nectar sources. Behaviour Ecological Sociobiology 46: 129-140.

**Biesmeijer J.C., Smeets, M.J.A.P., Richter, J.A.P., Sommeijer, M.J., 1999c**. Nectar foraging by stingless bees in Costa Rica: botanical and climatological influences on sugar concentration of nectar collected by *Melipona*. Apidologie 30:43-55

Coffey, M.F., Breen, J. 1997. Pollen and nectar sources of honey bees in Ireland

Crane, E., Walker, P., Day, R., 1984. Directory of important world honey sources.

**Daniel, T.W., meyn R.L., Moore, R.R., 1979**. Reineke's stand density index in tabular form in English and metric units wit hits applications. Research report 37, Utah Agricultural experiment station, Utah state university Logan.

**Diccionario 1985**. Geografico de El Salvador Tomo I y II, Instituto Geografica Nacional, San Salvador

**Engel, M.S., Dingemans-Bakels, F., 1980**. Nectar and pollen resources for stingless bees (Meliponinae, hymenoptera) in Suriname (South America). Apidologie 11(4): 341-350.

Espina, D., Ordetx, G.S., 1983. Flora Apicola Tropical (143). Editorial Technologica de Costa Rica

Frankie, G.W., Vinson, S.B., Rizzardi, M.A., Griswold, T.L., O'Keefe, S., Snelling, R.R.,
1997. Diversity and abundance of bees visiting a mass flowering tree species in disturbed seasonal dry forest, Costa Rica. Journal of the Kansas entomological society 70(4): 281-296

**Gracie, C., 1993**. Pollination of Cyphomandra endopogon var. endopogon by Eufriesea spp. (Euglossini) in French Guiana. Brittonia 45(1): 39-46.

Hsiang Liow, L., Sodhi, N.S., Elmqvist, T. 2001. Bee diversity along a disturbance gradient in tropical lowland forests of south-east Asia. Journal of Applied Ecology 38, 180-192

**Kearns, C.A., Inouye, D.W., 1993**. Techniques for pollination biologists. University press of Colorado. The United States of America.

Landaverde V, Sánchez L y Ruano C (2003) Dominancia temporal de polen de plantas nectapoliníferas colectado por *Melipona beecheii* en El Salvador y de plantas poliníferas por *Tetragonisca angustula* y *M. beecheii* en Costa Rica. En: III Seminario Mesoamericano sobre Abejas sin Aguijón, Tapachula México, pp87-90

Manly, B.F.J., McDonald, L.L., Thomas, D.L., 1993. Resource selection by animals. Statistical design and analysis for field studies.

**McCall, C., Primack, R.B., 1992**. Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. American Journal of Botany 79(4): 434-442.

**Ministerio de medio ambiente y recursos naturales 2000a**. Estudio geologico y propuesta para la recuperacion de la carcava el zompopero y el Cerro Pelon. Microregion La Palma. El salvador, Centro America.

**Ministerio de medio ambiente y recursos naturales 2000b**. Estudio geologico y propuesta para la recuperacion de la carcava el zompopero y el Cerro Pelon. Diagnostico: Zona de influencia Carcava El Zompopero, La Palma, San Ignacio, Chalatenango. El salvador, Centro America.

**Nagamitsu, T., Momose, K., Inoue, T., Roubik, D.W. 1999**. Preference in flower visits and partitioning in pollen diets of stingless bees in an Asian tropical rain forest. Resources of Population Ecology 41, 195-202.

**Nieuwstadt, M.G.L., van, Ruano Iraheta, C.E., 1996**. Relation between size and foraging range in stingless bees (Apidae, Meliponinae). Apidologie 27: 219-228

**PROMABOS 2004**: Website: http://www.bio.uu.nl/promabos.

Ramalho, M., Kleinert-Giovannini, A., Imperatriz-Fonseca, V.L. 1989. Utilization of floral resources by species of *Melipona (Apidae, Meliponinae)* : floral preferences. Apidologie 20, 185-195.

**Roubik, D.W., 1995**. On optimal nectar foraging by some tropical bees (Hymenoptera: Apidae). Apidologie 26: 197-211.

**Şahin, A., Keskin, K., Biçer, O., Gul, S., 2003**. Diet selection by Awassi lambs fed individually in a cafeteria feeding system. Livestock Production Science 82, 163–170.

Sandker, M.A.A.H., 2003. Reforestation with major bee food trees in El Salvador. Internal report PROMABOS.

**Silveira, da F.A., 1991**. Influence of pollen grain volume on the estimation of the relative importance of its source to bees. Apidologie 22: 495-502.

**Smeets, A.J.P., 2002**. Promabos, the flora of La Palma (Chalatenango, El Salvador. CD-Rom Promabos@123.com.sv.

**SNET-Servicio Nacional de Estudios Territoriales 2004**. Data from the Meteorological station in La Palma, Chalatenango, El Salvador. Internet: www.snet.gob.sv

**Starr, F., Starr, K., Loope, L., 2003.** *Tibouchina longifolia* Longleaf glorytree Melastomataceae. United States Geological Survey-Biological Resources Division Haleakala Field Station, Maui, Hawai'I. Website: http://www.hear.org/starr/hiplants/reports/html/tibouchina\_longifolia.htm

**Sommeijer, M.J., M.J., Rooy G.A. de, Punt W, Bruijn L.L.M. de, 1983**. A comparative study of foraging behaviour and pollen resources of various stingless bees (Hym., Melioninae) and honeybees (Hum., Apinae) in Trinidad, West-Indies. Apidologie 14(3): 205-224.

**Thorp, R.W., 2000**. The collection of pollen by bees. Plant Systematics and Evolution 222: 211-223.

Vinson, S.B, Williams, H.J, Frankie, G.W, Shrum G, 1997. Floral lipid chemistry of *Byrsonima crassifolia* (Malpigheaceae) and a use of floral lipids by *Centris* bees (Hymenoptera: Apidae). Biotropica vol 29(1), pp76-83

Woodall, C.W., Fiedler, C.E. and Milner, K.S. 2003. Stand density index in uneven-aged ponderosa pine stands. Canadian Journal of forestry research. 33, 1, 96-100.

**Zeide, B., 2004**. How to measure stand density. School of Forest Resources, University of Arkansas at Monticello, United States of America.