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Author(s): Gretchen D. Jones and Vaughn M. Bryant
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Pollen Studies of East Texas Honey

Gretchen D. Jones[a] and Vaughn M. Bryant[b]*

[a]*United States Department of Agriculture, Agriculture Research Service (USDA-ARS), Areawide Pest Management Research Unit, College Station, Texas 77845, USA;* [b]*Palynology Laboratory, Department of Anthropology, Texas A&M University, College Station, Texas 77843-4352, USA*

Since the beginning of honey production, certain honey types have been favored because they taste better, are better for cooking or do not rapidly crystallize. Thus, they are preferred over others, are in high demand and are sold at higher prices. The pollen of 37 honey samples from East Texas was examined. Pollen was recovered from the honey by using an alcohol-dilution method. Overall, 431 taxa identified into 61 families, 104 genera and 85 species were found in the samples. The number of taxa per sample varied from 17–52. Half of the samples contained 31–40 taxa, indicating a high diversity in botanical origin. Three taxa were found in >50% of the samples and are the most important: *Berchemia scandens*, *Salix nigra* and *Toxicodendron radicans*. *Berchemia scandens* was found in 89% of the samples and was a predominant type in three samples and an important secondary type in 14. Both *Salix nigra* and *Toxicodendron radicans* pollen occurred in 83% of the samples and neither occurred as a predominant or secondary type. Three samples were *Berchemia scandens* unifloral honey. By examining the pollen in honey, it can determined which habitats honeybees visit, which plants honeybees use as food, if they visit row crops and orchards and their role in pollination. In order to differentiate honey from the United States of America (USA) from honey produced in other countries, the honey from each state must be analyzed. Only by analyzing the pollen in the honey of the USA can it be reliably differentiated from foreign honey that is being sold as produced in the USA.

Keywords: melissopalynology; pollen; honey; East Texas

1. Introduction

Social honey bees (*Apis* spp.) evolved in the Old World tropics and have been producing and storing honey for probably more than 20 million years (Crane 1980, 1983). Collectively, six or seven races of *A. mellifera* L., native to Europe, are called European Honeybees. They have been genetically selected for honey production, gentleness, tendency not to swarm and winter hardiness.

There were no indigenous honey-producing bees of major significance in the New World. The German honeybee, *A. mellifera* subsp. *mellifera* L., is believed to have been introduced into the New World by European settlers in the early 1600s (Jones & Bryant 1992). European honeybees were called 'white man's flies' by some Indian tribes and many Indians referred to the introduced white clover (*Trifolium repens* C. Linnaeus) as 'white man's foot' because it expanded its range into the same regions where the new European settlers walked (Crane 1975).

Melissopalynology is the study of pollen found in honey. Precision in interpreting pollen data recovered from honey has always been a primary goal of those who study pollen and honey. For example, when using pollen counts to determine the nectar sources of a honey sample, we recognize that the types and percentages of recovered pollen do not provide a one-to-one correlation with the true nectar sources in the honey. Nevertheless, it is still the fastest, least expensive and most common method of determining the origin of nectar contents in honey. The basis for conducting these types of studies is the fact that honey bees utilize certain natural raw materials which are identifiable in honey. These raw materials include pollen and nectar (Seedley 1985). Pollen, the bees' major source of proteins, fatty substances, minerals and vitamins, is essential for growth of the larvae and young adult bees (Dietz 1975; Gary 1975). The rearing of a single worker bee from hatching to adult requires 120 to 145 mg of pollen (Alfonsus 1933; Haydak 1935). An average colony collects about 44 to 125 lbs. of pollen a year (Armbruster 1921; Eckert 1933, 1942).

Nectar, a bee's source of carbohydrates, contains 5–80% sugar and is collected by foraging worker bees and carried back to the hive in their honey stomachs (Tan et al. 1989). Upon returning to their hive, the nectar is usually transferred to workers in the hive for processing into honey. Enzymes from the bee's hypohyaryngeal glands are added to break down the nectar into simple sugars. Water in the nectar is evaporated off the worker's tongue and the residue is placed into cells and fanned. Once the evaporation process is

*Corresponding author. Email: vbryant@tamu.edu

complete, the nectar is considered ripened and is called honey. A worker larva requires about 142 mg of honey for development (Winston 1987). To make 0.45 kg (1 pound) of honey, honeybees must visit about 2 million flowers. On the average, one worker produces about 1/12 of a teaspoon of honey in her lifetime (Armbruster 1921; Eckert 1933, 1942).

Pollen gets into the nectar and honey in various ways. First, pollen occurring in the nectar collected by the honeybee is regurgitated with the collected nectar and deposited into the honeycomb cells. Second, pollen can fall into the open cells when a honeybee grooms herself. Third, airborne pollen from taxa not visited by honeybees can enter the hive on air currents and fall into the open cells. Finally, pollen can fall onto the honeycomb as it is being removed by the beekeeper.

Pollen analyses of honey and bee loads are used to learn and understand honeybee foraging ecology, the habitat and vegetation visited, habitat composition, changes in honeybee food sources and the geographical region of the hive location (Maurizio 1951, 1975; Louveaux et al. 1970; Lieux 1972, 1980, 1981; Crane 1975, 1980; Agwu & Akanbi 1985; Moar 1985; Ramalho & Kleinert-Giovannini 1986; Feller-Demalsy & Parent 1989; Barth 1990; Diaz-Losada et al. 1998; Terrab et al. 2004). Pollen analyses of honey can also be used to monitor and ascertain changes in honeybee nectar and pollen sources and to verify changes in habitat composition both pre- and post-introduction of genetically modified (GM) crops. However, as yet, most GM pollen cannot be distinguished from non-GM pollen using light microscopy.

With the increase of GM plants, honeybee nectar and pollen resources could be altered because of changes in the composition of the plant communities and habitats in which the honeybees forage. Switching to sub-optimal nectar and pollen sources will change the quality and production of honey and drastically affect the hive dynamics and honeybee numbers. Any additional pressure on honeybee numbers would certainly limit the use of honeybees as pollinators and reduce honey production. As honeybee populations decline worldwide, pollen analyses of their honey can help determine changes in nectar and pollen sources and may help determine the causes of this decline.

Currently, among the major honey-producing countries in the world, only the United States of America (USA) has been almost totally neglected in terms of research related to pollen studies of the honey it produces, which is not true for other countries such as Brazil, Canada, China, France, Great Britain, New Zealand, Spain, Switzerland, Japan and the former USSR (Silitskaya 1966). Data collected by these other nations enable them to impose strict laws governing the importation and exportation of honey products (Johansson & Johansson 1968). Before honey products can be marketed, three types of certification are required. This certification includes verification of the honey's floral type, quality and precise place of origin. This type of certification requirement limits the exportation of US domestic honey types because there are no published data to comply with certification standards imposed by other nations, causing the USA to lag behind other nations for exportation and verification of honey.

Because there are no restrictions or certification for importing and exporting honey into the USA, less expensive honey from foreign countries can be marketed in the USA. In addition, with the advent of the free trade act between the United States, Canada and Mexico, there is a growing concern that cheaper foreign honey may be substituted for more expensive domestic honey. The National Honey Board, United States Department of Agriculture (USDA), and US beekeepers are interested in establishing quality standards for honey because of the concern that inexpensive honey from foreign countries may be substituted and labeled as domestic honey. This concern is so great that Senate bill 662 is currently in the US Congress focusing on this problem.

There is a vast amount of literature on pollen analyses of the honey of many countries including but not limited to: Argentina (Forcone et al. 2005; Forcone 2008; Vossler et al. 2010), Austria (Ruttner 1961), Brazil (Oliveira et al. 2010), Chile (Horn & Aira 1997), Mexico (Villanueva-Gutierrez et al. 2009), Finland (Salonen et al. 2009), France (Louveaux 1956; Vergeron 1964), Greece (Tsigouri et al. 2004), Hungary (Ruttner 1964), India (Bhusari et al. 2005; Datta et al. 2008), New Zealand (Moar 1985), Poland (Demianowicz 1964, 1966, 1968; Wozna 1966), Spain (Louveaux & Vergeron 1964; Seijo & Jato 1998; Terrab et al. 2004), Romania (Pelimon 1960; Tone 1966; Tone & Coteanu 1968; Dobre et al. 2013) and Yugoslavia (Maurizio 1960). Unfortunately, for a few countries, including the USA, little melissopalynological research has been pursued or published (Jones & Bryant 1992, 1993).

The first scientific investigation of US honey was Young's (1908) examination of honey from 32 states, including Hawaii, several US territories, Canada and Cuba. Young compared the pollen contents of the honey samples to the purported sources listed for the honey and made a key to the common pollen grains seen in the samples.

Oertel (1939) published a 7-year study on the sources and blooming periods of plants thought to be principal honeybee nectar sources in various regions of the USA. Unfortunately, he did not compare the pollen from the nectar producing plants to the pollen found in the honey.

Todd and Vansell's (1942) research on California honey was the first major study after Young (1908). They examined over 2600 nectar samples and demonstrated that equal amounts of nectar from different species contain different amounts of pollen. They also found that bees eliminate much of the pollen in the nectar before depositing the nectar in the hive.

Although Vorwohl (1970) examined 11 honey samples in Florida, Lieux (1972, 1975, 1977, 1978, 1981) conducted the first extensive pollen study of US honey by examining honey from Louisiana and Mississippi. Lieux was the first in the USA to produce detailed pollen analyses of honey samples, and the first in the USA to attempt identification of honey types as to the specific geographical regions based on a pollen spectrum. She was also one of the first to use acetolysis on a regular basis in melissopalynological studies (Lieux 1972; 1980) and to suggest the addition of tracer spores to calculate pollen concentration values (Lieux 1980).

White et al. (1991) examined the chemical and pollen properties of 11 honey samples collected from regions in Mexico, Texas and Arizona. Their focus was to examine problems in honey adulteration testing and to identify stable isotope levels in samples of unifloral honey coming from *Prosopis* spp. (mesquite) and *Acacia* spp. (cat claw) sources.

Because there is so little published melissopalynological research in the USA, and because most of that data is very old, the purpose of this research was to examine the honey from East Texas and compare it with the more recent data from the honey of Louisiana, Mississippi, Florida and Canada. It is expected that since the USA does not have detailed pollen analyses of its honey, analyses of East Texas honey will help in determining the honey of the continental USA. Furthermore, because of the importation of foreign honey into the USA, analyses of East Texas honey will help differentiate it from the foreign honey. The results of this research will be compared only to the previous US and Canadian honey analyses because of the similarity of habitats between the two countries.

2. Material and methods

2.1. Description of the study site (East Texas)

East Texas (Figure 1) measures about 21 million acres (Arbingerst & Kennamer 1963). The temperatures vary enough to produce a temperate climate with four distinct seasons (Nixon 1985). Rainfall averages 89 to > 127 cm annually and the elevation ranges from 0–230 m above mean sea level (Nixon 1985).

East Texas is the most mesophytic area of Texas and is the southwestern edge of the Eastern Deciduous Forest (Correll & Johnston 1979). It is characterized by rolling or hilly country. The habitats and vegetation of East Texas vary greatly due to topography, precipitation, soil and climate and have been described by various authors (Bray 1906; Tharp 1939; Braun 1950; Gould 1975; Ajilvsgi 1979; Nixon 1985; Hatch et al. 1990). Within East Texas are a variety of habitats including dry and mesic uplands, mesic and wet creek bottoms, bogs and seepage areas, river bottomlands, swamps, gulf prairies and marshes (Sullivan & Nixon 1971; Chambless & Nixon 1975; Nixon 1985). Tharp (1939) called East Texas a vegetational mosaic. Plants range from a variety of *Quercus* spp. (oaks) and *Pinus* spp. (pines) in the forests to insectivorous plants such as *Drosera brevifolia* Pursh (sundew) and *Sarracenia alata* Wood (pitcher plants) in the bogs.

Pellet (1930, 1976) considered East Texas a good beekeeping area yielding over 100 lbs. per colony per year. Major honeybee plants include *Berchemia scandens* (Hill) K. Koch (rattanvine), *Tetraneuris linearifolia* (Hook.) Greene var. *linearifolia* (sy = *Hymenoxys linearifolia* Hook.) (bitterweed), *Tilia* spp. (basswood) and *Monarda* spp. (horsemint) (Pellet 1976).

For this research, 'East Texas' followed political boundaries (Figure 1). As such, it includes all of Gould's (1975) Region 1 (Pineywoods), the northeastern counties of Region 2 (Gulf Prairies and Marshes) east of and including Chambers County, the northeastern counties of Region 3 (Blackland Prairies), and Region 4 (Post Oak Savannah) (Gould 1975; Nixon 1985).

2.2. Honey samples

Thirty-seven honey samples were donated by members of the Texas Beekeepers Association who keep honeybees in East Texas. All samples were reported by the beekeepers as being from mixed floral sources.

2.3. Honey processing

Each sample was heated in a microwave oven to 38°C and thoroughly stirred to insure a uniform mixing of the pollen in the honey. Ten grams of honey, the internationally preferred standard for testing pollen content in honey samples (Louveaux et al. 1978), was extracted from the sample and poured into a 150-mL beaker. Two *Lycopodium clavatum* C. Linnaeus tablets (each containing 11,300 + 300 spores) per sample were used as a marker. The tablets were dissolved in 5 mL of 5% hydrochloric acid (HCl). The dissolved spores and 10 mL of distilled water were added to the honey and the honey/spores were stirred well.

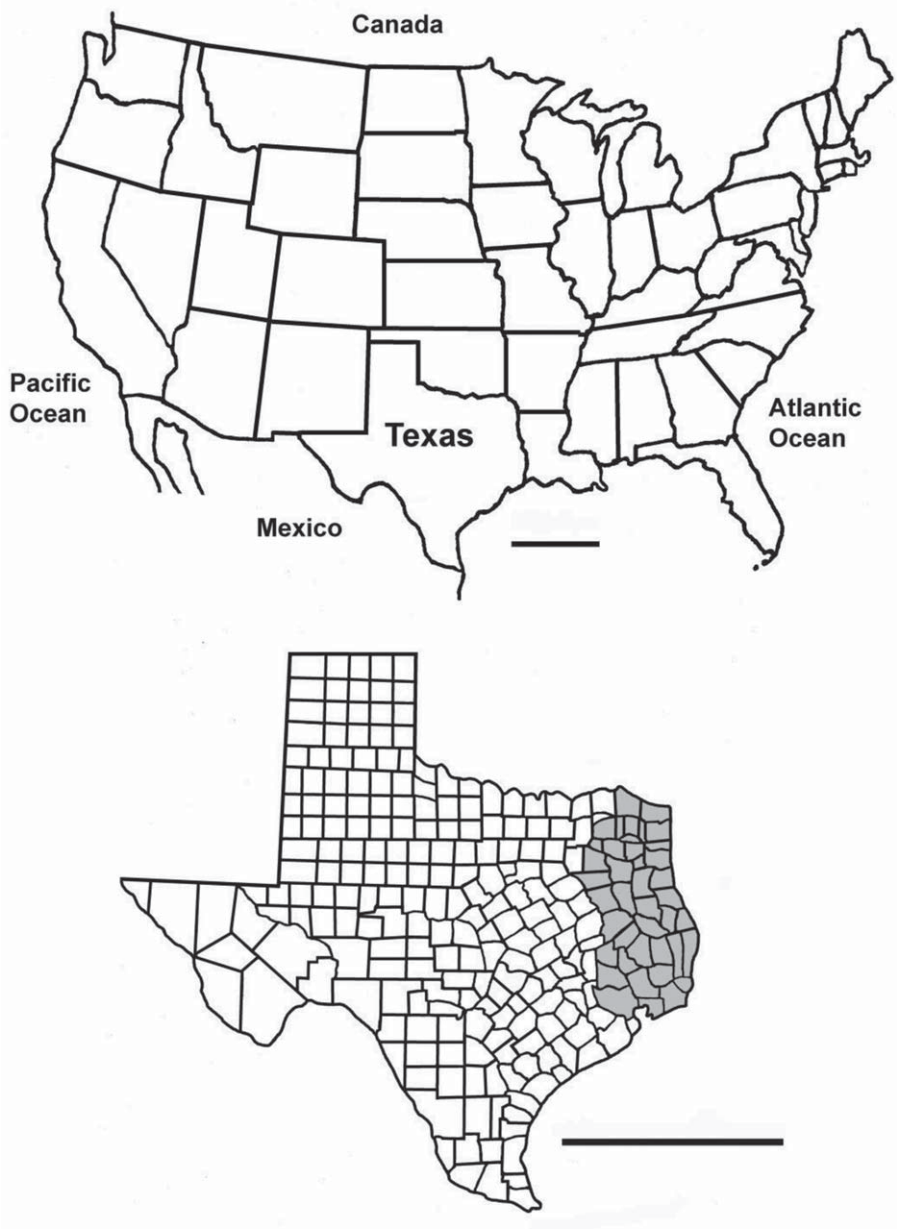

Figure 1. A map of the United States of America showing the location of Texas and a Texas County Map showing the counties of East Texas (shaded). Bars = 500 km. The direction north is at the top of both maps.

To lower the specific gravity of the solution, 100 mL of 95% ethyl alcohol (ETOH) was added to each sample and stirred well (Jones & Bryant 2004). The honey-ETOH solutions were kept warm on a hot plate at 20 °C and stirred occasionally to ensure continued mixing of the pollen and marker spores. Each sample was poured into a 12-mL centrifuge tube and centrifuged at 1060 × *g* for 3 min. After decanting the supernatant, the residue was vortexed for 30 s, and additional honey-ETOH solution was added to the residue. This processes of centrifuging, decanting, vortexing and adding more solution was repeated until the volume was reduced to only a residue. Finally, each beaker was rinsed three times with 95% ETOH and the rinse liquid was poured into the centrifuge tubes. Again, the samples were centrifuged, decanted and vortexed.

Each sample was acetolyzed (Erdtman 1960, 1963; Lieux 1980; Low et al. 1989; Jones & Bryant 2004) with a 9:1 ratio of acetic anhydride to sulfuric acid and placed into a preheated hot block set at 100 °C. After 12 min, 5 mL of glacial acetic acid was added to each

sample to stop the acetolysis reaction. The samples were rinsed twice with distilled water, centrifuging and decanting each time, and then once with 95% ETOH. The samples were stained with three drops of Safranin O and poured into marked 2-dram vials (Jones & Bryant 2004; Jones 2012). Seven drops of glycerin were added to each vial. The vials were placed on a hot plate (20 °C). Slides were made from one drop of the pollen residue of each sample the following day when the ETOH had evaporated.

2.4. Pollen analyses

We counted 200 pollen grains for all samples. We could have conducted higher pollen counts per sample but found this unnecessary based on our earlier research (Jones & Bryant 1998). *Lycopodium* spores were also counted but their number was kept separate from the pollen count. Pollen concentration values (PCV), the number of taxa, pollen percentages, relative abundance and floral frequency classes were calculated for each sample. Percentages and floral frequency classes were calculated by totaling the number of pollen grains of a particular taxon and dividing by the total number of grains counted (Louveaux et al. 1970, 1978). Predominant taxa are those that occurred >45%; secondary taxa were between 45 and 16%; important minor taxa occurred between 15–3% and less important taxa occurred below 3% (Louveaux et al. 1970). If one taxon of pollen predominated (>45%), the honey was categorized as unifloral. If no taxa predominated, the honey was considered 'mixed'.

Pollen concentration values per 10 g for each sample were calculated by computing the ratio of marker spores to counted pollen grains using the following formula:

$$\frac{(\#\text{ of pollen grains counted})(\#\text{ of } \textit{Lycopodium} \text{ spores added})}{(\#\text{ of } \textit{Lycopodium} \text{ counted})} \quad (1)$$

Pollen concentration values per 10 grams of < 20,000 were considered 'very poor', 20,000–100,000 'intermediate', 100,000–500,000 'rich', and 500,000–1,000,000 'very rich' (Feller-Demalsy et al. 1989).

For this study, we determined the frequency of occurrence by calculating the total number of samples in which a taxon occurred and then divided that number by the total number of samples. The result was then multiplied by 100 to obtain a percent. Frequency distribution of a taxon was classified as 'rare' if it occurred in less than 10%; 'infrequent', 10–20%; 'frequent', 20–50%, and 'very frequent', more than 50%.

Relative frequency was calculated by totaling the number of samples in which a taxon occurred and dividing by the total number of taxa. The resultant was then multiplied by 100 to obtain a percent.

2.5. Pollen identification

Floral studies were conducted in East Texas to obtain voucher specimens of nectar and pollen plants and plants on which honeybees were observed foraging. Flowers and voucher specimens were collected for plant identification and for scanning electron microscopy (SEM) and light microscopy (LM). The flowers were acetolyzed as above but without adding *Lycopodium* spores (Jones & Bryant 2004). Prior to the addition of glycerin, a drop of each taxon's pollen residue was placed onto a marked stub for SEM. This pollen drop was allowed to dry, then coated with 400 Å of gold palladium and examined using a JEOL T330-A scanning electron microscope. Many of the SEM micrographs of the voucher taxa were used in a pollen atlas, *Pollen of the southeastern United States* (Jones et al. 1995), and are on the United States Department of Agriculture, Agriculture Research Service Unit (USDA-ARS) pollen website (http://pollen.usda.gov).

The pollen in each honey sample was identified, counted and photographed using an ausJENA Jenaval compound light microscope using bright field, phase contrast and Nomarski phase techniques. In each honey sample, all pollen grains that remained questionable as to their precise identity were photographed in several diagnostic positions and prints were made for comparison with similar photos from other honey samples we examined. For each major taxon we found during these analyses we made both LM and SEM micrographs, which we put into plastic sheets and placed into notebooks by aperturation type for comparisons and pollen identifications.

In many melissopalynology studies, palynologists do not identify the majority of taxa beyond either the family or genus level. This is often caused by a failure to process the honey samples using acetolysis, thereby removing surface lipids and cytoplasm that often obscure details. Another reason for not assigning species to most taxa found in honey samples is created by an inability to recognize minute differences in structural morphology or ornamentation of taxa using only light microscopy. We found that by preparing modern pollen reference materials for most of the pollen taxa we found in our East Texas honey study using SEM, we could recognize far more taxa to the species level using LM. For example, after printing large SEM micrographs of each pollen taxon we encountered during our study, we could see small diagnostic characteristics that were not obvious using only LM. Once those diagnostic characteristics were known, we found we could then employ various contrast enhancement

techniques such as phase contrast, Nomarski phase and polarization to make many of these minute characteristics discernible with LM. Through careful attention to these diagnostic characteristics and our vouchered pollen reference collection, it was possible to differentiate the Fabaceae species of *Melilotus*, *Medicago* and *Trifolium*. In addition, it was also possible to differentiate *Toxicodendron radicans* (C. Linnaeus) K. E. O. Kuntze (SY = *Rhus radicans*) (poison-ivy) from *Rhus copallina* C. Linnaeus (winged sumac), *Nyssa sylvatica* H. Marshall (blackgum) from *N. aquatic* C. Linnaeus (tupelo) and *Gleditsia aquatica* H. Marshall (water locust) from *G. triacanthos* C. Linnaeus (honey locust), etc.

Pollen grains in the family Asteraceae are easily recognized, and were placed into several broad groups such as *Baccharis* type, *Helianthus* type, etc. Historically, the members of this family are often lumped together under a family category, or divided into three broad categories based on morphological differences: the fenestrate type, subfamily *Liguliflorae*, and two categories of the *Tubliflorae* based on the length of their processes (spines) (Wodehouse 1935; Martin 1963). Asteraceae pollen can be differentiated by the arrangement and number of the pores at the base of the processes (Sullivan 1975), which were easily seen with SEM. Once aware of the arrangement, it could also be recognized with LM. Except for some definite size distinctions and differences in the patterns of sub-tectum columellae, all grass pollen appear too similar to distinguish into specific genera, except for a few cultivated cereal grasses such as *Zea mays* C. Linnaeus, *Triticum, Secale, Hordeum* and *Avena*. Therefore, members of the family Poaceae were combined into one pollen type.

Likewise, pollen from the many species of oaks (*Quercus*) in Texas cannot be consistently distinguished. Oaks have an affinity to hybridize thus compounding the problem of species identification of the pollen. Therefore, all *Quercus* pollen grains were listed in a single pollen type. The pollen grains of the family Chenopodiaceae and the genus *Amaranthus* are nearly impossible to distinguish from one another and were lumped into a single category, (Cheno-Am), as suggested by Martin (1963).

3. Results

Pollen concentration values per 10 g of honey varied from 24,533 (Van Zandt Co.)–567,825 (Shelby Co.) (Table 1). Twenty-one samples (57%) had pollen concentration values between 100,000–500,000 pollen grains and are considered pollen 'rich' (Figure 2, Table 1, calculated from Table 1) (Feller-Demalsy et al. 1989). Only one sample (Shelby Co.) was 'very rich', containing over 500,000 pollen grains per 10 g of honey; no samples were 'very poor', having < 20,000 pollen grains (Figure 2, Table 1).

Table 1. The regions of East Texas, the number of pollen grains (Grains) and *Lycopodium clavatum* spores (Lyco) counted and the calculated pollen concentration values per 10 g of honey (PC), found in the East Texas honey samples arranged alphabetically by county. Multiple samples from the same county are separated by the beekeepers' initials. The highest and lowest PC values are in bold.

County	Region	Grains	Lyco	PC
Anderson	NW	202	17	268,541.18
Angelina	NE	200	220	51,363.64
Bowie	NE	210	186	25,516.13
Chambers	SW	201	44	103,240.91
Gregg-loc1	NW	200	45	100,444.44
Gregg-loc2	NW	200	42	107,619.05
Gregg-str	NW	200	64	70,652.00
Hardin/Tyler	SE	200	45	100,444.44
Harris-cej	SW	202	94	121,414.89
Harris-mmg	SW	200	354	31,920.90
Harris-sj	SW	200	74	152,701.70
Harrison-pt1	NE	210	174	27,120.00
Harrison-pt2	NE	216	38	128,463.16
Harrison-ws	NE	200	291	38,831.62
Henderson	NW	205	68	68,132.35
Jefferson-es	SE	205	151	76,705.30
Jefferson-pa	SE	208	92	127,739.13
Liberty	SW	201	15	302,840.00
Marion-br1	NE	214	45	107,475.56
Marion-br2	NE	207	45	103,960.00
Montgomery	SW	200	67	67,462.69
Newton	SE	203	38	120,731.58
Orange-dt	SE	200	169	66,863.91
Orange-hal	SE	200	108	41,851.85
Orange-law	SE	200	95	47,578.95
Panola	NE	200	43	105,116.28
Polk	SW	201	66	68,827.27
Red River	NW	203	12	382,316.67
Rusk	NE	201	32	141,956.25
San Jacinto	SW	200	49	92,244.90
Shelby	NE	201	8	**567,825.00**
Smith-bra	NW	201	40	129,788.57
Smith-hut	NW	200	23	196,521.74
Smith-rth	NW	211	82	145,384.15
Van Zandt	NW	203	187	**24,533.69**
Walker	SW	200	53	213,207.55
Wood	NW	208	35	134,308.57

The number of taxa per East Texas honey sample varied between 17 (Gregg-str) and 52 (Chambers and Liberty Co.) (Table 1, Figure 3). Over 50% of the samples contained between 30 and 39 taxa (calculated from Table 2, Figure 3). Three honey samples contained over 50 different pollen taxa (Figure 3, Table 2). Only two samples contained fewer than 20 taxa (Gregg-loc2 and Gregg-str Co.) (Figure 3, Table 2).

Overall, 431 taxa were found and were identified into 61 families, 104 genera and 85 species (Table 2).

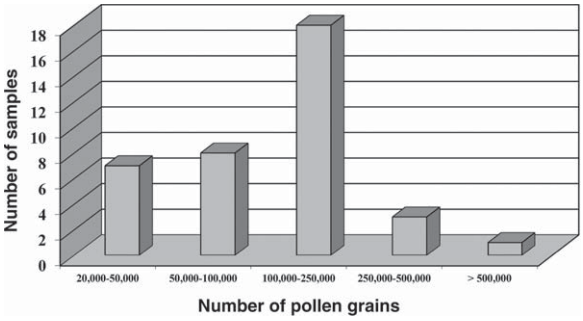

Figure 2. The number of pollen grains per 10 g of East Texas honey.

No one honey sample contained the greatest number of taxa identified to the family, genus and species rankings. Honey from Chambers and Orange-law contained the greatest number of taxa identified to the family ranking (23, Table 2). The pollen from the Orange-law honey also was identified into the greatest number of genera (25) (Table 2), while pollen was identified to the most species rankings in the Smith-hut honey (22, Table 2).

Berchemia scandens (Rhamnaceae) was found in the greatest number of samples (33) and had a higher frequency of occurrence (FOC) and relative frequency (RF) than any other taxon (Table 3). Both *Toxicodendron radicans* and *Salix nigra* were next and were found in 31 samples (Table 3). Within the 10 taxa found in the greatest number of samples, three were Rhamnaceae, *Berchemia scandens*, *Ceanothus americanus* and *Rhamnus caroliniana* (Table 3). Of the 431 taxa found in the honey samples, 174 (42.6%) of them only occurred in one sample.

Of the identified pollen, the Fabaceae had the greatest number of taxa (36) followed by the Asteraceae (16), and then the Vitaceae (10) (Table 4). Of the 61 families identified in the samples, 29 (78%) were represented by only a single taxon (calculated from Table 4).

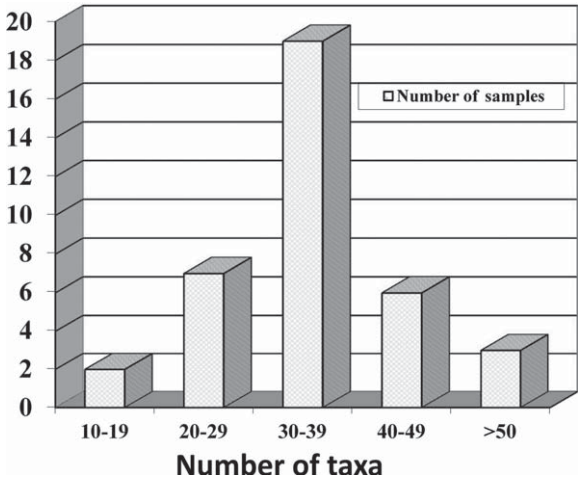

Figure 3. The number of taxa recovered in the East Texas honey samples.

More pollen grains were identified as Rhamnaceae (1746 grains, 23%) than any other family (Table 4). Following the Rhamnaceae in the number of pollen grains were Fabaceae with 775 grains, and the Salicaceae with 236 grains. Seven families (19%) were represented by a single pollen grain (calculated from Table 4).

Fabaceae had the highest frequency of occurrence (FOC) (94.59) and relative frequency (RF) (57.38) (Table 5). Following the Fabaceae were Rhamnaceae (91.89 and 55.74 respectively), Salicaceae (86.49, 52.46 respectively) and Anacardiaceae (83.78, 50.82 respectively) (Table 5).

Three of the 37 honey samples from East Texas, Gregg (Grg-str), Harris-pt1 (Hrr-pt1) and San Jacinto (SJa) contained *Berchemia scandens* as a predominant taxon (> 45%) (Table 6). *Berchemia scandens* occurred in 17 of the 37 samples (46%) either as a predominant (>45%) or a secondary pollen type (16–45%) (Table 6). The only other secondary taxa were Apiaceae #5 in a Jefferson Co. (Jef-pa), *Solidago* #3 in the Angelina Co. and *Mimosa strigillosa* from Harris Co. (Har-mmg), and both honey samples from Jefferson Co.

4. Discussion

Because the alcohol-dilution method was used to process the honey, a greater number and diversity of pollen was recovered than in the honey from SW Texas, Louisiana, Mississippi or Florida (Vorwohl 1970; Lieux 1972, 1981; White et al. 1991; Jones & Bryant 2004) (Table 1). More than 50% of the East Texas honey samples were in the 'rich' or 'very rich' categories, containing over 100,000 pollen grains per 10 g (Table 1). None of the honey from SW Texas contained over 100,000 pollen grains and three contained fewer than 20,000 (White et al. 1991). Of the honey from Florida, 38% contained over 100,000 grains, but none contained over 500,000 pollen grains. In Louisiana, 22% of the honey contained over 100,000 pollen grains (Lieux 1972), and in Mississippi 7% contained more than 100,000 pollen grains (Lieux 1981). Interestingly, 68% of the honey in Mississippi and 52% of the honey in Louisiana contained fewer than 20,000 pollen grains (Lieux 1972, 1981).

Similar to the East Texas honey, some Canadian honey contained a greater number of pollen grains than did SW Texas, Louisiana or Mississippi honey. In Alberta and Saskatchewan, Canada, the majority of honey samples were classified as 'rich', containing 100,000–500,000 pollen grains per 10 g (53 and 57%

Table 2. The number of taxa, families, genera and species of the identified pollen by county of East Texas honey, and the overall total of each for East Texas. Samples from the same county are separated by the beekeepers' initials.

	Taxa	Family	Genus	Species
Anderson	33	13	14	9
Angelina	39	17	24	16
Bowie	37	17	14	14
Chambers	52	23	24	15
Gregg-loc1	30	15	15	11
Gregg-loc2	19	6	10	9
Gregg-str	17	6	9	9
Hardin/Tyler	46	21	23	18
Harris-cej	27	13	17	13
Harris- mmg	33	15	16	12
Harris, -sj	39	14	17	12
Harrison-pt1	30	13	15	12
Harrison-pt2	31	18	17	10
Harrison-ws	32	15	16	9
Henderson	51	17	22	19
Jefferson-es	32	14	18	11
Jefferson-pa	31	15	19	10
Liberty	52	21	16	14
Marion-br1	42	20	22	16
Marion-br2	31	13	15	11
Montgomery	40	19	20	16
Newton	26	10	8	6
Orange, dt	25	10	11	7
Orange, hal	35	14	13	10
Orange, law	44	23	25	11
Panola	31	10	17	20
Polk	40	11	12	9
Red River	35	12	16	17
Rusk	29	13	16	11
San Jacinto	25	8	14	8
Shelby	26	13	17	15
Smith-bra	32	11	15	11
Smith-hut	49	16	23	22
Smith-rth	37	14	16	14
Van Zandt	30	17	16	9
Walker	27	14	16	13
Wood	37	16	22	16
East Texas	431	61	104	85

Table 3. The scientific name, family, number of samples in which the pollen occurred, the frequency of occurrence and relative frequency of identified pollen taxa found in 37 East Texas honey samples. Only taxa found in 10 or more samples are listed. Samples = the number of samples in which the pollen from each taxon occurred, FOC = frequency of occurrence, and RF = relative frequency. Family Taxon Samples, FOC RF.

Family	Taxon	Samples	FOC	RF
Rhamnaceae	*Berchemia scandens*	33	89.19	7.66
Anacardiaceae	*Toxicodendron radicans*	31	83.78	7.19
Salicaceae	*Salix nigra*	31	83.78	7.19
Poaceae	various genera	29	78.38	6.73
Nyssaceae	*Nyssa sylvatica*	19	51.35	4.41
Rhamnaceae	*Ceanothus americanus*	18	48.65	4.18
Fabaceae	*Trifolium repens*	17	45.95	3.94
Polygonaceae	*Brunnichia ovata*	17	45.95	3.94
Rhamnaceae	*Rhamnus caroliniana*	17	45.95	3.94
Fabaceae	*Trifolium incarnatum*	16	43.24	3.71
Fagaceae	*Quercus* spp.	16	43.24	3.71
Fabaceae	*Mimosa strigillosa*	15	40.54	3.48
Apiaceae	various genera	14	37.84	3.25
Verbenaceae	*Callicarpa americana*	14	37.84	3.25
Vitaceae	*Vitis* spp.	14	37.84	3.25
Asteraceae	*Ambrosia*	11	29.73	2.55
Cyperaceae	*Cyperaceae*	11	29.73	2.55
Fabaceae	*Crotalaria*	11	29.73	2.55
Saururaceae	*Saururus cernuus*	11	29.73	2.55
Aquifoliaceae	*Ilex* spp.	10	27.03	2.32
Euphorbiaceae	*Stillengia* sp.	10	27.03	2.32

respectively) (Feller-Demalsy et al. 1987a, 1987b). In Manitoba, the majority of samples (65%) had an intermediate number of pollen grains (50,000–100,000) (Feller-Demalsy et al. 1989). Honey from Québec had very little pollen. Only four percent (4%) of the samples contained over 100,000 pollen grains, while 55% contained less than 20,000 grains (Feller-Demalsy & Lamontagne 1979).

East Texas honey also appears to contain more pollen than many European honeys. European honeys that are unfiltered and extracted by a rotary extractor generally contain 20,000–100,000 grains per 10-g sample (Maurizio 1951; Lieux 1972, 1981). Being able to construct precise pollen concentration values using *Lycopodium* spores as markers gives a better determination of the number of pollen grains per 10 g (Benninghof 1962; Stockmarr 1971). Unfortunately, the majority of honey examined is processed using methods that mix the honey with water and centrifuge at low speeds for 3–10 min (Louveaux 1956, 1964, 1966; Louveaux & Maurizio 1963). Jones and Bryant (2004) compared those methods using ethyl alcohol (ETOH) to dilute the honey and showed that the pollen recovery using the ETOH was greater than other methods. Not only did they get more pollen, but they also got a greater pollen diversity (Jones & Bryant 2004). The problem is that pollen in honey has a specific gravity close to or less than 1.0, the specific gravity of water (Flenley 1971; Jemmett & Owen 1990; Jones & Bryant 2004). When water is added to dilute the honey, the specific gravity of the solution is greater than 1.0 and some of the pollen grains can float to the top even when centrifuged. When ETOH is used to dilute the honey, the specific gravity of the solution is well below 1.0 and pollen grains with a specific gravity less than 1.0 sink to the bottom of the centrifuge tube especially when centrifuged (Jones & Bryant 2004). This is

Table 4. The number of taxa (Taxa), samples (SA) and pollen grains counted (Grains). Percent total (% total) is the total number of pollen grains counted by family as a percentage of total pollen identified in the East Texas honey. The family with the greatest number in each category is bolded.

Family	Taxa	SA	Grains	% total
Acanthaceae	1	2	3	0.04
Aceraceae	3	4	6	0.08
Alismataceae	1	1	4	0.05
Amaranthaceae	2	2	2	0.03
Anacardiaceae	3	31	206	2.74
Apiaceae	9	14	111	1.48
Aquifoliaceae	8	20	64	0.85
Araliaceae	1	2	11	0.15
Asteraceae	16	20	211	2.81
Betulaceae	2	4	6	0.08
Bignoniaceae	1	1	1	0.01
Brassicaceae	2	3	9	0.12
Campanulaceae	1	7	22	0.29
Caprifoliaceae	2	9	27	0.36
Celastraceae	1	2	4	0.05
Cheno-Am	3	6	13	0.17
Clusiaceae	1	1	4	0.05
Commelinaceae	1	2	2	0.03
Cornaceae	4	12	41	0.55
Cupressaceae	1	8	18	0.24
Cyperaceae	7	11	22	0.29
Ebanaceae	3	3	7	0.09
Euphorbiaceae	4	19	106	1.41
Fabaceae	**36**	**35**	775	10.32
Fagaceae	2	18	48	0.64
Grossulariaceae	1	1	1	0.01
Hammeliadaceae	1	5	6	0.08
Hippocastinaceae	1	2	3	0.04
Hydroleaceae	2	10	23	0.31
Juglandaceae	2	2	8	0.11
Lamiaceae	5	6	27	0.36
Lythraceae	3	7	37	0.49
Magnoliaceae	1	1	1	0.01
Meliaceae	1	3	5	0.07
Moraceae	1	1	1	0.01
Myricaceae	1	1	3	0.04
Nyssaceae	2	23	106	1.41
Oleaceae	3	12	17	0.23
Onagraceae	2	2	5	0.07
Pinaceae	1	2	3	0.04
Platanaceae	1	1	1	0.01
Poaceae	1	29	184	2.45
Polygonaceae	4	19	76	1.01
Pontedariaceae	1	1	2	0.03
Rhamnaceae	5	34	**1746**	**23.24**
Rosaceae	3	13	45	0.60
Rutaceae	2	9	14	0.19
Salicaceae	3	32	236	3.14
Sapotaceae	1	1	1	0.01
Saururaceae	1	11	20	0.27
Scrophulariaceae	1	3	7	0.09
Smilacaceae	1	6	15	0.20
Solanaceae	2	9	25	0.33
Tamaraceae	1	7	12	0.16
Taxodiaceae	1	4	8	0.11
Theaceae	1	1	2	0.03
Tiliaceae	1	1	5	0.07
Ulmaceae	6	8	38	0.51
Verbenaceae	3	18	76	1.01
Violiaceae	1	1	1	0.01
Vitaceae	10	21	129	1.72

extremely important when trying to recover pollen from honey because it is necessary to obtain all of the pollen grains within the honey to make accurate determinations and honey classifications.

Over 430 different taxa were found in the 37 honey samples from East Texas (Table 2). Poor pollen diversity occurred in SW Texas, Louisiana, Mississippi and Florida honey samples (Vorwohl 1970; Lieux 1972, 1981; White et al. 1991). In SW Texas, 32 taxa were recovered, 54 types in Louisiana, 84 types in Mississippi and 70 in Florida (Vorwohl 1970; Lieux 1972, 1981).

Half of the East Texas honey samples contained 31–40 taxa, indicating a high diversity in botanical origin (Table 2). All of the honey samples from SW Texas contained 6–14 taxa (White et al. 1991). The majority of Louisiana honey (76%) contained between 6–15 types (Lieux 1972). In Mississippi, 37% of the samples contained 16–20 taxa, indicating a slightly better botanical diversity than Louisiana honey (Lieux 1981). Florida honey contained between 11–15 taxa (Vorwohl 1970).

In Québec, 60 taxa were identified into 24 families and 46 genera, while in another study, 56 types were found (Feller-Demalsy & Lamontagne 1979). Pollen in the honey from Alberta, Canada, contained 46 taxa; however, no more than 17 types were found in an individual sample (Feller-Demalsy et al. 1987a). Honey from Manitoba contained 50 types with 90% of the samples having between 6–15 types, although some did have as many as 25 types (Feller-Demalsy et al. 1989). In Saskatchewan honey, only 37 taxa were identified and most samples contained only 6–10 taxa (Feller-Demalsy et al. 1987b).

The lack of pollen diversity in SW Texas, Louisiana, Mississippi and Canadian honeys may be caused by many factors. First, the level of taxonomic differentiation of pollen differs substantially among the studies (Vorwohl 1970; Lieux 1972, 1975, 1981; Feller-Demalsy et al. 1987a, 1987b, 1989; White et al. 1991). When taxa are combined and classified only at the family ranking, most of the diversity and the actual resources are lost. To identify a pollen type to the species ranking such as *Trifolium repens* C. Linnaeus tells the researcher more than identifying the pollen grain as Fabaceae. The better the pollen identification, the better the information is for honeybee resources utilized,

Table 5. The frequency of occurrence (FOC) and the relative frequency (RF) of the plant families identified in the East Texas honey. The five families with the highest frequencies are bolded.

Family	FOC	RF
Acanthaceae	5.41	3.28
Aceraceae	10.81	6.56
Alismataceae	2.70	1.64
Amaranthaceae	5.41	3.28
Anacardiaceae	**83.78**	**50.82**
Apiaceae	37.84	22.95
Aquifoliaceae	54.05	32.79
Araliaceae	5.41	3.28
Asteraceae	54.05	32.79
Betulaceae	10.81	6.56
Bignoniaceae	2.70	1.64
Brassicaceae	8.11	4.92
Campanulaceae	18.92	11.48
Caprifoliaceae	24.32	14.75
Celastraceae	5.41	3.28
Cheno-AM	16.22	9.84
Clusiaceae	2.70	1.64
Commelinaceae	5.41	3.28
Cornaceae	32.43	19.67
Cupressaceae	21.62	13.11
Cyperaceae	29.73	18.03
Ebanaceae	8.11	4.92
Euphorbiaceae	51.35	31.15
Fabaceae	**94.59**	**57.38**
Fagaceae	48.65	29.51
Grossulariaceae	2.70	1.64
Hammeliadaceae	13.51	8.20
Hippocastinaceae	5.41	3.28
Hydroleaceae	27.03	16.39
Juglandaceae	5.41	3.28
Lamiaceae	16.22	9.84
Lythraceae	18.92	11.48
Magnoliaceae	2.70	1.64
Meliaceae	8.11	4.92
Moraceae	2.70	1.64
Myricaceae	2.70	1.64
Nyssaceae	62.16	37.70
Oleaceae	32.43	19.67
Onagraceae	5.41	3.28
Pinaceae	5.41	3.28
Platanaceae	2.70	1.64
Poaceae	**78.38**	**47.54**
Polygonaceae	51.35	31.15
Pontedariaceae	2.70	1.64
Rhamnaceae	**91.89**	**55.74**
Rosaceae	35.14	21.31
Rutaceae	24.32	14.75
Salicaceae	**86.49**	**52.46**
Sapotaceae	2.70	1.64
Saururaceae	29.73	18.03
Scrophulariaceae	8.11	4.92
Smilacaceae	16.22	9.84
Solanaceae	24.32	14.75
Tamaraceae	18.92	11.48
Taxodiaceae	10.81	6.56
Theaceae	2.70	1.64
Tiliaceae	2.70	1.64
Ulmaceae	21.62	13.11
Verbenaceae	48.65	29.51
Violiaceae	2.70	1.64
Vitaceae	56.76	34.43

habitats visited, time of day visited, distance from the hive, etc.

In Louisiana and Mississippi honey, pollen grains of *Melilotus* spp., *Medicago* spp., *Trifolium* spp. (those different from *Trifolium incarnatum* C. Linnaeus) and any other pollen grain that looked similar to these types were combined into the single taxon *T. repens* (Lieux 1969, 1972, 1975, 1981). This is similar in the Florida honey where many of the identified taxa are actually groups of taxa (Vorwohl 1970). In the Canadian studies, taxa were divided more into genera but each genus had multiple types which were not separated out (Feller-Demalsy & Lamontagne 1979; Feller-Demalsy et al. 1987a, 1987b; Feller-Demalsy et al. 1989; Parent et al. 1990). Furthermore, in the Louisiana and Mississippi studies, all types of Asteraceae were combined into a single type (Lieux 1972, 1975, 1981). However, in the SW Texas, Florida and Canada honey, the Asteraceae were divided into more than one type (Vorwohl 1970; Feller-Demalsy & Lamontagne 1979; Feller-Demalsy et al. 1987a, 1987b; Feller-Demalsy et al. 1989; Parent et al. 1990; White et al. 1991).

There are five major factors that have often made pollen analyses of honey difficult. First, the Asteraceae is the largest plant family in many regions, such as Texas (Correll & Johnston 1979; Jones et al. 1997). Not only does it include the greatest number of taxa in the state, but it also competes with the family Poaceae in having the greatest number of individual plants growing during each season. In Texas, the Asteraceae can be herbs, shrubs or vines, and are important foraging sources for honeybees for both pollen and nectar. Asteraceae occur in all habitats, and can be found in flower throughout the year in most of the state. Abundant honey has been obtained from *Aster* spp., *Baccharis* spp., *Liatris* spp., *Eupatorium* spp. and *Solidago* spp. (Pellett 1930, 1976). To combine all of the taxa of Asteraceae would lose important information on the floral resources of East Texas and the resources utilized by honeybees. Unfortunately, differentiating the pollen of the Asteraceae is difficult and time consuming.

Second, the optics in today's microscopes are far superior to those in the microscopes of the 1960s and 1970s. This enhances the ability to look for and find the small differences that separate taxa. Furthermore,

Table 6. Frequency classes of the East Texas honey for taxa >3% in at least one sample. D = predominant pollen (>45%), S = secondary pollen (16–45%), M = important minor pollen (3–15%), and L = lesser important minor pollen (<3%). Taxa that never occurred >3% are not included in the table. County names with their three-letter code are listed at the end of the table.

Family	County* / Taxon	And	Ang	Bow	Cha	Grg loc1	Grg loc2	Grg str	HaT	Har cej	Har mmg	Har sj	Hen	Hrr pt2	Hrr pt1	Hrr ws	Jef es	Jef pa	Lib
Anacardiaceae	*Rhus copallina*										L								
Anacardiaceae	*Toxicodendron radicans*	M	L	L	L	L	L		L	M		M	L	L	L	L	L		M
Anacardiaceae	*Toxicodendron toxicarium*											L	M		L				
Apiaceae	Apiaceae #5								M									S	
Apiaceae	Apiaceae, #158								L									L	
Apiaceae	Apiaceae, #396					M													L
Aquifoliaceae	*Ilex* #6				L														
Araliaceae	*Aralia spinosa*					M													
Asteraceae	*Ambrosia* #1		M															L	
Asteraceae	*Ambrosia* #3				M				L					L					
Asteraceae	*Eupatorium* #2		M			L													
Asteraceae	*Solidago* #3		S		L				L					M					
Brassicaceae	Brassicaceae #3												M						
Campanulaceae	*Lobelia* sp.												L						M
Caprifoliaceae	*Sambucus canadensis*	L	L							L			L	L					
Cornaceae	*Cornus foemina*	M																	
Cupressaceae	*Juniperus* sp.	L							L							L			L
Euphorbiaceae	*Sebastiana fruticosa*								L										L
Euphorbiaceae	*Stillingia* sp., Un #99										M	L				L	L	L	L
Fabaceae	*Cercis canadensis*		M																
Fabaceae	*Crotalaria spectabilis*										M				L	L			
Fabaceae	*Glycine max*																		L
Fabaceae	*Medicago* sp.									L									
Fabaceae	*Melilotus albus*						L		L				M						
Fabaceae	*Melilotus indicus*			L	L								M						
Fabaceae	*Melilotus officinalis*			L									M		L				
Fabaceae	*Mimosa strigillosa*				M	L			L		S		L			M	S	S	M
Fabaceae	*Robinia hispida*																		
Fabaceae	*Schrankia occidentalis*									M								L	
Fabaceae	*Tephrosia onobrychoides*																		
Fabaceae	*Trifolium campestre*			M					L										
Fabaceae	*Trifolium carolinianum*							L	L				L						
Fabaceae	*Trifolium incarnatum*	L	L	L			M	M						M	M				
Fabaceae	*Trifolium repens*	L		M		L		L	L		M	L	M					L	
Fabaceae	*Trifolium resupinatum*			M					L					L	L				L
Fagaceae	*Quercus* spp.	L			L				L		L	L	L	L	L	L	L		
Hydroleaceae	*Hydrolea uniflora*								L			L	L					L	L
Juglandaceae	*Carya* #2										M								
Lamiaceae	Lamiaceae #5																		
Lamiaceae	*Salvia* sp.																		
Lythraceae	*Lagerstroemia indica*										L						M	M	
Nyssaceae	*Nyssa aquatica*							L			L			L					L
Nyssaceae	*Nyssa sylvatica*	L	L	M			L		L	L	L				L				L

(*continued*)

Table 6. (*Continued*)

Family	County* Taxon	And	Ang	Bow	Cha	Grg loc1	Grg loc2	Grg str	HaT	Har cej	Har mmg	Har sj	Hen	Hrr pt2	Hrr pt1	Hrr ws	Jef es	Jef pa	Lib
Poaceae	various types		M	L	L	L	L		L		L	L	L	M	L	L	M	L	L
Polygonaceae	*Brunnichia ovata*		L		L					M		L		L			L	L	
Rhamnaceae	*Berchemia scandens*	S	M	S	L	L	S	D	M	S	L	L		M	D	L	L		M
Rhamnaceae	*Ceanothus americanus*		M				M	M		M					M				
Rhamnaceae	*Rhamnus caroliniana*					L	S	M	L									L	
Rhamnaceae	*Ziziphus jujuba*							M				L							
Rosaceae	*Prunus* #1												L					L	
Rosaceae	*Prunus serotina*		M				L						L						
Salicaceae	*Salix nigra*	M	L	M	L	L	M	L	L	L	L	L	L	L	L	L		L	M
Smilacaceae	*Smilax* sp				M				L					L					L
Solanaceae	*Solanum* sp.												M						L
Ulmaceae	*Ulmus americana*												M						L
Ulmaceae	*Ulmus crassifolia*																		
Verbenaceae	*Callicarpa americana*	L	M	L								L	L	L		L	L		
Verbenaceae	*Phyla* #2										L								M
Vitaceae	*Ampelopsis arborea*		L							L		L					L		
Vitaceae	*Parthenocissus quinquefolia*		L							L		L		M					
Vitaceae	*Vitis* #3				L														
Vitaceae	*Vitis* #4																		

from examining vouchered pollen samples with SEM, it was later possible to differentiate the pollen of many species using LM and avoid having to combine all of them into single genera categories (i.e., *Melilotus*, *Medicago*, *Trifolium*, *Toxicodendron radicans*, *Rhus copallina*, *Nyssa sylvatica*, *N. aquatica, Gleditsia aquatica*, *G. triacanthos*, etc.)

Third, Texas contains over 5000 species of plants that produce pollen and spores. Plants flower in most of Texas throughout the year. Because the winters are mild in most of East Texas, honeybee plants are in bloom year-round and honeybees can be found on those plants year-round. Many of these plants are utilized by honeybees when optimal flowers are unavailable, and thus will be in the honey.

Fourth, the techniques used to recover pollen from honey impacts all data generated from the honey sample including the number of pollen grains, number of taxa, frequency of occurrence, relative frequency, frequency classes and the classification of the honey (unifloral vs. multifloral). Non-acetolyzed pollen often has lipids and waxes on the surface that can obscure critical ornamentation features and thus make precise identification more difficult (Low et al. 1989). Acetolysis dissolves most of the tissue and organic debris, and removes the proteins, lipids and carbohydrates from the surface of the pollen grains (Erdtman 1960; Low et al. 1989). This makes the pollen grains easier to stain, photograph and identify. Without acetolysis, pollen from *Salix* (willow) and *Brassica* (rapeseed) can be virtually identical and impossible to differentiate (Low et al. 1989). However, when acetolysis is used on the pollen of the two taxa, the differences between pollen from the two taxa are easily seen (Low et al. 1989). Furthermore, when ETOH is used to dilute honey, the pollen recovery, the numbers of types and pollen grains are increased (Jones & Bryant 2004).

Finally, melissopalynologists recognized that not all taxa contribute equally to the production of honey. Thus, corrective values for certain taxa called pollen coefficient values (PC) were developed and can be used to compensate for taxa that are under or over represented in the relative pollen counts of honey (Bryant & Jones 2001). The use of PC values assists in the verification and sale of premium honey types because many premium honeys are not easily confirmed as being from a single floral-source (unifloral). Unfortunately, not everyone accepts the use of PC values, and there are many problems with PC values because of the techniques used to generate PC values and to recover pollen from honey (Bryant & Jones 2001).

Some studies eliminate the anemophilous taxa from the honey taxa list; however, we believe that this

is a grave mistake. It has often been assumed that anemophilous taxa such as Poaceae, *Juniperus*, *Quercus* and *Sagittaria*, etc. are accidental honey contaminants that have fallen or been blown into the nectar or honey. However, honeybees visit and forage on the flowers of anemophilous taxa (grasses, maples, willow, ashes, *Sagittaria*, etc.) during times when 'preferred' flowers are unavailable. Honeybees are even known to collect pollen from male catkins and honeydew from oak galls (Lovell 1966; Lieux 1978). Pellett (1976) considers many anemophilous taxa such as oak, willow, maples, etc. as valuable sources of pollen.

Anemophilous taxa formed a large part of the spring pollen collection in England (Synge 1947), Wisconsin (Severson & Parry 1981) and at four of five apiaries investigated in Arizona (O'Neal & Waller 1984). In the spring months in New Zealand (September and October), 82% of the pollen (eight of 21 species) were anemophilous (Pearson & Braide 1990). Among the New Zealand taxa were members of the families Poaceae, Cyperaceae and Cupressaceae. If honey bees forage on these plants, they should not be dismissed as unimportant foraging resources, and should be viewed as an integral part of the foraging resources of honey bees, and the geographical location of the honey.

Three taxa were found in $> 50\%$ of the samples and are the most important (Tables 3 and 5). In decreasing percentages they are *Berchemia scandens*, *Salix nigra* and *Toxicodendron radicans*. *Berchemia scandens* was found in 89% of the samples (34 of 37). It was the predominant type in three samples and an important secondary type in 14. This is similar to the honey of Louisiana and Mississippi. In Louisiana, *B. scandens* had the highest frequency of occurrence (96%) and was found in 52 of the 54 honey samples (Lieux 1972, 1975, 1977, 1978). It was a predominant type in seven honey samples and an important secondary in 14 (Lieux 1972, 1975, 1977). In Mississippi, *B. scandens* has a frequency of occurrence of 79% in the honey samples and was a predominant pollen type in seven samples and an important secondary in 16 (Lieux 1981).

Pellett (1976) lists *B. scandens* as one of the most important foraging sources for surplus honey in East Texas. *Berchemia scandens* is a vine with inconspicuous green flowers that can be found in East, Southeast, North Central and South Central Texas (Correll & Johnston 1979). It grows from Virginia west to Missouri and south to Florida and Texas. Because of the importance of this taxon for honeybees, it probably contributes to honey throughout its range. Nevertheless, Vorwohl (1970) did not identify it as occurring in Florida honey.

Both *Salix nigra* and *Toxicodendron radicans* pollen were the second most important taxa, having a frequency of occurrence of 83%. Each was found in 30 of the 36 samples, and neither was a predominant or secondary type. Pellett (1976) did not list *Salix nigra* as a honeybee plant in Texas, but did report its importance as a foraging source in Louisiana. In Louisiana, *S. nigra* occurred in 89% of the honey samples and was a predominant pollen type in four Louisiana samples and an important secondary in 13 (Lieux 1972, 1975, 1977). In Mississippi, willow (mostly *S. nigra*) had a frequency of occurrence of 94% and was found in 64 of the 68 examined samples. Willow was a predominant type in 11 samples and a secondary in 17 (Lieux 1981). Willow (including *S. nigra*) was considered as one of the four major honey-producing plants in Louisiana, and one of the most important honey plants in Mississippi.

In Florida, *Salix* occurred in 54% of the samples, and in all samples but one occurred in less than 3% of the pollen count (Vorwohl 1970). In one Florida honey, *Salix* was a secondary type occurring in between 16 and 45% of the pollen grain count (Vorwohl 1970). Pellet (1976) does not list *Salix* as a honeybee plant in Florida, but says that it is a valuable resource in the Gulf States, of which Florida is considered one.

Toxicodendron radicans pollen was present in 30 of the 37 East Texas honey samples (83%), Although not listed as a major honey-producing plant in Louisiana, Lieux (1972, 1975) found *T. radicans* pollen in 79% of her honey samples. In one sample, it is listed as a secondary type, but in all other samples it is only a minor type. In Mississippi, *T. radicans* pollen had a frequency of occurrence of 92% and occurred in 63 of the 68 samples (Lieux 1981). *T. radicans* was one of the top four honey-producing plants in Mississippi (Lieux 1981). *T. radicans* is not listed as occurring in the Florida honey (Vorwohl 1970). Dominant pollen in Florida honey included a '*Rhus vernix*-Form'. Whether or not one of those taxa was *T. radicans* is unknown. Pellett (1976) noted that when conditions are right *T. radicans* can be the source of abundant, surplus honey. The flowers are inconspicuous, but secrete nectar abundantly (Lieux 1972).

According to Zander's (1935) classification, the category called 'predominant pollen (more than 45%)' denotes a unifloral honey. When using those criteria outlined by Zander, we found that three of the 37 honey samples are *Berchemia scandens* unifloral honey (Table 6). The rest of the honey examined was a mixed floral type. This is quite different from the reported honey in Louisiana, Mississippi, Florida and Canada (Vorwohl 1970; Lieux 1972, 1981; Feller-Demalsy & Lamontagne 1979; Feller-Demalsy 1983; Feller-Demalsy et al. 1987a, 1987b, 1989; Parent et al. 1990). In Louisiana, 57% of the honey was unifloral, three of which were *B. scandens* unifloral (Lieux 1972, 1981). In

Mississippi, 53% of the honey examined was unifloral, and *B. scandens* was the unifloral type in seven of those (Lieux 1981). In Florida, 85% of the samples were unifloral and the majority of Florida unifloral honey was *Ilex* (Vorwohl 1970).

The majority of honeys examined in Canada are unifloral. In Alberta 92% were unifloral; in Manitoba, 93%, and in Saskatchewan, 93% (Feller-Demalsy et al. 1987a, 1987b, 1989). The high number of unifloral honeys from Canada may be due in part to the lumping of taxa into broad categories. Very few taxa were identified to species. Most are identified only to family or genus. This makes pollen identification much easier and less time consuming. However, by lumping taxa together, there is a loss in foraging resource information. For example, one species of a plant group may be a primary foraging resource while others in the same group may be only minor resources or not visited at all. Without differentiating the pollen species, a beekeeper might erroneously place the honeybee hives near a plant species which is not visited, thus wasting time and money and possibly losing the colony. Furthermore, differentiating the pollen species also helps in determining the geographical region of the honey.

The determination of the geographical origin of honey is traditionally a function of pollen analyses of honey. Important considerations that are used to determination of the geographical origin of honey include the types of predominant pollen, secondary pollen, minor taxa, overall pollen spectra and the percentages of each pollen type (Maurizio 1951; Maurizio & Louveaux 1965; Lieux 1972). Unfortunately, only three of the 37 honey samples had predominant pollen. The majority of East Texas honey was multifloral.

Honey types collected from the different East Texas counties are not easily distinguished from one another. Honey samples from northern counties appear to be similar, as do the honey samples from the southern counties. By joining the northern counties together and the southern counties together, differences in the geographical origin of the honey can be more easily recognized.

Honey from the northern counties is characterized by large amounts of pollen from *Berchemia scandens*, *Ceanothus americanus*, *Nyssa sylvatica*, *Rhamnus caroliniana* and *Salix nigra*. The northern counties are dominated by the eastern deciduous forest with bottomland hardwoods and pine-hardwood uplands. *Berchemia scandens* is to be expected in these honeys because it is occurs mainly in bottomland hardwood forest habitats. *Ceanothus americanus* C. Linnaeus, *Nyssa sylvatica*, *Rhamnus caroliniana* T. Walter and *Salix nigra* are common in the eastern deciduous forest and are found in the pine-hardwood uplands. Three of these species (*Berchemia scandens*, *Ceanothus americanus* and *Rhamnus caroliniana*) are members of the family Rhamnaceae, indicating the importance of the plant family for honeybees in the northern counties of East Texas.

Honey from the southern counties is characterized by the presence of *Mimosa strigillosa* J. Torrey and A. Gray, small amounts of *Berchemia scandens* and traces of Poaceae pollen. *M. strigillosa* is abundant in the coastal prairies growing in wet areas, on lawns and along roadsides. *M. strigillosa* occurs in higher percentages in the honey from the southern counties than from the northern counties. *Berchemia scandens* was also found in honey from the southern counties because it grows in the bottomland hardwood forested regions in the forests along the banks of the Trinity, Nueches, Sabine and San Jacinto Rivers, and areas within the Sam Houston National Forest. However, *B. scandens* pollen was found in much lower frequencies in the honey from the southern counties.

5. Conclusion

It would be difficult to differentiate honey of East Texas from the honey of Louisiana and Mississippi. Superficially, East Texas honey seems to be very different from Florida honey. *Berchemia scandens* and *Salix nigra* are key taxa found in all East Texas, Louisiana and Mississippi honey, but not Florida honey (Vorwohl 1970; Lieux 1972, 1975, 1981). The same is true for *Toxicodendron radicans* which grows throughout East Texas, Louisiana, and Mississippi. However, *Rhamnus caroliniana* was not found in the honey from either Louisiana or Mississippi. This taxon does occur in Louisiana and Mississippi but was not identified in the honey. It is unknown if this taxon did not occur near the hives where the honey was collected or if the pollen type was not separated from *Berchemia scandens* (Lieux 1972, 1975, 1981).

To differentiate the honey of the lower 48 states of the USA from other countries of the world, the honey from each state will have to be analyzed. Prior to this, a decision must be made about lumping taxa together. If beekeepers want to classify honey as a particular type, they have to decide how particular they want to be. For example, will the honey be classified as clover honey, or will it be classified as red clover with the scientific name attached to it so that there is no doubt of the floral source? Florida has begun this process by classifying tupelo honey as having *Nyssa aquatica* as the predominant pollen type, and citrus honey having predominately *Citrus* spp. pollen. Similarly, beekeepers from North Carolina are doing the same thing for sourwood [*Oxydendrum arboreum* (L.) de Candole] honey. Classification of honey will not only help the beekeepers sell their honey at a premium but will also help prevent the influx of honey from other countries from being sold as US domestic honey.

Author biographies

GRETCHEN D. JONES is currently retired; she was previously a research biologist for the United States Department of Agriculture (USDA), Agriculture Research Service (ARS), and worked in the Areawide Pest Management Research Unit at College Station, Texas. She received her masters in botany from Stephen F. Austin State University and a PhD in botany from Texas A&M University. For her PhD, Gretchen studied melissopalynology and worked with European and Africanised honey bees. Gretchen's research for USDA-ARS included studying the pollen on and in insect pests to determine what they eat besides our crops, the migration and dispersal of these insects, and what parts of the USA they originated from.

VAUGHN M. BRYANT received his degrees from the University of Texas at Austin (BA in geography; MA in anthropology; PhD in Botany), and currently is a Professor of Anthropology and the Director of the Texas A&M University Palynology Laboratory. He is also an adjunct Professor of Forensic Sciences at the University of Nebraska. Vaughn has been Managing Editor and President of AASP - The Palynological Society. He has been awarded the Distinguished Service Award, the Honorary Life Membership Award and the Medal for Excellence in Education by AASP - The Palynological Society. Currently he is Secretary and one of the trustees of the AASP Foundation.

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