Palynology Research Laboratory
Department of Anthropology
Texas A&M University
College Station, TX 77843-4352
(979) 845-5242 FAX (979) 845-4070

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Denise Altay
Killer Bees Honey
828 Evens Ridge Rd.
Lake Toxaway, NC 28747

Dear Denise,

I grew up in Hendersonville, and I well remember your area. My friends and I would often go to Lake Toxaway with our canoes and camp out on weekends during high school. Of course that was more than 50 years ago so I guess a lot has changed. Anyway, I do know your area and I remember it well.

I have completed the pollen study of the two honey samples you submitted for analysis. Specific details about the extraction and analysis procedures I used for these samples are mentioned below and are identical to those I normally use on other such samples. These procedures are outlined below.

**EXTRACTION PROCEDURE:**

To conduct a pollen study of raw honey we first must dilute it before the pollen can be removed for analysis. For our study, we use a 10g sample of raw honey for the analysis. The sample of raw honey is diluted with 10 ml of distilled water and 100 ml of ETOH, and then heated to 100$^{o}$ F to ensure a complete mixture. This is a technique that we developed and has now been adopted by most others (Jones and Bryant, 2004, **The use of ETOH for the dilution of honey** *Grana* 43: 174–182).

Next, we add one tablet containing a total of 18,583 ***Lycopodium*** spores to enable us to conduct a pollen concentration study for each sample. We use these lycopod spores because they are not utilized by bees for any purpose and thus we do not have to worry about these being found in natural honey sources. Once these initial stages are complete, the pollen sample is dehydrated with glacial acetic acid and then heated in a mixture of a sulfuric acid and acetic anhydride. This chemical treatment, called *acetolysis*, is designed to remove lipids, waxes, and cytoplasm thereby making the pollen easier to identify.

Once the acetolysis process is complete, each sample is again dehydrated in glacial acetic acid and treated with a series of distilled water rinses. The resulting pollen residue is stained to create contrast for microscopic analysis and photography. Finally, we mix a few drops of glycerin into the sample and mount one drop of it on each microscope slide for analysis. To ensure an accurate representation of the overall sample we stir the sample for one minute on a Vortex stirrer before removing each drop for analysis. Our laboratory experiments

and published results have demonstrated that this technique ensures that each drop is a true reflection of the original sample.

Analysis of a honey sample follows a two-step procedure. First, the sample is scanned at 400x under a microscope, initial identifications are made of each pollen type, and key photographic images are taken of each pollen type. During this procedure if a pollen grain is not one we are familiar with, we will compare it with our extensive modern pollen reference samples on file in our laboratory in hopes of finding a match. Second, a quantitative pollen count is conducted for each sample to determine the pollen types present and the frequency of each taxon.

A statistically valid quantitative pollen count of 200-300 pollen grains is conducted for each sample as originally recommended for honey specimens in 1978, by Louveaux, Maurizio, & Vorwohl (***Bee World*****,** 59:139-157). Quantitative counts are used because testing has shown that these offer an accuracy of greater than 95% as to the actual composition of pollen taxa within a given honey sample. The result of our pollen count for your sample is included below (Table 1). In 2004, Von Der Ohr et al. (***Apidologie*** 35:S18–S25) reaffirmed that for most honey types a unifloral should contain at least 45% pollen from one type, but he did point out there are exceptions.

We have followed the reporting system recommended by Louveaux *et al.* (op. cit.) and others who stress that pollen results should be listed according to percentage classes rather than actual percentages when counts of between 200-1200 grains per sample are conducted. We show the actual percentage counts for general reference but these are not deemed totally accurate for honey samples until a total count in excess of 1,200 pollen grains per sample is reached. We rarely count this many pollen grains for a honey sample because in most cases it is not needed and because larger counts add cost and time considerations.

**The recognized pollen percentage's classes used for honey analysis are**:

- A= >45%, called predominant pollen types
- B= 16-45%, called secondary pollen types
- C= 3-15%, called important minor pollen types
- D= <3%, called a minor pollen types

In making quantitative counts, each pollen type is identified to the family, genus, or in some cases species level. Sometimes the pollen types within one plant family (such as the **Amaranthaceae** [amaranths], **Liliaceae** [lilies], **Myrtaceae** [gum family], **Poaceae** [grasses], **Rhamnaceae** [buckthorns], **Brassicaceae** [mustards], **Rosaceae** [rose family] and **Ericaceae** [ericads]) are diagnostic at the family level yet often many of their individual genera cannot easily be separated into specific types because of their morphological similarity with one another. In addition, even within a single genus containing many species each pollen species will generally appear similar to the genus, yet the pollen of each species will also contain minor variations. In addition, the size of the pollen grains in a taxon is not a reliable way to differentiate types into specific genera or certain species. Many studies have demonstrated that within each taxon there is a range of size variation and within plant families size is not a reliable way to distinguish even one genus from another. Often many of the species within a single genus will overlap with other species in the same genus making that an unreliable way to

identify a specific species. In some large plant families, such as the **Fabaceae** (legumes) and the **Asteraceae** (composites), we are able to identify some taxa down to the generic level yet most of the others in these families produce pollen types that are too similar to one another to distinguish apart even at the genus level without extensive reference collections and studies at levels of higher resolution using scanning electron microscopy (SEM). Some of the advantages and disadvantages of using either light microscopy or scanning electron microscopy for pollen work are outlined in a published article I wrote for a journal, which I can send you if you wish.

A pollen concentration value (PC) of pollen grains per 10g of honey was calculated for your sample. This value usually ranges from a few thousand pollen grains to more than one million. As Maurizio (1975) has noted, the number of pollen grains in individual honey samples can vary greatly, therefore, she recommends using a set of concentration categories. Honey pollen counts in **Category I:** contain less than 20,000 grains/l0 g. Often, honey in this category represents samples that have been pressure-filtered, honey from floral sources that produce little pollen, honeys that were partly produced by sugar-feeding bees, or honey that has been adulterated by adding high-fructose syrup or adding highly-filtered honey with no pollen. Usually, honeydew honey samples also fall into this first category. Pollen concentration counts in **Category II:** contain between 20,000-100,000 grains/10 g, which includes the majority of honey produced in the world from most floral sources. **Category III:** pollen concentration values range from 100,000-500,000 grains/10 g and represent floral sources that are high pollen producers or indicate that some of the comb storage cells containing pure pollen may have been mixed with the extracted honey. **Category IV:** includes pollen concentrations between 500,000-1,000,000 grains/10 g. That category along with honey in **Category V:** (containing pollen concentrations of more than 1,000,000 grains/10 g) indicate honey that is produced from a few floral sources that are extremely rich in pollen (i.e., *Myosotis sylvatica, Cynoglossum officinale*, etc.).

Pollen concentration values are very important and useful because they give us a general idea of the amount of pollen present and also suggest the geographical location where the honey was produced. In some cases, adulterated honey samples that have been mixed with highly-filtered honey or with quantities of other sugars (i.e., cane sugar or corn syrup) will contain low pollen concentration values. Nevertheless, without chemical isotope testing for possible adulteration, pollen concentration values alone are generally not sufficient to warrant such a claim for added sugar adulteration.

We calculated our pollen concentration value using the formula

$$PC = \frac{(\#\text{ of } \textbf{Lycopodium} \text{ spores added}) \times (\#\text{ of pollen grains counted})}{(\#\text{ of } \textbf{Lycopodium} \text{ spores counted}) \times (\text{amount of honey (grams) processed})}$$

The complete pollen count for your samples is listed below. A summary of the pollen types found and the pollen concentration values is also noted.

## ANALYSIS

**Sample 1**

Based on the pollen counts in **Sample # 1**, the honey sample appears to be a Mixed Floral Honey because as you can see in the table below, it is not dominated by any one pollen and nectar type in a percentage greater than 45%, as required by the International Commission on Bee Biology for unifloral honey. Instead, it appears to be dominated by the pollen and nectar from holly and tulip tree with only a minor amount of nectar coming from sourwood and other sources. However, if we use pollen coefficient values to determine the true nectar value (TNV) for this sample, it reveals that it is almost a good sourwood honey (see attached article). Using the corrective values, it appears that the TNV of your honey is actually composed of about 38% sourwood nectar, which makes it close to being a unifloral sourwood honey.

Good sourwood honey should have a pollen concentration value of under 10,000 pollen grains per 10 grams of honey. Therefore, for good sourwood honey, the expected pollen concentration value should be in Category I, and the lower the concentration value generally the better the purity of sourwood honey. We also know that tulip tree pollen is underrepresented in honey and it also tends to decrease the pollen concentration value. That is why your pollen concentration value is under 10,000 pollen grains per 10 grams of honey even though it is not quite a sourwood honey.

**Sample 2**

Your **Sample 2** would be classified as a good Unifloral Holly Honey based on the basic pollen counts. However, by applying the pollen coefficient formula to this sample we find that it is actually a good sourwood sample with about 77% of the nectar coming from sourwood flowers. The pollen concentration value is nearly 13,000 pollen grains per 10 grams of honey suggests that even though you do have sourwood honey, it is not as pure as it might be if the total was under 10,000 pollen grains per 10 grams of honey.

**Relative Pollen Counts of Honey Samples AR & JW**

**Table 1**

**Killer Bees Honey 2017**

| Pollen Taxa | 1 | % | TNV | 2 | % | TNV |
|---|---|---|---|---|---|---|
| ***Acer*** **(maple)** | 2 | 1.0% | | 0 | 0.0% | |
| **ASTERACEAE (dandelion-type)** | 2 | 1.0% | | 1 | 0.5% | |
| **ASTERACEAE (sunflower-type)** | 0 | 0.0% | | 0 | 0.0% | |
| **BRASSICACEAE (mustard family)** | 0 | 0.0% | | 0 | 0.0% | |
| ***Castanea*** **(chestnut, chinquapin)** | 11 | 5.4% | | 0 | 0.0% | |
| ***Celtis*** **(hackberry)** | 0 | 0.0% | | 0 | 0.0% | |
| ***Centaurea*** **(thistle)** | 1 | 0.5% | | 0 | 0.0% | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Cornus* (dogwood) | 0 | 0.0% | | 1 | 0.5% | |
| *Diospyros* (persimmon) | 0 | 0.0% | | 0 | 0.0% | |
| *Euphorbia* (euphorbia) | 0 | 0.0% | | 0 | 0.0% | |
| *Fraxinus* (ash) | 1 | 0.5% | | 0 | 0.0% | |
| *Ilex* (holly) | 33 | 16.3% | | 123 | 61.2% | 11% |
| LAMIACEAE (mint family) | 0 | 0.0% | | 0 | 0.0% | |
| *Ligustrum* (privet) | 0 | 0.0% | | 0 | 0.0% | |
| *Liriodendron* (tulip tree) | 83 | 41.1% | 40% | 5 | 2.5% | |
| *Lonicera* (honeysuckle) | 1 | 0.5% | | 0 | 0.0% | |
| *Magnolia* (magnolia) | 2 | 1.0% | | 3 | 1.5% | |
| *Nyssa* (tupelo) | 7 | 3.5% | | 1 | 0.5% | |
| *Oxydendrum arboreum* (sourwood) | 4 | 2.0% | 38% | 17 | 8.5% | 77% |
| *Parthenocissus* (Virginia creeper) | 0 | 0.0% | | 1 | 0.5% | |
| *Pinus* (pine) | 1 | 0.5% | | 0 | 0.0% | |
| *Plantago* (plantain) | 6 | 3.0% | | 2 | 1.0% | |
| *Quercus* (oak) | 7 | 3.5% | | 10 | 5.0% | |
| RANUNCULACEAE (buttercups) | 2 | 1.0% | | 1 | 0.5% | |
| RHAMNACEAE (buckthorn) | 0 | 0.0% | | 0 | 0.0% | |
| *Rhododendron/Kalmia* (laurel) | 3 | 1.5% | | 6 | 3.0% | |
| *Robinia (*locust) | 6 | 3.0% | | 4 | 2.0% | |
| ROSACEAE (rose family) | 9 | 4.5% | | 7 | 3.5% | |
| *Prunus* (plum, peach, cherry) | 0 | 0.0% | | 0 | 0.0% | |
| *Rubus* (blackberry, dewberry) | 7 | 3.5% | | 6 | 3.0% | |
| *Salix* (willow) | 1 | 0.5% | | 1 | 0.5% | |
| *Rhus/Toxicodendron* (poison ivy, sumac) | 0 | 0.0% | | 0 | 0.0% | |
| *Trifolium/Melilotus* (clover) | 10 | 5.0% | | 12 | 6.0% | |
| *Ulmus* (elm) | 0 | 0.0% | | 0 | 0.0% | |
| *Vitis* (grape) | 0 | 0.0% | | 0 | 0.0% | |
| *Zanthoxylum* (prickly ash) | 0 | 0.0% | | 0 | 0.0% | |
| *other pollen types total* | | | 22% | | | 12% |
| Unknown pollen | 3 | 1.5% | | 0 | 0.0% | |
| Totals | 202 | 100% | 100% | 201 | 100.0% | 100% |
| **Lycopodium spores counted** | 480 | | | 325 | | |
| **Pollen conc. per 10 g of honey** | 8,773 | | | 12,893 | | |

**Honey Pollen Categories** **Honey Pollen Concentration Categories**

A= >45% predominant pollen type
B= 16-45% secondary pollen type
C= 3-15% important minor pollen type
D= <3% minor pollen type

| | |
|---|---|
| Category I | 0-20,000/10 g |
| Category II | 20,000-100,000/10 g |
| Category III | 100,000-500,000/10 g |
| Category IV | 500,000-1,000,000/10 g |
| Category V | over 1,000,000/10 g |

Should you desire additional clarification of this report please let me know. If we can assist you in the future, please let us know. We did get your check. Thank you.

Sincerely,

Vaughn M. Bryant, Jr.
Professor and Director