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Ms. Julie O'Steen
2900 East Park Ave
Tallahassee, Florida 32301

Dear Julie,

I have completed the pollen study of your honey sample submitted for analysis. Specific details about the sample are outlined below and a quantitative report sheet of the pollen is attached. The **extraction** and **analysis** procedures I used for these samples are identical to those I normally use on other such samples. These 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 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 150 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. 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 a 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 one 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+ pollen grains is conducted for each sample as originally recommended for honey specimens in 1978, by Louveaux, Maurizio, & Vorwohl (***Bee World,* Vol. 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 results of our pollen count for your sample are included below. 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. Actual percentage counts are not deemed 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 of the added cost and time considerations needed.

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 **Asteraceae** [composites]; **Liliaceae** [lilies], **Poaceae** [grasses], **Rhamnaceae** [buckthorns], **Rosaceae** [rose family] and **Ericaceae** [ericades]) are diagnostic at the family level yet often many of their genera are not easily separated into specific types or species because of their morphological similarity with one another. In some other large plant families, such as the **Fabaceae** (legumes), we are often able to identify some taxa to the generic level yet others in this family produce pollen types that are too similar to distinguish at the genus level without extensive reference collections and studies at levels of higher resolution using scanning electron microscopy (SEM).

A pollen concentration value (PC) of pollen grains per 10g of honey was calculated for your samples. 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 honey 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. 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 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 different floral sources that are extremely rich in pollen (i.e., *Myosotis sylvatica*, *Cynoglossum officinale*).

Pollen concentration values are useful because they give us a general idea of the amount of pollen present. In some cases, adulterated honey samples that have been mixed 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 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 sample is listed below. A summary of the pollen types found and the pollen concentration values are also noted.

ANALYSIS

Sample # 1

The complete pollen count for this sample is listed in Table 1. I have also included a few photos of the pollen types in your two honey samples (Figure 1).

Overall, this is one of the "purest" examples of superior tupelo honey I have examined. Often good tupelo honey may contain 50% or 60% pollen from tupelo flowers, but your sample is exceptional in the amount of tupelo pollen, and by inference nectar, in your honey. Essentially, your sample is about as pure a **Unifloral Tupelo Honey** as you can get. As you can see, 90% of the pollen, and by inference the nectar used in making this honey, is from *Nyssa* (tupelo) flowers. There are a few other pollen types in your sample, but none of them are of important significance when compared to your percentages of tupelo nectar and pollen. There are a few pollen grains, and by suggestion some nectar in your sample coming from *Ilex*, which is probably from gallberry, holly, or perhaps some yaupon bushes. It is difficult to be certain of the species based only on light microscopy of the pollen, but the genus is certain.

The pollen concentration value of 116,143 pollen grains per 10 grams of honey places the sample in Category III, which is the appropriate category for honey similar to yours. *Nyssa* trees produce copious amounts of nectar and pollen, both of which are incorporated into the production of honey. Since honeybees tend to remove pollen from their honey stomachs on the flight back to the hive, such high concentration values suggest that your hives were very close to the sources of tupelo nectar and thus little pollen was removed during the short flight back to the hive.

I hope this summary gives you a better idea about the composition of the honey sample you sent for analysis. 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 will invoice you separately for the cost of this analysis.

Table 1

Relative Pollen Counts of the Honey Sample and Frequency Classes

Honey Pak Sample

Pollen Taxa	# 1		
Carya **(pecan, hickory)**	1	0.5%	D
Ilex **(holly)**	12	6.0%	C
Liriodendron **(tulip tree)**	1	0.5%	D
Magnolia **(magnolia)**	2	1.0%	D
Myrica **(was myrtle)**	1	0.5%	D
Nyssa **(tupelo)**	180	90.0%	A
Quercus **(oak)**	1	0.5%	D
ROSACEAE (rose family)	1	0.5%	D
Rubus **(blackberry, dewberry)**	1	0.5%	D
Unknown pollen	0	0.0%	
Totals	200	100.0%	
Lycopodium spores counted	32		
Pollen concentration per 10 g of honey	116,143		

Honey Pollen Categories

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

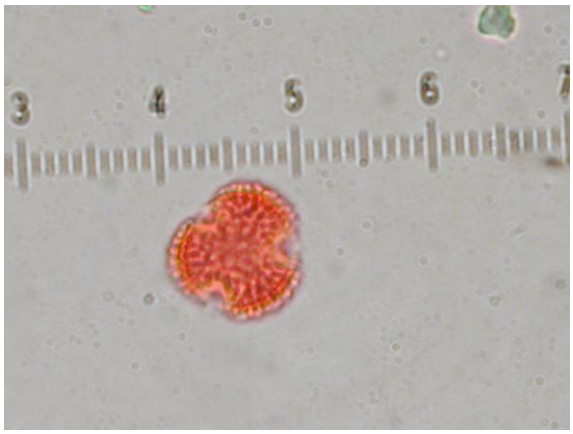
Honey Pollen Concentration Categories

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

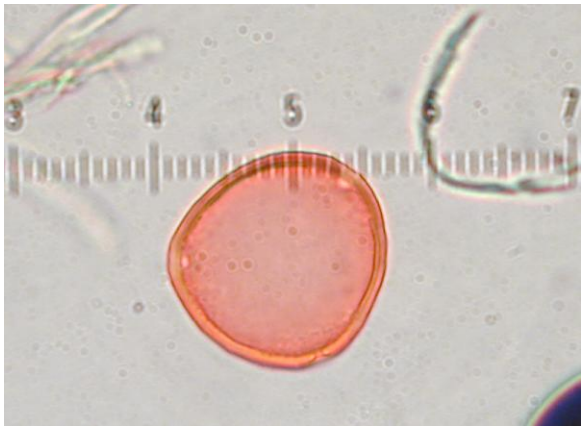
Sincerely,

Vaughn M. Bryant, Jr.
Professor and Director

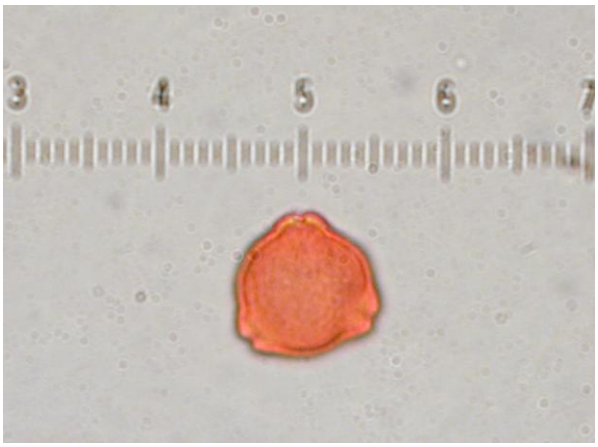
Key Pollen Types in your Samples
(Scale is in microns; 25 um between numbers)



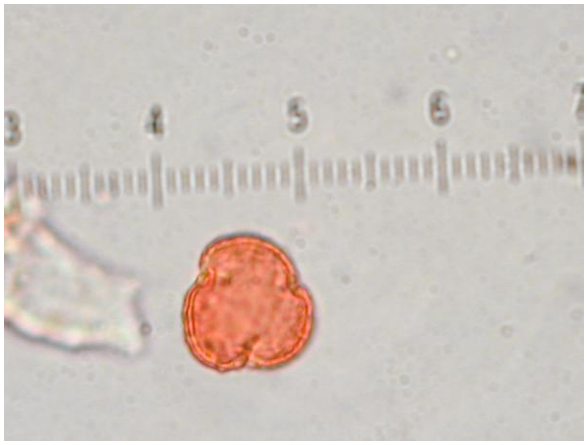
Ilex



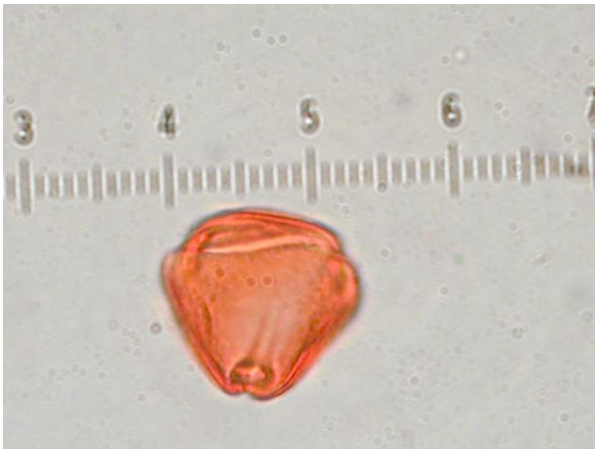
Carya



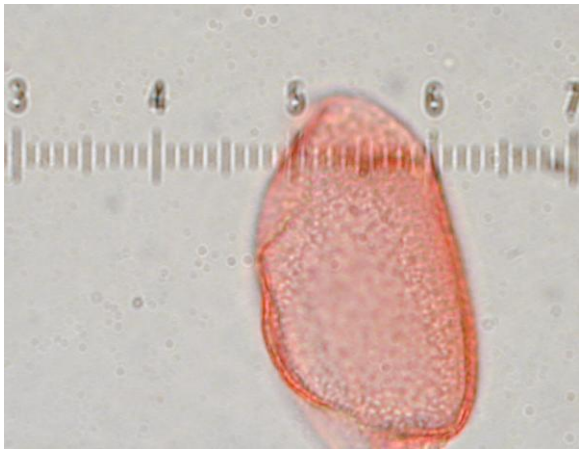
Myrica



Quercus



Nyssa



Magnolia