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Dear Curtis,

I finished the analysis of the honey sample you sent and I have included the information in this report. The extraction procedure is also listed for your information.

EXTRACTION PROCEDURE:

To conduct a pollen study of raw honey we first dilute it. For our study, we use a 10 g sample of raw honey for the analysis. We dilute the raw honey with 10 ml of distilled water and 100 ml of 95% ETOH, and then heat it for one minute to 80° C 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 or two tablets containing around 20,000 ***Lycopodium*** spores to enable us to conduct a pollen concentration study for each sample. We use these *Lycopodium* spores because bees do not collect them for any purpose; therefore, we do not have to worry about these originating in natural honey sources. Once these initial stages are complete, we dehydrate the pollen sample with glacial acetic acid and then heat it in a mixture of a sulfuric acid and acetic anhydride. This chemical treatment, called *acetolysis*, removes lipids, waxes, and cytoplasm thereby making the pollen morphology easier to identify.

Once the acetolysis process is complete, we dehydrate each sample in glacial acetic acid and then with a series of distilled water rinses. 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 using 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 (Jones, Gretchen D., & Bryant, Vaughn M. 2001. Is one drop enough?; *In:* Goodman, D.K., & Clarke, RT. (eds.), ***Proceedings of the IX International Palynological Congress, Houston, Texas, U.S.A., 1996;*** American Association of Stratigraphic Palynologists Foundation, Dallas, TX. p. 483-487).

Analysis of a honey sample follows a two-step procedure. First, we scan the sample at 400x under a microscope making initial identifications of each pollen type, and key photographic

images are sometimes taken of unknown pollen types. 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, we conduct a quantitative pollen count for each sample to determine the pollen types present and the frequency of each taxon.

We conduct a statistically valid quantitative pollen count of 200-300 pollen grains as originally recommended for honey specimens in 1978, by Louveaux, Maurizio, & Vorwohl (Commission Internationale de Botanique Apicole de L'U.I.S.B. ***Apidologie*** 1(2):211-227). We use quantitative counts 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 (Jones & Bryant *op. cit.*). The result of our pollen count for your sample or samples 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 recognize that Louveaux *et al.* (*op. cit.*) has stressed that pollen results should be listed in actual percentages only when counts are between 500-1200 grains per sample. Although we count fewer pollen grains than those just mentioned, statistically our counts are 95% accurate for honey. We rarely count more than 200-300 pollen grains for a honey sample because in most cases it is superfluous 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, we identify each pollen type to the family, genus, or in some cases species level. Sometimes the pollen types within one plant family (such as the **Amaranthaceae** [amaranths, goosefoot], **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 be unique in morphology. This is why we can say that no two pollen species produce exactly the same pollen. 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 in size 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. Nevertheless, most of the others in these families produce pollen types that are too similar to one another making it difficult to distinguish them apart 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 in honey analysis are outlined in an article we wrote (Jones& Bryant. 2007. A comparison of pollen counts: light versus scanning electron microscopy. ***Grana,*** 46: 20–33).

We calculated the pollen concentration value (PC) of pollen grains per 10 g of honey for your sample. This value usually ranges from a few thousand pollen grains to more than one million. The number of pollen grains in individual honey samples can vary greatly, therefore, according to methods outlines by Louveaux, Maurizio, & Vorwohl (*op cit.*) they recommend using a set of concentration categories. Honey pollen counts in **Category I:** contain less than 20,000 grains/10 g. Often, honey in this category represents samples that have been highly 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 were 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 comes from a few floral sources that are extremely rich in pollen such as, *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 suggest the geographical location where the honey originated. In some cases, adulterated honey samples that were 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 sample or samples is listed below. A summary of the pollen types found and the pollen concentration values is also noted.

ANALYSIS

Sample

Your honey would normally be called a Holly Honey based on the relative pollen percentages. However, as you may know, sourwood pollen is highly underrepresented in honey samples. Therefore, if we use pollen coefficient values to determine the true nectar value (TNV) for your sample, it reveals that it is a sourwood honey with about 51% sourwood nectar. As with all good examples of sourwood honey, your sample also has a low pollen concentration value of 8,106 pollen grains per 10 grams of honey. Anything under 10,000 pollen grains per 10 grams of

honey is good and the lower the concentration the purer the honey, meaning that it is a good example of sourwood honey.

Relative Pollen Counts of the Honey Sample
Table 1

Wooten Honey 2019
August

Pollen Taxa	# 1	%	TNV
Acer (maple)	6	2.2%	
APIACEAE (umbel family)	4	1.5%	
ASTERACEAE (dandelion-type)	0	0.0%	
ASTERACEAE (ragweed-type)	0	0.0%	
ASTERACEAE (sunflower-type)	5	1.8%	
BRASSICACEAE (mustard family)	0	0.0%	
Castanea (chestnut, chinquapin)	0	0.0%	
Centaurea (thistle)	0	0.0%	
Convolvulus (bindweed)	0	0.0%	
Diospyros (persimmon)	0	0.0%	
ERICACEAE (ericads)	0	0.0%	
Ilex (holly, gallberry)	141	51.6%	16.0%
Juglans (walnut)	1	0.4%	
Lagerstroemia (crepe myrtle)	0	0.0%	
Ligustrum (privet)	0	0.0%	
Liriodendron (tulip tree)	0	0.0%	
Lonicera (honeysuckle)	0	0.0%	
Magnolia (magnolia)	4	1.5%	
Melilotus (clover)	0	0.0%	
Mimosa (various species)	0	0.0%	
Nyssa sylvatica (black gum)	59	21.6%	16.0%
ONAGRACEAE	0	0.0%	
Oxydendrum arboreum (sourwood)	9	3.3%	51.0%
Parthenocissus (Virginia creeper)	0	0.0%	
Pinus (pine)	0	0.0%	
Plantago (plantain)	0	0.0%	
POACEAE (grass family)	1	0.4%	
Prunus (plum, peach, cherry)	0	0.0%	
Quercus (oak)	0	0.0%	

Rhododendron/Kalmia **(laurel)**	1	0.4%	
Rhus /Toxicodendron **(sumac, poison ivy)**	8	2.9%	
ROSACEAE (rose family)	7	2.6%	
Rubus **(blackberry, dewberry)**	8	2.9%	
Salix **(willow)**	7	2.6%	
Tilia **(basswood, linden)**	0	0.0%	
Trifolium/Melilotus **(clover)**	5	1.8%	
Vitis **(grape)**	16	5.9%	
Zea mays **(maize)**	1	0.4%	
all other nectar sources combined			**17.0%**
Unknown pollen	0	0.0%	
Totals	**273**	**100.0%**	**100.0%**
Lycopodium spores counted	**651**		
Pollen concentration per 10 grams of honey		**8,106**	

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

I hope this summary gives you an idea of the nectar source in sample. Should you have any questions or desire additional clarification of this report please let me know. We did get your check, thank you.