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Dear Jaime

I have completed the pollen study of the five honey samples you submitted for analysis. Specific details about the extraction and analysis procedures I used for this sample 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+ 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 result of our pollen count for your sample is included below (Table 1).

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 **Asteraceae** [composites]; **Liliaceae** [lilies], **Myrtaceae** [gum family], **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 able to identify some taxa to the generic level yet others in this family produce pollen types that are too similar to one another 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 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

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

A summary of the pollen types and pollen concentration values for your samples is noted below.

ANALYSIS

Sample 1 Fall/Winter (dark):

Your Fall/Winter sample would be classified as a multifloral wildflower honey because it is not dominated by one pollen and nectar type in a percentage greater than 45% (Table 1). By definition, which was established more than 50 years ago by the International Bee Commission, a unifloral honey should contain at least 45% pollen and nectar from a single source, but there are a few exceptions. Essentially, it appears that the main pollen and nectar sources for this sample are coming from mesquite, phacelia, and several different types in the Brassicaceae family. As noted in Table 1 there are also other minor pollen and nectar sources indicated in this sample.

The pollen concentration value for this sample is over 337,000 pollen grains per 10 grams of honey, which is far above the expected or normal range for typical wildflower honey. Therefore, I suspect that the extra and added pollen may have come from ruptured pollen storage cells that were accidently included with the honey when it was being extracted. The excess pollen does not hurt the honey.

Sample 2A (Spring/Summer Light) and 2B (Spring/Summer dark):

Both of these samples would be classified as being unifloral mesquite honey samples. As noted in Table 1, the percentage of mesquite pollen ranges from 64% to 75% in these two samples. You noted that one of these samples (2A) was light while the other one (2B) was dark. I honestly do not know why some honey ends up light and some ends up dark but it must have something to do with the nectar sources that are collected and used to make the honey. If you look at Table 1 and compare the 2A and the 2B samples you will see that they are very similar except one has a bit more screwbean mesquite, more creosote, and more salt cedar pollen and by inference nectar from those sources. We can separate out the screwbean mesquite pollen from the other types but the rest of the mesquite species produce pollen that is essentially very similar.

As noted below, the pollen concentration for the 2B sample is twice as much as in the 2A sample, which might also be a factor in why the 2B sample is darker. As noted in your letter, Sample 1 was also rated at being dark and it had a lot of pollen in it as well. Actually, both pollen amounts (2A and 2B) are normal and within the expected range for unifloral mesquite honey which can vary greatly from around 40,000 up to over 100,000 pollen grains per 10 grams of honey. Many factors will determine how much pollen actually ends up in mesquite honey and thus the wide variations exist within the expected range.

Relative Pollen Count of the Honey Samples 1, 2, 2B
Table 1

ReZoNation Honey 2016

Pollen Taxa	1	%	2A	%	2B	%
Acacia **(acacia)**	1	0.5%	0	0.0%	0	0.0%
Alternanthera **(joyweed)**	1	0.5%	0	0.0%	0	0.0%
AMARANTHACEAE (amaranth & goosefoot)	3	1.5%	0	0.0%	0	0.0%
ASTERACEAE (dandelion-type)	0	0.0%	0	0.0%	0	0.0%
ASTERACEAE (sunflower-type)	11	5.4%	0	0.0%	0	0.0%
BRASSICACEAE (mustard family)	21	10.3%	0	0.0%	0	0.0%
CACTACEAE (cactus but not Opuntia)	0	0.0%	0	0.0%	0	0.0%
Celtis **(hackberry)**	2	1.0%	4	2.0%	6	2.9%
Chilopsis linearis **(desert willow)**	0	0.0%	2	1.0%	0	0.0%
Erodium **(stork's bill)**	1	0.5%	0	0.0%	0	0.0%
Eucalyptus/Melaleuca/Eugenia **(gum)**	2	1.0%	1	0.5%	0	0.0%
Fraxinus **(ash)**	3	1.5%	0	0.0%	4	1.9%
LAMIACEAE (mint family)	15	7.4%	0	0.0%	3	1.4%
Larrea **(creosote bush)**	13	6.4%	7	3.5%	13	6.3%
Linaria **(toadflax)**	4	2.0%	0	0.0%	0	0.0%
Opuntia **(prickly pear cactus)**	1	0.5%	1	0.5%	0	0.0%

Parkinsonia **(paloverde)**	0	0.0%	0	0.0%	0	0.0%
Phacelia **(phacelia)**	81	39.9%	1	0.5%	1	0.5%
Polygonum **(knotweed)**	0	0.0%	0	0.0%	0	0.0%
Lonicera **(honeysuckle)**	0	0.0%	0	0.0%	0	0.0%
Populus **(aspen, cottonwood)**	0	0.0%	0	0.0%	1	0.5%
Prosopis pubescens **(screwbean)**	2	1.0%	18	8.9%	24	11.5%
Prosopis **(mesquite)**	30	14.8%	151	74.8%	137	65.9%
RANUNCULACEAE (buttercups)	0	0.0%	0	0.0%	1	0.5%
RHAMNACEAE (buckthorn family)	0	0.0%	0	0.0%	0	0.0%
Rhus /Toxicodendron **(sumac, poison ivy)**	0	0.0%	1	0.5%	0	0.0%
ROSACEAE (rose family)	5	2.5%	5	2.5%	3	1.4%
Salix **(willow)**	0	0.0%	0	0.0%	0	0.0%
Sphaeralcea **(globe mallow)**	1	0.5%	0	0.0%	1	0.5%
Tamarix **(salt cedar)**	0	0.0%	6	3.0%	10	4.8%
Ulmus **(elm)**	1	0.5%	0	0.0%	0	0.0%
Vicia **(vetch)**	1	0.5%	0	0.0%	0	0.0%
Zea mays **(maize)**	0	0.0%	0	0.0%	0	0.0%
Unknown pollen	4	2.0%	5	2.5%	4	1.9%
Totals	203	100%	202	100.0%	208	100.0%
Lycopodium spores counted	10		71		35	
Pollen concentration per 10 grams of honey	337,234		52,869		110,436	

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

Sample 3 (SXCF) May 2016 (Light) and 500 E. via Estancia (Dos Manos Apiaries):

As noted in Table 2, both of these samples would be classified as being unifloral mesquite honey because both contain more than 45% mesquite pollen and nectar. Many of the minor pollen types in these two samples are different. For example the Dos Manos sample is the only one that contained agave and sotol pollen as well as some other pollen type from a member of the lily family, paloverde, and also mimosa pollen. The pollen concentration value

for Sample 3 is twice as much as the pollen concentration value for the Dos Manos sample, but both were within the excepted range for unifloral mesquite honey.

Relative Pollen Count of the Honey Samples 3 & Dos Manos
Table 2

ReZoNation Honey 2016

Pollen Taxa	3	%	Dos Manos	%
Acacia (acacia)	1	0.5%	2	1.0%
Agave (agave)	0	0.0%	7	3.5%
Alternanthera (joyweed)	0	0.0%	0	0.0%
AMARANTHACEAE (amaranth & goosefoot)	0	0.0%	0	0.0%
ASTERACEAE (dandelion-type)	0	0.0%	0	0.0%
ASTERACEAE (sunflower-type)	2	1.0%	0	0.0%
BRASSICACEAE (mustard family)	0	0.0%	0	0.0%
CACTACEAE (cactus but not Opuntia)	2	1.0%	1	0.5%
Celtis (hackberry)	0	0.0%	0	0.0%
Chilopsis linearis (desert willow)	0	0.0%	0	0.0%
Dasylirion (sotol)	0	0.0%	6	3.0%
Erodium (stork's bill)	0	0.0%	0	0.0%
Eucalyptus/Melaleuca/Eugenia (gum)	0	0.0%	1	0.5%
Fraxinus (ash)	0	0.0%	0	0.0%
LAMIACEAE (mint family)	0	0.0%	0	0.0%
LILIACEAE (lily)	0	0.0%	6	3.0%
Larrea (creosote bush)	6	2.9%	0	0.0%
Linaria (toadflax)	5	2.5%	0	0.0%
Mimosa (various mimosa)	0	0.0%	2	1.0%
Opuntia (prickly pear cactus)	0	0.0%	0	0.0%
Parkinsonia (paloverde)	0	0.0%	3	1.5%
Phacelia (phacelia)	2	1.0%	0	0.0%
Polygonum (knotweed)	0	0.0%	0	0.0%
Lonicera (honeysuckle)	0	0.0%	0	0.0%
Populus (aspen, cottonwood)	0	0.0%	0	0.0%
Prosopis pubescens (screwbean)	12	5.9%	7	3.5%
Prosopis (mesquite)	149	73.0%	154	76.6%
RANUNCULACEAE (buttercups)	0	0.0%	0	0.0%
RHAMNACEAE (buckthorn family)	0	0.0%	0	0.0%

Rhus /Toxicodendron **(sumac, poison ivy)**	**2**	**1.0%**	**0**	**0.0%**
ROSACEAE (rose family)	**3**	**1.5%**	**6**	**3.0%**
Salix **(willow)**	**5**	**2.5%**	**0**	**0.0%**
Sphaeralcea **(globe mallow)**	**1**	**0.5%**	**0**	**0.0%**
Tamarix **(salt cedar)**	**7**	**3.4%**	**0**	**0.0%**
Ulmus **(elm)**	**0**	**0.0%**	**0**	**0.0%**
Vicia **(vetch)**	**0**	**0.0%**	**0**	**0.0%**
Zea mays **(maize)**	**0**	**0.0%**	**0**	**0.0%**
Unknown pollen	**7**	**3.4%**	**6**	**3.0%**
Totals	**204**	**100%**	**201**	**100.0%**
Lycopodium spores counted	**36**		**90**	
Pollen concentration per 10 grams of honey	**105,303**		**41,502**	

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 possible nectar sources in your samples. Should you have any questions or desire additional clarification of this report please let me know. We did receive your check, thank you.

If we can assist you in the future, please let us know.

Sincerely,

Vaughn M. Bryant, Jr.
Professor and Director