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Spencer Wingfield
Wingfield Honey Co.
P.O Box 1104
Nevada City, California 95959

Dear Spencer,

I recently finished the analysis of your three honey samples and the results are shown below. I have also included the extraction procedure we used to prepare your samples for analysis. These are also listed below.

EXTRACTION PROCEDURE:

To conduct a pollen study of raw honey we first dilute it so that the pollen can be removed for analysis. For our study, we use a 10g sample of raw honey. The sample of raw honey is diluted with 10 ml of distilled water and 100 ml of ETOH. This is a technique that we developed and is now adopted by many others (Jones and Bryant, 2001, **Is one drop enough?**; *In:* Goodman, D.K., and Clarke, RT. [eds.], ***Proceedings of the IX International Palynological Congress, Houston, Texas, U.S.A., 1996****;* American Association of Stratigraphic Palynologists Foundation, p. 483-487).

Next, we add one or more tablets of spores to get a total of around 20,000 ***Lycopodium*** spores, which will enable us to conduct a pollen concentration study for each sample. We use these lycopodium spores because bees do not use them for any purpose; therefore, we do not have to worry about these spores coming from natural nectar sources. Once these initial stages are complete, the pollen sample is dehydrated with glacial acetic acid. Next, we heat the residue in a mixture of sulfuric acid and acetic anhydride at a ratio of 1:9 (called ***acetolysis***). We heat this mixture at 80^{o} C for ten minutes in a heating block to ensure a complete removal of lipids, waxes, and cytoplasm thereby making the pollen easier to identify during analysis.

Once the acetolysis process is complete, we dehydrate each sample with glacial acetic acid and then treat each with three distilled water rinses. The resulting pollen residue is stained, if needed, 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 (Jones and Bryant, 2004. **The use of ETOH for the dilution of honey.** ***Grana*** 43: 174–182).

Analysis of a honey sample follows a two-step procedure. First, we scan the sample at 400x under a microscope. During that procedure, we make initial identifications of each pollen type, and we take key photographic images of each pollen type, if needed. If we see a pollen grain that we are not familiar with, then we compare it with our extensive modern pollen reference samples 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 count a statistically valid quantitative amount of pollen (200-300 pollen grains) for each sample as originally recommended for honey specimens in 1978, by Louveaux, Maurizio, & Vorwohl (***Bee World*****,** 59:139-157). We use these quantitative counts because testing has shown that these offer an accuracy of greater than 95% in terms of the actual composition of pollen taxa within a given honey sample. The result of our pollen count for your honey 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 they did point out there are a few exceptions. We compiled a summary of these and other known exceptions in a published article (Bryant and Jones 2001. **The R-Values of Honey: Pollen Coefficients**. ***Palynology*** Vol. 25:11-28).

We have followed the reporting system recommended by Louveaux *et al.* (op. cit.) and others who stress that pollen concentration values should be listed according to frequency groups. Others have said that pollen percentages in honey are not accurate beyond 95% unless one counts between 500-1200 pollen grains per sample. We show the actual percentage counts in our reports for general reference but we recognize that these counts are not deemed accurate until we count over 1,000 pollen grains per sample. We rarely count that many pollen grains because in most cases it is not necessary and because larger counts add cost and time considerations. We also know that statistically the difference between lower counts and counts of over 1,000 pollen grains increase the accuracy by only an additional 2-4%. We published the results of our study that validate this point (Jones and Bryant, 1998. **Are all counts created equal?**; *In:* Bryant, V.M. and Wrenn, J.H. [eds.], ***New Developments in Palynomorph Sampling, Extraction, and Analysis****;* American Association of Stratigraphic Palynologists Foundation, Contributions Series Number 33:115-120).

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], **Liliaceae** [lilies], **Myrtaceae** [eucalyptus 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 frequently all species appear similar to the genus, yet the pollen of each species will contain minor variations that can be seen only using a scanning electron microscope (Jones & Bryant. 2007. **A comparison of pollen counts: Light versus scanning electron microscopy**. ***Grana*** 46: 20–33). 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 and within each plant family, there is a range of size variation, therefore, size alone 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), pollen identification to genus is often nearly impossible without an adequate reference collection of the plants close to where the honey originated. Pollen grains in the family Asteraceae, for example are easily recognized because the majority have spines of some type on the surface. Members of the composite family occur in several broad subfamilies based on morphological differences. Several of these include the fenestrate type, subfamily **Cichorioideae**, which includes dandelions, and the subfamily Asteroideae**,** that includes pollen grain types that mostly have spines and are insect-pollinated. This second subfamily make up more than 70% of the total species in this large plant family.

We calculated the pollen concentration value (PC) per 10g of honey 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/10 g. Often, honey in this category represents samples that have been highly filtered, honey from floral sources that produce little pollen, honey that came from sugar-feeding bees, or honey that has been adulterated by adding high-fructose syrup or adding 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 produced from a few floral sources that are extremely rich in pollen (i.e., ***Myosotis sylvatica, Cynoglossum officinale, Leptospermum scoparium*** etc*).*

Pollen concentration values are very important and useful because they give us a general idea of the amount of pollen present and often suggest the geographical location where the honey was made. In some cases, adulterated honey samples, mixed with 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 can only suggest it occurred because that alone is not sufficient to warrant such a claim for adulteration.

We calculated our pollen concentration value using the formula

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

We have listed the complete pollen count for your sample or samples below. A summary of the pollen types found, and the pollen concentration value is also noted.

ANALYSIS

Samples 2019:

All three of your honey samples are a Multifloral or Wildflower Honey. In order to be a unifloral honey it must be dominated by one pollen type in a percentage over 45%. Two of the major types of pollen, and by inference the nectar, come from two or three different genera or species in the buckthorn family (Rhamnaceae). California is the "buckthorn Capital" of the US with more genera and species than any other state. Whenever I analyze California honey, it always has some buckthorn pollen and nectar. Some of the major genera of buckthorns in California include ***Ceanothus, Rhamnus, Condalia***, and ***Ziziphus***. Each of those major genera have many different species in California. However, trying to distinguish one of the many genera or species from the rest is quite difficult since all of the members of this family have very similar morphology. From my observation, I believe many of them are from either Ceanothus or Rhamnus. I do not think they are from ***Ziziphus*** because I am familiar with that pollen type from my work in the Middle East.

In all three samples there is quite a bit of rose pollen and nectar in the samples as well as considerable amounts from ***Prunus*** (which includes cherries, plums and some other types that are both cultivated and wild. ***Prunus*** also has a wide variety of species). The biggest problem with pollen in the rose family is that there are over 85 different genera and more than 3,000 different species in that family. In your region, of California there are many different genera and species in the rose family. Each of those species produces a "unique" pollen type but ALL OF THE TYPES look extremely similar to one another making precise identification very difficult without an adequate pollen reference collection of the rose family types in your specific region. Another problem is that rose pollen grains tend to fold and crumple easily making it even more difficult to decipher the precise morphology and thus identify the correct genus in the rose family. Therefore, we tend to record most of them at the family level. In looking at your samples I can see that some of the main types of rose pollen look very similar to ones I have seen in other samples from California that include ***Amelanchier, Crataegus, Rosa, Malus,*** and ***Potentilla***. Usually, we can separate the pollen in the genus ***Rubus*** and the pollen in the genus ***Prunus*** from the others but if they are crumpled then it is more of a guess than accuracy.

The alternative to better identification could come from the use of the higher resolution possible using a scanning electron microscope. For example, ***Malus*** (apples) pollen and several species of ***Crataegus*** (hawthorn) pollen look nearly identical. In addition, both of these genera have different species, and each species look only slightly different from the other species in the same genus. Therefore, for most of the rose pollen, except for ***Rubus*** and ***Prunus*** types I have

lumped all pollen and nectar from members of the Rosaceae family into that one category in the table below.

The pollen concentration value for your three honey samples is over 170,000 pollen grains per 10 grams of honey in each sample placing them in Category III. Those amounts far exceed the typical amounts in normal wildflower honey. Therefore, I suspect that when the honey from the combs was being extracted some of the nearby storage cells with pollen were probably ruptured and that extra pollen was added to the honey. It does not hurt the honey but I just wanted to mention it.

Relative Pollen Counts of the 2019 Honey Sample

Table 1

Wingfield Honey 2019

Pollen Taxa	Sample 1	%	Sample 2	%	Sample 3	%
Acer **(maple)**	0	0.0%	2	0.9%	0	0.0%
Aesculus **(horse chestnut)**	2	0.9%	0	0.0%	2	1.0%
Alnus (alder)	0	0.0%	0	0.0%	0	0.0%
AMARANTHACEAE (amaranth & goosefoot)	0	0.0%	0	0.0%	0	0.0%
Amsinckia **(fiddlenecks)**	0	0.0%	0	0.0%	0	0.0%
ANACARDIACEAE (sumac family)	0	0.0%	0	0.0%	0	0.0%
APIACEAE (umbel family)	0	0.0%	0	0.0%	0	0.0%
ARECACEAE (palms)	0	0.0%	0	0.0%	0	0.0%
Artemisia **(sagebrush)**	0	0.0%	0	0.0%	0	0.0%
ASTERACEAE (dandelion-type)	2	0.9%	0	0.0%	0	0.0%
ASTERACEAE (ragweed-type)	0	0.0%	0	0.0%	0	0.0%
ASTERACEAE (sunflower-type)	0	0.0%	0	0.0%	0	0.0%
Astragalus **(milk vetch)**	0	0.0%	0	0.0%	0	0.0%
BRASSICACEAE (mustard family)	6	2.7%	4	1.8%	4	2.0%
Carya **(pecan, hickory)**	0	0.0%	0	0.0%	0	0.0%
CARYOPHYLLACEAE (carnation family)	0	0.0%	0	0.0%	0	0.0%
Ceanothus **(ceanothus)**	0	0.0%	0	0.0%	0	0.0%
Centaurea **(thistle)**	0	0.0%	1	0.5%	0	0.0%
Chrysolepis **(chestnut, chinquapins)**	0	0.0%	6	2.7%	3	1.5%
Cirsium **(thistle)**	0	0.0%	0	0.0%	1	0.5%
Citrullus **(watermelon)**	0	0.0%	0	0.0%	0	0.0%
Citrus **(orange, lemon, etc.)**	0	0.0%	0	0.0%	0	0.0%
Cornus **(dogwood)**	0	0.0%	0	0.0%	0	0.0%

Convolvulus (bindweed)	0	0.0%	0	0.0%	0	0.0%
CYPERACEAE (sedge)	0	0.0%	0	0.0%	0	0.0%
Dalea (prairie clover)	0	0.0%	0	0.0%	1	0.5%
Echium (blue weed)	0	0.0%	0	0.0%	0	0.0%
Elaeagnus (autumn olive)	0	0.0%	0	0.0%	0	0.0%
ERICACEAE (ericads)	1	0.4%	1	0.5%	1	0.5%
Eucalyptus/Melaleuca/Eugenia (gum)	1	0.4%	0	0.0%	1	0.5%
FABACEAE (various legume types)	0	0.0%	0	0.0%	0	0.0%
Fagopyrum (buckwheat)	0	0.0%	0	0.0%	0	0.0%
Fraxinus (ash)	1	0.4%	0	0.0%	0	0.0%
Gleditsia (honey locust)	0	0.0%	0	0.0%	0	0.0%
Glycine max (soybean)	0	0.0%	0	0.0%	0	0.0%
Ilex (holly, yaupon, gallberry)	0	0.0%	0	0.0%	0	0.0%
Impatiens (touch-me-not)	0	0.0%	0	0.0%	0	0.0%
Juglans (walnut)	1	0.4%	1	0.5%	1	0.5%
Lagerstroemia (crepe myrtle)	0	0.0%	0	0.0%	0	0.0%
LAMIACEAE (cf. *Salvia*) (mint family)	1	0.4%	1	0.5%	0	0.0%
Liquidambar (sweetgum)	0	0.0%	0	0.0%	1	0.5%
Ligustrum (privet)	0	0.0%	0	0.0%	0	0.0%
Lonicera (honeysuckle)	0	0.0%	0	0.0%	0	0.0%
Lotus (trefoil)	0	0.0%	0	0.0%	0	0.0%
Magnolia (magnolia)	0	0.0%	0	0.0%	0	0.0%
Medicago (alfalfa)	0	0.0%	0	0.0%	0	0.0%
Mimosa (various mimosa)	0	0.0%	0	0.0%	0	0.0%
Mimosa pudica type (sensitive plant)	0	0.0%	0	0.0%	0	0.0%
Morus (mulberry)	0	0.0%	0	0.0%	1	0.5%
Nuphar (cowlily)	0	0.0%	1	0.5%	0	0.0%
Nymphaea (water lily)	0	0.0%	1	0.5%	0	0.0%
Nyssa (tupelo)	0	0.0%	0	0.0%	1	0.5%
Ligustrum (privet)	0	0.0%	0	0.0%	0	0.0%
Ocimum basilicum (sweet basil)	0	0.0%	1	0.5%	0	0.0%
Olea (olive)	0	0.0%	0	0.0%	0	0.0%
Oenothera (evening primrose)	0	0.0%	0	0.0%	0	0.0%
Parthenocissus (creeper)	0	0.0%	0	0.0%	0	0.0%
Phacelia (phacelia)	0	0.0%	0	0.0%	0	0.0%
Plantago (plantain)	2	0.9%	0	0.0%	0	0.0%
Prosopis (mesquite)	0	0.0%	0	0.0%	0	0.0%
POACEAE (grass)	0	0.0%	0	0.0%	0	0.0%
Polygonum (knotweed)	0	0.0%	0	0.0%	0	0.0%
Prunus (plum, peach, cherry)	36	16.0%	27	12.3%	29	14.1%

Quercus **(oak)**	7	3.1%	5	2.3%	6	2.9%
RANUNCULACEAE (buttercups)	0	0.0%	0	0.0%	0	0.0%
RHAMNACEAE (buckthorn family)	44	19.6%	64	29.2%	59	28.8%
Rhus /Toxicodendron **(sumac, poison ivy)**	9	4.0%	6	2.7%	0	0.0%
ROSACEAE (rose family)	79	35.1%	67	30.6%	63	30.7%
Rubus **(blackberry, dewberry)**	5	2.2%	3	1.4%	10	4.9%
Rumex **(dock)**	0	0.0%	0	0.0%	0	0.0%
Salix **(willow)**	19	8.4%	15	6.8%	15	7.3%
Sarcobatus (greasewood)	0	0.0%	0	0.0%	0	0.0%
SCROPHULARIACEAE	0	0.0%	0	0.0%	0	0.0%
Silene **(catchfly)**	1	0.4%	0	0.0%	0	0.0%
SOLANACEAE (nightshade)	0	0.0%	0	0.0%	0	0.0%
Tamarix **(salt cedar)**	0	0.0%	0	0.0%	1	0.5%
Triadica sebifera **(tallow tree)**	0	0.0%	0	0.0%	0	0.0%
Trifolium/Melilotus **(clover)**	6	2.7%	9	4.1%	0	0.0%
Typha angustifolia **(cattail)**	0	0.0%	0	0.0%	0	0.0%
Ulmus **(elm)**	0	0.0%	0	0.0%	0	0.0%
Vicia **(vetch)**	0	0.0%	1	0.5%	1	0.5%
Vitis **(grape)**	0	0.0%	0	0.0%	0	0.0%
Zea mays **(maize)**	0	0.0%	0	0.0%	1	0.5%
Unknown pollen	2	0.9%	3	1.4%	3	1.5%
Totals	225	100%	219	100%	205	100%
Lycopodium spores counted	25		16		15	
Pollen concentration per 10 grams of honey	173,998		264,606		264,204	

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 will invoice you; thank you.

Sincerely,

Vaughn M. Bryant, Jr.
Regents Professor and Director