THE R-VALUES OF HONEY: POLLEN COEFFICIENTS

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Abstract

One of the goals of melissopalynology is to determine the floral sources utilized by honeybees in the production of honey. Because some types of commercial honey are preferred over others, the preferred types are in high demand and are sold at much higher prices. Verification of these preferred (premium) types of honey is often difficult because many of them come from plant sources that are either weak pollen producers or have pollen that is under-represented in honey. In an effort to verify these premium honey types, researchers developed various methods for correcting the pollen data. These methods produce what are known as *pollen coefficient (PC) values*. Pollen coefficient values are used to verify honey types produced from floral sources that are over or under-represented in the relative pollen counts of a honey sample. We examine the historical development of PC values, the reliability of PC data, the flaws inherent in the development of various types of PC data, and the steps needed to formulate new types of PC values that would become universally accepted for the verification of honey types.

INTRODUCTION

Precision in interpreting pollen data has always been a primary goal of palynologists. For example, when using pollen counts to reconstruct current or past vegetations or determine the nectar sources of a honey sample, the types and percentages of recover pollen are rarely considered an accurate one-to-one correlation with the floral types they represent. Since the development of pollen analytical techniques during the early 1900s, many advances and improvements have been made in sample collection techniques, in laboratory methods for pollen extraction from matrix materials, and in microscope optics. Nevertheless, once the pollen analyses are completed and the data tabulated, the palynologist must rely on other factors before assigning meaningful interpretations to the data. First, pollen distribution is initially affected by one or more innate characteristics such as pollen mass, pollen morphology, pollen production, and the method of dispersal. Second, methods of pollen dispersal vary. Some pollen types are released from their anthers into the air (anemophilous), become airborne, and are dispersed various distances from their source. The distance of dispersal depends on many factors including air temperature, humidity, pollen sinking speed, changes in surface topography, and the force and direction of the prevailing winds. Other pollen types are zoophilous and rely upon insects, bats, birds, or other small mammals to disperse their pollen. The methods of collection, transportation and storage of these insect and animal pollinating vectors play important roles in any subsequent

interpretation of the importance of the types and abundance of collected pollen (Pendleton *et al.,* 1996). Finally, once pollen is stored by insects or deposited on the ground as part of the pollen rain, its rate of deterioration will be affected by microbial activity, cycles of wetting and drying, pH, chemical oxidation, and mechanical breakdown of the cellulose and sporopollenin portions of the pollen wall (Bryant & Hall, 1993).

When examining honey samples palynologists encounter many of the same types of problems that confront those who examine sediment samples. There are a number of major variables that affect the accurate determination of where a honey sample was produced and the type of nectar sources that were used to produce the honey. First, we have learned that field identification of nectar sources used by bees in the production of honey is most often incorrect. After examining 1,100 honey samples from hives in the United States we have discovered that more than 60% of those identifications made by the beekeeper or honey producer are incorrect as to the purported nectar sources. Second, experimental data reveal that honeybees are able to remove a vast amount of pollen during their return flight to the hive from the nectar sources they collect. In addition, tests reveal that all honeybees are not equally efficient in removing pollen from nectar sources. The size and shape of a pollen grain also determines how efficiently honeybees can remove that pollen type from nectar sources. Third, a growing number of bee keepers and honey producers partially or completely filter their comb honey before selling it. Fourth, we have examined a number of standard processing techniques currently used to extract pollen from honey and have found flaws in each method. Finally, even when honey samples are correctly processed and their pollen contents are carefully noted, the resulting relative pollen data may not provide an accurate view of the primary nectar sources used to produce the honey.

R-Values

Lennart von Post's early pollen studies in 1916 are widely recognized as the beginning of pollen analysis (Davis and Faegri, 1967). In his initial studies of Flandrian peat bogs in southern Sweden, von Post recognized that relative pollen counts did not precisely reflect the vegetations they represented yet it was Margaret Davis who was the first to try and quantitatively match relative pollen counts and the vegetation composition they represented. She addressed this concept in her paper entitled, "*On the Theory of Pollen Analysis*" (Davis, 1963). Her classic paper examined the relationship between the actual vegetation and the resulting pollen rain produced by, and distributed within a single vegetational area. Through this type of detailed study she hoped to discover a direct and statistical relationship between recovered fossil pollen percentages and the exact types of vegetation those data represented.

Her research resulted in the construction of R-values (ratios) for each plant taxon growing in the region she studied. Each R-value was calculated by dividing the relative percentage of a pollen type recovered in surface samples by the exact percentage that same plant occurred in the regional vegetation. Thus, if a plant species composed 40% of the living, local vegetation, but its pollen in the region's surface samples accounted for 80% of the total pollen rain, then that plant taxon was assigned an R-value of two (80/40 = 2). Likewise, if a different plant taxon covered 50% of the total vegetation, but its pollen was weakly represented in surface samples by only 5%, then that taxon was assigned the R-value of 0.1 (5/50). In theory, once all the taxa within a given region were assigned corrective R-values, then the actual vegetational composition for any area in that region could be inferred from the surface pollen data by dividing each pollen type's by its R-value.

The importance of Davis' research is that it demonstrates the use of a statistical method to "correct" the percentages of pollen recovered from any buried or surface sample. Nevertheless, Davis acknowledged that the assignment of R-values for taxa within a given region was valid **only** for that region and **only** at one point in time. The migration or death of a single plant, changes in rainfall, colder winters, hotter summers, or any other event that changes the composition of vegetational structure within the region would require new ecological studies, the collection and counting of new surface pollen samples, and the possible assignment of new R-values for each plant taxon.

Davis's main contribution to palynology was not the idea that finally there was a way to utilize

relative pollen counts and derived R-values to reconstruct the precise composition of present or past plant communities. Instead, her main contribution was that it alerted palynologists to the complexities of interpreting vegetational regimes based on uncorrected, relative pollen counts. Her use of statistical data and the development of the concept of R-values encouraged others to experiment with other statistical techniques in an effort to understand the intricate and critical relationship between each plant taxon and how its pollen can become either over or under-represented in relative pollen counts.

Some of the same problems that for decades have perplexed palynologists who try to use pollen data for vegetational reconstructions have also plagued melissopalynologists (palynologists who try to identify the precise floral sources used by bees in the production of honey). In both cases, the challenge is to understand the relationship between the pollen sources and the recovered pollen data. In the study of honey, the focus has been on the correct assignment of floral sources and the determination of unifloral honey types.

During the early 1940s, two scientists working for the USDA in California, Frank Todd and George Vansell, did for melissopalynology what Margaret Davis did for terrestrial palynology. Todd and Vansell examined the relationship between the pollen in the floral sources utilized by honeybees and the importance and recovery of those same pollen types in honey (Todd and Vansell, 1942). Their research began when they discovered that bee colonies survived, but would not reproduce when fed only sugar syrup. Once pollen was added to the syrup, the bees began egg laying within 12 hours. Their research was restricted to the plants and honey produced in California, because their laboratory was located there and they could get assistance from botanical experts at the University of California at Berkeley. Their research began by collecting and examining over 2,600 individual samples of nectar. They had three major goals. The first goal was to determine the number of pollen grains one should expect to find in one cubic centimeter of nectar from various plant species. The second goal was to determine if the number of pollen grains naturally occurring in nectar samples matched the number of grains found in the honey stomachs of the bees that foraged on the same nectar types. The third goal was to discover how efficiently honeybees removed pollen from the nectars they collected. Although Todd and Vansell did not propose a table of statistical “R-values” to compensate for the over or under representation of pollen types in honey, their data showed that not all plant sources contribute pollen equally to nectar and honey. They effectively demonstrated that there is not a 1:1 relationship between a honeybee’s use of a plant’s nectar and the percentages of pollen contributed by that nectar source to the produced honey. Their research became the foundation for the later development of *pollen coefficient values* in melissopalynology, which are the statistical equivalents of R-values.

Pollen in Honey

Pollen is a honeybee's major source of proteins, fatty substances, minerals, and vitamins (Gary, 1992). It is essential for the growth of larvae and young adult bees (Herbert, 1992). Honeybees remove pollen from an anther by using their tongue and mandibles. While crawling over flowers, pollen adheres to their "hairy" legs and body. The honeybee combs pollen from her head, body, and forward appendages, mixes it with nectar from her mouth, and transfers it to the *corbiculae*, or "pollen basket", on her posterior pair of legs (Hodges, 1974). When "loaded" with pollen, she will return to her hive. Once at the hive, workers pack the pollen into special comb cells located in the central portion of the hive surrounding the brood area (Dietz, 1992). To prevent bacterial growth and delay pollen germination, a phytocidal acid is added to the pollen as it is packed into the comb. Other enzymes produced by worker bees are also added to prevent anaerobic metabolism and fermentation; thereby enhancing the longevity of the stored pollen. Once completely processed for storage, the pollen comb referred to as "bee bread," is ready for later consumption by the bees. The protein source needed for rearing one worker bee from larval to adult stage requires approximately 120 to 145 mg of pollen (Alfonsus, 1933; Haydak, 1935). An average bee colony will collect about 44 to 125 pounds of pollen a year (Armbruster, 1921; Eckert, 1942).

In most cases, the primary foraging sources for pollen are the various insect-pollinated (entomophilous) plants honeybees visit for nectar. However, honeybees will also visit a number of species of anemophilous plants to collect pollen. Anemophilous taxa such as *Salix* spp. (willow), *Quercus* spp. (oak), *Celtis* spp. (hackberry), many species of grasses (Poaceae), and wind-pollinated types of the composites (Asteraceae) are all important pollen sources for foraging honeybees (Teale, 1942).

Melissopalynology is the study of pollen in honey. For over 100 years the literature pertaining to the study of pollen in honey has been termed or spelled several ways, including: mellissopalynology, mellittopalynology, and melittopalynology. According to the 1868 edition of *Paxton's Botanical Dictionary,* both "melissa" and "melitta" mean "a bee." The scientific name of the honeybee is *Apis mellifera* L. The word "melliferous" comes from the Latin word *mellifer* (honey) and the suffix -ous meaning, "having, full of, or characterized by." The International Commission for Bee Research uses "melissopalynology", which is, therefore, the term we adopted.

Pollen can be incorporated into honey in a number of ways. When a honeybee lands on a flower in search of nectar, some of the flower's pollen is dislodged and falls into the nectar that is sucked up by the bee and stored in her stomach. At the same time, other pollen grains can become attached to the "hairs", legs, antenna, and even the eyes of visiting bees. Later, some of the pollen that was sucked into her stomach will be regurgitated with the collected nectar and deposited into open comb cells of the hive. While still in the hive that same honeybee may groom her body in an effort to remove the entangled pollen on her body. During that process pollen can fall directly into open comb cells or onto areas of the hive where other bees may track it into regions of the hive where unripe honey is still exposed. Airborne pollen is another potential source of pollen in honey. Airborne pollen produced by anemophilous plants not usually visited by honeybees can enter a hive on wind currents. These anemophilous pollen grains are usually few in number, when compared to the pollen carried into the hive by worker bees; nevertheless, those pollen types regularly enter a hive on air currents and can settle out in areas where open comb cells are being filled with nectar. Sometimes airborne pollen is deposited into ripened honey when it is being removed by the beekeeper.

The pollen rain for various regions consists mainly of airborne pollen, and is important in forensics, archaeology, and ecology to identify a specific geographic region. As informational data, anemophilous pollen types are not as useful in melissopalynology because they generally form only a minor fraction of the total pollen spectra found in honey.

Pollen is an essential tool in the analyses of honey. The taxa of pollen indicate the floral sources utilized by bees to produce honey (Lieux, 1975, 1977, 1978; Louveaux *et al*., 1970; Moar, 1985; Sawyer, 1988). As a result, pollen frequency is often used to reveal and label a honey sample as to the major and minor plant foraging sources that were used by the honeybees. This information has important commercial value because consumers prefer honey made from some plants and those types command a premium price (i.e., acacia, sourwood, sage, tupelo, buckwheat, or citrus honey). Even non-premium grades of honey often need to be examined for legal reasons because they must be correctly labeled as to type before being marketed. Only by identifying and quantifying the pollen in honey can the full range of plant taxa be identified and the honey's actual foraging resources be correctly labeled. Another reason that pollen analyses of honey are often required is to determine the honey's geographical origin. The combination of anemophilous and entomophilous taxa found in a honey sample will often produce a pollen spectrum that is unique for a specific geographical region. Because of trade agreements, import tariffs, and legal trade restrictions, most of the leading honey-producing nations of the world require accurate labeling of honey before it can be sold.

Establishing Pollen Coefficient Values

Early melissopalynologists Whitcomb and Wilson (1929) were studying dysentery in honeybees when they noticed that the bee feces were filled with pollen grains. They determined that these pollen grains had been sucked into the bee's honey stomach along with nectar during foraging activities. They

also noticed that once nectar enters a bee's honey stomach it is filtered. Within about 10 minutes, this filtering process removes most of the pollen in the nectar and leaves mostly pure nectar in the honey stomach. The ability of a bee to filter nectar in her honey stomach is important because it is the primary way of removing unwanted debris from nectar, such as pollen and fungal spores, which might germinate and spoil the gathered nectar as it is converted into honey.

The honeybee's filtering process is rapid and effective (Snodgrass and Erickson, 1992). The bee sucks nectar into a slender tube that ends in the bee's abdomen where it becomes an enlarged thin-walled sac called the *honey stomach*. This honey stomach is greatly distensible and can expand to hold large amounts of nectar. Once in the honey stomach, the nectar flows over the proventriculus that serves as a regulatory apparatus filtering and controlling the entrance of food into the bee's stomach. The anterior end of the proventriculus, called the *honey stopper*, projects into the bee's honey stomach like the neck of a bottle. At its anterior end is an x-shaped opening consisting of four, thick, triangular-shaped, muscle-controlled lips. Nectar in the honey stomach is drawn back and forth into the funnel-shaped proventriculus. This process filters the nectar and removes debris such as pollen grains and the fungal spores that cause foul brood. The posterior end of the proventriculus extends into the anterior end of the ventriculus, the part of the bee's alimentary canal (mid gut) where digestion and food absorption occurs. A valve at the bottom of the proventriculus prevents the filtered nectar from entering the bee's digestive system, but allows the debris removed from the nectar to pass into the bee's alimentary canal and into the intestines where it is first stored then later voided from the rectum. From time to time people get alarmed about a phenomenon referred to as "yellow rain" (Newman, 1984). When large numbers of bees forage on nectars that are laden with pollen, the rapid removal of those pollen grains from their honey stomachs and the resulting defecation by those swarms of bees can appear as "yellow rain" spots on leaves, cars, sidewalks, or buildings.

Todd and Vansell (1942) conducted many experiments to determine the efficiency of pollen removal from the nectar in a bee's honey stomach, which was noted during earlier research by Whitcomb and Wilson (1929). Todd and Vansell goals included determination of the effectiveness of pollen removal from the honey stomach, the duration of the removal process, and the equality of pollen removal by the filtering process of a bee's honey stopper. In one experiment, honeybees in a caged laboratory hive were fed only diluted, unifloral star thistle (*Centaurea* sp.) honey that had been produced by other honeybees foraging in the wild. The star thistle pollen concentration in the honey-water mixture was measured as being 5,200 pollen grains per cc of fluid. Later, the sealed honeycomb cells produced by the caged honeybees were removed and examined. They found that instead of the original count, the newly produced honey contained an average of only 1,200 pollen grains per cc. Those data surprised Todd and Vansell because they expected the pollen concentration of the newly produced honey to be higher. They believed that the concentration of star thistle pollen would increase as the excess water was removed during the evaporation process in the hive that forms honey. However, the opposite was true.

In another experiment, Todd and Vansell mixed three grams of pure pollen (the pollen type is not mentioned) with 100 cc of a water and syrup solution. When measured, the pollen concentration level of the syrup-water solution was 750,000 pollen grains per cc of fluid. After allowing caged honeybees to feed only on that mixture, the honeycomb cells made from that syrup-water solution were removed. They discovered that the newly produced honey's pollen concentration value was only 25,000 pollen grains per cc of honey. In other words, the honeybees fed on a diluted syrup-water solution containing a pollen concentration of 750,000 pollen grains per cc, but, most of the pollen was removed by the honey stomach filtration system before the solution was emptied from their honey stomachs into new comb cells and made into honey. Todd and Vansell were surprised when they realized that the newly produced honey had a pollen concentration value that was only 1/30th of the original pollen concentration of the syrup-water fluid. The only logical conclusion they could reach from their data was that the significant reduction in pollen concentration was the result of the internal filtration system in a bee's honey stomach, which was far more effective than most researchers realized.

The detailed research efforts and quantitative results published by Todd and Vansell went virtually unnoticed for years after their study was completed. More than a decade later, other researchers,

when trying to determine which pollen types are over or under-represented in honey samples, realized the importance of Todd and Vansell's initial research efforts. Although Todd and Vansell's initial goals were not to develop pollen coefficient tables, their pioneering effort led others to use the ideas and experimental data to compile lists of plants that are over or under-represented by their relative pollen counts in honey samples (Maurizio, 1949, 1955, 1958; Berner, 1952; Pritsch, 1956; Deans, 1957; Demianowicz, 1961, 1964; and Sawyer, 1988).

Using some of the ideas developed by Todd and Vansell (1942), Demianowicz (1961, 1964) became one of the earliest melissopalynologists who worked tirelessly for many (13) years trying to resolve the problem of identifying unifloral honey types based on pollen contents. After examining many honey samples, Demianowicz realized that the relative pollen counts in honey did not always reflect the primary floral and nectar sources (Table 1). Demianowicz's summarized data appeared in her 1964 publication where she identified the pollen characteristics of 46 different types of unifloral honey common to various regions of Eastern Europe. To develop the data for each unifloral type, she used caged hives of only 300-400 workers bees and one queen. Fresh, open flowers of a single species were brought to the caged bees several times a day. Under these controlled conditions, Demianowicz believed that the honey produced by each hive was a valid representation of the expected *absolute pollen concentration* (APC) for the flower species being examined. Based on this research, she developed 19 different categories of plants ranked on the basis of whether their APC values in honey were under or over represented (Table 1). Each category was assigned an "average number" that was determined by averaging the totals of each type in that category. Each of these 19 categories was called a "pollen coefficient class." She was the first to use that term and she believed that the newly established pollen coefficient values could be used as a guide for determining the true unifloral nature of honey samples from any region, regardless of the data implied by the relative pollen counts.

In Demianowicz's table of values (Table 1), the expected APC of all pollen types in a "class 0 unifloral type" should not be expected to be any higher than 740 pollen grains per 10 g of honey. Her key example of a highly under-represented plant type in class 0 is *Asclepias* sp. (milkweed) which she determined to have a pollen coefficient value of 32 per g of honey (i.e., 320 pollen grains per 10 g of honey). Each additional pollen coefficient class is represented by APC values that are twice as high as the previous class, and each class has an assigned generalized pollen coefficient value. In class 1 of her unifloral honey types, each taxon in the group should average between 750-1,500 pollen grains per 10 g of honey. Plant examples in that class include *Robinia pseudoacacia* (white acacia, locust), *Cucumis* sp. (cucumber), and *"Chamaenerion* sp.*"* (now known as *Epilobium* sp. [fireweed]). Although she calculated the potential APC values for 19 different classes of plants, her data from the caged bee experiments covered only 12 of the 19 classes. In our Table 1, we list the 12 classes for which she provided APC data. Her last APC category is class 18 and is characterized by prolific pollen producing plants such as *Myosotis sp.* (forget-me-not), which produce unifloral honeys containing between 98,304,001 to nearly 200 million pollen grains per 10 g (Table 1).

TABLE 1

Honey class types based on the absolute pollen concentrations (APC) of 46 plant taxa that are under or over represented by their relative pollen counts in honey. These data were compiled from experiments that produced unifloral honey in the hives of caged bees that were allowed to forage only on the flowers of a single plant species. The information in this table is based on the experiments and APC calculations conducted and reported by Demianowicz (1964).

Class	Type of unifloral honey	Expected APC range pollen gains/10 g	Pollen coefficient per 10 g pollen gains/10 g
Class 0	*Asclepias syriaca*	0-740	320

Class 1	*Cucumis sativus*	750-1,500	1,125
	Epilobium angustifolium	750-1,500	1,125
	Robinia pseudoacacia	750-1,500	1,125
Class 2	*Tilia cordata*	1,501-3,000	2,250
	Althaea officinalis,	1,501-3,000	2,250
	Centaurea jacea	1,501-3,000	2,250
	Salvia nemorosa	1,501-3,000	2,250
	Scrophularia nodosa	1,501-3,000	2,250
	Echinops commutatus	1,501-3,000	2,250
	Borago officinalis	1,501-3,000	2,250
	Hyssopus officinalis	1,501-3,000	2,250
	Helianthus annuus	1,501-3,000	2,250
	Lamium album	1,501-3,000	2,250
Class 3	*Dracocephalum moldavicum*	3,001-6,000	4,500
	Ribes vulgare	3,001-6,000	4,500
	Anchusa officinalis	3,001-6,000	4,500
	Salvia officinalis	3,001-6,000	4,500
Class 4	*Centaurea cyanus*	6,001-12,000	9,000
	Polemonium coeruleum	6,001-12,000	9,000
	Solidago serotina	6,001-12,000	9,000
	Sinapis alba	6,001-12,000	9,000
	Allium cepa	6,001-12,000	9,000
	Geranium pratense	6,001-12,000	9,000
Class 5	*Onobrythis viciaefolia*	12,001-24,000	18,000
	Taraxacum officinale	12,001-24,000	18,000
	Trifolium repens	12,001-24,000	18,000
	Digitalis purpurea	12,001-24,000	18,000
	Leanorus cardiaca var. *villosus*	12,001-24,000	18,000
	Malus domestica	12,001-24,000	18,000
Class 6	*Marrubium vulgare*	24,001-48,000	36,000
	Coriandrum sativum	24,001-48,000	36,000
	Helenium autumnale,	24,001-48,000	36,000
	Echium vulgare	24,001-48,000	36,000
	Ruta graveolens	24,001-48,000	36,000
	Fagopyrum esculentum	24,001-48,000	36,000
Class 7	*Melilotus albus*	48,001-96,000	72,000
	Brassica napus	48,001-96,000	72,000
	Rubus idaeus	48,001-96,000	72,000
	Phacelia tanacaetifolia	48,001-96,000	72,000
Class 8	*Lythrum salicaria*	96,001-192,000	144,000
Class 9	*Lotus corniculatus*	192,001-384,000	288,000
	Archangelica officinale	192,001-384,000	288,000

	Reseda lutea	192,001-384,000	288,000
Class 13	*Cynoglossium officinale*	3,072,001-6,144,000	4,608,000
Class 18	*Myosotis silvatica*	98,304,001-196,608,000	147,456,000

Since Demianowicz's work, other melissopalynologists have tried to refine the techniques that use pollen contents to classify various types of unifloral honey. Maurizio (1953) published a report of research conducted by the International Commission for Bee Botany. This report was a series of technical recommendations for the microscopic analysis of pollen in honey samples. During the 1960s Maurizio and Louveaux (1965) suggested additional recommendations and defined many terms relating to pollen analyses such as "controlled unifloral honey, experimental unifloral honey, etc." Louveaux *et al*. (1978). This followed Maurizio's (1975) suggestion, which was to establish 45% as the minimal amount of a single pollen type in a honey sample needed for unifloral classification. Although honey is rarely ever derived from a single botanical source, the term "unifloral" is used to describe honey that is produced mainly from the nectar of one plant species (Anklam, 1998).

Recently, others have produced tables listing various plants with the "expected" pollen percentages needed to indicate unifloral honeys from those plants. Moar (1985) questions the technique used by Louveaux *et al*. (1970, 1978) and others for determining unifloral honey types. First, he points out that there should be exceptions to the Louveaux *et al.* (1970, 1978) statement that 45% is the minimal amount for a single pollen type in a unifloral honey. Moar agrees that this might be true for pollen types in honey samples containing between 20,000-100,000 pollen grains per 10 g of honey, but should be adjusted for honey samples when concentration values are less than 20,000 pollen grains per 10 g. Second, he agrees that white clover (*Trifolium repens*) should be considered the baseline for determining coefficient values for other pollen types in honey. However, Moar questions the APC value of 18,000 for *T. repens*, as calculated from the experiment with caged bees by Demianowicz (1964). Instead, Moar uses an APC value of 23,116 grains per 10 g of honey for *T. repens*. He fails to explain how he established that APC value, but we suspect he determined it after studying the contents of 55 purported white clover, unifloral honey samples from hives in various locales in New Zealand. Perhaps the difference between the two APC values for *T. repens* (Moar vs. Demianowicz) results from the different ways the bees were allowed to collect honey (free vs. caged) or different methods each used to calculate the APC value. Third, Moar proposes new ways to establish baseline APC ratios for various pollen taxa in honey samples produced by honeybees that are allowed to forage freely, when observations reveal that they are visiting mainly the flowers of one plant type.

In his summary, Moar (1985) presents an example of how to calculate APC values for various plant taxa in New Zealand. He then uses those data to produce pollen coefficient values for those pollen types. He notes, for example, that New Zealand thyme (*Thymus* sp.) honey is considered a premium commercial type, but based on Louveaux *et al*.'s (1970, 1978) unifloral measuring standards, none of the New Zealand thyme honeys would be accepted as unifloral. Moar notes that in the New Zealand honey samples, thyme pollen rarely reaches the minimally needed total of 45% because its pollen is under-represented. Other melissopalynologists including Demianowicz (1964) and Sawyer (1988) also report that thyme and many other pollen types in the mint family are under-represented in honey samples. In view of those data, it is noteworthy that Tsigouri and Passaloglou-Katrali (2000) report that they found relative percentages of thyme as high as 80% in some unifloral honey samples from Greece. Those authors did not calculate the APC values for thyme pollen but they do mention that the pollen concentration values for the 20 samples they examined ranged from 5,000-85,000 per 10 g of honey.

In Moar's (1985) study, he examined honey samples that were produced by beehives located in or

close to abundant fields of blooming thyme. The honey produced in those hives was then examined to ensure that it had the traditional color and taste of thyme honey. Four separate honeycomb samples from various hives that fit these criteria were then processed to extract the pollen. The average relative pollen percentage of thyme in the four samples was 42%. He then compared his pollen counts of thyme against the number of tracer spores counted (Stockmarr, 1971) in each sample. The result was an average APC value of 5,415/10 g of honey for thyme pollen. Moar reasoned that because the relative pollen percentage of thyme was less than the standard 45% needed for unifloral classification, the thyme pollen's APC (5,415) could be adjusted by multiplying it times .45 (the needed percentage). Next, to calculate the "corrected APC" value for thyme at the 45% level, Moar divided the resulting sum by .42, which is the average relative pollen frequency (42%) of thyme pollen in the four samples. These calculations increased the number of "expected" thyme pollen in 10 g of honey to 5,801, which Moar considered to be the appropriate "corrected APC" for thyme pollen at the internationally accepted unifloral level of 45%.

Next, Moar (1985) notes that because thyme pollen is considered under-represented in honey (i.e., any taxon with an APC under 20,000 per 10 g of honey), new calculations were necessary to determine what the minimum percentage of thyme pollen in a honey sample should be in order to classify that sample as being a unifloral thyme honey. Because the APC of white clover is considered the baseline standard for honey studies (Sawyer, 1988), Moar uses the ratio of thyme's actual APC of 5,801 against the baseline APC of white clover (23,116) in order to determine the minimal percentage of thyme needed in a unifloral honey. By examining his calculations, we see that Moar divides the APC for thyme (5,801) by the combined APC of thyme and white clover (5,801 + 23,116). The quotient becomes two-tenths (0.2), which he then multiplies by 100 to convert it to a percentage (20%). From these calculations Moar states that in New Zealand 20% thyme pollen qualifies a sample as being unifloral thyme honey. Finally, Moar proposes that by using his technique any melissopalynologist can determine the minimal amount of pollen needed for unifloral classification of any under-represented floral source in any region.

Rex Sawyer, one of the foremost early melissopalynologists in the United Kingdom, began his lifelong interest in pollen studies during the 1930s after meeting Harry Godwin, a British pioneer in the study of pollen analysis. Sawyer began raising bees, studying beekeeping, and later helped Deans (1957) compile a detailed study of pollen composition of honey types produced in the U.K. After years of melissopalynology research, Sawyer published several books on pollen and honey (Sawyer, 1981, 1988). Included in his last book is a table listing the numerical pollen coefficient (PC) values that he developed for a number of nectar sources found mostly in the U.K. and Europe (Table 2). He also says that he believes his PC values can be applied to samples from almost any region of the world in order to "correct" the relative pollen percentages in honey (Sawyer, 1988). The basis for developing his PC values come from data he generated as well as the published data reported by other melissopalynologists (Todd and Vansell, 1942; Maurizio, 1949, 1955, 1958; Berner, 1952; and Pritsch, 1956; Demianowicz, 1961, 1964).

The primary difference between Sawyer's PC values and other published PC values is that Sawyer's values are not expressed as the *expected APC* for each pollen type per 10 g of honey. Instead, his PC values are expressed as the *expected number of pollen grains per gram* of honey. Nevertheless, by following Sawyer's formula and applying his PC values to the relative pollen percentages found in any honey sample, one can determine the "*actual*" floral identities and "*true*" unifloral or multifloral characteristics. Since Sawyer did not determine the PC values for all known pollen types found in honey, he recommends using a PC value of 50 (i.e., the value he assigned to the baseline pollen taxon of *Trifolium repens*) for percentages of unidentified pollen and pollen taxa that occur in sporadic and low frequencies.

As Sawyer and others have argued, by using pollen coefficient tables melissopalynologists can confirm that some honey samples should be classified as unifloral even if their relative pollen counts do not verify that classification. This is especially true for nectar sources that come from plants with low pollen yields, or from plants that produce pollen types that are quickly removed by the filtering actions of a honeybee's honey stopper. As noted in Sawyer's PC list (Table 2), some of the pollen types that are rarely found in high frequencies will benefit the most from using pollen coefficient values. For example,

fireweed, basswood (*Tilia* sp.), alfalfa (*Medicago sativa*), sourwood (*Oxydendron* sp.), orange blossom (*Citrus* sp.), buckwheat (*Eriogonum* sp.), mint (*Salvia* sp.) and locust (*Robinia pseudoacacia*) are some of the pollen types that normally do not reach unifloral levels of 45% in most relative pollen counts. However, when pollen coefficient tables (Table 2) are used to correct the normally low relative pollen percentages (i.e., 5-20%) of these types in honey samples, the result becomes a validation that the honey should indeed be classified as unifloral.

Table 2

Pollen coefficients of various pollen types as calculated by Sawyer (1988).

Plant Pollen Type	Pollen Coefficient Value per gram
Asclepias syriaca, milkweed	0.3
Epilobium angustifolium, willowherb, fireweed	0.3
Oxydendron arboreum, sourwood	1
Acacia dealbata (type), mimosa, wattle	5
Lamiaceae, thyme, rosemary, sage, mint	5
Medicago sativa, alfalfa	5
Cirsium spp. (type), thistles	10
Erica spp., heaths, bell heathers	10
Helianthus annuus, sunflower	10
Robinia pseudoacacia, white acacia, locust	10
Tilia spp., lime tree, basswood	10
Calluna vulgaris, heather, ling	12
Nyssa ogeche, tupelo	20
Liriodendron tulipifera, tulip poplar	20
Citrus spp., orange, lime, grapefruit, etc.	25
Ligustrum, privet	25
Lotus spp., birdsfoot trefoil	25
Prunus spp. and *Pyrus* spp. (type), peach, plum, pear	25
Trifolium incarnatum, crimson clover	25
Trifolium pratense, red clover	25
Vicia faba, vetch, field or broad bean	35
Ilex spp., holly, gallberry	50
Rubus spp., chokeberry, raspberry, dewberry	50
Trifolium repens, white clover	50
Burseraceae (*Bursera, Canarium, Protium,* etc.)	75
Eucalyptus spp., gum	75
Fagopyrum esculentum, buckwheat	75
Melilotus spp., sweet clover	75
Mimosa pudica, sensitive plant	75
Onobrychis, sainfoin	75
Brassic spp., oil-seed, rape, canola	150
Echum spp., viper's bugloss, blueweed	250
Leptospermum scoparium, manuka	250
Castanea sativa, sweet chestnut	1000
Eucryphia lucida, leatherwood	1000
Myosotis spp., forget-me-not	5000

An example of how Sawyer's (1988) PC values can be used in an actual honey analysis is shown in Table 3. The data in Table 3 represent the analysis of a typical unifloral fireweed honey sample produced near Fairbanks in central Alaska. Based on the relative percentages of pollen determined from a 200-300 grain count, this sample would be classified as a "unifloral rapeseed /canola" honey. The relative percentage of fireweed pollen in this sample is only 6.3% (Table 3). When the relative pollen percentages are adjusted using Sawyer's PC values, the primary nectar source of this honey becomes fireweed flowers (95%), not rapeseed/canola flowers (1.9%). These pollen data suggest that in spite of the low relative percentage of fireweed pollen, the dominant nectar source that produced this honey was blooming fireweed flowers. Note that the 28.3% relative pollen count of clover suggests that less than 2% of the nectar source of this honey was actually derived from clover (Table 3).

TABLE 3

Pollen analysis of a honey sample produced in central Alaska. *

Pollen Type	Relative Pollen %	(PC) Coefficient Value	(RQ) Relative Quantity	Adjusted Percentage
Apiaceae	00.6	50.0	00.012	00.05
Brassica sp.	62.8	150.0	00.419	01.9
Epilobium sp.	06.3	0.3	21.000	95.9
Melilotus sp.	28.3	75.0	00.377	01.7
Taraxacum sp.	00.6	10.0	00.060	00.27
Other minor types	01.4	50.0	00.028	00.128
Total	100.0%		21.896	100.0%

* Following Sawyer (1988), prior to using pollen coefficient tables to determine the actual or expected nectar composition of each plant taxon in a honey sample, the relative pollen spectrum must first be calculated. Then the relative percentage of each pollen type is divided by its PC value. The resulting value for each pollen type is the taxon's "relative quantity (RQ)." Finally, each RQ value is divided by the sum of all RQ values to determine what percentage of the honey's nectar was actually derived from each plant type.

Statistical Baselines for Pollen Coefficient Values

One of the current problems of using pollen coefficient values is the disagreement among melissopalynologists on which PC values should be assigned to the nectar or pollen source of each plant species. If the pollen data in Table 3 is reexamined using the PC values published by Demianowicz (1964), then the adjusted percentages for the nectar sources of each taxon change (Table 4). In our PC formula below, we have followed Sawyer's (1988) recommendation of using the baseline PC value of *Trifolium repens* (i.e., 18,000 as reported by Demianowicz) for unknown pollen types or pollen types for which no PC values were determined by Demianowicz (1964).

TABLE 4.

The relative pollen percentage of a honey sample produced in North Pole, Alaska. These data are then used to construct adjusted nectar source percentages based on pollen coefficient values developed by Demianowicz (1964) and by Sawyer (1988).

Taxon	**Relative %**	**Demianowicz's Adjusted %**	**Sawyer's Adjusted %**
Apiaceae	00.6	00.47	00.50
Brassica sp.	62.8	12.44	01.90
Epilobium sp.	06.3	79.90	95.90
Melilotus sp.	28.3	05.60	01.70
Taraxacum sp.	00.6	01.09	00.27
Unknown	01.4	00.47	00.128
Total %		100.00	100.00%

$$\mathbf{AP}a = \frac{a/PCa}{(a/PCa + b/PCb + c/PCc + d/PCd + \cdots n/PCn)}$$

$$\mathbf{AP}a = \frac{.006/18{,}000}{(.000033 + .000872 + .005600 + .000393 + .000033 + .000077)}$$

$$\mathbf{AP}a = \frac{.000033}{.007008}$$

$$\mathbf{AP}a = .0047 = .47\%$$

Where "**AP**" represents the "adjusted percentage" for each pollen taxon. The small letters represent each pollen type found in the honey sample (a = Apiaceae, b = Brassica, c = *Epilobium*, d = *Melilotus*, e = *Taraxacum*, f = unknown). We have illustrated the calculations for one pollen type in Table 4, to demonstrate how the **AP** value for each pollen taxon should be determined.

Minor differences in the "adjusted percentages" can be seen when comparing the adjusted percentages derived from Sawyer and Demianowicz; nevertheless, in terms of over or under representation, both sets of calculations indicate the same general conclusions for each pollen taxon. Sawyer's PC values indicate that over 95% of the actual nectar source came from fireweed flowers, but Demianowicz's values indicate that only 80% of the nectar source came from fireweed. Although, there is a difference of 15% between the two calculations, both emphasize that fireweed is a pollen type that is

highly under-represented in honey samples and both calculations verify that the sample is a unifloral fireweed honey.

It is difficult to determine which PC data set (Sawyer vs. Demianowicz) is more nearly accurate. The precise method Sawyer used to construct his PC values is not published. Data and methods Demianowicz (1961, 1964) used to construct her PC values are published and are based on more than 13 years of research in which caged bees were fed only the nectar from one type of flower. However, Demianowicz's method may contain errors created by her method of determining pollen concentration values. Demianowicz's method for determining pollen concentration values consisted of collecting small amounts of honey (often less than 5 or 10 grams) and then diluting the sample with water. From the diluted solution, she extracted one drop, put it onto a microscope slide, and then counted the pollen. Using those counts, she projected what the APC value for each pollen taxon should be in 10 g of honey. In contrast, and as noted in Moar's (1985) PC study, today most melissopalynologists calculate pollen concentration values by comparing the ratio of pollen grains found in 10 g of honey against the ratio of a know number of tracer spores that are added to the honey prior to processing and counting. Depending on the number of tracer spores added, this system provides more reliable pollen concentration data than the system Demianowicz used.

Other melissopalynologists (D'Albore, 1998; van der Ham, *et al*., 1999) have not proposed using PC values. Instead, they prefer to include generalized information on which nectar and pollen types are traditionally over or under-represented in honey. D'Albore (1998) and van der Ham et al. (1999) have placed various taxa in different categories depending on the "*expected*" pollen yield in honey samples. For example, based on the research of van der Ham *et al*. (1999), in the Netherlands honey types are now considered unifloral if their relative pollen counts reveal they contain a minimum of: 1) 10% *Borago* spp. (borage); 2) 20% *Robinia pseudoacacia* (white acacia, locust), *Tilia* spp. (linden, basswood), or *Carduus* spp. (thistle); or 3) 30% *Crambe* spp. (sea kale) or *Calluna* spp. (heather). Van der Ham *et al*. (1999) also state that the relative percentages of pollen from some plants are so prolific in honey that those honeys should not be considered unifloral unless they contain far more pollen than the normally required minimum of 45%. Some of those types in the Neatherlands include: 1) *Salix* spp. (willow) 70%; and 2) *Phacelia* spp. (bluecurls), *Myosotis* spp. (forget-me-not) and, *Castanea* spp. (chestnut, chinkapin), each at 90%.

D'Albore (1998) in his study of Mediterranean honey types does not list the expected percentage levels that should be used as a guide for determining unifloral honeys. Nevertheless, he includes some practical advice about certain plant taxa and how over or under-represented those pollen types can be in honey samples. For example, he states that there are two reasons most plants producing large pollen grains (>40 ❍m) will be significantly under-represented in the honey produced from those nectar sources. First, most plants that produce large pollen grains do not produce large amounts of nectar. Second, honeybees are more efficient at filtering out large pollen grains in their honey stomachs during their return flight to the hive. He points out that the opposite is true for tiny pollen grains, which are usually over represented in honey. Pollen grains from plant taxa such as *Echium* spp. (canaria), *Eucalyptus* spp. (gum), *Amorpha* spp. (indigo), *Castanea* spp. (chestnut), and *Tamarix* spp. (salt cedar), are very small and are usually produced in larger numbers. Because of their small size and prolific numbers, they are only partially filtered out in the honey stomachs of honeybees. Finally, D'Albore (1998) lists plants that he determined are either over or under-represented in honey samples because of other factors such as: 1) flowers from some species produce small amounts of pollen (i.e., *Citrus, Robinia, Salvia*); 2) some species are monoecious, thus only the male flowers produce pollen (i.e., *Citrullus* spp. [watermelon], *Cucumis* spp. [cucumber], *Cucurbita* spp. [pumpkin or gourd], *Bryonia* spp. [bryony]); 3) some species have flowers that are morphologically unfavorable for pollen collection (i.e., *Asphodelus* spp. [affofill], *Epilobium* spp. [fireweed], *Abutilon* spp.[mallow], *Datura* spp. [datura], *Digitalis* spp. [foxglove]); and 4) flowers from some species present special pollen and nectar gathering problems for honeybees or have flowers that are difficult for bees to enter (i.e., *Agrostemma* spp. [corn cockle], *Cestrum* spp. [cestrum], *Nicotiana* spp. [tobacco], *Medicago* spp. [bur clover, alfalfa]).

In the United States, Todd and Vansell's (1942) pioneering research focused on another very

important variable that influences the amount and types of pollen that occur in honey samples. In one experiment, a group of honeybees were starved, then allowed to feed freely from solutions of sugar syrup mixed with various amounts of pollen. After feeding, one group of bees was immediately sacrificed and dissected. The content of their honey stomachs was carefully removed and examined for pollen. These were the control group of honeybees. Their honey stomachs revealed that the syrup-pollen mixtures they ate contained an average of 248,666 pollen grains per cc of fluid (Table 5). In the same experiment using starved bees that were fed the same syrup-pollen solution, a second group was allowed to fly around freely for 15 minutes. Then, that group was caught and dissected. Todd and Vansell found that nearly one-half (44%) of the honeybees collected as part of the second group had been able to remove and excrete more than 90% of the pollen they had consumed as part of the syrup-pollen solution they drank (Table 5). The other one-half of bees in the second group removed much of the pollen they had consumed in the syrup-pollen, but their averages varied and all were less than 90% (Table 5).

TABLE 5

Average number of pollen grains recovered from the honey stomachs of starved bees allowed to feed on solutions of sugar syrup containing pollen. These data are based on experiments and calculations reported by Todd and Vansell (1942).

	Percentage of honeybees	Average number of pollen grains remaining per cc
Control set	100.0	248,666
Set 2	44.0	0-20,000
	15.0	21-40,000
	17.0	41-60,000
	10.4	61-80,000
	00.2	81-100,000
	13.4	over 100,000

The data compiled by these two researchers suggest that some honeybees are more efficient at removing pollen with their honey stoppers than are others. This variable alone sheds doubt on the reliability of using PC values in melissopalynology. Without knowing what percentage of pollen each bee in a hive is able to remove from the nectar each collects, and without knowing how far or how long each honeybee travels between the time nectar collection begins and the time she returns to the hive, the raw database for determining accurate PC values is too imprecise to use.

Todd and Vansell (1942) repeated the same experiment using different pollen concentrations in different sugar syrup solutions fed to bees. In all their tests, they found that the amount of pollen present in the honey stomachs of bees that were allowed to fly freely for 15 minutes after feeding was drastically reduced. Although their results revealed that not all bees are identical in their efficiency of removing pollen from the nectar they drink, the data reveal that nearly one-half of the tested bees could remove approximately 90% of the pollen from the fluids in their honey stomach within 15 minutes after feeding.

Another important contribution of Todd and Vansell's (1942) experiments was the development of a list of plants documenting the number of pollen grains that occur naturally in their nectars. Because most flower nectar sources are located near a flower's dehiscing anthers, some pollen will fall into the

nectar. Later, honeybees might gather the nectar containing the pollen. Knowing this, Todd and Vansell carefully collected the nectar from more than 2,600 flowers representing 73 different plant taxa that grew in California. Some of those nectar samples were collected from the honey stomachs of bees that were captured and dissected immediately after feeding on the nectar of a specific plant. Other nectar samples were carefully collected by hand from the flower's nectaries. After all the samples were examined and the pollen concentration values for each nectar source was averaged, they produced a list of nectar types and the amount of pollen expected to be in each nectar source. That list is important because it provides a perspective of which nectar sources contain vast amounts of pollen and which contain little if any pollen. For example, Todd and Vansell report that 30 honeybees were captured and dissected immediately after each had finished feeding on the nectar of navel orange blossoms (*Citrus sinensis*). A second set of 32 bees was collected immediately after each had fed on the nectar of cotton flowers (*Gossypium hirsutum*). To their amazement, they could not find even one pollen grain in the full honey stomachs of any of those 62 bees. At the other extreme, they reported finding an average of 103,330 pollen grains per cc of fluid in the honey stomachs of 24 bees captured immediately after each had completed feeding on the nectar of white mustard (*Brassica alba*) (Table 6).

TABLE 6

Mean number of pollen grains per cc of fluid recovered from nectaries or the honey stomachs of bees allowed to feed on the nectar of various plants before being captured and dissected immediately after feeding. These data are based on experiments and calculations reported by Todd and Vansell (1942).

Plant Source	Number of samples	Pollen grains per cc
Gossypium hirsutum, cotton	32	none
Citrus sinensis, navel orange	32	none
Epilobium angustifolium, fireweed	27	220
Linum usitatissimum, flax	13	310
Salvia apiana, white sage	50	800
Trichostema lanceolatum, bluecurls	49	900
Eriogonuin fasciculatum var. *polifolium,* desert buckwheat	78	1,390
Prunus persica, peach	20	1,800
Convolvulus arvensis, morning glory	41	1,760
Cydonia oblonga, quince	28	1,930
Persea americana, avocado	18	2,000
Vicia villosa, hairy vetch	25	2,100
llemizonia pungens, spikeweed	20	2,400
Prunus domestica, prune	84	2,410
Salvia mellifera, black sage	33	2,430
Salvia leucophylla, purple sage	57	2,560
Catalpa speciosa, catalpa	58	2,620
Robinia pseudoacacia, black locust	21	2,670
Hemizonia fitchii, spikeweed	18	2,780
Lippia canescens, lippia	38	2,890
Silybum marianum, milk thistle,	37	2,920
Prunus spp., plum	29	2,970
Eriogonuin cicutarium, red filarce	42	3,250
Chamaebatia foliolosa, chamaebatia	60	3,600

Medicago sativa, alfalfa	114	3,790
Salvia carduacea, thistle sage	6	3,830
Eriogonuin fasciculatum, flat-top buckwheat	67	3,850
Prunus avium, cherry	111	3,950
Lotus scoparius, wild alfalfa	32	4,380
Salvia sonomensis, creeping sage	20	4,700
Prunus armeniaea, apricot	19	4,840
Vicia atropurpurea, purple vetch	28	6,070
Ligustrum sp., privet	31	6,130
Umbellularia californica, California bay	6	6,330
Citrus sinensis, Valencia orange	47	6,770
Chrysothamnus nauseosus, rabbitbrush	38	7,100
Photinia arbutifolia, toyon	42	7,140
Nemophia sp., nemophilia	15	8,000
Phacelia tanacetifolia, phacelia	5	8,800
Calandrinia caulescens, redmaids	38	9,030
Stellaria media, chickweed	6	10,000
Trifolium repens, white clover	34	10,290
Bassica nigra, black mustard	36	11,940
Taraxacum officinale, dandelion	61	12,260
Pyrus ma1us, pear or winter Nelis	75	12,300
*Prunu*s sp., wild Plum	7	12,850
Allium cepa, onion	21	13,330
Pyrus malus, apple	75	13,800
Marrubium vulgare, hoarhound	30	13,920
Eucalyptus globulus, blue gum	45	15,200
Tamarix articulata, tamarisk or salt cedar	30	18,000
Oxalis sp., oxalis	13	19,230
Rhamnus californica, casara	29	20,170
Genista monosperma, genista	20	21,110
Brassica kaber var. *pinnatifidum,* Charlock mustard	57	21,520
Rubus occidenialis, raspberry	18	21,670
Centaurea solstitialis, star thistle	25	24,000
Aesculus californica, buckeye	48	24,690
Rubus procerus, blackberry	22	33,640
Asparagus officinalis, asparagus	21	33,810
Meliotus alba, white sweet clover	77	41,040
Trifolium repens, dutch clover	56	45,000
Raphanus satirus, wild radish	14	47,500
Salix spp., willow	27	47,780
Daucus carota, carrot	30	52,330
Vitus vinifera, grape	41	55,610
Eriodictyon californicum, yerba-santa	27	61,110
Brassica rapa, turnip	18	61,660
Trifolium hybridum, alsike clover	46	64,780
Amsinckia spp., amsinckia	46	66,520
Brassica campestris, common mustard	23	86,520
Brassica alba, white mustard	24	103,330

In another part of their research, Todd and Vansell (1942) tried to determine what happens to pollen between the time it is collected by honeybees in the nectar until it becomes part of comb honey sealed in chambers of a hive. As in previous experiments, they caged a hive of honeybees, deprived them of stored honey, but allowed them to feed freely from trays filled with a solution of sugar syrup mixed with pollen. This time they used two groups of honeybees. One group was fed a sugar syrup solution with pollen added and the other group was allowed to feed on a mixture of star-thistle honey that was diluted with water. Testing revealed that the syrup-pollen solution had a pollen concentration value of 750,000 pollen grains per cc and the diluted star-thistle honey contained 5,200 pollen grains per cc. Honey in the sealed comb cells made from these two solutions revealed a pollen concentration value of 25,300 pollen grains/cc for the honey made from the syrup-pollen solution and 1,200 pollen grains/cc for the honey made from diluted star-thistle honey. Todd and Vansell concluded that only 3.1% of the pollen placed in the syrup-pollen feeding trays actually appeared in the honey made from that feeding source. For the honey made from diluted star-thistle honey, only 23% of the pollen from that feeding source appeared in the new comb cells of honey.

DISCUSSION

Since the beginning of honey production, certain types of honey have sold for premium prices because they taste better, produce better mead, are better for cooking, or do not crystallize rapidly. One of the goals of melissopalynological research is to search for data that can be used to compensate for errors produced by pollen types that are over or under-represented in the relative pollen counts in honey samples. Similar types of problems also occur during the reconstruction of vegetational histories using fossil pollen data. Although not the first to recognize this problem, Margaret Davis (1963) was one of the first to try to develop a precise list of values that could be used to compensate for the over or under representation of various pollen types in the fossil record. The result of her effort was the construction of a table of pollen ratios she called *R-values,* which she then used in a formula to adjust relative pollen counts. Her goal was to try to find a way to use pollen data to reflect the precise vegetation it represented.

From the beginning, some melissopalynologists recognized, as did Davis (1963), that not all pollen types contribute equally to either the natural pollen rain or to the production of honey. However, instead of developing R-values for the plants that bees utilize in making honey, melissopalynologists began generating lists of corrective values for certain pollen types. These became known as *pollen coefficient values*. Similar to R-values, pollen coefficient (PC) values are used to compensate for pollen types that are under or over represented in the relative pollen counts of honey (i.e., *Asclepias*, *Epilobium*, *Oxydendron arboreum*, *Salvia*, *Robinia pseudoacacia*, *Nyssa*, *Myosotis*, etc.).

One of the main reasons to develop and use PC values is to assist beekeepers in the verification and sale of premium honey types. Many premium honeys are not easily confirmed as being from a single floral-source (unifloral) because the species they come from often contribute only minimal amounts of pollen. However, those premium types of honey can be verified as being unifloral by applying the use of pollen coefficient values, and thus can qualify under the internationally accepted standard that requires a unifloral honey to contain a minimum of a 45% from its primary nectar source.

The use of PC tables is one way to explain why some of premium unifloral types are not dominated by the pollen from the unifloral source. But, everyone does not accept the use of PC values because of questions about the techniques used to generate PC values. As we have shown, the same relative pollen data from a honey sample can be adjusted using different sets of published PC values. The result is a different conclusion concerning the percentages of probable nectar sources used in the production of that honey sample.

Current arguments against the use and acceptance of PC values as a valid way to correct relative pollen counts in honey focus on the many variables that influence the calibration of PC values. As early

as the 1920s, scientists knew that the longer nectar remains in a honeybee's honey stomach, the greater the potential for that bee to remove most or all of the pollen in that nectar, regardless of the pollen type (Whitcomb and Wilson, 1929). Therefore, the time period between when a honeybee begins to forage and when she returns to the hive will determine the amount of pollen remaining in her honey stomach, which becomes a potential component of the resulting production of honey. Experiments by Todd and Vansell (1942) revealed two additional factors that can control the potential amount of pollen in honey. First, some honeybees are more efficient at removing pollen from their honey stomachs than are others from the same hive. Second, if nectar sources are located far from the hive, honeybees can effectively remove as much as 90% of the pollen they collected in the nectar on their return trip to the hive. Later research by Demianowicz (1961, 1964) documented yet another variable associated with the pollen collected in nectar. She noted that honeybees are more efficient at removing large pollen grains than smaller ones from the nectar in their honey stomachs during return flights to the hive.

Even if melissopalynologists find a method to adjust for these variables associated with how honeybees collect and transport nectar from its source areas to hives, only one of the major problems associated with the generation of PC values is solved. Other problems focus on the current techniques used to extract pollen from honey samples and on the methods used to determine absolute pollen concentration (APC) values for pollen types in honey.

Historically, the liquid medium used to dilute honey for analyses is water. Although 10 g of honey diluted with 20 ml of water is the recommended standard procedure (Louveaux *et al*., 1970, 1978), various published procedures use other ratios that vary from 1-20 g of honey diluted with 20 - 100 ml of water (Erdtman, 1935; Maurizio, 1951; Deans, 1957; Louveaux and Maurizio, 1963; Lieux, 1972; Gadbin, 1979; Agwu and Akanbi, 1985; Low *et al.*, 1989; White *et al*., 1991). The reasoning often cited for increasing the dilution ratio is the potential for better pollen recovery from honey. Honey has a specific gravity of around 1.44. Therefore, the specific gravity of any honey-water solution will range between 1.0 and 1.44. A honey-water solution containing 10 g of honey and 20 ml water will has a specific gravity of about 1.3 at 20^{o}C (Low *et al*., 1989). By diluting the solution with more water, the specific gravity of the solution will gradually approach one.

To test the reliability of these processing methods we constructed our own experiment. We prepared three identical groups of sub samples from each of 10 different honey samples so that each sub set group could be processed in a different manner. Each of the honey samples in each sub set consisted of 10 g extracted from a larger sample that was heated in a microwave oven to 38^{o} C and then thoroughly stirred to ensure a uniform mixing of the pollen before any sub samples were collected. Next, we poured each sub sample into a 600 ml beaker and slowly stirred it until it was fully dissolved in a solution of either water or alcohol, depending on which sub group it represented. Two sets were diluted using only 100 ml of water and then were centrifuged for different lengths of time at the same speed (4,000 rpm). For one of the two sets we used a short spin time of one minute as recommended by Deans (1957) and White *et al.* (1991). For the other sub set we used a long spin of 10 minutes as recommended by Low *et al.* (1989). The third sub set of samples was diluted with 95% ETOH and for those samples we used a spin time of three minutes at 4,000 rpm. We discovered that honey will not dissolve directly in 95% ETOH, so we first had to add 10 ml of distilled water to the 10 grams of honey. Once mixed we could then add an additional 100 ml of 95% ETOH. We checked the specific gravity of all alcohol sub samples before any further processing. We found that their specific gravity ranged between 0.83-0.88. This variation occurred because of the slight variations in the specific gravity of the individual raw honey samples.

To calculate an accurate pollen concentration value for each of the sub samples we added 67,500 tracer *Lycopodium* spores. After mixing each sub sample in a beaker, we reduced the volume of the liquid in each beaker by centrifuging in 12 ml Pyrex tubes. After each centrifuging and decanting, the residue was vortexed before adding more solution. When all the liquid was removed, each beakers was thoroughly rinsed with 95% ETOH.

Each sub sample was acetolyzed (Erdtman, 1960) for 12 minutes at 50^{O} C in a heating block. When finished, each sample was rinsed in glacial acetic acid and then twice with distilled water before a

final rinse with 95% ETOH. The residue for each sub sample was stained with Saffarin and poured into a 1 dram, glass vial. Five drops of glycerin were added to each vial and the vials were placed on a warm hot plate (30^{O} C) until the remaining alcohol evaporated. Next, a few drops of additional glycerin was added to each sample before it was vortexed for 30 seconds. After mixing, one drop of pollen residue was placed onto a glass slide and the residue was spread in all directions with a wooden toothpick to ensure that all pollen taxa would become evenly distributed under the cover slip. Each cover slip was sealed around the edges with a coating of fingernail polish to prevent evaporation.

When based on 200 grain pollen counts, we found that there was a significant differences in the pollen concentration values recovered in samples using each of the three techniques (**ANOVA**, n = 30, F = 14.24, P = 0.0001). Pollen concentration values in the alcohol-diluted sub samples were significantly higher than those recovered from the same honey samples diluted with water, regardless of the centrifuge spin time (< 0.05, Tukey's studentized range test). We also discovered that the pollen concentration values for all of the water-diluted sub samples were not significantly different (**ANOVA**, P > 0.05) from one another regardless of centrifuge spin time. On average, the alcohol-diluted sub samples had pollen concentration values that were 426% higher than those found in the water-diluted, short spin sub samples, and 226% higher than those found in the water-diluted, long spin sub samples.

In addition, we discovered there was a marked difference in the total number of pollen taxa recovered in each of the various sub sample groups. All together, we found 258 different pollen taxa in these 10 honey samples, a number that reflects the rich flora potentially available to honeybees foraging in the mixed deciduous forests of east Texas. Of the total 258 taxa, we found 161 of them in the 10 alcohol sub samples. We found a total of 152 of them in the 10 water-diluted, long spin sub samples, and 148 of the total pollen taxa in the 10 water-diluted, short spin sub samples.

From this experiment we discovered (Jones and Bryant, 2001) that regardless of how much water is used to dilute honey, there will often be a significant loss of pollen during the centrifugation process. Therefore, we recommend diluting honey with ethyl alcohol in order to recover *all* the potential pollen in a honey sample. Our data (Jones and Bryant, 1998) also reveal that the alcohol-dilution method will achieve comparable results with honey samples that are processed using a filtration system designed to capture **all** pollen.

Until now the researchers who have produced PC values for honey have based their conclusions on samples that were diluted only with water. Therefore, as far as we can determine, all of the existing PC data derived from honey are suspect.

The final flaw with existing PC values focuses on the methods researchers have used to determine APC values. Todd and Vansell (1942) are not clear about how they determined the APC or pollen concentration values they reported. Later, from the detailed and pioneering efforts of Demianowicz (1961, 1964), we know that she diluted various quantities of honey with water and then counted a small drop of the resulting solution to derive her APC values. That technique is subject to counting and calculation errors. For example, even a minor counting error will be greatly magnified when the APC value for the entire solution is calculated. More recently, researchers such as Moar (1985) have added “tracer spores” with the diluted honey samples and then determined APC values for pollen taxa based on the ratio of counted tracer spores to pollen grains. That technique has the potential to be more nearly accurate than the calculation method used by Demianowicz. Nevertheless, our own experiments with honey samples reveal that unless a large number of tracer spores are added to a honey sample, the potential for calculation errors in computing APC values can be great.

The original concept of trying to find ways to correct the relative pollen counts of honey samples so that the resulting interpretations are more realistic and meaningful is as important today as it was nearly a century ago. Because of the added value placed on premium types of honey, and because of laws requiring truth in labeling, beekeepers, honey distributors, and buyers need to have methods available that will validate the unifloral contents of the honey products they buy and sell. Sometimes validations can be assured by determining the calibration of the types of sugars in a honey sample. However, like PC values, tables outlining the precise isotopic ranges for sugars, such as those developed by White *et al*. (1962), are not available for all regions nor many of the types of premium, unifloral honey

types.

CONCLUSIONS

What is needed most in the field of melissopalynology is a new series of experiments to determine the precise PC values for many of the nectar sources used to produce premium types of honey. This type of research, however, will need to be conducted under controlled conditions that will satisfy skeptics and will result in PC data that will be accepted by melissopalynologists.

For each floral type a separate experiment will need to be conducted. First, an isolated hive of honeybees needs to be caged to prevent outside contamination. Second, the caged bees should be starved, then allowed to feed freely on the flowers of only one type of plant until a measurable amount of honey from that single floral source is produced. Third, during the experiment, a selected numbers of bees should be trapped and examined immediately after they have filled their honey stomachs to determine the original APC in the nectar. Fourth, at various times during the experiment, the opening to the hive should be sealed for periods ranging from 5-15 minutes while the bees are feeding. Before allowing the bees to re-enter the hive, some bees should be captured and the contents of their honey stomachs must be examined to determine how effectively they have been at removing pollen from the nectar. Fifth, once honey is produced from the experimental feeding process, several honey samples must be collected, processed correctly using new pollen extraction techniques, and several pollen counts of over 1,000 grains need to be tabulated for each sample to determine the APC.

A critical aspect of any future experiments designed to calculate new PC values must include new processing techniques that include either a filtration process similar to the one described by Lutier and Vaissiere (1993), or an alcohol-dilution technique similar to the one described by us (Jones and Bryant, 1996, 2001). Our experimental tests have confirmed that both the filtration and alcohol-dilution techniques are comparable in the amounts of pollen recovered from honey samples. Those same tests revealed that both the filtration and alcohol processing methods increased pollen recovery from honey samples by an average of more than 200% over water dilution processing methods currently in use by melissopalynologists. Finally, a large quantity of tracer spores must be added to each honey sample prior to processing to ensure an accurate ratio of tracer spores to pollen. Maher (1981) provides an excellent discussion of how one can determine how many tracer spores need to be added to a pollen sample in order to ensure reliable statistical results when calculating pollen concentration values. He notes that the ideal ratio of pollen to tracer spores in a sample is 1:1. Because counting pollen and tracer spores is time consuming, Maher investigated the reliability of other ratios. His data suggest that a pollen to tracer spore ratio of 2:1 produces what he says is a “good level of precision for concentration estimates, and reduces the total counting time (Maher, 1981:157).”

In tests of honey samples Jones (1993) states that she found no statistical difference in pollen concentration values when she added 67,500 tracer spores per 10 g of honey, and then completed four separate pollen counts from each of five different honey samples. Her five samples ranged in average concentration levels from a low of 72,000 in one sample to a high of 132,000 in another of the five samples. Based on her experimental evidence and similar tests in our laboratory, we recommend that for most honey samples the addition of from 65,000-100,000 tracer spores per 10 g sample should produce concentration values that are statistically reliable.

SUMMARY

The type of new research we have proposed in this article will be expensive and time consuming. However, if our recommended steps are taken and if the research efforts are completed successfully, then the resulting data can be used with confidence to construct APC values for honey

samples that should become universally accepted, even by the most ardent skeptics. One final benefit of the new research will be that those same honey samples could serve as unique opportunities for complementary chemical testing to determine precise sugar types and expected ranges for various types of sugar isotopes.

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