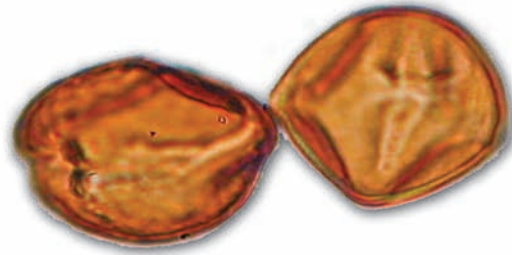


THE STUDY OF POLLEN AND ITS ROLE IN THE HONEY MARKET



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Melissopalynology (the study of pollen in honey) is an important tool in determining the geographical origin and floral sources upon which the bees foraged to produce honey. Each flower species has a unique pollen grain which, using proper techniques, may be studied to determine the geographical origin and major floral sources of the honey. For some areas of the world we have a number of pollen atlases and photographic keys to pollen types (Europe, North Africa and USA) while other areas are deficient in pollen reference material necessary for a complete analysis and geographic origin. This has implications in the worldwide market when issues such as "transshipment", unifloral sources, and "honey laundering" (removal of all pollen grains) are addressed. A basic step-by-step process for sample preparation, counting methods, discussion of floral types that are over or under represented in honey samples and, the potential for value-added honey due to geographic origin, floral sources (e.g. manuka honey) or product uniqueness are examined. SE Asia, frequently the victim of transshipped honey (relabeling the country of origin), needs to build a pollen data base to assist melissopalynologists and maintain national pride in their own unique products.

Introduction

Beekeeping in a broad sense may be defined as the management of honey bee colonies (*Apis* spp.) so as to take advantage of a large adult foraging population at the beginning of the regional nectar flow. No matter where bees are kept, beekeepers face three common problems that must be addressed; define the local nectar/pollen plants and their bloom times (phenology), take the appropriate management steps to maximize

adult foraging populations, and decide what to do with the honey bee colonies in times of non-nectar flow or dearths – which can be winter in temperate regions or rainy/dry seasons in tropical environments.

Most of the "how to" books on beekeeping deal with *Apis mellifera*, a species with origins in Europe, the Mid-East and Africa but, at the present day, has world-wide distribution. There are eight other species of honey bees (all found in Asia) which produce honey, but do not necessarily conform to the Western methods of honey bee management.

Honey bees can broadly be differentiated into two groups; single-comb, open-nesting

(e.g. *Micrapis* and *Megapis* [Maa 1953]), and multi-comb, cavity-nesting (e.g. *Apis cerana*, *mellifera*, *koschevnikovi*, *nuluensis*, *nigrocincta*, etc.); almost all of these species are exploited for their honey stores and some are managed at different levels. There are two axioms that are germane to honey bee management regardless of the location – "the best bee is the one that survives your management" and "adapt your management to the bees – not the bees to your management." If the bees are migratory by nature, then adopt a management plan that takes their seasonal activities into perspective.

During periods of dearth (winter in temperate areas or a rainy/dry season in the



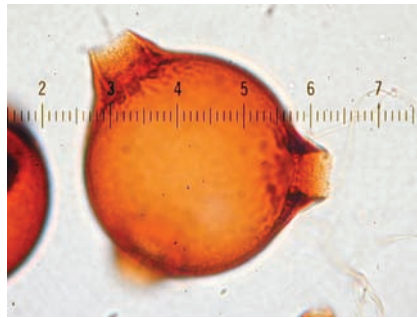
Dr. Vaughn M. Bryant, America's only melissopalynologist, at his microscope at Texas A&M University. (Photo courtesy of VM Bryant, Texas A&M University)

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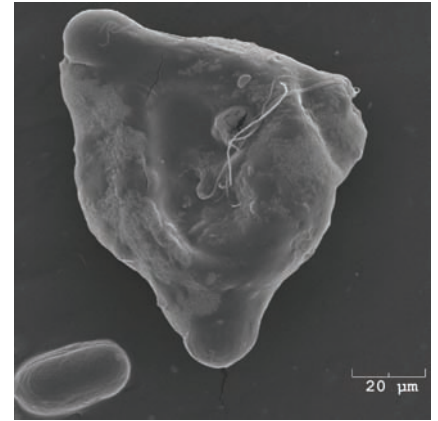
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Pollen grains of the wild rose (*Rosa acicularis*) a major source of pollen for colonies in Interior Alaska during June. In this light microscope photograph each tick mark is 2.5 micron (μm) so the grain is approximately 25-30 μm long. (Photo courtesy of VM Bryant, Texas A&M University)



Tricolpate (three lobed) grains of fireweed pollen (*Chamerion angustifolium*) are some 72-91 μm in diameter. (Photo courtesy of VM Bryant, Texas A&M University)



A Scanning Electron Microscope (SEM) photograph of fireweed (*Chamerion angustifolium*) (Photo courtesy of Tina Buxbaum, University of AK)

tropics) most tropical bees will migrate over either distance or elevation to take advantage of differing floral assemblages; the cues bees use to find these “greener pastures” are poorly understood. Some migrations can be rather predictable, e.g. *A. dorsata* bees in West Kalimantan, Borneo migrate from the surrounding highlands, which they inhabit during the dry season, to the flooded forests of the region during the rainy season; similarly bees migrate from the surrounding Elephant and Cardamon Mountains to the coastline *Melaleuca* forest in southern Cambodia. Other migration attractants such as the mass flowering events in the *Dipterocarp* dominated rain forests are less predictable. In some areas local beekeepers have adapted their management styles to that of the bees - providing them with artificial nesting sites (called rafter beekeeping) and harvesting only the honey portion of the colony (Petersen 2005, 2010).

By any measure, the pollination efforts of honey bees (managed or wild colonies) is their most important characteristic, not only from the agricultural aspect (one-third of our food resources are dependent on honey bees), but also in the natural environment – approximately one-third of tropical rainforest flora are dependent on the pollinating efforts of social honey bees (Oldroyd & Wongsiri 2006).

A thorough understanding of the plant communities in a region is a critical factor in bee management decisions, which includes agriculture and Non Timber Forest Product (NTFP) management and sustainable use plans, and the economics/marketing of honey bee products. A comprehensive bee botany and phenology study will allow planners, developers, and beekeepers to:

1. Develop a beekeeping calendar that integrates floral phenology with bee activity (swarming, migrations, absconding), human activities (agricultural pursuits such as rice harvest/planting), bee-related chores (e.g., placing swarm traps or

- rafters out in anticipation of the bees' arrival) and the local weather.
2. Identify plants in the local area, which are significant contributors to the bee colony build up or honey flow (bee plants).
3. Identify plants that may be the source of a desirable unifloral honey thus increasing the market share or return to the producers.
4. Identify a floral spectrum that may be unique to a particular region, which may subsequently identify the geographic origin of the honey.
5. Allow the *certification* of a honey from a particular floral source or region adding value to the product (e.g., *manuka* honey - *Leptospermum scoparium*).

The Role of Melissopalynology in Honey Analysis

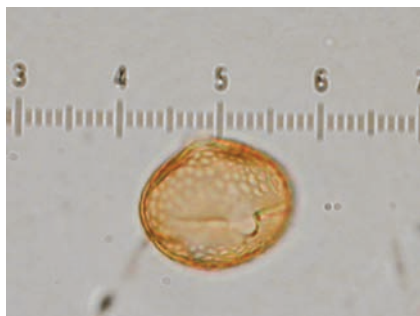
Melissopalynology (the study of pollen in honey) is an important tool in determining the floral sources upon which the bees foraged to produce honey. Each flower species has a unique pollen grain which, using proper techniques, may be studied to determine the geographical origin and major floral sources of the honey.

There are set procedures and laboratory guidelines for the study of pollen in honey and their relationship to the floral resources (Jones & Bryant 1996; Bryant & Jones 2001). Typically a 10 gram sample of raw honey (strained but not filtered) is diluted partly with warm water and partly with alcohol (ETOH), centrifuged and, the resulting residue examined under a light microscope (typically at 400X but in some cases 1000X). A known number of *Ly-copodium* spores are added to assist in calculating the pollen concentration value per 10 gr. of honey, which is an essential factor in determining the accurate floral sources of the collected nectar. Pollen grains are identified to the plant family level, frequently to the genus level, and sometimes (with unique pollen grains) to the species level. The level of identification is dependent on how unique a pollen type might be. For example, in some families, such as the grasses (Poaceae), it is nearly impossible at the light microscope level to distinguish different

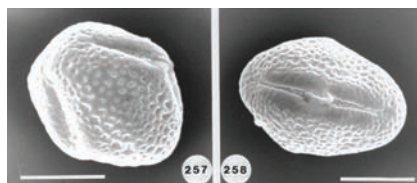
genera based on their pollen types. At least 200 or more pollen grains are counted in the typical sample analysis. Known corrective values or the “pollen coefficient” are then applied to the counts to ascertain the relative contribution to the honey from any particular floral source. In some cases pollen counts need to be corrected for over-abundant pollen from some plants, such as clover, and from under-represented pollen from other floral sources such as acacia or fireweed honey. These coefficient values have been established by a number of different tests, but they exist only for a few of the major honey nectar sources in the world; a great deal of added research effort is still needed in this area.

In order for an accurate and valid analysis of a particular honey to be made, there are several criteria that must be met:

1. There must be a broad-based (botanically and geographically speaking) pollen reference slide collection available to the technician. This involves collecting and preparing samples from living or herbarium flower voucher specimens that have been correctly identified by trained personnel or experienced taxonomists. Efforts should focus on known honey and pollen source plants in a region.
2. In order to achieve the highest degree of accuracy the “pollen coefficient” for a particular bee-friendly plant species must be determined. This is perhaps the most difficult aspect of the procedures as samples of “pure” unifloral honey must be obtained to establish the pollen coefficient for that particular floral source. Most of the pollen coefficient values that have been established are for temperate European and North American bee forage plants, but some countries have done an excellent job of local pollen studies, e.g. (Sajwani et al 2007).
3. There must be trained personnel and adequate laboratory facilities. The laboratory procedures are fairly straight forward: normal laboratory safety precautions



An alsike clover pollen grain (*Trifolium* spp.), each tic mark is 2.5 microns (μm); note the net-like surface of the grain (reticulate) – this and other factors help in identification. (Photo courtesy of VM Bryant, Texas A&M University)



A Scanning Electron Microscope (SEM) photo of alsike clover (*Trifolium hybridum*); note the increased detail especially of the surface of the pollen grain obtainable using a SEM. The process, however, is much more expensive and time-consuming in sample preparation than for a light microscope. (SEM photo courtesy of VM Bryant, Texas A&M University)



Another type of clover (*Melilotus* spp.) – it is often difficult to determine to the species level unless there are some unique characteristics. (Photo courtesy of VM Bryant, Texas A&M University)

must be followed, especially during the use of various acids needed to prepare pollen grains for analysis; there must be access to quality microscopes; there must be a pertinent pollen reference slide collection; and the technician must have experience in the field of palynology (the study of pollen).

4. The honey sample under scrutiny must be “raw” or at least only strained to remove wax bits, bee legs, and debris; samples that have been “ultra-filtered” so as to remove material the size of pollen grains (pollen size varies from about 5-200 microns) are worthless to study because they can only produce inaccurate results.
5. To save lab time and energy an indication of the purported nectar source and geographical origin of the sample is helpful.

How Does Honey Certification Affect the Market for the Product?

The honey market can be divided into two channels—the generic, large-volume world honey market, produced predominately by *Apis mellifera*, and the specialty honeys which are either unifloral, or are produced by different *Apis* species, or come from a particular geographic region, have substantiated health benefits, or meet a customer’s purchase criteria such as organic, Fair-Trade, sustainably produced, assisting in conservation efforts, or in poverty alleviation/sustainable lifestyles. Countries listed below accounted for almost 65% of the global honey output from *Apis mellifera* honey production (Workman 2007).

1. China – 298K metric tons (21.5% of global honey production)
2. Turkey – 82.3K metric tons (5.9%)
3. Argentina – 80K metric tons (5.8%)
4. United States – 79.2K metric tons (5.7%)
5. Ukraine – 71.5K metric tons (5.5%)
6. Russia – 52.1K metric tons (3.8%)
7. India – 52K metric tons (3.7%)
8. Mexico – 50.6K metric tons (3.6%)
9. Ethiopia – 39K metric tons (2.8%)
10. Spain – 37K metric tons (2.7%)

In 2004, the following nations exported the most honey by weight (Workman 2007).

1. China – 81.3K metric tons (24.2% of top ten totals).
2. Argentina – 65.2K metric tons (18.6%)
3. Mexico – 23.4K metric tons (6.9%)
4. Germany – 22.4K metric tons (6.6%)
5. Brazil – 21K metric tons (6.2%)
6. Vietnam – 15.6K metric tons (4.6%)
7. Hungary – 15K metric tons (4.4%)
8. Canada – 14K metric tons (4.2%)
9. Uruguay – 13.4K metric tons (4%)
10. India – 13.4K metric tons (4%)

Although Chinese authorities are implementing stricter controls, Chinese honey has, in the past, been associated with chloramphenicol – an antibiotic used in some honey bee colonies. Banned from food processing in many countries, chloramphenicol can cause the fatal blood condition aplastic anemia. The chloramphenicol scare motivated the United States to temporarily ban Chinese honey in 2001. The European Union (EU) outlawed People’s Republic honey from 2002 to 2003. The EU has accused about 10 honey exporters including Argentina, Vietnam, Hungary and India of re-labeling and re-shipping contaminated honey originally from China. Re-labeled honey with chloramphenicol has been found in European ports, shipments to honey blending companies and grocery stores. Light colored Chinese honey, re-labeled as Vietnamese honey (normally darker colored), also raised alarm bells. Chinese honey garners the lowest price in global honey trade; US\$ 1,094 per metric ton in 2004. Re-labeled as Argentine honey, the unit price per ton almost doubles to US\$ 1,927/ton; New Zealand honey dictates the highest prices on the world market – US\$ 6,813/ton in 2004 (Workman 2007).

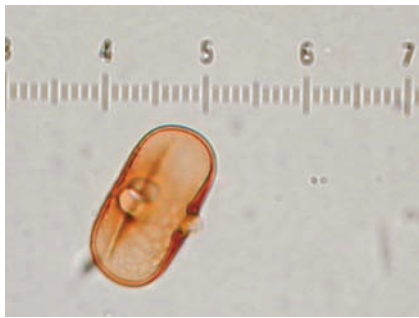
There has been considerable flurry in the United States recently (2009 & 2010) on the illegal circumvention of tariffs and the transshipments/re-labeling of honey to disguise the country of origin. Frequently, the honey is “ultra-filtered” removing all the pollen

(and sometimes adding rice or corn sweeteners to “extend” the product) making a melissopalynological examination to determine the geographical origin of the honey impossible. Firms have received substantial fines and individuals have been sentenced to time in jail as a result of their market activities.

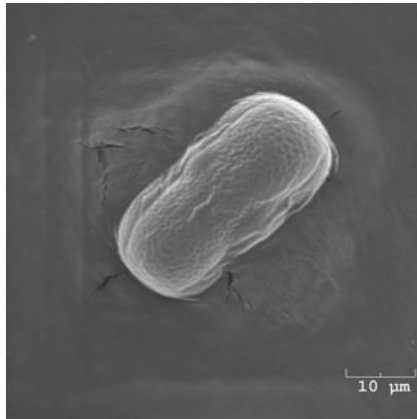
At the other end of the economic spectrum are the specialty honeys that target a particular market and whose price is based primarily on customer perception and supply/demand for the product. Perhaps the two most famous specialty honeys are *sidr* (from *Ziziphus spina-christi*) produced in the desert areas of the Mid-East – (Yemen and Oman being major sources) and *manuka* (*Leptospermum scoparium* produced in New Zealand and the subject of extensive scientific research into its healing properties). Billed as the world’s most expensive honey, these honeys command premium prices at the retail level (e.g. *sidr* honey \approx \$160 USD/Kg.; *manuka*, depending on the Unique Manuka Factor [UMF] level, from \$50-\$180 USD/kg).

Some regional honeys command a high retail price derived from several factors. For example, fireweed honey from Alaska (*Chamerion angustifolium*), is perceived as coming from a wild and pure environment (the Alaskan wilderness). It is visually (extra water white) and organoleptically stunning (Swanson & Lewis 1993), and has been described as the “purest fireweed honey I’ve ever seen” (Bryant 2009). It is notoriously difficult to predict the potential flows and its limited production allows it to command a high price, e.g. \$35USD/kg. (Petersen & Bryant 2010).

Other honeys can command a premium price even though they may not be of superior quality simply by addressing public perceptions of ecological, sustainably harvested, aiding in protecting endangered species, or sustaining indigenous lifestyles. Examples would be honey marketed through the auspices of the International Gorilla Conservation Program (IGCP) in Uganda and Rwanda, Mondulkiri Wild Honey sustaining indigenous peoples in



Pollen grain of *Vicia* spp. whose common name is vetch, each tic mark is 2.5 μ m (Photo courtesy of VM Bryant, Texas A&M University)



This pollen grain is a SEM photograph of *Vicia cracca* (Photo courtesy of Tina Buxbaum, University of AK)



A pollen grain of dandelion (*Taraxacum officinale*) under a SEM; when viewed with a light microscope one can observe vacuoles filled with a yellow oil, the stuff that make dandelion pollen stain everything. (Photo courtesy of Tina Buxbaum, University of AK)

eastern Cambodia, or Dorsata Honey from the Denau Sentarum National Park in West Kalimantan certified as organic by Indonesia's Bio Cert Agency. All of these honeys command a price well above the world-market price for bulk *mellifera*-produced honeys.

In fact, development beekeeping projects should channel development dollars into marketing of specialty honeys instead of trying to compete with the world's mega producers of *mellifera* honey. The problem lies in the verification/certification of purported characteristics, whether the claims made are organic, unifloral or, sustainably harvested.

Few countries outside Europe have the expertise and facilities for a melissopalynology laboratory – so they are left with sending samples off to foreign experts; frequently a major expense not likely to be funded by development programs. Foreign experts are at a loss because few accessible pollen reference collections are available for many parts of the world. Most European importers require some sort of pollen identification to confirm geographical origin and the floral components purported to be the source of honey so the consumer may be confident in their purchase (Persano-Oddo & Piro 2004). There are no such regulations in the United States—as a result domestic honey is frequently “mislabelled” (perhaps through no intention on the producer/packers part) as to its true floral source. After examining more than 1,100 samples of American honey, it was found that more than 60% of the time the floral sources identified by the producers or beekeepers were incorrect (Bryant & Jones 2001).

What do we need?

The world honey industry is facing a crisis – honey bee populations are on the decline worldwide through disease, loss of habitat, and application of agro-chemicals; unscrupulous traders are transshipping honey re-labeling it as to country of origin; honey is being found contaminated with banned chemicals and adulterants; and globalization has upset the paradigm of trade – frequently honey is produced in lesser-developed countries at a fraction of the domes-

tic Western production costs. National pride and reputation suffer when “funny honey” is transshipped through a third-party country. Thus, is it fair to the mid-point country to have their reputation and that of their domestically produced honey ruined by circumvention of trade regulations?

As beekeepers are fond of saying, “There is no honey like your own.” In order to develop this pride and allow entry into quality markets, claims must be able to be verified. Unfortunately, honey has been the source of unverified and often wild claims in the past (if it really did cure baldness why am I losing my hair?). Especially important is the education of the honey-consuming public—just because honey is beginning to crystallize does not mean sugar has been added (a common perception).

One of the major steps that must be taken is the establishment of a pollen reference data base; research is needed to establish unifloral pollen coefficient values for important honey nectar source plants. Technicians who are competent in melissopalynology need to be trained (there is only *one* melissopalynologist in the United States!). Beekeepers must be trained in proper management and sanitary harvesting techniques, and research done on techniques to reduce chemical dependence in managed colonies.

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