Feral Honey Bees in the Sonoran Desert: Propolis Sources other than Poplars (*Populus* spp.)

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In Central Europe and other temperate regions the lipophilic bee hive product propolis normally originates from the bud exudate of poplar trees that is collected by the bees. Based on bee observations, various other sources have been discussed in particular for tropical regions, but in only few cases the origin has been proved by analytical methods. We have analyzed propolis samples from managed honey-bees as well as from feral bee colonies in the Sonoran Desert. Propolis collected in hives out of flight reach of poplars contained flavonoid aglycones and other phenolics that point to specific plants as the source of propolis in this area, namely *Ambrosia deltoidea* and *Encelia farinosa*.

Introduction

Propolis or bee-glue is a resinous or sometimes wax-like bee hive product that has been used by man since ancient times for its pharmaceutical properties. It is still used as a remedy in folk medicine (Ghisalberti, 1979), as a constituent of "biocosmetics" and for numerous further purposes (Vanhaelen and Vanhaelen-Fastré, 1979; Hausen *et al.,* 1987; Wollenweber *et al.,* 1990). Bees use this material to seal hive walls and its entrance, to strengthen the border of the combs, to embalm dead invaders. It is now well known and it has been confirmed by many studies that at least in Central Europe the bees almost exclusively collect this material from the bud exudate of poplar trees, in particular of black poplar, *Populus nigra*. Bankova *et al.* (1992) have shown that also in Mongolia poplar buds (*Populus suaveolens*) are the source of propolis material. Deviant vegetable propolis sources have been reported from Russia, South Africa, Australia, Hawaii etc. Bees are even said to collect materials like asphalt, mineral oil or paint as a substitute in case of need (König 1985 a, 1985 b). Many of these statements, however, are based on bee observations only and not on the chemical analysis of propolis.

The best indicator for the origin of European propolis is the pattern of flavonoid aglycones present in this lipophilic material (Hausen *et al.,* 1987; Wollenweber *et al.,* 1990). Normally the flavonoid pattern ("fingerprint") of propolis can hardly or not at all be discerned from that of poplar bud exudate (cf. Wollenweber, 1975), the latter being its source. During a plant collection trip in Arizona, the senior author wondered where bees living in the Sonoran Desert might get their propolis material. Which plant source would they use in this area? Are they desperately searching for the scarce cottonwood trees, or have they adopted to collect lipophilic material from other plant sources? Analysis of propolis flavonoids should allow us to answer this question.

The first propolis samples from the Sonoran Desert area that we studied came from bees hives in the vicinity of Tucson/Arizona, where the scattered vegetation is dominated by xeromorphic shrubs and cacti, whereas cottonwood (*Populus*) is very scarce. A study of the flavonoid aglycones of these samples revealed that even in that region the bees must have access to poplar trees, as the propolis flavonoid patterns were typical for *Populus* bud exudates (Wollenweber and Egger, 1971; Wollenweber, 1975; Wollenweber *et al.,* 1987a).

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Fremont cottonwood, *Populus fremontii* S. Wats. occurs, indeed, naturally in the Arizona deserts (Benson and Darrow, 1981). The "fingerprint" of these propolis samples matches with bud exudate of *P. fremontii,* although this poplar is extremely rare in the Tucson area where it grows only sporadically in watercourse beds (Wollenweber *et al.,* 1990). Only one flavonoid was detected (xanthomicrol, 5,4′-dihydroxy-6,7,8-trimethoxy flavone) that cannot be derived from *Populus*, but might originate from the leaf exudate of an asteraceous desert shrub such as an *Ambrosia* species (Wollenweber *et al.,* 1987b; 1995) or a *Baccharis* species (Wollenweber *et al.,* 1986; 1989). Finally, propolis samples were obtained from colonies of feral bees found in rock outcrops beyond the flight range of any poplar trees. The insects were observed collecting material from the leaf surfaces of Asteraceae belonging to the genera *Ambrosia, Baccharis, Encelia* and *Gutierrezia*. One would also expect that the bees use the abundant leaf resin of the most common shurb in that area, the creosotebush *Larrea divaricata* (Moc. and Sessé) Cov. The resin of the elephant tree, *Bursera microphylla* A. Gray, is another possible source that one might think of. Analysis of these feral bees' propolis samples was, therefore, of high interest. In the following we report the results of a study on this subject, done with propolis from the hives of cultivated bees as well as from the nests of feral bees out of flight range from any poplars.

Material and Methods

Aerial parts of *Ambrosia deltoidea* were collected west of Tucson city limits. Aerial parts of *Encelia farinosa* were collected in the foothills of Mount Lemon near Tucson/AZ (G. Yatskievych, May 1990). Air-dried material of both plants was briefly rinsed with acetone to dissolve the lipophilic exudates. Identification of flavonoid aglycones in *Ambrosia deltoidea* has been reported previously (Wollenweber *et al.,* 1987 b). For the present study samples of the acetone-wash were used for direct comparisons with propolis samples.

The following collection data refer to the propolis samples now studied. They were collected from the hives of cultivated bees, i.e. managed European honey bees (*Apis mellifera ligustica*) and from the nests of feral bees (wild bees of the same race/subspecies). The samples were collected before the Arizona Africanized honey bee invasion, so do not represent propolis from that race.

A) Propolis from managed *colony* within easy flight range (about 0.5 mile) of Rillito River and some poplar trees. The colony has been in the same spot for about 6 years and was full of propolis. Coll. H. G. Spangler (Carl Hayden Bee Research Center, Tucson, Arizona), June 10, 1988.

B) Propolis from the Carl Hayden Bee Research Center. Mixed managed colonies within easy flight range of the Rillito River poplar trees. Coll. S. Buchmann, December 16, 1990.

C) Propolis and melted wax from small entrance around mouth of Colony No. DS-2. Colony dead when sampled. Dripping Springs, Organ Pipe Cactus National Monument, Ajo, Arizona. Coll. S. Buchmann, February 2, 1989.

D) Dark propolis floor sample. Feral colony in rock wall of wash at the Arthropod Discovery Center, Sonoran Arthropod Studies, Institute, Tucson Mountains, Tucson Mountain Park. Live colony above. Coll. S. Buchmann, August 23, 1990.

E) Propolis from feral colony OWL-1. Colony on ledge behind saguaro at Owl Head Buttles, Pima Co., Arizona.. Coll. John Edwards, March 12, 1990.

F) Sample from feral colony TORT-1, Honey Bee Canyon, Rancho Vistoso, Pima Co., Arizona. Life colony. Coll. John Edwards, March 12, 1990.

G) Scrapings from very large debris midden. Organ Pipe Cactus National Monument, Ajo, Arizona. Copper Mountain Col. no. 1. Colony long dead. Coll. S. Buchmann, 1989.

H) Propolis sample from "Artificial Swarm Scale Colony Experiment." Arthropod Discovery Center, Tucson Mountains, Pima Co. Sonoran Arthropod Studies, Institute, Tucson, AZ. Large amounts of *Ambrosia deltoidea* nearby, probably no *Populus* trees within flight range. (Propolis is only from March – November of 1990). Coll. S. Buchmann, November 8, 1990.

Propolis samples J – N came from feral honey bee colonies living within a Schmidt/Thoenes "swarm trap" located within Organ Pipe Cactus National Monument, Ajo, Arizona. They were all collected by Steven C. Thoenes. Specific locations are as follows:

J) Colony 1.5 miles from Senita Basin. February 14, 1991.

K) Mesquite bosque approx. 1 mile from Quitobaquito Springs. February 14, 1991.

L) Quitobaquito Springs area. February 14, 1991.

M) Burro Springs near Quitobaquito Springs area. February 14, 1991. N) Dripping Springs. No cottonwood trees in area. January 15, 1992.

The phenolic portions of ground propolis samples were extracted with acetone and with warm ethanol, respectively. Flavonoid aglycones were identified by direct TLC comparison with markers, based on the senior author's experience with the analysis of flavonoid aglycones in general, and with poplar bud exudates and with propolis in particular (Wollenweber, 1975; Wollenweber *et al.*, 1987a; Wollenweber *et al.*, 1990). Propolis samples and plant exudates were also compared directly by TLC. Thin layer chromatograms were run on polyamide DC-11 with solvents A) petrol 100–140° – toluene – methylethylketone – methanol 12:6:1:1 (v/v), B) toluene – petrol 100–140° – methylethylketone – methanol 12:6:2:1 (v/v), and C) toluene – methylethylketone – methanol 12:5:3 (v/v), sometimes also on silica with toluene – MeCOEt 9:1 (v/v) and with toluene – dioxane – HOAc 18:5:1 (v/v). Chromatograms were viewed under UV_{366} before and after spraying with "Naturstoffreagenz A" (Nat. A). Authentic flavonoid aglycones and coumarins for comparison purposes were available in E. W.'s lab.

Results and Discussion

Propolis samples A and B, collected from managed *Apis mellifera* colonies, exhibit the characteristic flavonoid patterns of poplar bud exudates, although it is not completely identical with that of *Populus fremontii*. The hives are within flight range of Fremont cottonwood.

The collection sites of samples C – J are all out of flight range of any cottonwood tree. These samples show different wax contents, different colors, and somewhat different solublility. In sample G ("colony long dead") the flavonoid content is very low. In each case the flavonoid pattern does not match that of poplar bud exudate. After isolation of two major flavonoids and identification of some minor flavonoids, a data base search for their distribution revealed that the flavonoid pattern corresponds to that found in the exudate of *Ambrosia deltoidea*. The characteristic exudate flavonoids of this plant are the major products, 3′-desmethoxysudachitin (5,7,4′-trihydroxy-6,8-dimethoxy flavone) and xanthomicrol (5,4′-dihydroxy-6,7,8-trimethoxy flavone), and the minor components, scutellarein-6-methyl ether (hispidulin), luteolin-7-methyl ether, 6-methoxy luteolin (nepetin), sideritiflavone (5,3′,4′-trihydroxy-6,7,8-trimethoxy flavone), kaempferol, quercetin, rhamnetin (quercetin-7-me), rhamnazin (quercetin-7,3′-diMe) and ombuin (quercetin-7,4′-diMe). The presence of two coumarins, scopoletin and ayapin, is also typical for the "fingerprint" of *Ambrosia deltoidea* on TLC, where they appear as light blue fluorescent spots (Wollenweber *et al.*, 1987b). Thus, their presence in these propolis samples confirms this plant source. Triangular leaf bur sage, *Ambrosia deltoidea* (Torrey) Payne, is, in fact, omnipresent in most Sonoran Desert sites. The presence of *Ambrosia* exudate constituents in propolis was further confirmed by GC/MS analysis of several of our samples. Apart from the flavonoid aglycones it revealed identical major peaks in both, propolis and *Ambrosia* exudate, which do not correspond to flavonoids. The relevant products were not identified, but nonetheless this result has additional value as evidence.

Sample K originates from a feral European *Apis mellifera* colony in a swarm trap. This sample exhibits flavonoids derived from *Populus* bud exudate as well as flavonoids derived from *Ambrosia deltoidea* leaf exudate. Obviously the bees have collected both materials.

Samples L and M, also from feral bee colonies, exhibit neither the flavonoid pattern of *Populus*, nor the flavonoid pattern of *Ambrosia deltoidea*, although both sources are present within the bees flight range. Curiously enough, we here found some highly lipophilic products that are characteristic of another asteraceous plant, namely the brittle bush *Encelia farinosa* A. Gray. This species occurs on rocky or gravelly slopes and mesas in the Arizona deserts and adjacent areas (Benson and Darrow, 1981). Chromenes (benzopyrans) and benzofurans are common in *Encelia* species like in many other Asteraceae (Proksch and Rodriguez, 1982). These compounds are non-polar products that fluoresce on irradiation with long wave UV-

light (Proksch *et al.,* 1984). They might, therefore, well correspond to the non-polar fluorescent spots observed on TLC in *Encelia farinosa* leaf wash as well as in propolis samples L and M. According to Proksch *et al.* (1984) the accumulation of chromenes and benzofurans is strictly correlated to the presence of resin ducts. With the help of fluorescence microscopy they have proved that in *Encelia farinosa* these compounds are stored exclusively in the resin ducts and the surrounding cells. It appears unlikely, however, that bees pierce the stems of brittle bush to suck the resin, so we checked the localization once more. When undamaged aerial parts are rinsed with acetone, the solution actually exhibits exactly those non-polar spots on TLC that we observed in propolis samples L and M. It is assumed, therefore, that at least a small amount of these products is also excreted by and deposited on leaves and stems and this material is used by bees as a source of propolis. With regard to the function of propolis, the insecticidal and antimicrobial effects of these phenolics (Proksch *et al.,* 1983; Isman and Proksch, 1985) might be a welcome property.

Sample N exhibits not only the flavonoid pattern of *Ambrosia deltoidea*. In addition, it shows the typical *Encelia farinosa* products. Obviously these bees have collected their propolis material from both Asteraceae species.

In view of the observations mentioned in the Introduction we assume that other propolis samples might contain flavonoid aglycones that are characteristic for the leaf exudates of *Baccharis* and of *Gutierrezia* species among other plant sources. By contrast, it is unlikely that *Larrea* is used as a source for propolis because considering its ubiquity in the area, its phenolic constituents should already have been found in the samples analyzed so far.

Tomás-Barberán *et al.* (1993) studied a total of 38 propolis samples from tropical Venezuela by HPLC analysis. 29 samples were produced by imported *Apis mellifera*, the others by five indigenous species of stingless bees. Most of these tropical propolis samples showed a rather uniform phenolic profile that is characterized by the presence of polyprenylated benzophenones, while some samples were devoid of any phenolic compound. The uniform phenolic pattern pointed to a single plant species or plant genus as the source. As a matter of fact, *Clusia minor* and *Clusia major* (Guttiferae) were found to produce the same polyprenylated benzophenones in their flowers and bees have been observed collecting this resin. For comparison purposes the same authors analyzed propolis from temperate regions: Spain, Bulgaria, Canada, Arizona, New Zealand. In each case the typical flavonoids found in the bud exudate of *Populus* sect. Aigairos were abundant. On the other hand, only a few of the tropical samples showed trace amounts of flavonoids which in one of them could be identified to be 6-methoxylated flavones. This type of flavones is often found in exudate on the leaf surfaces of Asteraceae (Wollenweber and Valant-Vetschera, 1995) and of Labiatae (Tomás-Barberán and Wollenweber, 1990). Hence we assume that such plants might be the source in a few tropical propolis samples.

Bonvehí and Coll (1994) analyzed 15 samples of propolis originating from China, from Brazil and from Uruguay. They identified 24 phenolic compounds among which benzoic acid and benzaldehyde derivatives as well as flavonoids were abundant. Several of the phenolic compounds identified by these authors might be collected from poplar bud exudates (chrysin and tectochrysin, galangin, pinocembrin and pinostrobin, vanillin). The preponderance of apigenin and apigenin-4′-methyl ether, however, indicates marked differences between propolis from Central Europe and propolis from China and South Ameria and points to different sources. The origin of glycosides such as rutin, hesperetin and naringin has not been clarified; the same is true e. g. for gallic acid.

A recent study on antimicrobial compounds in Brazilian propolis (Aga *et al.,* 1994) reports three typical compounds: 3,5-diprenyl-4-hydroxycinnamic acid, 3-prenyl-4-dihydrocinnamoloxycinnamic acid and 2,2,-dimethyl-6-carboxyethenyl-2H-1-benzopyran. Again the origin of these compounds, i.e. the plant source is not known. It is assumed, though, that they might come from *Baccharis* and *Flourensia* species that have been shown previously to produce the same prenylated cinnamic acid derivatives (cf. Bankova *et al.,* 1995).

Bankova *et al.* (1995) also report on the antibacterial activity of Brazilian propolis. Major constituents that they identified comprise diprenyl acetophenone, 2*Z*,6*E*-farnesol, a sesquiterpene alcohol, dihydrocinnamic acid and *p*-coumaric acid. Only

traces of an unidentified flavone and of an unidentified flavanone were detected. It is obvious that the propolis samples studied here were not collected from poplar buds. Bankova *et al.* (1995) in their Brazilian propolis samples did not find the polyprenylated benzophenones that Tomás-Barberán *et al.* (1993) had found in material from Venezuela nor did they encounter prenylated cinnamic acid derivatives as they have been reported by Aga *et al.* (1994) for propolis from Brazil. Two of the samples studied, however, contained considerable amounts of prenyl acetophenone and diprenyl acetophenone, respectivly. This might point to *Flourensia heterolepis*, a plant producing prenylated hydroxyacetophenones (Bankova *et al.*, 1995).

In summary it can be stated that among the papers discussed above, only the one by Tomás-Barberán *et al.* (1993) brings direct proof for a plant source of propolis not derived from poplar bud exudate. Our findings on *Encelia farinosa* resin as a source are likewise based on the presence of several compounds in both materials. The present results with cottonwood and triangular leaf bur sage are even more convincing as they are based on completely identical sets of flavonoids ("fingerprint") in both, *Populus fremontii* and *Ambrosia deltoidea* exudates and propolis.

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