

Analysis of pollen and nectar of *Arbutus unedo* as a food Source for *Bombus terrestris* (Hymenoptera: Apidae)

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Abstract: The mineral, total amino acid, and sterol compositions of pollen collected by *Apis mellifera* L. were compared with the pollen of a plant consumed by *Bombus terrestris* (L.): *Arbutus unedo* L. This plant provides the predominant food resource for the main autumn generation of *B. terrestris* in southern France. Honey bees also forage on this plant, although only for nectar. The mineral composition of 30 pollen samples collected by honey bees is close to the presently known requirements of *A. mellifera*, except for Cu and Mn, which are substantially lower. The total amino acid mean composition of a set of 54 pollen samples fits the basic requirements of honey bees except for valine, isoleucine, and methionine, which are present in lower concentrations in all the samples. For pollen of *A. unedo*, the amino acid balance is not very different from that of the survey. The main sterolic component in pollen of *A. unedo*, β -sitosterol, is known to have antifeedant effects on *A. mellifera*. Honey bees cannot dealkylate C₂₉ sterols like β -sitosterol or 85-avenasterol to obtain C₂₇ cholesterol and ecdysteroids. Because these phytosterols as well as cholesterol are nearly absent from pollen of *A. unedo*, the metabolic capabilities of *Apis* seem unadapted to this plant. On the contrary, pollen of *A. unedo* is freely consumed by *B. terrestris*, which develops huge autumn populations solely on this food. These data indicate that the sterolic metabolisms of *B. terrestris* and *A. mellifera* differ, allowing separation in foraging activity.

Keywords: *Bombus* – *arbutus* – amino acids – sterols – pollination.

The dietetics of bumblebees is still poorly understood. The only food sources used in commercial rearing are sucrose or invert sugar solutions (as substitutes for nectar) and frozen pollen collected by honey bees. This diet seems to provide adequate nutrition for good development of colonies (Plowright and Jay 1966, Rio seler 1985).

For commercial purposes, the nutritional requirements of bumblebees are generally considered equivalent to those of *Apis mellifera* (L.), which have been investigated in detail. Several studies have described *A. mellifera* basic requirements for proteins and amino acids (De Groot 1953), minerals (Nation and Robinson 1968, Herbert and Shimanuki 1978b, Herbert 1979), and sterols (Herbert *et al.* 1980; Svoboda *et al.* 1980, 1991; Feldlaufer *et al.* 1985, 1986a, b; Feldlaufer and Svoboda 1988; Svoboda and Feldlaufer 1991). Requirements for vitamins have been provided by Nation and Robinson (1968), Haydak and Dietz (1972), and Herbert and Shimanuki (1978c, d). Older overall nutritional requirements have been reviewed by Chauvin (1968).

Under field conditions, however, bumblebees and honey bees do not forage on exactly the same range of flowers, with some flowers intensively exploited by the former and ignored by the latter (Fussel and Corbet 1991).

Until now, the foraging literature in pollination systems has been dominated by assessing economic decisions made in nectar foraging (Heinrich 1979, 1983; Cibula and Zimmerman 1984; Cartar 1991; Chittka and Thomson 1997).

Few studies have emphasized the role of dietary proteins, lipids, vitamins, or minerals in the foraging strategy (Baker and Baker, 1975, 1983; Prys-Jones and Willmer 1992). Because foraging and cost-benefit studies have rarely taken nonsugar components of nectar and pollen into account (Waddington 1987), it is not known how these dietetic parameters could affect behavioral choices (Galen and Plowright 1985).

Bombus terrestris (L.) is commercially reared by several companies for pollinating glasshouse fruit (De Wael *et al.* 1990, Navez and Budin 1990, Banda and Paxton 1991, Kevan *et al.* 1991, van Ravestijn and van der Sande 1991).

At the University of Mons-Hainaut, we have been rearing *B. terrestris* since 1990, and it now is obvious that some kinds of pollen are better than others (Ribeiro *et al.* 1996). For example, sunflower, *Helianthus annuum* L., pollen does not allow good colony development. Other pollen, such as from willow (*Salix* spp.) or rape (*Brassica* spp.), is valuable.

In Mediterranean areas, *B. terrestris* is polyvoltine with an autumn generation (Ferton 1901, Krausse 1910, Rasmont 1985, Ricciardelli d'Albore 1986, Maciel de Almeida Correia 1991, Duhayon and Rasmont 1993). During winter, few

plants are available for feeding and in some areas, as in the "Massif des Maures" hills (southeastern France), the lowers of the strawberry tree, *Arbutus unedo* L., provide the only source of pollen and nectar for *B. terrestris* (Rasmont 1985). The emergence of young queens from estivation in the end of September is synchronized with the blooming of strawberry trees. The queens, workers, and eventually the males forage nearly exclusively on this tree during one generation. The disappearance of this bumblebee during a short hibernation in January and February is correlated with the end of the lowering period of this plant (Duhayon and Rasmont 1993). From 1991 to 1995, we have made 7,195 field observations of the autumn generation of *B. terrestris* on flowers, and in the wild areas, 7,173 of these observations involved foraging on *A. unedo*. That it is nearly the only food available for the whole autumn generation seems to indicate that nectar and pollen of *A. unedo* satisfy the needs of *B. terrestris*. The high population density of *B. terrestris* in scrub of the Massif des Maures (between 1000 and 10,000 bumblebees/ha) (Duhayon 1992, 1993) is further evidence that *A. unedo* provides a well balanced diet for the bumblebees.

During autumn, honey bees also forage on strawberry trees side by side with *B. terrestris* (Rasmont 1985). They only were observed to collect nectar, not pollen. Although the pollen of *A. unedo* seems an adequate diet for *B. terrestris*, it is not used by honey bees.

In this study, pollen and nectar of *A. unedo* have been used as models for a first evaluation of dietary requirements of *B. terrestris*. The objective of this study is to analyze the composition of sugars, proteins, amino acids, sterols, and minerals of the pollen and nectar of *A. unedo* and to compare them with pollen collected by honey bees.

MATERIALS AND METHODS

Sampling of Pollen of *A. unedo*

Pollen and nectar of strawberry trees were collected in southeastern France matorrals (Var, Collobrières, Jas de la Moutte, 43° 17' N 6° 23' E, 652 m in altitude) in November and December 1991 and 1992. Foraging bumblebee workers were collected with an insect net. Pollen was collected with tweezers and conserved in sterile plastic containers. Each evening, the containers were deep frozen. We collected 2.5 g of pollen, corresponding to the load of 242 *B. terrestris* workers in 4 d. In November and December 1991 and 1992, during sampling, the temperature varied from frost to $\approx 10^{\circ}\text{C}$, and *B. terrestris* was seen lying regardless of weather.

Commercial Pollen

Fifty-five pollen samples collected from honey bees and provided by various commercial beekeepers also were analyzed. All pollen came from southern France. The botanical composition of all commercial pollen was determined by the apicultural workgroup of the Université Catholique de Louvain (P. Lebrun, E. Bruneau). This composition was characterized by their most abundant fraction: Brassicaceae (mainly *Brassica napus* L.), 22 samples; Ranunculaceae, 12 samples; *Salix* spp., seven samples; Rosaceae, five samples; orchards, four samples; *Castanea sativa* L., three samples; and *Trifolium* spp., two samples.

Sampling *A. unedo* Nectar

A. unedo nectar was collected from two regions of southern France. The first was at the same station as that for pollen. Capillary tubes (20 μl) were used to extract nectar from the nectaries. In 4 d in early December 1992, 101 tubes were filled (2-ml final volume). In 2004, additional samples were collected from south central France near Montpellier. In October 2004, three trees were sampled in the mountain range L'Arboussas (Hérault, La Boissière, 43° 40' N, 3° 37' E, ≈ 200 m in altitude) with a further plant tree sampled at 43° 37' N, 3° 37' E (Hérault, St-Paul-et-Valmalle, ≈ 200 m in altitude). For each plant, nectar volume of nine or 10 individual flowers was measured using 5- μl glass capillaries. Sugar content was measured using a hand-held refractometer (ATAGO HSR 500, ATAGO Co. Ltd., Tokyo, Japan). Finally, a return visit was made to L'Arboussas (Mas de Navas; 43° 37' N, 3° 37' E, 200 m in altitude) in late December 2004, and two more lowers were evaluated for nectar concentration. This was done because the October 2004 and December 1992 measurements differed strongly in sucrose concentration (see below), and we wanted to ascertain whether these differences were an effect of climatic conditions (December measurements were made after nocturnal frosts).

Pollen Analysis

The very low quantity of pollen and nectar of *A. unedo* did not allow us to replicate the characterization techniques. However, for each analysis, all measurements have been repeated to ensure significant precision. The methods are summarized here, and the explanations are sufficiently complete to allow the duplication of the analysis.

Sugar concentration of pollen was determined by a colorimetric method (Dubois *et al.* 1956). A solution of pollen sugars was prepared by extracting them with demineralized water in a Potter homogenizer. Reaction of this extract with phenol-H₂SO₄ reactant yields a brown-yellow solution that was analyzed spectrophotometrically at 480 nm. The sugar concentrations were determined with D-glucose as reference.

Protein concentration of pollen was determined by micro-Kjeldahl analysis, which measures the amount of nitrogen (Kirk 1950). This titrimetric method is based on the mineralization of the pollen sample in H₂SO₄, leading to the release of the pollen nitrogen in a mineral form. The total protein is estimated as 6.25 times the nitrogen content (Rabie *et al.* 1983).

Amino acid analyses were obtained with an automatic amino acid analyzer (Alpha Plus II, Pfizer, Inc., Täby, Sweden) after hydrolysis of the sample in 6 M HCl at 110°C for 24 h. The results were expressed for fresh and dry matter. The dry matter amount was determined after drying the pollen for 24 h at 103°C. The entire process has been repeated two times for each sample.

Mineral concentration of pollen was determined by atomic absorption spectrometry (Welz 1985) by using an atomic absorption spectrophotometer with an air-acetylene burner (for Ca, Mg, Fe, and Zn) or graphite furnace (for Cu and Mn). A solution of mineralized pollen was obtained by processing the dry matter in a furnace (6 h, 400°C). The ashes are then treated with HNO₃ (1:1). The final solution is 1% HNO₃. For Na and K, we used a flame photometric method with a typical conical burner (air-acetylene). The minimal sample used for this analysis was 319 mg (for *A. unedo*). The measurements were repeated three times.

For sterols, the method of analysis used was adapted from Lognay *et al.* (1992). Before each analysis, the collected pollen was lyophilized and homogenized carefully. Two hundred milligrams of pollen was saponified with 20 ml of a 2 M KOH methanolic solution for 1 h at 75°C. After addition of 1 ml of internal standard (ethanolic solution of 0.25 mg/ml betulin), the solutions were diluted with 20 ml of water and extracted three times with 20 ml of diethyl ether. The pooled ethereal extracts (crude unsaponifiable matter) were washed three times with 20 ml of distilled water and then concentrated until dryness at 35°C under reduced pressure. After silylation, the sterols were identified means of gas liquid chromatography as trimethylsilyl ethers with betulin as internal standard. The entire process was conducted three times.

Nectar Analysis

The methods used for the analysis of *A. unedo* nectar were identical to those used for the pollen, except for the 2004 samples, where a hand-held refractometer was used to measure sugar content. The protein level of nectar was determined according to the Bradford technique (Peterson 1983, Delobette *et al.* 1991). The amino acid analyses were determined by direct nectar injection in the amino acid analyzer. All measurements were repeated at least three times.

A. mellifera Requirements

Many studies have dealt with honey bee nutrition; however, it is not yet possible to feed honey bees colonies with fully synthetic diet for more than a few months. On the basis of the present state of knowledge, *A. mellifera* basic requirements used here as reference are to be taken as indicative.

RESULTS

The composition of *A. unedo* nectar is presented in Table 1. This nectar seems essentially a solution of sugars in water: concentrations of amino acids and lipids are nearly nil; the mineral matter is only 0.48% with Zn, Cu, and Mn in low concentrations. The K:Ca ratio is low at 0.25. Nectar standing crop (nectar volume per flower) was extraordinarily high. Flowers from different trees contained, on average, between 3.6 and 7.1 µl of nectar. In October 2004, sugar concentration ranged from 31 to 43%, whereas in December, after frosts, the concentration was 10% in 2004 and 16.5% in 1992 (Table 2).

Mineral content of 30 of the 54 studied samples and in pollen of *A. unedo* is given in Table 3, compared with honey bee requirements. The mean total mineral content of the 30 samples exceeds that of honey bee requirements. For *A. unedo*, this mineral content is lower but remains in the range of the estimated requirements given by Nation and Robinson (1971), Herbert and Shimanuki (1978a), and Herbert (1979). For both series, the Ca and K concentrations exceed *Apis* requirements, but the K:Ca ratio falls in the range. The Cu and Mn concentrations are clearly below the estimated requirements for nearly all the pollen studied, including *A. unedo* (Cu: 30 deficiencies on 30 samples; Mn: 23/30). Slight deficiencies also may occur for Zn (18 deficiencies on 30 samples and in *A. unedo*), Fe (18/30 and in *A. unedo*), and Na (17/30).

Table 1. Composition of the nectar of *A. unedo* (mean of three measurements).

	Concn in fresh matter
Dry matter	17.1%
pH	6.0
Total sugar	16.5%
Mineral matter	0.48%
Total amino acids	Trace
β -Sitosterol	Trace
Ca	370 ppm
K	88 ppm
Na	253 ppm
Mg	69 ppm
Fe	10 ppm
Zn	5 ppm
Cu	2 ppm
Mn	Trace
K:Ca ratio	0.25

Table 2. Nectar volume and sugar content of flowers sampled near Montpellier in October and December 2004.

Date	Plant	Vol (μ l)	Sugar content (%)
Oct	A	3.9 \pm 0.6 (10)	41.0 (1)
	B	4.8 \pm 0.4 (10)	31.0 (1)
	C	4.4 \pm 0.5 (10)	43.0 (1)
	D	7.1 \pm 1.1 (10)	31.9 \pm 2.3 (9)
Dec.	E	3.6 \pm 0.6 (2)	10.0 \pm 0.0 (2)

Mean \pm SE, numbers in parentheses indicate the numbers of flowers sampled.

Table 4 presents the results of amino acid analysis. The *A. mellifera* requirements (De Groot 1953) are compared with the measured concentrations by using a relative value of 3.0 for threonine to allow scanning for relative deficiencies (McCaughey *et al.* 1980). Methionine, isoleucine, and valine are deficient in nearly all pollen, including that of *A. unedo*. The methionine concentration is especially low for *A. unedo*. Leucine is deficient in nine of the 54 pollen species studied, histidine in three samples, and phenylalanine in one. These three amino acids are not deficient in pollen of *A. unedo*.

For the sterols, reference is made to a complete survey of a large number of honey bee pollen sources published by Simal *et al.* (1988) (Table 5). Pollen of *A. unedo*, foraged by *B. terrestris*, shows a huge concentration of β -sitosterol. The survey of Simal *et al.* (1988) shows a great concentration of 24-methylenecholesterol + campesterol fraction, but it is generally 85-avenasterol that is the most abundant.

Table 3. Minerals in pollen of *A. unedo* and in 30 pollen samples from southern France compared with *A. mellifera* satisfying balance.

Mineral matter	Concn in pollen of <i>A. unedo</i>	Mean concn in 30 pollen samples	Satisfying balance for <i>A. mellifera</i>	
			A	B
Dry matter	75.0%	75.3%		
Total mineral matter	1.7%	3.5%	3.0%	1.0%
Ca	1836 ppm	1573 ppm	1000 ppm	500 ppm
K	5543 ppm	5528 ppm	5000 ppm	1000ppm
Na	431 ppm	54 ppm	200 ppm	50 ppm
Mg	698 ppm	971 ppm	1000 ppm	300 ppm
Fe	44 ppm	59 ppm		50 ppm
Zn	28 ppm	50 ppm		50 ppm
Cu	6 ppm	7 ppm		50 ppm
Mn	13 ppm	37 ppm		50 ppm
K:Ca ratio	3.1	3.5	5.0	2.0

Potential deficiencies are in bold. All measurements have been repeated three times for each sample. All concentrations are given for fresh matter. Data are from Herbert and Shimanuki (1978a) and Herbert (1979) (A) and Nation and Robinson (1971) (B).

DISCUSSION

The normal concentration of sugars in nectar extends from 10 to 72% (wt:wt) (Percival 1961, South-wick *et al.* 1981, Roubik 1989); a typical value is 30% (Heinrich 1979). The nectar of *A. unedo* falls within this range but varies greatly over time. In late October, when queens are establishing colonies, *A. unedo* nectar has a high sugar content (31-41%) (Table 2), which is supplied in relatively large volumes; 3.6-7.1 μ l per flower (Table 2) is an energetically important resource. This is confirmed by a previous study in Sardinia (Chittka *et al.* 2004), where *A. unedo* lower visitors were excluded for fixed amounts of time to measure nectar production rates. Nectar production was found to 490 μ g per sugar per lower per day, one of the highest production rates in Sardinia.

However, it seems that nectar quality falls over time to between 10 and 16.5% in December, when colonies are producing males and new queens. It is likely that this change is related to the lower daytime temperatures and increase in frosty nights during December. However, it is surprising that at this time, *A. unedo* nectar, one of the sole resources that *B. terrestris* can forage on at low late autumn temperatures, is energetically so poor. It seems possible, therefore, that due to its high sugar concentration (33.4%), pollen of *A. unedo*, not nectar, is the main carbohydrate source for *B. terrestris* in the late stages of the Mediterranean autumn generation.

Baker and Baker (1973, 1975) hypothesize that nectar could be a significant nitrogen source for some pollinators. Because the content of amino acids and ammonia in strawberry tree nectar is nearly nil, this hypothesis is not supported for *B. terrestris* in southern France.

Insects, in general, and honey bees in particular, require a K:Ca ratio >2 and near 4 (Beck *et al.* 1968, Herbert and Shimanuki 1978b, Herbert 1979). The ratio in nectar of *A. unedo* is inverted (0.25); however, this is probably not a problem because of the low mineral concentration of the nectar and the normal K:Ca ratio in the pollen of *A. unedo*.

The survey of 30 pollen types collected from *A. mellifera* shows an apparent deficiency in Cu and Mn in most pollen compared with *A. mellifera* satisfying balance estimated by Herbert and Shimanuki (1978b) and Herbert (1979) (Table 3). These studies and Nation and Robinson (1968) report that mineral content of natural pollen is often suboptimal. However, Herbert and Shimanuki (1978b) indicated that their honey bee requirement estimates were approximations. Our data indicate a lower value for Cu and Mn.

Table 4. Total amino acids (a.a.) in pollen of *A. unedo* and of 54 honey bee pollen samples from southern France compared with amino acid satisfying *A. mellifera*.

<i>A. unedo</i> pollen (mean of 2 processes)		Mean content of 54 pollen samples (mean of 2 processes for each sample)		<i>A. mellifera</i> satisfying balance (De Groot 1954)	
Dry matter	75.2%		75.3%		
Total carbohydrates	33.4% ^a		N.S.		
Total amino acids	16.4% ^a		20.6% ^a	17-23%	
Total amino acids	% Total a.a.	Threonine 3% scale	% Total a.a.	Threonine 3% scale	Threonine 3% scale
Arg ^b	8.9	5.6	4.7	3.2	3.0 ^{c,d}
His ^b	2.6	1.6	2.4	1.7	1.5 ^c
Lys ^b	7.6	4.8	7.1	4.9	3.0 ^c
Try ^b	N.S.	N.S.	N.S.	N.S.	1.0 ^{c,d}
Phe ^b	4.9	3.1	4.2	2.9	2.5 ³
Met ^b	1.0	0.7	1.6	1.1	1.5 ^c
Thr ^b	4.8	3.0	4.4	3.0	3.0 ^c
Leu ^b	7.9	5.0	6.8	4.7	4.5 ^c
Ile ^b	4.4	2.8	4.0	2.8	4.0 ^c
Val ^b	5.5	3.5	4.7	3.2	4.0 ^c
Ser	6.0	3.7	5.2	3.6	+ ^e
Gly	5.1	3.2	4.5	3.1	+ ^e
Pro	5.6	3.5	8.7	6.0	+ ^e
Cystine	0.8	0.5	1.0	0.7	
Asp + Asn	10.4	6.5	9.8	6.7	
Glu + Gln	15.0	9.5	10.1	7.4	
Ala	6.1	3.8	5.2	3.6	
Tyr	3.1	2.0	2.9	2.0	
α -Amino butyric acid			0.4	0.3	
% Identified	99.7		88.2		

The process has been repeated two times for each sample. Potential deficiencies are in bold. Strong and frequent deficiencies: Ile (54 deficiencies on 54 samples), Val (54/54), and Met (53/54); low and occasional deficiencies: Leu (9/54), His (3/54), and Phe (1/54). N.S., not studied.

^a Concentration in fresh matter.

^b Essential amino acids.

^c Essential amino acids balance determined by De Groot (1953).

^d Phagostimulant effect (Chalmers 1980).

^e High concentrations increase *A. mellifera* weight (Chauvin 1968).

Table 5. Sterols in the pollen of *A. unedo* compared with a survey of 35 pollen collected from honey bee (Simal *et al.* 1988).

Sterol	<i>A. unedo</i> (mean of 3 processes)	Mean of 35 <i>A. mellifera</i> pollen (Simal <i>et al.</i> 1988)
Cholesterol	30 $\mu\text{g/g}$ (0.8%)	4.0%
Brassicasterol	8 $\mu\text{g/g}$ (0.2%)	
24-Methylenecholesterol + Campesterol ^a	149 $\mu\text{g/g}$ (4.0%)	29.8%
Stigmasterol	19 $\mu\text{g/g}$ (0.5%)	
δ 7-Campesterol	4 $\mu\text{g/g}$ (0.1%)	
β -Sitosterol	1,363 $\mu\text{g/g}$ (37.3%)	16.7%
Stigmastanol	4 $\mu\text{g/g}$ (0.1%)	
δ 5-Avenasterol	653 $\mu\text{g/g}$ (17.9%)	46.4%
δ 7-Stigmasterol	316 $\mu\text{g/g}$ (8.6%)	
δ 7-Avenasterol	738 $\mu\text{g/g}$ (20.2%)	1.2%
Others	376 $\mu\text{g/g}$ (10.3%)	1.9%
Total sterols	3660 $\mu\text{g/g}$ ^b	1440 $\mu\text{g/g}$ ^c

^a under the analytical conditions, campesterol and 24-methylenecholesterol are nearly impossible to separate; the results are therefore pooled.

^b Expressed in fresh matter.

^c Total ether extracted and unsaponifiable fraction of a commercial dried pollen.

As for the other pollen surveyed, that of *A. unedo* has Cu and Mn levels clearly lower than *A. mellifera* needs, as indicated by Herbert and Shimanuki (1978b) and Herbert (1979). This mineral balance, nevertheless, sustains large *B. terrestris* populations. We cannot exclude that some minerals can be obtained elsewhere.

Total mineral matter of *A. unedo* (1.7%) is near the optimal level for *A. mellifera* (Herbert 1979) (Table 3). The K:Ca ratio of *A. unedo* and of the other pollen types its basic insect requirements.

The amino acid content and balance of honey bee pollen surveyed here are congruent with the basic requirements of bees as defined by De Groot (1953). Levels of valine, isoleucine, and methionine, however, seem to be deficient in most samples. Because it is unlikely that honey bees forage consistently for nutritionally deficient food, it is possible that the estimates by De Groot for these three amino acids are too high. Nevertheless, that study clearly defines methionine as a principal limiting factor for *A. mellifera*. Other deficiencies may occur for some amino acids such as leucine, histidine, and phenylalanine.

The total amino acid content in pollen of *A. unedo* is very low for *A. mellifera* (Herbert *et al.* 1977), but the sugar level is high. Possibly, the high sugar concentration in pollen may compensate for the low sugar level in nectar. We can suggest, in this condition, that the total amount of pollen consumed by *B. terrestris* colonies must be high, and it is therefore understandable to observe a very low amino acid level.

As for nearly all the other pollen surveyed, valine, isoleucine, and methionine levels in *A. unedo* are below the basic requirements defined by De Groot (1953), who could have overestimated them. The amino acid balance of *A. unedo* pollen seems to be compatible with the honey bee requirements, and it is also appropriate to sustain *B. terrestris* populations. It is surprising that honey bees never forage on *Arbutus* for pollen, despite its nearly correct amino acid and mineral balances and the lack of other local winter resources.

Herbert *et al.* (1980) demonstrated that 24-methylenecholesterol and cholesterol are easily used by honey bees but that campesterol, stigmasterol, and β -sitosterol are not. In their experiments, stigmasterol and β -sitosterol had a clear antifeedant effect.

Phytosterols are not directly used by most insects; most phytophagous species dealkylate them through one of the several pathways: 1) from sitosterol through fucosterol to cholesterol; 2) from campesterol through 24-

methylenecholesterol to cholesterol; or 3) from stigmasterol to cholesterol (Svoboda *et al.* 1991, Svoboda and Feldlaufer 1991). The cholesterol is then metabolized to obtain ecdysteroids, the major insect molting hormones.

Svoboda *et al.* (1980) demonstrated that 24-methylenecholesterol is the primary sterol present in larval honey bees. Honey bees receiving a diet supplemented with sitosterol do not increase their cholesterol concentration, and Svoboda *et al.* (1980) interpreted this finding as a strong evidence that they cannot dealkylate sitosterol to cholesterol. The isofucosterol ($\approx \delta 5$ -avenasterol) concentration of honey bee larval tissues also seems not correlated with a β -sitosterol diet, suggesting that isofucosterol is not the result of dealkylation of β -sitosterol. Svoboda *et al.* (1983) demonstrated that metabolism of *A. mellifera* lacks all possibilities for phytosterol dealkylation, preventing use of sterols such as $\delta 5$ -avenasterol, β -sitosterol, and other C₂₈ and C₂₉ sterols to obtain C₂₇ cholesterol and ecdysones.

Campesterol (normally converted into 24-methyl-enecholesterol) is related in *A. mellifera* to a 28-carbon molting hormone: makisterone A (Feldlaufer *et al.* 1985, 1986a, b; Feldlaufer and Svoboda 1988). Because *A. mellifera* lacks a dealkylation mechanism and uses such an uncommon ecdysteroid, these studies suggest that a special metabolic pathway exists in this insect, probably also including 24-methylenecholesterol. These considerations could explain why *A. mellifera* is unable to use stigmasterol or β -sitosterol. It also gives an explanation to the antifeedant effect of β -sitosterol observed by Herbert *et al.* (1980).

The sterol composition of *A. unedo* pollen does not seem to meet the metabolic requirements of *A. mellifera* (Table 5). This may explain why the honey bee does not forage on the pollen of strawberry trees but only on their nectar. That during winter, in the Mediterranean region (Spain, France, Italy, Greece, and Turkey, personal observation), *B. terrestris* often feeds exclusively on *A. unedo*, lacking 24-methylenecholesterol, yet develops large populations, is strong evidence that its sterol metabolic pathways may not be the same as those of *A. mellifera*. We plan to verify this hypothesis through an analysis of sterol content of honey bee and bumblebee larvae fed with controlled sterol diets.

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