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Original article

Pollen analysis of honeybee rectum as a method to record the bee pollen flora of an area*

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Abstract – Pollen is very important for the bees' nutrition and it is necessary for their survival and reproduction. In this work we studied the possibility of recording the pollen flora of an area by examining the pollen content of the rectum of honeybees (*Apis mellifera* L.) and comparing the results to these coming from pollen traps. We concluded that the pollen analysis of the rectum of honeybees could be used as a fast screening method of the bee flora of an area. However, for quantitative results, additional methods such as pollen pellet analysis should be applied.

***Apis mellifera* / nurse bees / pollen consumption / melissopalynology**

1. INTRODUCTION

Pollen is very important for the bees' diet and it is necessary for their survival and reproduction (Dietz, 1978). Pollen consumption is significantly correlated to the age and the activities of the honeybee (Dietz, 1978; Eischen et al., 1984; Crailsheim et al., 1992; Hrassnigg and Crailsheim, 1998; Naiem et al., 1999; Loidl and Crailsheim, 2001). Worker bees start to consume pollen just a few hours after emerging and this amount reaches a maximum when the bees are four to nine days old (Zherebkin, 1965; Hagedorn and Moeller, 1967; Haydak, 1970; Crailsheim et al., 1992). Pollen consumption varies with different worker-larvae ratios. More pollen is consumed when more brood is present (Al-Tikrity et al., 1972; Pearson and Braiden, 1990; Camazine, 1993; Hrassnigg and Crailsheim, 1998; Pankiw et al., 1998; Dreller et al., 1999; Dreller and Tappy, 2000). Generally, nurse bees consume high quantities of pollen compared to foragers or drones (Crailsheim et al., 1992; Szolderits and Crailsheim, 1993; Naiem et al., 1999).

Considering that pollen is required for growth of honey bee colonies, the knowledge of bee flora in an area constitutes a basic tool for the development of apiculture. Pollen traps have been used widely as a method to record the pollen flora of an area (Severson and Parry, 1981; Biesmeijer et al., 1992; Telleria, 1993; Pearson and Braiden, 1990; Coffey and Breen, 1997; Andrada and Telleria, 2005; Dimou and Thrasyvoulou, 2007). However, the use of pollen traps is not always feasible for some bee species nor desirable, since it may influence the nectar foraging behaviour of the colony in a negative way (McLellan, 1974; Webster et al., 1985; Duff and Furgala, 1986; Fewell and Winston, 1992; Dreller et al., 1999).

Pollen is transported to the honey stomach and then removed into the midgut where digestion and absorption occur. Posterior to this is the rectum where water and feces are stored until the bee exits the hive and defecates (Seeley, 1995). Thus, pollen analysis of the rectum of the bees could be used as an alternative method to record the bee flora of an area. However, the use of this method

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is limited (Ramírez-Arriaga and Martínez-Hernández, 1998; Oliveira et al., 2002) and no data about its validation exists.

In this research, we examined the possibility to study the bee pollen flora of an area by analysis of the pollen content from the rectum of honeybees. To validate this method, we compared the pollen data collected from the bee rectum to that coming from the pollen trap analysis and we investigated the sampling procedure considering the amount of pollen consumed by the honeybees.

2. MATERIALS AND METHODS

The experiments took place at an apiary located on the farm of the Aristotle University of Thessaloniki (Greece).We fitted two pollen traps (A and B) in the entrance of two respective hives of *Apis mellifera* L., during spring. After a few days, we marked several newly emerged bees from each colony. The same procedure was repeated two days later. We collected 40 marked nurse bees from each colony when they were seven-days-old and examined their rectum content for pollen. We removed and weighed the rectum and we mixed it by vortex with 1 mL distilled water in a test tube. We then spread 50 μL of the solution onto a 22×22 mm area on a slide. Ten fields of view distributed uniformly over the area were analysed at a magnification of 400X using light microscopy, counting on average 753 ± 342 (mean ± sd) pollen grains per slide.

To minimize and simplify the sampling procedure, we also randomly collected twenty honeybees of no particular age from one hive. The pollen content of the rectum of these honeybees was compared to the pollen content of the rectum of a respective number of seven-days-old nurse bees from the same hive to investigate whether the higher pollen consumption of nurse bees could affect the results of the study. We removed and weighed the rectum of the bees, we mixed it with 1 mL distilled water and examined 50 μL of the solution as mentioned above under a microscope, counting on average 688 ± 379 (mean ± sd) pollen grains per slide.

In spring, when the brood area expands, the amount of pollen collected increases as does pollen consumption. On the contrary, in autumn the bees consume much less pollen. Thus, to ensure that the amount of pollen consumed by the honeybees did not affect the results of the study, the same experiments were repeated during autumn.

Finally, during the experiments, we collected daily the trapped pollen from the two hives. We thoroughly mixed 10% of the pollen loads of the initial trapped amount and then diluted it in distilled water (1:10) (Dimou et al., 2006). The pollen grains of 50 μL of the solution were analysed as mentioned above, counting on average 645 ± 228 (mean ± sd) pollen grains per slide.

The identification of the pollen types was carried out using the reference slide collection of the studied area from the Laboratory of Apiculture and Sericulture of Aristotle University (Dimou and Trasyvoulou, 2007).

The experiments were restricted to a short time period to avoid major changes in the pollen flow that could have an effect on the differences between the pollen pellets and the bee rectum analysis. Indeed, the daily pollen abundance collected from the colonies did not show statistically significant differences (minimum observed value $P = 0.105$).

The nonparametric Mann-Whitney test was used to compare the results between the colonies, between the pollen trap and the bee rectum analysis, and between the group of the nurse bees and the group of the various-age bees. The non-parametric test was preferred since the normality and homogeneity of variance assumptions did not hold in all cases. The normality assumption was tested using the Kolmogorov-Smirnov test and the homogeneity of variance was tested using Levene's test. The observed significance level (P-value) of the nonparametric tests was estimated by Monte-Carlo simulations (Mehta and Patel, 1996). The analyses were carried out using SPSS V.12 enhanced with the module Exact Tests. The significance level of all the statistical tests was set at $\alpha = 0.05$.

3. RESULTS

The weight of the rectum of the nurse honeybees during spring (26.9 ± 13.3 mg) was not statistically significantly different from the weight in autumn (28.0 ± 12.3 mg) $P = 0.727$). However, we found statistically significant differences in the rectum weight between the sample of nurse bees and the sample of various-age honeybees ($P < 0.001$). The mean weight and standard deviation of the rectum at the last group was 8.9 ± 3.1 mg in spring and 9.5 ± 2.8 mg in autumn.

During spring we recorded 18 pollen types in the rectum of the nurse bees and 17 pollen

Table I. Frequency of pollen types in pollen traps and nurse bee rectums during spring.

Pollen type	Percentage (%) of pollen grains in	
	Pollen trap (mean ± sd)	Rectum (mean ± sd)
Adonis Type	11.92 ± 4.66	14.89 ± 8.32
Campsis radicans (Bignoniaceae)	1.07 ± 1.44	2.24 ± 4.22
Carduus Type	0.02 ± 0.03	0.12 ± 0.37
Chenopodiaceae	2.04 ± 4.16	1.10 ± 2.34
Cistus sp. (Cistaceae)	1.56 ± 2.59	0
Convolvulus arvensis (Convolvulaceae)	2.28 ± 0.97	1.94 ± 2.51
Daucus carota (Apiaceae)	4.10 ± 4.30	3.92 ± 5.63
Elaeagnus angustifolia (Elaeagnaceae)	0.52 ± 0.38	0.91 ± 1.78
*Ligustrum japonicum** (Oleaceae)	3.04 ± 2.27	6.80 ± 7.92
*Olea europea** (Oleaceae)	0.84 ± 0.94	16.63 ± 14.81
*Parthenocissus inserta** (Vitaceae)	17.53 ± 9.15	1.04 ± 1.52
*Pastinaca sativa** (Apiaceae)	9.00 ± 7.95	0.65 ± 1.15
Pinus sp. (Pinaceae)	0	0.73 ± 3.02
Sisymbrium irio (Brassicaceae)	10.71 ± 3.59	11.18 ± 8.08
*Taraxacum officinale** (Asteraceae)	2.38 ± 1.25	0.29 ± 0.44
Thymus Type	0	0.04 ± 0.17
Tilia intermedia (Tiliaceae)	3.41 ± 1.69	3.43 ± 2.02
Tribulus terrestris (Zygophyllaceae)	0.30 ± 0.32	0.15 ± 0.41
Trifolium sp.* (Fabaceae)	29.30 ± 4.83	1.22 ± 1.26
Unidentified	0	32.71 ± 10.52

* Statistically significantly different according to Mann–Whitney test ($\alpha = 0.05$).

types in the trap. Most important with regards to their percentage frequency in the traps and/or the rectum were: *Adonis* Type, *Ligustrum japonicum*, *Olea europea*, *Parthenocissus inserta*, *Sisymbrium irio* and *Trifolium* sp. (Tab. I, Fig. 1). All these pollen types were found both in bee rectum and pollen trap analysis. *Cistus* sp. was located only in the pollen trap; and *Thymus* Type and *Pinus* sp. were recorded only in the rectum analysis (Tab. I). Statistical analysis showed significant differences with respect to the percentage frequency of the pollen types between honeybee rectum and pollen trap analysis in several cases: *Olea europea*, *Ligustrum japonicum*, *Parthenocissus inserta*, *Pastinaca sativa*, *Taraxacum officinale* and *Trifolium* sp. (Tab. I).

During autumn we recorded 16 pollen types in the rectum of the nurse bees and 18 pollen types in the trap. Most important with regards to their percentage frequency in the traps and/or the rectum were: *Erica manipuliflora*, *Hedera helix*, *Phoenix* Type, *Polygonum aviculare* and *Sisymbrium irio* (Tab. II, Fig. 1). All these pollen types were found both in bee rectum and pollen trap analysis. The two pollen types (*Citrus* Type and *Lagerstroemia indica*) found only in the trap were present in very small concentrations (< 1%) (Tab. II). Statistical analysis showed significant differences with respect to the percentage frequency of pollen types between honeybee rectum and pollen trap analysis in six cases (Tab. II).

The pollen preferences of honey bees can be genetically influenced and thus vary among the colonies of an apiary (Page et al., 1995; Pankiw et al., 2002). In this study, both colonies collected the same pollen spectra except one taxon with minor abundance (*Citrus* Type) which was collected only from one hive. Statistical analysis between the colonies relative to the percentage frequency of each pollen type showed no significant differences in spring (minimum observed $P = 0.095$); while there were three cases (*Rubus ulmifolius*, *Scholmus hispanicus* and *Sonchus* Type) involving minor pollen sources during autumn (Fig. 2).

Figure 1. Main pollen taxa found in pollen loads and bee rectum: (a) *Erica manipuliflora,* (b) *Polygonum aviculare*, (c) *Ligustrum japonicum*, (d) *Parthenocissus inserta*, (e) *Sisymbrium irio*, (f) *Olea europea*. All the plates are in the same scale.

Table II. Frequency of pollen types in pollen traps and nurse bee rectums in autumn.

Pollen type	Percentage (%) of pollen grains in	
	Pollen trap (mean ± sd)	Rectum (mean ± sd)
Ocimum bassilicum (Lamiaceae)	0.04 ± 0.08	0.02 ± 0.05
Chenopodiaceae	0.53 ± 0.54	0.53 ± 1.12
Citrus Type	0.05 ± 0.10	0
Daucus carota (Apiaceae)	0.04 ± 0.15	0.41 ± 1.79
*Erica manipuliflora** (Ericaceae)	15.41 ± 11.23	3.38 ± 4.29
Hedera helix (Araliaceae)	10.39 ± 5.93	16.64 ± 16.98
Liliaceae*	0.08 ± 0.14	14.98 ± 17.29
Lagerstroemia indica (Lythraceae)	0.15 ± 0.36	0
Malva sylvestris (Malvaveae)	0.04 ± 0.07	0.01 ± 0.05
Phoenix Type	9.47 ± 11.84	10.55 ± 12.26
*Polygonum aviculare** (Polygonaceae)	28.82 ± 19.02	15.36 ± 11.79
*Portulaca oleraceae** (Portulacaceae)	0.54 ± 0.83	0.06 ± 0.21
*Rubus ulmifolius** (Rosaceae)	0.21 ± 0.45	2.56 ± 3.46
Scholmus hispanicus (Asteraceae)	1.52 ± 1.85	0.68 ± 1.50
Sisymbrium irio (Brassicaceae)	31.40 ± 10.60	28.54 ± 10.09
Sonchus Type*	1.13 ± 1.12	0.28 ± 0.69
Tribulus terrestris (Zygophyllaceae)	0.19 ± 0.24	0.23 ± 0.52
Zea mays (Poaceae)	0.02 ± 0.11	0.03 ± 0.07
Unidentified	0	5.73 ± 3.69

* Statistically significantly different according to Mann–Whitney test ($\alpha = 0.05$).

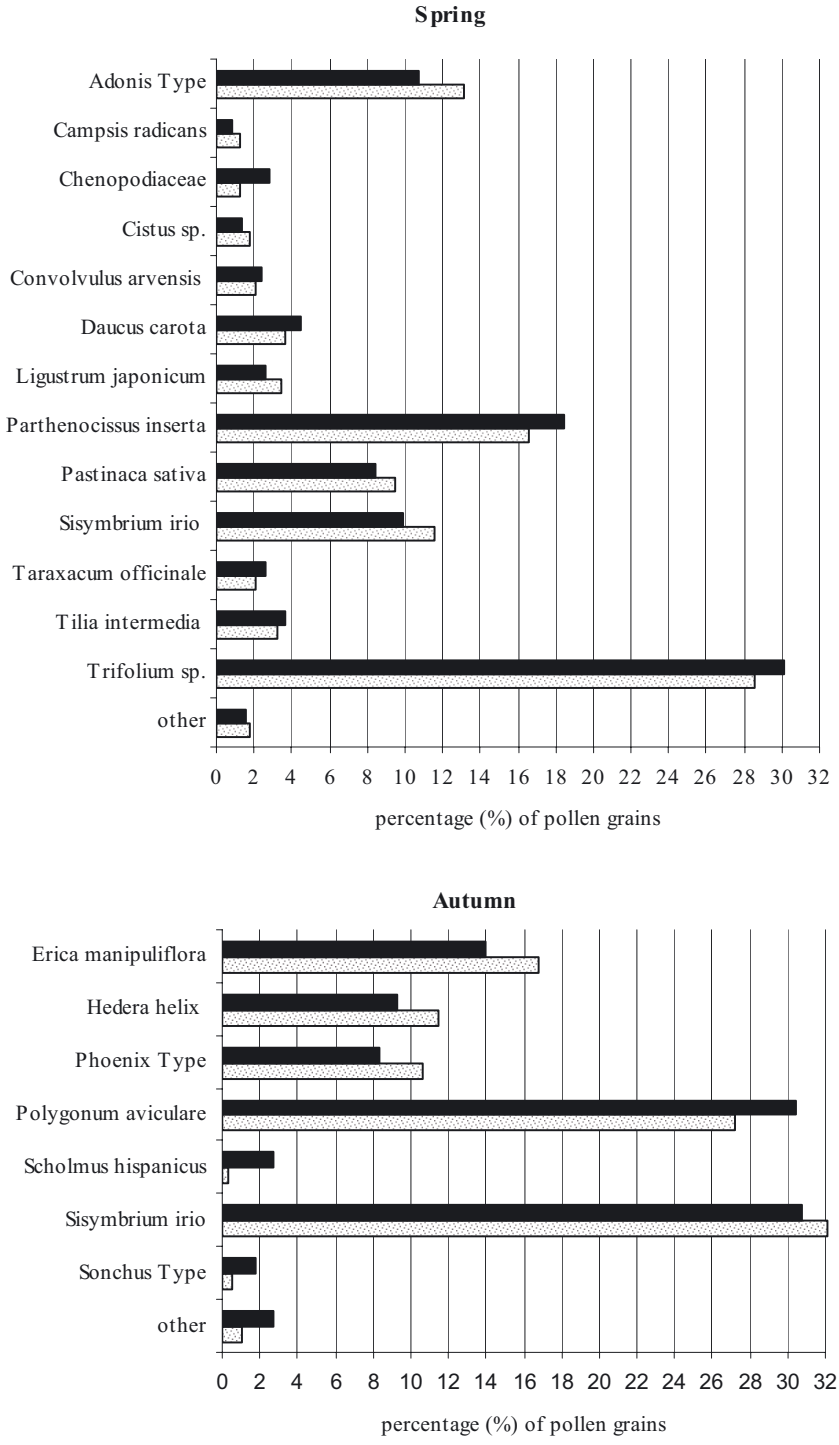

Figure 2. Frequency of pollen types collected by the two colonies (trap A (■), trap B (□)) in percentages over 1% in spring and in autumn.

Table III. Frequency of pollen types in the rectum of nurse bees and various-age bees in autumn.

Pollen type*	Percentage (%) of pollen grains	
	Nurse bees (mean ± sd)	Various-age bees (mean ± sd)
Ocimum bassilicum (Lamiaceae)	0.01 ± 0.03	0.02 ± 0.04
Chenopodiaceae	0.62 ± 1.52	0.79 ± 1.52
Daucus carota (Apiaceae)	0.53 ± 1.94	0.42 ± 1.94
Erica manipuliflora (Ericaceae)	3.93 ± 4.10	4.50 ± 2.82
Hedera helix (Araliaceae)	17.24 ± 6.12	14.70 ± 3.66
Liliaceae	15.01 ± 12.61	15.82 ± 16.26
Malva sylvestris (Malvaveae)	0.01 ± 0.04	0.02 ± 0.23
Phoenix Type	9.01 ± 8.56	8.76 ± 9.21
Polygonum aviculare (Polygonaceae)	12.10 ± 10.34	14.59 ± 6.80
Portulaca oleraceae (Portulacaceae)	0.04 ± 0.24	0.02 ± 0.18
Rubus ulmifolius (Rosaceae)	2.77 ± 3.87	2.38 ± 4.19
Scholmus hispanicus (Asteraceae)	0.45 ± 0.76	0.84 ± 0.79
Sisymbrium irio (Brassicaceae)	31.83 ± 13.44	31.81 ± 15.08
Sonchus Type	0.03 ± 0.09	0.07 ± 0.12
Tribulus terrestris (Zygophyllaceae)	0.40 ± 0.87	0.32 ± 0.96
Zea mays (Poaceae)	0.04 ± 0.06	0.02 ± 0.07
Unidentified	5.98 ± 3.53	4.90 ± 2.86

* No statistically significant differences were observed according to Mann–Whitney test ($\alpha = 0.05$).

The statistical analysis showed no significant differences between the two groups of honeybees (nurse bees and various-age bees) in autumn concerning the percentage frequency of the pollen types in their rectum (minimum observed ($P = 0.083$) (Tab. III). The same pollen types were also recorded in both groups in spring. Statistical analysis showed significant differences only in two cases: *Adonis* Type and *Parthenocissus inserta* (Tab. IV). The percentage frequency of the pollen grains of *Adonis* Type was higher in the rectum of the nurse bees compared to the various-age bees (22.7 ± 5.8% and 15.8 ± 2.9% respectively). In contrast, the percentage frequency of the pollen grains of *P. inserta* was higher in the rectum of the various-age bees compared to the nurse bees (4.6 ± 2.1% and 2.5 ± 1.1% respectively).

4. DISCUSSION

The higher amount of pollen collected in spring did not influence the total weight of the rectum, and consequently the amount of pollen in the rectum of the nurse bees, compared to autumn. This suggests that the use of this method is suitable throughout the year.

Foraging age bees not only consume less amount of pollen, but they also fly and empty their rectum more frequently compared to nurse bees (Crailsheim et al., 1992; Naiem et al., 1999). In this study we found that there were statistically significant differences with respect to the weight of the rectum between the group of the nurse bees and the various-age bees. However, the difference in the weight of the rectum, and consequently the amount of pollen content, did not influence the results. We found no statistically significant differences with respect to the percentage frequency of the pollen types in the rectum between the two groups in autumn and only in two cases (*Adonis* Type and *P. inserta*) in spring.

During the days before the introduction of the nurse bees to the hives we observed that the amount of pollen loads in the pollen traps of *Adonis* Type pollen was increasing, while the amount of *P. inserta* pollen was diminishing. Both this observation and the statistical results mentioned above are in accordance to the conclusions of other authors: nurse bees tend to consume mainly fresh pollen (Doull, 1974).

Table IV. Frequency of pollen types in the rectum of nurse bees and various-age bees in spring.

Pollen type	Percentage (%) of pollen grains in	
	Pollen trap (mean ± sd)	Rectum (mean ± sd)
Adonis Type*	22.66 ± 5.75	15.80 ± 2.87
Campsis radicans (Bignoniaceae)	0.17 ± 0.31	0.12 ± 0.23
Carduus Type	0.04 ± 0.02	0.02 ± 0.03
Chenopodiaceae	0.47 ± 0.56	0.89 ± 0.66
Convolvulus arvensis (Convolvulaceae)	0.94 ± 0.84	0.68 ± 0.64
Daucus carota (Apiaceae)	6.08 ± 3.94	7.59 ± 6.64
Elaeagnus angustifolia (Elaeagnaceae)	1.18 ± 1.02	0.84 ± 1.32
Ligustrum japonicum (Oleaceae)	5.13 ± 1.13	6.66 ± 3.02
Olea europea (Oleaceae)	11.51 ± 7.96	14.79 ± 10.12
*Parthenocissus inserta** (Vitaceae)	2.52 ± 1.10	4.67 ± 2.12
Pastinaca sativa (Apiaceae)	0.84 ± 0.94	0.52 ± 0.64
Pinus sp. (Pinaceae)	0.04 ± 0.27	0.01 ± 0.03
Sisymbrium irio (Brassicaceae)	10.79 ± 4.45	8.45 ± 4.16
Taraxacum officinale (Asteraceae)	0.73 ± 0.78	0.90 ± 1.55
Thymus Type	0.09 ± 0.22	0.33 ± 0.64
Tilia intermedia (Tiliaceae)	2.22 ± 1.36	1.57 ± 0.91
Tribulus terrestris (Zygophyllaceae)	0.19 ± 0.27	0.02 ± 0.06
Trifolium sp. (Fabaceae)	1.04 ± 0.56	0.82 ± 0.51
Unidentified	33.38 ± 4.95	35.32 ± 6.85

* Statistically significantly different according to Mann–Whitney test ($\alpha = 0.05$).

However, the same pollen types were recorded in both groups. Thus, any-age bees of a colony could be used to reveal the bee pollen spectra of an area for a specific time period. Practically, samples of honeybees could be easily collected randomly without requiring previous preparations or colony disturbance at any time during the year and then have their rectums examined for pollen content in the laboratory.

On the other hand, statistical analysis showed significant differences among the two methods that were used to record the pollen flora (pollen trap and rectum analysis), with respect of the percentage frequency of the pollen types in most cases. Most of the pollen types found in the samples came from both polleniferous and nectariferous sources as it has been reported by Crane et al. (1984). In contrast to pollen traps, that only record only the pollen flora, rectum analysis can also reveal pollen grains coming from the nectar (Todd and Vansell, 1942). However, the contribution of pollen from nectar is extremely low compared to directly consumed pollen. A likely explanation for these differences between the two methods was the high percentage of unidentified pollen grains of the rectum analysis. During digestion, a great number of pollen grains can break or shrink. Hence, pollen identification becomes very difficult. Crailsheim et al. (1992) have reported that the number of shrunken and empty pollen grains in the rectum of nurse bees can often be higher than 70%. Similar results have also been reported by Oliveira et al. (2002).

The degree of digestion is affected by the botanical origin, the exine and the intene of the pollen grains (Klungess and Peng, 1984; Peng et al., 1985, 1986; Dobson and Peng, 1997). For example, pollen grains of dandelion are less digested compared to pollen grains with thin wall (Kroon et al., 1974; Peng et al., 1985). On the other hand, pollen identification is mainly based on the sculpture of the exine, the size and the shape of the pollen grains. Since during digestion most of the pollen grains break or shrink, the exine sculpture is critical for the identification. Consequently, pollen grains with distinctive exine ornamentation such as Asteraceae can more

easily be identified after digestion than other pollens, whose exine surface is smooth.

In this study the percentage of the unidentified pollen grains in the bee rectums was much higher in spring than autumn. Although it is known that during digestion enzymes are more active during spring than autumn (Zherebkin, 1965), the botanical origin of the pollen was also a very likely explanation for this difference. A high percentage of pollen grains of *Trifolium* sp., were recorded in the traps but not in the rectum during spring. Due to the morphology of the pollen grain, the degree of digestion of *Trifolium* sp. is relatively high (Crailsheim et al., 1992) making identification difficult. Consequently, the pollen spectra of an area during the year could significantly affect the accuracy of the results coming from the bee rectum analysis.

Although the percentage of unidentified pollen grains found during rectum content examination of honeybees can be relatively high, the great number of pollen grains and the different degree of digestion among the same pollen type (Crailsheim et al., 1992; Fernandes-da-Silva and Serrão, 2000), still allows the analyst to identify and record the full pollen spectra collected by the honeybees in an area. The results accomplished from the analysis of the rectum of the nurse bees and the various age bees were similar to those obtained from pollen pellet analysis with respect to the number of pollen taxa.

In conclusion, the pollen analysis of the rectum of the honeybees can give qualitative information for the bee flora of an area and can be used as a fast screening method, considering the simplicity and time-saving sampling procedure. However, for quantitative results, additional methods such as pollen pellet analysis should be applied.

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Analyse pollinique du rectum de l'Abeille domestique comme méthode pour déterminer les plantes pollinifères d'une région utilisées par les abeilles.

***Apis mellifera* / consommation alimentaire / pollen / mélissopalynologie / abeille nourrice / flore pollinifère**

Zusammenfassung – Pollenanalyse des Enddarms der Honigbienen als Methode zur Bestimmung der Bienenpollenpflanzen in einer Gegend. Die Kenntnis der Bienenpflanzen in einer Gegend ist von grundlegender Bedeutung für die Entwicklung der Imkerei. Pollen ist für die Ernährung der Bienen, ihr Überleben und ihre Reproduktion von essentieller Wichtigkeit. Als Methode der Erfassung der Pollenpflanzen in einer Gegend werden weithin Pollenfallen verwendet, für einige Bienenarten ist dies allerdings nicht praktikabel oder nicht wünschenswert, da die Pollenfallen das Nektarsammeln der Völker negativ beeinflussen. Hier untersuchten wir die Möglichkeit, die Pollenpflanzen in einer Gegend anhand des Pollengehaltes im Enddarm (*Apis mellifera* L.) zu erfassen und die Ergebnisse mit denen von Pollenfallen zu vergleichen. Wir untersuchten den Pollengehalt des Enddarms von Ammenbienen und Bienen sowie die aus Pollenfallen gewonnenen Pollenladungen aus zwei Völkern im Frühjahr und im Herbst. Die Ergebnisse der Untersuchung zeigten keine Unterschiede in der Häufigkeit verschiedener Pollen zwischen den Ammenbienen und den Bienen unterschiedlichen Alters. Dagegen zeigte eine statistische Analyse in den meisten Fällen signifikante Unterschiede zwischen den beiden Methoden zur Erfassung des Pollens (Pollenfallen und Enddarmanalyse). Wir schließen daraus, dass die Pollenanalyse des Enddarms von Honigbienen als rasches Sichtungsverfahren der Bienenflora in einer Region verwendet werden könnte. Allerdings sollten für eine quantitative Analyse zusätzliche Methoden wie die Untersuchung von Pollenhöschen Verwendung finden.

***Apis mellifera* / Ammenbienen / Pollenverbrauch / Melissopalynologie**

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