Differences of pollen and pollenkitt attractiveness of some cultivated and ornamental apples for honeybees

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Summary: Differences in the attractiveness of pollen according to plant families and species for the honeybees had been examined by researchers, but pollen samples of cultivars were not tested yet. This examination attempts to evaluate the differences in attractiveness of pollen of some cultivated and ornamental apples to honeybees. We applied biotest with equal amounts of hand collected pollen samples and by a nucleus of bees with brood. The evaluation was based on the number of visitation by foraging honeybees on the samples during the time of the experiment which was determined. Pollenkitt samples were also tested in a separate experiment.
The data were analysed by variance analysis and significant differences were found according to the number of bee visitation in the attractiveness of the pollen and pollenkitt samples of apple cultivars.
The visualisation of pollenkitt was done by light microscope and SEM.

Introduction

There have been intensive research activities in the topic of insect pollination of crops in the past few decates, but we have found quite a few studies concerning the attractiveness pollen and pollenkitt for the honey bees. Most of the experiments reported in the literature were carried out in the orchards and based on observations in the field (*Benedek* et al., 1994, 1995, 1996). The objects of these experiments were to study nectar quality and quantity and the number and formation of the reproductive organs of the flower. These studies are very important in describing the characters of the flowers from the point of view of insect pollination of apple cultivars. *Malus* species have been examined as pollinators in apple orchards and *Malus* pollinating system was worked out for Hungarian condition (*Gyúró* et al., 1979). Pollination biology of related various *Malus* taxa (*Soltész* et al., 1982) and their effect on the quality of apple (*Tóth*, 1985) was examined. The structure and production of nectarium was examined by *Gulyás* et al. (1989) and *Orosz-Kovács* (1989, 1990). In the international literature there have been reported about the phagostimulativ effect of pollen for the honeybees and experiments were set up to identify the materials responsible for this effect (*Lepage* et al., 1968, *Doull,* 1974, *Boch,* 1982, *Dobson,* 1984). In most of these experiments honeybee collected pollen was used as experimental material from different plant species.

Among the characteristic features of flowers influencing the honeybee visitation the most important is the odour, according to *DeGrandy-Hoffmann* (1987), and the structure of the flower and the odour according to *Halmágyi* et al., (1991). In the composition of flower aromas there are different terpenes, alcohols, ketons and esters. The odour of the flower is mainly emitted by the petals and the pollen, sometimes the leaves and the produced nectar may be aromatic (*Williams,* 1983, *Dobson,* 1987). According to these results we focused our studies on the pollen grains.

In the plant family of *Rosaceae* and *Compositae* it often happens that the pollen grain itself has a separate odour. These aromatic components are localized in the oily surface of the exine which is called pollenkitt connected to lipids (*Dobson,* 1984, 1988, *Pham-Delegue* et al., 1987). In our study we wanted to find the answer to the following questions:

- Are there any differences in the attractiveness of pollen from different apple cultivars for the honeybees?
- Does the oily surface of the pollen grain (pollenkitt) influence the pollen collecting activity of the honeybees?
- How can the pollenkitt visually observed by microscope?

We consider this question to be quite important, because the blooming time of the apple coincides with the brooding period of the honeybee colony and in this time the pollen collecting activity is high. We also have to note that the pollination efficiency of different collecting activities of the honey bees (pollen collection, nectar collection, mixed behaviour) are different according to varieties, and it is depending on the position of the anthers and the pistils, and the actual need, activity and development of honeybee colony (*Williams* et al., 1985, *Benedek,* 1996). In the case of apple, the pollen collectors are the most effective pollinators because during the collection of pollen they touch the stigma as well. The top nectar collectors are also effective, but the side workers are not considered as good pollinators. (*Kuhn* et al., 1982, *Benedek* et al., 1996).

Methods

Hand collected pollen samples of 12 apple cultivars were examined in a biotest with honeybees, in a shaded green house, regarding the attractiveness of pollen. The apple cultivars were chosen and collected at Szigetcsép, (Experiment field of the University). The new intensive varieties were: 'Elstar', 'Jonagold', 'Jonagored' and good pollinator varieties like ornamental *Malus* species and Delicious varieties (*G. Tóth,* 1985, 1995). The biotest was carried out according to *Doull & Standifer* (1969). One nucleus of honey bees (4 frames, 1500 bees with brood) were placed in an empty green house (located in the SZIE Arboretum). During the experiment 2–2 g of hand-collected pollen samples were offered to the foraging honeybees for 15 minutes in four repetition. The number of bee-visitations were counted on the samples during the experiment. In one experiment four different apple cultivars were tested at the same time. Data were evaluated by analysis of variance by method of Statgraf/anova.

Pollen and pollenkitt samples of two apple and two sunflower cultivars were also tested to evaluate the importance of pollenkitt to elicit foraging behavior. We have prepared the pollen samples and carried out biotest according to *Dobson* (1988). The pollen grains were briefly washed in organic solvents and the pollenkitt fractions were incorporated by cellulose powder. The differences in the attractiveness of pollenkitt fractions and washed pollen grains were evaluated according to the number of bee visitation during the experiment.

Visualisation of the pollenkitt on the surface of the exine was made by light and scanning electron microscope. The exine wall was dyed by basic Fucsin, and the pollenkitt can be seen as yellow oily drops on the exine surface. The Sudan IV is suitable to detect fats and oils was used to give a color reaction with pollenkitt. We could apply UV light with HBO lamp that indicate bright red colouring of the oily pollenkitt.

The application of SEM was successful either using gold (*Stellemann,* 1978) covering or cryo-technic (*Sargent,* 1988).

Results and discussion

Attractiveness of pollen samples

There had been significant differences among the apple cultivars tested as for their attractiveness to the honeybees. According to the average number of visitation a relative order can be set *(Table 1.; Figure 1.).* During the experiment it was clear that the honeybees preferred one or two cultivars according to their visitation and they have collected the preferred samples after the experiment time.

We could establish that the pollen sample of *'Redspur Delicious'* was the most attractive according to the number of bee visitation at $P_{5\%}$ level of significance. There have significant differences between *'Redspur Delicious'* and *Malus x purpurea 'Aldehamensis',* between *'Redspur Delicious'* and *'Jonagored'* and *'Jonagold',* between *'Elstar'* and *Malus x purpurea 'Aldehamensis'.*

Table 1 The average number of bee visitation and homogenous groups

Cultivars	Experiment No.	Repetition	Average	Homogenous groups
1. Malus x purpurea	1	4	8.25	a
2. Golden Delicious	1	4	17.00	b
3. Jonagold	1	4	23.25	b
4. Redspur Delicious	1	4	50.75	c
5. Malus x purpurea	2	2	28.00	a
6. Jonagored	2	2	56.50	ab
7. Elstar	2	2	85.00	c
8. Redspur Delicious	2	2	91.50	c
9. Jonathan	3	4	41.75	a
10. Jonagored	3	4	46.25	a
11. Idared	3	4	64.00	ab
12. Redspur Delicious	3	4	80.75	c

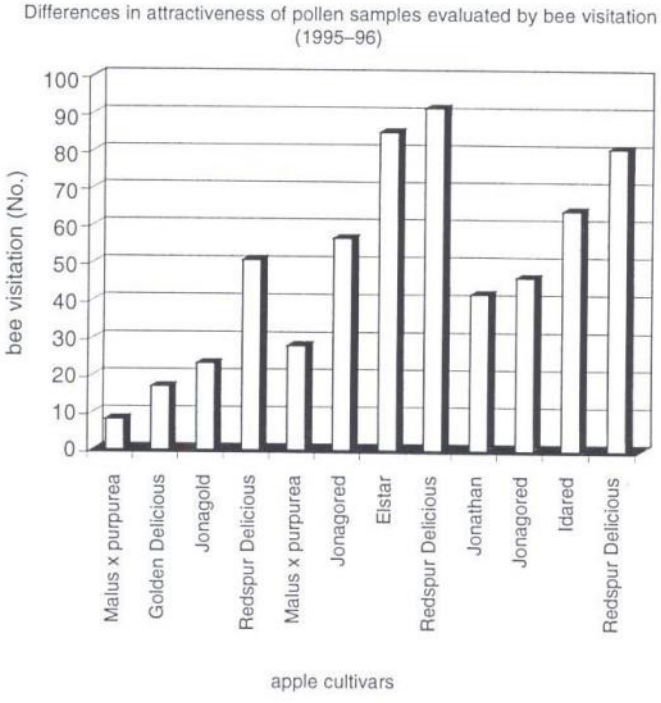

Fig. 1 The attractiveness of pollen samples of apple cultivars according to the average number of bee visitation

Attractiveness of pollenkitt samples

Table 2 Testing the attractiveness the samples of pollen and pollenkitt according to the number of bee visitation. (x= arithmetical mean, Σx=summa x, sd=standard deviation)

Samples	Pollen			Pollenkitt		
Cultivars	x	Σx	sd	x	Σx	sd
1. Golden Delicious	**52.33**	157	16.6	**27.33**	82	2.08
2. *Malus* x 'Baskatong'	**48.66**	146	8.02	**7.33**	22	2.51
3. *Helianthus a.* 'Frankasol'	**12.0**	36	2.64	**31.0**	93	3.60
4. *Helianthus a.* 'Sunbread'	**5.33**	16	1.57	**22.33**	67	4.04
5. Total	**118.32**	355		**87.99**	264	

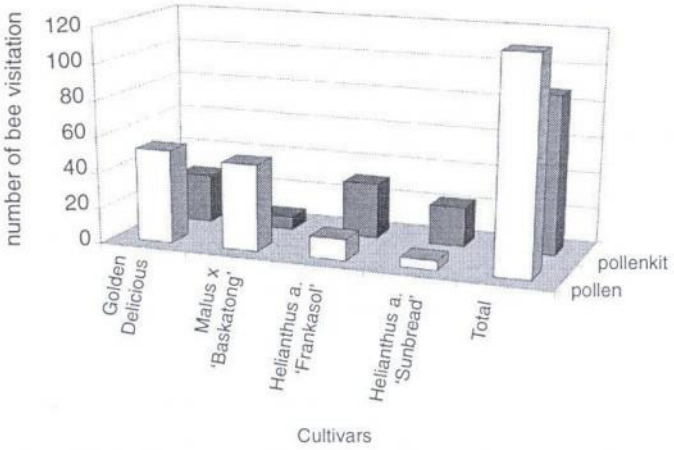

Figure 2 Attractiveness of pollen and pollenkitt samples according to the average number of bee visitation

Table 2. and Figure 2. show the attractiveness of the pollen and pollenkitt samples of the same cultivars. The data show that the pollen and pollenkitt sample of *'Golden Delicious'* was the most attractive from the apples. There were sunflower hybrids in the experiment too, and their pollenkitt samples were more attractive than those of the pollen samples.

The total number of the bee visitation on pollen and pollenkitt samples was similar, which indicates the importance of the pollenkitt in the attraction of the honeybees.

Table 3 Testing the attractiveness of different fractions of pollenkitt and pollen samples according to the number of bee visitation. (x=arithmetical mean, Σx=summa x, sd=standard deviation)

Samples	Golden *Delicious*	*Malus x pupurea'* Baskatong'	*Helianthus annuus* 'Frankasol	*Helianthus annuus* 'Sunbread'	Total
1. pollen x	**20.66**	**4.99**	**12.0**	**8.66**	**46.1**
Σx	62	15	36	26	139
sd	4.72	1.73	5.29	2.51	
2. pollenkitt x	**3.0**	**3.0**	**11.9**	**7.33**	**25.2**
(I.F.) Σx	9	9	36	22	77.0
sd	1.0	1.73	1.73	3.05	
3. pollenkitt x	**5.66**	**3.33**	**3.0**	**2.66**	**14.5**
(II. F.) Σx	17.0	10.0	9.0	8.0	44.0
sd	2.08	1.0	1.0	0.57	
4. treated pollen	–	–	–	–	–
5. cellulose powder	–	–	–	–	–

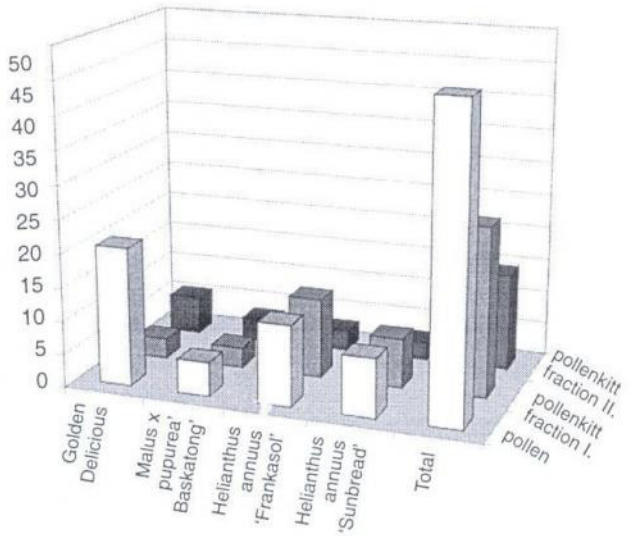

Figure 3 Attractiveness of pollen and pollenkitt fractions according to the bee visitation

Table 3. and *Figure 3.* show that the different fractions of pollenkitt received different number of bee visitation. In most cases the easily soluble part of the pollenkitt (F. I.) was the most attractive. But in the case of *'Golden Delicious'* the slowly washable components (F. II.) were more attractive. The washed pollen samples and the cellulose powder as carrier material did not attract the honeybees. It was noticeable that the total number of bee visitation was quite similar on the pollen samples and on the two pollenkitt fractions. The bee visitation of the pollen samples and pollenkitt fractions of the tested cultivars was different.

Attractiveness of the pollen and pollenkitt samples of apple cultivars

Comparing our results with the results of *Doull* (1974) and *Doull* et al. (1969), they have established a relative order of pollen attractiveness for honeybees using bee collected pollen, from plant species important in the honeybee pasture. In this experiment pollen of species from *Compositae, Cruciferae, Fabaceae* and *Rosaceae* family were the most attractive for the honeybees. The experiment material was bee-collected pollen, which could have modified the result, because of the added sugar content and the excrete of the mandibular glands of the honeybees. We used hand collected pollen because the quality of it is more similar what the bees can find in the flowers. We did not find reports in related literature concerning the pollen attractiveness of cultivars, only species, so it was difficult to make comparison.

We can compare our result with the experiment of *Dobson* (1988); she used hand collected pollen in biotest, and she also examined the attractiveness of pollenkitt with bees. In her experiment the number of bee visitations was different according to plant species and it was similar on the same pollen and pollenkitt samples. In our experiment the number of bee visitation was different according to cultivars, but it was similar on the pollen and pollenkitt of the same one.

Fig. 4 Apple *(Malus domestica)* pollen grains coloured by basic fucsin (M:750). On the surface of the exine the light-yellow oily drops are the pollenkitt

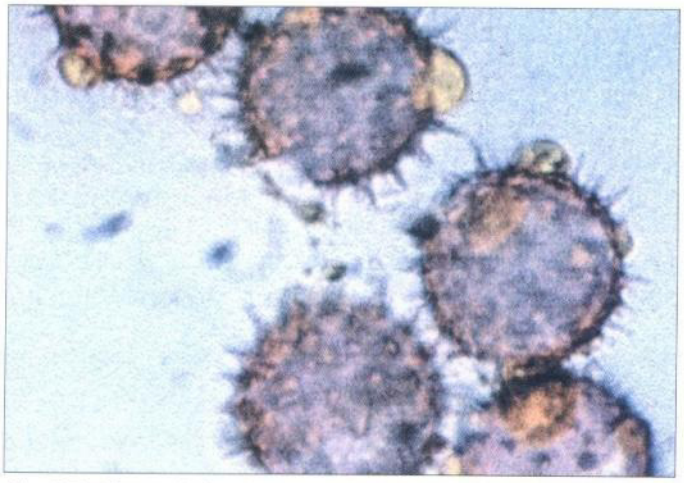

Fig. 5 Sunflower (*Helianhus annuus* L.) pollen grains coloured by basic fucsin (M:450). On the surface of the exine the -yellow oily drops and coverings are the pollenkitt

Examination of pollenkitt by light microscope and SEM

The light microscope examinations clearly showed that in the case of the examined species the pollenkitt appears on the exine surface as oily drops or covering and its colour was different kind of yellow according to species. The Sudan IV which is suitable to show fat and oil reacted with the

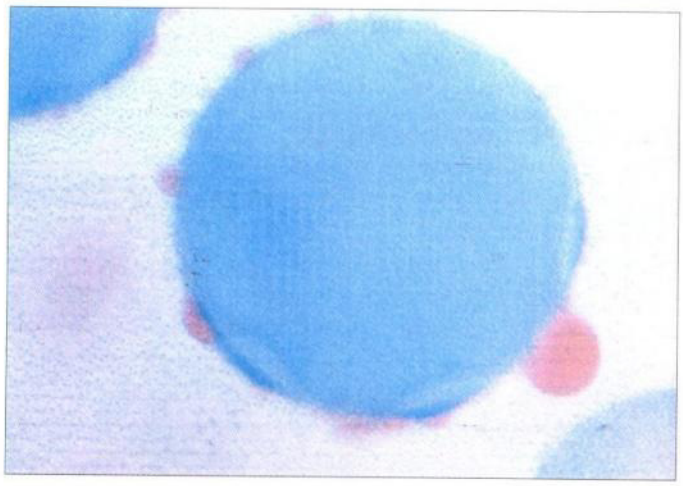

Fig: 7 Pumpkin *(Cucurbita pepo)* pollen grains in UV light (M:430). The pollenkitt appears as bright red droplets on the exine

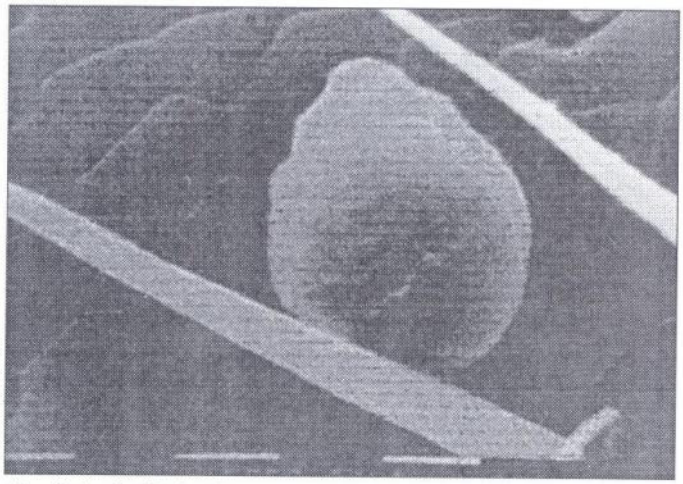

Fig. 8. Apple *(Malus domestica)* pollen grain attached on the hair of the hind leg of the honey bee. (Gold covered sample, M:1560)

pollenkitt and show brown-red coloring reaction. The UV light induced the pollenkitt to show bright red colour.

The SEM examinations resulted to see the real appearance of the pollenkitt. In the case of the apple the patterns of the exine can be seen, and the pollenkitt located in the deepness of the exine. In the case oft he sunflower the pollenkitt covered the whole surface of the exine, with decreasing amount to tip of the spins (2–3n), the pattern of the exine could not be seen. On the SEM photos, the connection of the pollen grains to the body hairs of honeybee, by the help of the pollenkitt was visualised.

Conclusions

Attractiveness of the pollen samples of apple cultivars

The results show that during the biotest the honeybees can discriminate between the pollen samples of tested apple cultivars. During the experiment one or two samples were more often visited by the bees, than the others. Among the

Fig. 9 Sunflower *(Helianhtus annuus L)* pollen grain attached to the hairs of hind leg above the corbicula. (cryo-technic sample, examined on –160 C, M: 1150, 1690)

tested varieties the *'Redspur Delicious'* and *'Elstar'* were the most preferred. Pollen samples of *Malus x purpurea* 'Aldehamensis' and other *Malus* species were less visited by the honeybees.

The practical application of the results: These results can contribute to our knowledge in the pollination behaviour of the honeybees on apple. The data about the different pollen attractiveness of apple cultivars give further information about their pollination biological characteristics.

We consider that our results need further experiments with the involvement of new apple cultivars and pollinator varieties as well.

Attractiveness of pollenkitt

It was established that the pollenkitt – solved from the pollen grain surface – produces similar intensity of bee visitation than the pollen samples of the same cultivar. This result is supporting the statement that the pollenkitt, which is covering the exine has a fagostimulativ effect for the pollen-eating insects.

After removing the pollenkitt from the exine the pollen grain itself did not elicit bee visitation.

The pollenkitt samples of the tested cultivars produced a different number of bee visitation. The two fractions of the pollenkitt also elicited different number of bee visitation. These results refer to the species and cultivar specific character of the pollenkitt, and that the two fractions of the pollenkitt are containing different components in quality and in quantity. We could establish that the pollenkitt has an important phagostimulative effect for the pollen-eating insects.

Examination of pollenkitt by light microscope and SEM

The pollenkitt was visually examined by microscope, and could be detected by microscopic dies, and UV light. SEM examinations gave good images of the appearance of pollenkitt and their function as natural glue in pollination. Differences were detected in the natural colour, and amount of the pollenkitt at the species examined, that support our previous conclusions.

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