# THE INFLUENCE OF ENERGY FOOD INTAKE AND QUANTITY OF UNCOVER LARVAE ON POLLEN COLLECTION EFFICIENCY

**A.A. Prelipcean**

*U.Ş.A.M.V. Iaşi*
*e-mail: andrewprelipcean@yahoo.com*

**Abstract**

*The purpose of the paper is to observe the interrelations that are established between the quantity of pollen collected by bees and some internal factors of the bees family: the amount of energy food, cover and uncovered brood. The methods used consisted of observing the internal situation of the bees families, taking pictures, putting pollen collectors, collecting raw data, statistical interpretation of obtained data. Four eperimental plots were organized, each with different contributions of honey and uncovered brood. Colonies had the same power. The collection period using pollen collectors was 24 hours. Pollen from each family was linked to its internal situation. The internal situation of the family was expressed both in cell number and percentage. Numerical results have given quite large variations due to the multitude of internal fluctuating factors of any family. The quantities of pollen obtained were between 25.60 and 262.40 grams. We applied Fisher and Tukey tests. Multiple correlation test revealed positive correlation between the amount of brood and pollen intake, and negative correlation between the amount of honey in the hive and pollen intake. There were also situations in which the absence of juveniles lead to increased intake of honey and pollen.*

**Key words**: pollen, uncovered brood, honey, interrelations

## INTRODUCTION

Pollen collected by bees is a food with a high biological value [13]. It is also the only source of natural protein for the bees. In beekeeping, pollen is a important economical factor in income supplementing and is a key product in the life of bees for optimal development of the colony. The primary factors influencing pollen collecting are pollen stocks and quantity of uncovered juvenile present in the colony [4]. The bees collect pollen from the available plants, then return to the hive and store the cargo in cells [1], [8], [9]. Harvest intensity is also controlled by quantitative changes in phenols content of pollen [7], bees showing preferences for certain types of pollen over others [16]. In the colony, the pollen stored, young juvenile, and free cells are direct stimuli that affect the gathering behavior [5]. Experiments made showed that the gathering of pollen and nectar are to some extent influenced independently, and if the pollen reserves are large, bees will move to the nectar gathering. Bees foraging for pollen are directly influenced by juveniles pheromones [14], resulting in increasing or decreasing in the number of foragers as well as the individual efforts of each bee [6]. The experiment performed is to relate information about the internal situation of the colonies with pollen obtained by collectors. It can provide information on the organization of bees families.

## MATERIAL AND METHOD

The experiment was conducted in May 2009, preceding the period of Tilia sp. flowering. Location of the experiment was Barnova forest. In the apiary, a total of 40 families of were checked. Depending on their power (number of frames covered with bees) families whose power was average (5,8-6 frames covered with bees) were selected [13]. The next step was the quantitative assessment of the number of cells with honey and uncovered brood. The Netz classical method provides a overlapping frame over the hive

frame and a quantitative assessment of cell numbers in each frame. Knowing that 1 $cm^2$ contains 4 cells, 5 $cm^2$ there will contain 100 cells.

For a more accurate reserves determination of the juvenile amount, digital pictures were taken with each of the hive frames. They were made perpendiculary, on both sides of the frame with a fotodigital camera Samsung Digimax 420. The pictures have been centralized on directories with the number of each hive. The photographs were analyzed with the program Digimizer v.3.6.0. to determine the number of cells for each parameter considered: uncovered juvenile cells, covered juvenile cells the honey cells. The amount of free cells and stored pollen was calculated by difference (3200 cells - (honey cells no. + uncovered cell no. + covered cell no.).

From the total of analyzed hives, 12 families were selected in which the experimental plots were prepared as follows:

Mt - good intake of honey (nectar) and large amount of uncovered brood

E1 - good intake of honey / nectar and small amount of uncovered brood

E2 - low intake (the limit) of honey / nectar and large amount of uncovered brood

E3 - low intake (the limit) of honey / nectar and small amount of uncovered brood

We set the following:

good intake of honey - over 45% of cells

low intake (the limit) of honey - under 40% of cells

large amount of uncovered brood - over 15% of cells

small amount of uncovered brood - under 15% of cells

After establishing the experimental lots - after 2 days – the pollen collectors were fixed at the entrance of each hive. Effective pollen collection period was 48 hours. Then, the collected pollen were weighed and the data placed in tables containing the properly quantified internal situation of the hive.

Data obtained for each family and lots were processed using descriptive statistics (Instat v. 3.036). A series of analitical statistics have been applied in order to observe any differences between the analyzed lots. To compare averages between groups we used Fisher and Tukey tests. To observe possible correlations appeared between lots multiple correlation test was used within Microsoft Office - Excel.

## RESULTS AND DISCUSSION

The obtained datas were analyzed using descriptive statistics: mean, standard error of the mean, standard deviation, variance, coefficient of variability (Table 1). The standard deviations obtained in terms of track indices are sometimes very different because the unit to work with (number of cells) and that their number can vary normally between considerable limits. Even if the power of the hives is a close one or even the same, quantities of honey (energetical food), brood and pollen may fluctuate.

Even a single index variation causes noticeble observable differences in the scattering of the other indexes. This is because existing food stocks and the number of individuals in the colony (adults and larvae) are correlated factors. In addition there are other factors studied such as the health of each individual, experience and age, etc.

They all fluctuate so that through feedback, the development of the colony is always tending to a balanced state[15]. Analysis of the obtained values may be explained by the interrelated actions of several factors, according to a complex mechanisms of organization of bee families. A high number of juvenile cells will lead to increased activity in gathering pollen, a fact highlighted in many observations made [2], [3], [10], [11], [12], [19].

Table 1. Situation of studied lots

| Watched indicator | Statistical indicators | Mt | E1 | E2 | E3 |
|---|---|---|---|---|---|
| Honey cells (no. cel) | $\bar{x}\pm s_{\bar{x}}$ | 3484,67±223,69 | 3641,67±161,61 | 2028,00±130,78 | 1592,00±474,95 |
| | s | 387,45 | 279,91 | 226,52 | 822,64 |
| | $s^2$ | 150117 | 78350 | 51312 | 676732 |
| | V% | 11,12% | 7,69% | 11,17% | 51,67% |
| | Compared to Mt | 100% | 104,50% | 58,19% | 45,68% |
| Uncovered brood cells (no. cel) | $\bar{x}\pm s_{\bar{x}}$ | 1798,53±401,91 | 188,00±158,95 | 1064,57±58,38 | 418,67±44,52 |
| | s | 696,12 | 275,30 | 101,11 | 77,11 |
| | $s^2$ | 484586 | 75792 | 10224 | 5945,3 |
| | V% | 38,71% | 146,44% | 9,50% | 18,42% |
| | Compared to Mt | 100% | 10,45% | 59,19% | 23,27% |
| Covered brood cells (no. cel) | $\bar{x}\pm s_{\bar{x}}$ | 664,00±332,80 | 304,67±304,67 | 546,00±363,23 | 1514,00±640,30 |
| | s | 576,43 | 527,70 | 629,14 | 1109,03 |
| | $s^2$ | 332272 | 278465 | 395812 | 1,22996E6 |
| | V% | 86,81% | 173,21% | 115,23% | 73,25% |
| | Compared to Mt | 100% | 45,88% | 82,22% | 228,01% |

The average quantity of pollen produced (Table 2) from each lot ranged from values of 45.61 g for Mt (corresponding intake of honey and large amount of uncovered juvenile) to 135.37 g for E1 (corresponding intake of honey and low quantity of uncovered juvenile).

Table 2. Pollen obtained from experimental plots

| Watched indicator | | Mt | E1 | E2 | E3 |
|---|---|---|---|---|---|
| Obtained pollen from collectors (g) | $\bar{x}\pm s_{\bar{x}}$ | 45,61±13,11 | 135,37±63,52 | 74,01±10,64 | 121,12±68,18 |
| | s | 22,71 | 110,02 | 18,43 | 118,10 |
| | $s^2$ | 515,65 | 12105 | 339,64 | 13947 |
| | V% | 49,79% | 81,28% | 24,90% | 97,50% |
| | Compared to Mt | 100% | 296,79% | 162,26% | 265,55% |

For analysis of homogeneity of variances between groups test Fisher was used. It was applied on the average of each lot. Because the experiment involved the simultaneous comparison of several groups, each with different internal factors, Tukey test was used (Table 3).

Using the Fisher test significant differences (**) were obtained in the quantity of honey and uncovered brood in the four lots taken in the study. The differences were not significant (NS) for covered brood and pollen collected by collectors. For more precise observation of statistical significance between each two lots taken, Tukey test was applied for each factor analyzed.

Quantities of honey showed distinct significant differences between experimental lots: Mt and E3; E1 and E3. Comparisons between all other groups have highlighted significant differences. According to statistics applied, there is a significant difference between lots in which the quantity of honey and uncovered brood amount is different, but also between those with different intake of honey, when the amount of brood is reduced (in both groups).

Table 3. Analytical statistics - Fisher and Tukey tests

| Watched indicator | Compared lots | Fisher test | | | | Tukey | | Statistical significance | |
|---|---|---|---|---|---|---|---|---|---|
| | | F | Fcr | n | Significance | q | w | Lots | Significance |
| Honey (cel. no.) | Mt, e1, e2, e3 | 13,30236 | 7,590992 | 0,01 | ** | 6,20 | 1750,43 | MtE1 | NS |
| | | | | | | | | MtE2 | NS |
| | | | | | | | | MtE3 | ** |
| | | | | | | | | E1E2 | NS |
| | | | | | | | | E1E3 | ** |
| | | | | | | | | E2E3 | NS |
| Uncovered brood (cel. no.) | Mt, e1, e2, e3 | 10,8843 | 7,590992 | 0,01 | ** | 6,20 | 1358,99 | MtE1 | ** |
| | | | | | | | | MtE2 | NS |
| | | | | | | | | MtE3 | ** |
| | | | | | | | | E1E2 | NS |
| | | | | | | | | E1E3 | NS |
| | | | | | | | | E2E3 | NS |
| Covered brood (cel. no.) | Mt, e1, e2, e3 | 3,508552 | 4,066181 | 0,05 | NS | 4,53 | 2205,73 | MtE1 | NS |
| | | | | | | | | MtE2 | NS |
| | | | | | | | | MtE3 | * |
| | | | | | | | | E1E2 | NS |
| | | | | | | | | E1E3 | * |
| | | | | | | | | E2E3 | NS |
| Collected pollen (grams) | Mt, e1, e2, e3 | 0,771301 | 4,066181 | 0,05 | NS | 4,53 | 214,5 | MtE1 | NS |
| | | | | | | | | MtE2 | NS |
| | | | | | | | | MtE3 | NS |
| | | | | | | | | E1E2 | NS |
| | | | | | | | | E1E3 | NS |
| | | | | | | | | E2E3 | NS |

Quantities of cells with uncovered juvenile showed significant differences between MT and E1, E3 and Mt. The differences in MtE1 confirmes the uncovered brood differences in the choice of lots, when the intake of honey is appropriate. Significant differences were observed also when the honey stocks were different. The amount of covered brood only differ significantly between Mt and E3, E1 and E3.

Regarding pollen collected, the differences were significant between all groups.

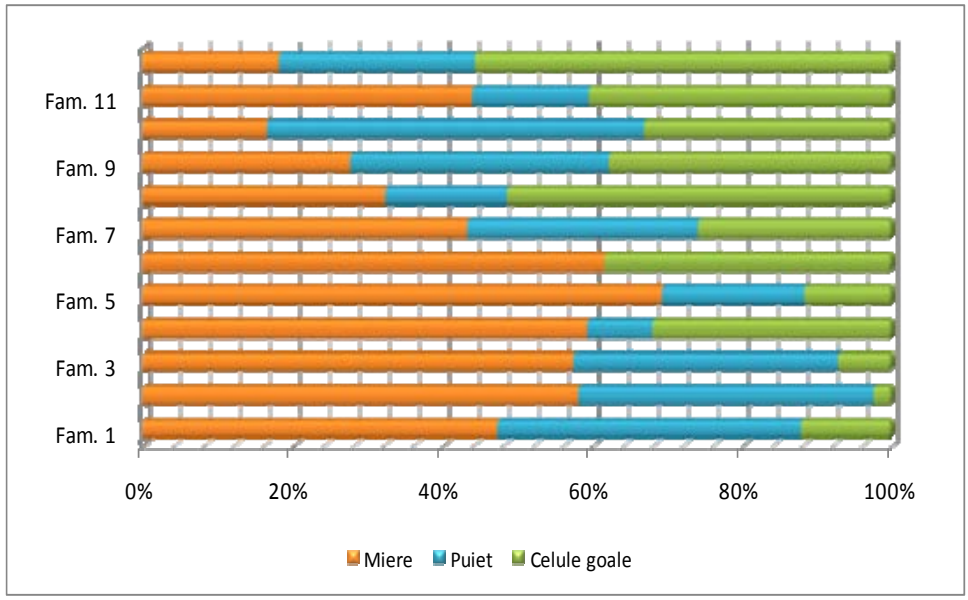
Statistical analysis of data by applying descriptive statistics: Fisher and Tukey tests don`t seem to provide conclusive results on the interrelations. Therefore we used a correlation test. For this, it has been used absolute values of the parameters (Table 4) and the total number of cells per hive. We also overlayed data on uncovered and covered brood and in a single set of values: total brood cells (Table 4).

Table 4. Absolute values of tracked parameters - total number of cells

| Lots | Family no. | Total honey cell no. | Total uncovered brood no. | Totalcovered brood no. | Total brood no. | Total empty cell no. | Pollen obtained (g) |
|---|---|---|---|---|---|---|---|
| Mt | 1 | 30380 | 25956 | 0 | 25956 | 7664 | 70,41 |
| | 2 | 37300 | 14900 | 10360 | 25260 | 1440 | 25,84 |
| | 3 | 36860 | 13100 | 9560 | 22660 | 4480 | 40,57 |
| E1 | 4 | 34446 | 5040 | 0 | 5040 | 18414 | 70,21 |
| | 5 | 35560 | 600 | 9140 | 9740 | 5900 | 73,5 |
| | 6 | 39544 | 0 | 0 | 0 | 24459 | 262,4 |
| E2 | 7 | 22240 | 11757 | 4040 | 15797 | 13163 | 61,24 |
| | 8 | 20800 | 10400 | 0 | 10400 | 32800 | 65,66 |
| | 9 | 17800 | 9780 | 12340 | 22120 | 24080 | 95,14 |
| E3 | 10 | 10660 | 5060 | 27200 | 32260 | 21077 | 253,17 |
| | 11 | 25400 | 3600 | 5380 | 8980 | 23220 | 84,6 |
| | 12 | 11700 | 3900 | 12840 | 16740 | 35560 | 25,6 |

Figure 1 shows the situation recorded for each family: the quantity of honey, brood and empty cells, all expressed in percentages reported to the total number of cells.

Figure 1. Situation of studied families in percent




According to literature, the mutual dependence between two or more random variables is that they vary to an extent given by their covariance. To test the interdependence of several factors, multiple correlation was used [17]. The test was applied to each lot to determine any correlations that may appear to determine the meaning of variation of each factor.

In the Mt lot (tab.5) negative correlations were obtained between the quantity of honey and brood; the quantity of honey and pollen collected. He obtained a positive correlation between the amount of brood and the pollen intake collected.

Table 5. Multiple correlation test

| Mt | Honey | Brood | Collected pollen |
|---|---|---|---|
| Honey | 1 | | |
| Brood | -0,619 | 1 | |
| Collected pollen | -0,962 | 0,384 | 1 |

| E1 | Honey | Brood | Collected pollen |
|---|---|---|---|
| Honey | 1 | | |
| Brood | -0,756 | 1 | |
| Collected pollen | 0,981 | -0,868 | 1 |

| E2 | Honey | Brood | Collected pollen |
|---|---|---|---|
| Honey | 1 | | |
| Brood | -0,695 | 1 | |
| Collected pollen | -0,979 | 0,826 | 1 |

| E3 | Honey | Brood | Collected pollen |
|---|---|---|---|
| Honey | 1 | | |
| Brood | -0,795 | 1 | |
| Collected pollen | -0,328 | 0,833 | 1 |

In E1 group negative correlations were obtained between the quantities of honey and brood; brood and pollen collected. The correlation between the quantity of honey and pollen collected was positive. To note that for this group, the colony number 6 did not had any brood cells, instead here has obtained the highest quantity of pollen (Table 4). In group E2 negative correlations were obtained between the amount of honey and brood; honey and collected pollen.

In group E2 negative correlations were obtained between the amount of honey and brood, honey and collected pollen.The correlation between the amount of brood and pollen collected was positive. For the E3 group negative correlations were obtained between the amount of honey and brood, honey and collectde pollen. The correlation between the amount of brood and pollen collected was positive.

The pollen stored in combs affects the number of pollen foraging bees. A high amount of pollen stored will result in decreased activity of gathering [8], [9].

Some experiments [15] have shown that bees respond to deficiencies in the quantity and quality of pollen. This is made by increasing the intake of pollen brought to the colony, rather than specializing harvest for a particular type of pollen with higher protein content. Individualy, the pollen bees foragers appear to be unaware of the quality of pollen collected, indicating the feedback need of the colony to control the protein flow to and in the colony.

Individual decisions of bees also create fluctuations in the contribution of nectar and pollen.

The experiment performed confirms that the nutrients flow in the colony is controlled by interrelations between internal factors of the colony. Following interpretation of the correlation test and direct observation of the situation of each family in part, confirms that the need for pollen, the pollen collectors default contribution increases with the amount of brood in the hive, especially with the number of uncovered brood.

As the pollen quantity increases, the greater the frequency of young larvae feeding. A high frequency and a greater total duration of feeding larvae will provide a higher protein level in juveniles. By contrast, the frequency of feeding older larvae (4 days), depends on the amount of pollen or the ration of pollen and larvae, even after a few days in reduceing the amount of pollen. The amount of honey stored was not correlated with feeding frequency in any category of juvenile [18].

The correlation between the amount of stored honey and the juvenile was found to be negative for all analyzed groups: the stock growth in honey will generate in colony fewer brood cells.

We also observed that the correlations between the amount of stored honey and pollen collected is negative, indicating that although the activities of gathering nectar and pollen is manifested almost the same way, they have different control mechanisms. Thus with increasing amount of stored honey, pollen collected tend to fall. Data obtained show that when the intake of pollen is increasing, the tendency of nectar harvest will decline.

Although in this paper we showed the main correlations between the intake of pollen, the amount of brood, and the quantity of honey, other researches are also needed to determine more precisely the quantitative correlations between internal factors in the bee families.

## CONCLUSIONS

In the colonies, there are correlations between fluctuating quantities of honey, brood and pollen. We observed a direct correlation between the amount of juveniles (particularly uncovered one) and the contribution of pollen brought to the hive.

Under normal conditions, with increasing tendency of nectar harvest, the pollen harvesting activity falls and also the quantity of layed larvae. The mechanism of pollen and nectar harvest works the same, but is controlled by the family at opposite feedbacks. The experiment revealed that with increasing amount of juvenile, the tendency of gathering pollen grows and the nectar intake decreases.

In extreme cases, in wich there is no queen, the amount of juveniles is decreasing, or it`s absent, the bees will show an increasing tendency for picking the protein food (pollen) and energy food (nectar).

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