EVALUATION OF VARIOUS PROTEIN PRODUCTS FOR USE AS POLLEN SUBSTITUTES APPROPRIATE FOR HONEYBEES

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Introduction

The great push back of natural vegetation as well as expansion of agricultural monoculture has led to drastic gaps of pollen plants during the bee season (Wille 1973). Cold snaps show a clear negative effect on the development of bee colonies due to the interruption of pollen intake (Dustmann/von der Ohe in press). Due to the high protein requirements of bee colonies especially during springtime and late summer/autumn the pollen deficiency in the colonies has to be compensated to avoid loss of colonies.

The continual search for pollen substitutes and supplements turned out that at the present time there is no optimal one available on the market (Doull 1975, Haydak 1933, Herbert 1979, Herbert/Shimanuki 1980, Nelson 1976, Wahl 1963). It is not the correct way to feed pollen substitutes mixed in candy paste inside the hive, because too many older bees but not enough nurse bees will get this protein paste (Wahl 1982). It is far more better to give the substitutes outside the hives, so that the normal behavioural way is not interrupted (Johannson/Johannson 1977). But by missing the attractiveness to pollen foragers these substitutes have no satisfactory influence on the development of colonies by feeding them outside the hives (Wahl 1982).

To look for a right pollen substitute adequate for bees means to ascertain both, the nutritive and the behavioural physiological value of the protein products. Several products based on soybean flour were tested in preliminary experiments. In this paper we present our results of experiments with two protein sources. The first one is Sojapoll (soybean flour plus brewer yeast), this product is already as a pollen substitute on the German market. The second one is Chlorella-G-Powder, dried cells of Chlorella ellipsoidea, an unicellular freshwater algae. Some algae are used in breeding of some diptera and Bombyx mori (Foote 1981, Hou/Chen 1981).

Material and Methods

Lab-Cages-Experiments:

The target was a pollen substitute, which is usable for nutritive

value and attractive for bees. The nutritive value was investigated by the physiological condition of young bees kept in lab-cages. In one "Liebefelder" lab-cage (Maurizio 1945) 50 just emerged bees were kept queenless for 14 days fed ad libitum with protein diets and water. The diets were 200 g sucrose, 50 ml water and protein sources according to 6 g protein. Along with a Chlorella- and Sojapoll-diet there were two controls: pollen-diet as a positive control and sugar without any protein as a negative control. The cages were kept in an air-conditioned chamber. In one trial there were 6 cages per diet group. 10 trials were carried out. Food consumption, diagnosis of diseases, mortality and the physiological condition of the 14 day old bees were analysed. The criteria of the physiological condition were the hypopharyngeal glands, the fat body and the hemolymph proteins. The grade of the development of the hypopharyngeal glands was determined by the width of the acini. The store of protein in the fat body was valued in 5 steps (Maurizio 1954). With the micromethod of Bradford (1976) the protein content and with the cellulose-acetate-electrophoresis (Engels 1972) the vitellogenin content of the hemolymph were measured.

Outdoor-Cage-Experiments:

In 3 trials (1983, 1984, 1985) colonies were kept 6 weeks in outdoor-cages with artificial feeders outside the hive. Inside each cage 1 colony was placed with ad libitum supply of water, 1,5 M sucrose solution and the respective protein source (pollen, Chlorella, Sojapoll — in each cage another one). Daily food consumption, build up of colonies by weekly measuring the brood and protein storage area and in the last week of experiments the physiological condition of just emerged and nurse bees were measured. For the investigation of the physiological condition we used the same criteria as in the lab-cage experiments.

The food consumption, pollen packing behaviour, the efficiency of gathering and the development of the brood nest were used to ascertain the behavioural physiological value of the substitutes.

Preference-behaviour:

Bio-assays carried out to look for the preference behaviour under standardized conditions in a flight-room.In those experiments foragers had the choice between the respective different protein sources (pollen, Chlorella, Sojapoll) and these products treated with different odors (Citral/Geraniol, Anethole and diethyl ether extracts of pollen, Chlorella and cucumber). The decrease of the protein sources in one hour were ascertained.

Results

Lab-Cage-Experiments:

All lab-cage experiments ran satisfactory and without any complications. In spite of a relative equal food consumption, there were

striking differences in protein content and vitellogenin-titer of the hemolymph and in the grade of development of the hypopharyngeal glands and fat body between the protein diets. These marks and the criteria "Nosematose infection" and "mortality" showed that the value of Chlorella as a polen substitute comes up to the value of pollen. Especially the acini width of hypopharyngeal glands of Sojapoll provided bees were smaller than those from other diet groups. The bees of the Sojapoll group were affected with Nosema spores.

Outdoor-Cage-Experiments:

During the outdoor-cage experiments the behaviour of the bees was generally quite normal. Nevertheless it was conspicious that all colonies provided with Sojapoll were extremely affected with spores of Nosema apis, whereas the other colonies showed no infections.

In all experiments there was more pollen off than Chlorella and still considerable more than Sojapoll. The number of foragers in the pollen feeders was significant higher than in the pollen substitute feeders. The Chlorella pellets were larger than in the pollen and Sojapoll pellets. The Sojapoll pellets were very small and instable. A high mortality was symptomatic for Sojapoll foragers. They were not able in any case to clean their body from the Sojapoll flour. Often the flour was fixed between the hairs.

The area of unsealed brood is not an optimal criterion to evaluate the state of a colony, because in many cases cannibalism takes place. A better way is to ascertain the area of sealed brood. In the first and second trial the colonies provided with pollen have the largest areas of sealed brood, followed by the Chlorella provided colonies and at the end the Sojapoll provided colonies. In the third trial there were many difficulties with the colonies and queens in the pollen group. Due to this, there were no significant differences.

The nurse bees of the Chlorella colonies are nearly as well developed as the nurse bees of the pollen colonies, whereas the nurse bees of the Sojapoll colonies showed up pretty bad: This could be seen particulary in the width of the acini of the hypopharyngeal glands. The grade of development of the fat body is the most important criterion on examination of just emerged bees. The fat bodies of young bees from Chlorella colonies are better developed than the fat bodies of bees from pollen colonies. The bees of Sojapoll colonies showed in all experiments a poorly developed fat body.

Corresponding to this the provision of food to larvae must be in the Chlorella colonies as good as in the pollen colonies. Reared bees and developed nurse bees of Chlorella provided colonies are nearly in the same good shape as bees of pollen provided colonies.

Preference-Behaviour:

In the bio-tests there was more

Chlorella taken off than pollen and extremly more than Sojapoll. Between Chlorella and Chlorella plus a pollen-ether-extract there were no significant differences. Pollen <250 μm was not gathered as well as pollen <100 μm. Furthermore there was only an insignificant increase by adding volatile substances like cucumber-, pollen-, Chlorella-ether-extracts, Citral/Geraniol or Anethole to Sojapoll flour.

Altogether there was much less Sojapoll gathered than in case of the other two protein sources. The Chlorella foragers were much faster than the other foragers in forming the pellets. After a short contact with the very dusty Chlorella powder they hovered over the feeders forming the pellets. The Sojapoll foragers showed no hovering-behaviour and needed more time to form the pellets. The Sojapoll pellets were smaller and not as stable as the Chlorella pellets. Chlorella foragers were working more efficient.

Discussion and Conclusion

Pollen lacks constrained beekeepers to use pollen substitutes since many years. Though research for pollen substitutes and supplements has been done for decades, it is still a current target, because the previous substitutes showed no satisfactory success (Wahl 1982). Next to the proteins the essential amino acids (Bieberdorf et al. 1961, de Groot 1953/54), the B-vitamine complex especially pyridoxine (Anderson/Dietz 1976, Haydak/Dietz 1972) and a sufficient mineral content (Herbert/Shimanuki 1978) of pollen are of tremendous value for the nutrition and by that for the development of honeybees. Chlorella-G- Powder meets all these requirements. This is not true to all the soybean products.

The evaluation of the nutritive value took place in the lab-cage experiments. The analyses concerned to those criteria, which are of great importance for the physiological condition of nurse bees and which depend on the pollen nutrition.

Forteen days old bees, fed only with a pollen or Chlorella diet since they emerged, were well developed. The differences between these groups were insignificant. There were no great differences in diet consumption. This shows that Chlorella has the neccessary nutrients in an adequate quality and quantity as pollen and the bees are able to utilize these nutrients.

The comparison of 0 day and 14 days old bees out of the pollen and the sugar diet groups showed that the development of the ascertained criteria continued in the pollen group during the first 14 days of the bee life, whereas in the sugar group it comes to degenerations. Based on these results the pollen nutrition during the nurse bee polyethism stage is important. Chlorella as a pollen substitute shows corresponding results and the nutritive value has to be con-

sidered equivalent to pollen.

The present results of the outdoor-cage experiments are interesting, because the feeding of the protein sources took place only outside and not inside the hives.

The pollen and Chlorella provided colonies had larger areas of sealed brood than Sojapoll provided colonies. Gathering pollen or Chlorella is more efficient for foragers than gathering Sojapoll. Chlorella foragers form bigger and more solid pellets in a shorter time. Larger pellets could have a positive effect to the population dynamics (Loper et al. 1984). Sojapoll foragers lost a lot of the pellets during their flight back to the hives. The flat scale of Sojapoll flour adheres to the hairs of the foragers. Not any bee is able to comb these scales out of the hair and these bees died by asphyxia.

Despite of protein reserves in the fat bodies the hypopharyngeals glands of the nurse bees are inferior developed than those of nurse bees from free flying colonies. The results of Brouwers (1983) allowed the interpretation that the decreasing area of unsealed brood was a limiting factor for the acini width. Honeybees can transfer a surplus nutriment stored in the fat body over the metamorphosis to the imaginal stage. The nutrient storage in the fat body of just emerged bees were evaluated for supply with nutriments during the larval stage of these bees. The results showed a really better fat body outfit of young bees from Chlorella provided colonies, followed by pollen provided colonies and in the end Sojapoll provided colonies.

The supply of nurse bees with proteins determined the development of the larvae. A further factor is the relation of nurse bees to larvae. If the number of larvae provided by one nurse bee did increase, the weight and lifespan of these progeny were decreasing (Eischen et al. 1983).

In contrast to Sojapoll Chlorella-G-Powder shows a quite similar positive influence on the build up of bee colonies as pollen.

V. Frisch (1965) presumed that the attractiveness of pollen to honeybees is the own odor of the pollen and not the scent of the flowers. The nutritive value has no influence on the attractiveness (Levin/Bohart 1955). Lepage/Boch (1968) presumed that there is an attractive substance in the pollenkitt and they found a fatty acid and a lutein ester. Octadeca-trans-2, cis-9, cis-12-tionicacid was isolated and seems to be very attractive to pollen foragers (Boch et al. 1973, Hopkins et al. 1969, Starrat/Boch 1971). Campana/Moeller (1977) and Schmidt (1982) found preference- and discrimination-behaviour of bees to different pollen species. On the supposition that there are volatile attractive substances, the bio-tests in the flightroom were carried out. Chlorella is as attractive to foragers as pollen. The attractivenes can't be put down to volatile substances. Using volatile extracts of pollen the gathering of the treated pollen

substitutes does not increase respectively in an unessential level only.

Sojapoll is not attractive for bees. This verifies the results of Wahl (1963). A creation of attractiveness of Sojapoll by adding other odorous substances is not possible.

Indicated preferences of bees to pollen <100 μm in opposite to pollen <250 μm is a reason to discuss the factors grain size and form. Levin/Bohart (1955) supposed that the size of pollen has an influence to the attractiveness. Chlorella cells and agglomerations are of the same form and range of size as pollen. They have also a solid cover, probably depending on the drying process, corresponding to the pollen exine and these cells are also spherical. In opposite to Chlorella, Sojapoll consists of flat and fraied out scales.

Looking at the present results the attractiveness of pollen and Chlorella is not caused by one factor only. Against the opinion that the attractiveness is based on volatile substances of the pollenkitt turns the fact of viscinfilaments. Pollenkitt serves for fixing the pollen grains to the insect body. In many cases entomophil plant species have developed viscinfilaments analogous to pollenkitt (Hesse 1980).

The results of bio-tests clearly show high attractiveness of Chlorella-G-Powder to foragers, even without pollen odor.

Sojapoll and Chlorella-G-Powder were evaluated for their nutritive and behavioural physiological value. Commercial pollen substitutes on the basis of soybean flour are absolutely unsuitable. In contrast to that the use of Chlorella brought very good results in every respect. Chlorella is indeed a promising pollen substitute.

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