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Evaluation of the nutritive value of maize for honey bees

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ABSTRACT

In modern managed agro-ecosystems, the supply of adequate food from blooming crops is limited to brief periods. During periods of pollen deficiencies, bees are forced to forage on alternative crops, such as maize. However, pollen of maize is believed to be a minor food source for bees as it is thought to be lacking in proteins and essential amino acids. This study was conducted to verify this assumption. In maize, a strikingly low concentration of histidine was found, but the amount of all other essential amino acids was greater than that of mixed pollen. The performance and the immunocompetence of bees consuming a pure maize pollen diet (A) was compared to bees feeding on a polyfloral pollen diet (B) and to bees feeding on an artificial substitute of pollen (C). Consumption of diets A and C were linked to a reduction in brood rearing and lifespan. However, no immunological effects were observed based on two parameters of the humoral immunity.

1. Introduction

Honeybees have a great demand for amino acids and proteins. Free amino acids occur in nectar (Baker, 1977), but the amount is insufficient to meet a honeybee's nutritional requirement. Bee colonies rely mainly on pollen to satisfy their protein needs. A single colony consumes between 17 and 34 kg pollen per year (Crailsheim et al., 1992; Keller et al., 2005). Workers forage intensively on male inflorescences to collect pollen. Pollen from different plant species differ in their nutritional value (Standifer, 1967; Haydak, 1970; Crailsheim, 1990). For instance, high quality pollen is produced by diverse clover species (*Trifolium* spp.), oilseed rape (*Brassica napus*), pear (*Pyrus communis*), almond (*Prunus dulcis*), *Populus* spp. or lupin (*Lupinus angustifolius*) (Schmidt et al., 1987; Pernal and Currie, 2001; Somerville and Nicol, 2006). Pollen of less quality can come from sunflower (*Helianthus annuus*), blueberry (*Vaccinium* spp.), or *Typha* spp. (Schmidt et al., 1987). Despite their optical attractiveness for pollinating insects, even such blooming plants as dandelion (*Taraxacum* spp.), *Haplopappus* spp. or *Kallstroemia* spp., produce pollen of minor value for bees' nutrition. On the other hand there are examples of wind-pollinated plants (e.g. *Populus* spp.) which are better apt to satisfy the dietary demands of bees than pollen from animal pollinated plants (Maurizio, 1950; Schmidt et al., 1987). Bees are believed to use inflorescences from anemophilous plants as pollen resources, mainly during periods when pollen of blooming zoophilous plants are scarce (Severson and Parry, 1981; Baum et al., 2004).

In Central-Europe, shortages of high-quality pollen occur in early spring and in summer. During both periods, foragers collect the highly available pollen, irrespectively of the nutritive value. That is the case by collecting pollen from hazel or from maize (*Zea mays*) (Keller et al., 2005).

Bees collect maize pollen, but are unable to discriminate between high or low quality or even toxic pollen (Roulston et al., 2000; Pernal and Currie, 2001). Contradictory studies (Cook et al., 2003) are preliminary, because they inadequately address other feed-stimulating factors, such as color or odors. In the last years the supply of maize pollen increased as the maize growing area extended rapidly. More maize was grown as a fodder crop and as biofuel crop as well. The acreage of maize tends to increase as maize becomes a valuable crop for farmer as basis for feeding husbandry and as a fuel crop as well. In 2009, the cultivated area of maize reached approx. 160 million hectares worldwide (FAO, 2010) versus 130 million hectares in 1989. In Germany, within 20 years the acreage of maize doubled from 200,000 hectares in 1989, to more than 460,000 hectares in 2009. Maize is known to be a poor source of proteins for humans. Its biological value is low and there is a significant deficit of essential amino acids (FAO, 1993). Likewise, maize pollen can be suspected to contain low amounts of protein (Pernal and Currie, 2001; Somerville and Nicol, 2006) and to be deficient for some essential amino acids. Advisors of the Australian extension body claim a link between a huge consumption of

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maize pollen and an elevated rate of certain diseases (Stace, 1996). There is increasing evidence that the functionality of the immune system of insects depends on nutritional factors (Feder et al., 1997; Schmid-Hempel, 2005). Alaux et al. (2010) showed similar effects for honeybees.

Taking the above-mentioned four points together, the dietary low value of maize pollen, the blindness of foragers for qualitative traits of pollen, the abundance and availability of maize pollen in late summer and the putative linkage of immune function with nutritional factors, we hypothesize that maize pollen can be a risk for bees. This study aims to clarify this hypothetical threat for honeybee colonies. We measured the protein and the amino acid content of maize pollen and compared the biological productivity, the longevity and parameters of the immune system of honeybees fed with a pure maize pollen diet to honeybees fed with a supplement or a mixed pollen diet.

2. Material and methods

2.1. Pollen collection and pollen substitute

Mixed pollen was collected by bees in June 2009 during the off-bloom period of maize using commercial pollen traps. The pollen loads were removed daily in the evening and frozen to −18 °C. Before the pollen was fed to the bees (colonies and caged bees) the pollen loads were ground and later mixed with honeydew honey (fir tree) to create a paste (ratio 2.5:1, wt/wt). Maize pollen (variety "Athletico" KWS, Einbeck, Germany) was collected by hand, to get absolute and enough maize pollen, and frozen to −18 °C. To get a paste, the pollen was also mixed with honeydew (ratio 1.5:1, wt/wt).

The pollen substitute was a mixture of proteins, oil and sucrose syrup. The individual ingredients (calcium caseinate flour, whey protein flour, soya flour, linseed oil, beer yeast flour, sucrose solution 50% w/v) were mixed according to the description of van der Steen (2007). As pilot tests revealed that bees did not eat the pollen substitute readily, honeydew was added to the artificial diet (ratio 2.5:1, wt/wt).

Pollen of different plants is contented in all sorts of honey in a high ratio, with the exception of honeydew honey. Therefore, honeydew honey was used to avoid pollen of other plants, which could have an impact on the results.

2.2. Analysis of protein content and amino acids

The protein content was analyzed by the method of Kjeldahl as shown by Hoegger (1998). The content of free amino acids was measured by cation exchanger chromatography. Twenty milligrams pollen (dry weight) was extracted with 500 µl water for 30 min in an ultrasonic bath (EMAG, Emmi 20HC). The following procedure is described by Weiner et al. (2010).

2.3. Brood-rearing and pollen consumption

Nine Colonies (*Apis mellifera carnica*), with their respective queens, were transferred from their original hives to new small hives (Mini Plus®) at a standardized size of 4500 worker bees. The queens were caged. The colonies had no honey or pollen storage and, instead, constructed new combs. From each experimental group, three mini hives were placed in outdoor flight cages near Würzburg, Germany and observed for 3 weeks in summer 2009. The flight cages (4 × 4 × 2 m) contained no flowering plants, so bees foraged exclusively on a feeder with sucrose solution. The respective pollen diets were administered ad libitum from feeding devices in the bottom board of each hive. The consumption of sucrose solution and pollen was recorded.

Brood-rearing was measured by the Liebefelder method (Imdorf et al., 1987). With the help of a cross-haired frame (5 × 5 cm) the number of occupied cells can be estimated. The number of brood cells and the stage of development were recorded 5, 8 and 12 days after releasing the queen.

Following the method described by Schur et al. (2003), the brood development was recorded. Acetate sheets were used to mark at least 400 cells with eggs per colony. Three days later, all emerged larvae and 7 days later all sealed cells were recorded. All frames containing sealed bee brood were removed from the hives, placed in an incubator (35 °C, 65% rel. H.) and the emerging young bees were picked from the frames. The number of unhatched cells were recorded 21 days after marking the eggs. The emerged bees of these combs were used for the following longevity experiment and the measurement of immunocompetence. As bees fed with the artificial pollen supplement (van der Steen, 2007) did not raise sufficient brood, no test animals were available for the longevity experiment or the immunological studies.

2.4. Longevity experiment

Cages with 50 newly emerged bees were placed in an incubator at 27 °C and 65% humidity. Each cage contained a piece of comb foundation. The respective pollen paste was offered in small plastic vessels. To feed bees carbohydrates, the cages also contained a 5 ml syringe which provided a sucrose solution (ApiInvert®). Both pollen diets and sucrose solution were fed ad libitum. The mortality and pollen consumption was recorded daily for 45 days.

2.5. Measurement of immunocompetence

Cohorts of 15 bees aged between 1 and 3 days after eclosion were gathered in cages. These bees were supplied with sucrose solution (20% w/v, ad libitum), the respective diet ad libitum and water ad libitum. After 7 days, the bees were anesthetised on ice, intrathoracilly injected with 7.5 µl *Paenibacilus larvae* suspension with a Hamilton microsyringe (needle gauge 33), and returned to the cages for another 24 h. The volumes of injection solutions were adopted from the literature (Yang and Cox-Foster, 2005) and its suitability confirmed by own pilot experiments (unpublished).

P. larvae were cultivated on MYPGP agar plates (Oie, 2008). Colonies were floated off with saline (0.9% w/v) and with the help of a photometer the density was adjusted to OD = 1.5 at 600 nm. Controls were non-injected bees, bees wounded by puncturing with an injection needle and bees injected with 7.5 µl saline (0.9% w/v). Twenty four hours after injection, bees were killed by freezing and stored at −20 °C until RNA preparation.

From each experimental group, five biologically independent replicates were analyzed.

2.5.1. RNA extraction and real time PCR

Total RNA was extracted from pools of 10 bees per replicate using Rneasy silica columns according to the manufacturer's recommendations (Qiagen, Hilden). The bees for these measurements came from cage – experiments as described in the previous chapter. They were scarified for extraction between 9 and 11 days after eclosion. From each extract, 100 ng RNA was reverse transcribed using poly(dT) oligomers and the omniscript Rt kit (Qiagen, Hilden). CDNA of the hymenoptaecin target and the rp49 housekeeper was amplified with SYBR green based real time PCR protocols using the hymenoptaecin-primers according to Evans et al. (2006) and the rp 49-primers from de Miranda and Fries (2008). Both primer pairs hybridize to a region flanking an intron thus allowing the detection of contaminating genomic DNA. Reaction mixes of

25 μl contained 1× QuantiTect SYBR Green PCR Master mix (Qiagen, Hilden), 0.3 μM forward primer, 0.3 μM reverse primer and 2 μl of the cDNA. After an initial heating at 95 °C for 15 min a temperature scheme followed with 50 cycles of 94 °C for 15 s, 54 °C (rp49) or 56 °C (hymenoptaecin) for 30 s and 72 °C for 30 s. Data were collected at 72 °C. The threshold cycles (C_T) were determined by the maximum curvature approach (BioRAD MyiQ cycler). All PCR runs were performed in duplicate and followed by a melting curve analysis and gel electrophoresis of the PCR products. Length of amplicons from rp49-RNA was 205 bp and of the hymenoptaecin RNA 200 bp.

2.5.2. Inhibition zone assay (IZA)

For this measurement of the immunocompetence, hemolymph from immune stimulated bees was collected 24 h after injection and analyzed for bactericidal substances as described by Randolt et al. (2008) with the test bacteria *Micrococcus flavus*. Individual hemolymph samples of the same replicate were pooled. Nine microliters hemolymph was mixed with 1 μl phenylthiourea–aprotinin solution [0.1 mg/ml] and stored at −20 °C until analysis. After 24 h, the plates were scored for inhibition zones. Two technical repetitions per sample were performed.

2.5.3. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE is used to visualize the antimicrobial peptides (AMPs) and storage proteins in the hemolymph of bees. Hemolymph samples from immune-stimulated bees were diluted with 2× concentrated sample buffer, heated for 3 min and subjected to electrophoresis as described by Randolt et al. (2008). One microliter of the samples were loaded on a SDS–polyacrylamide gel to separate the proteins. Two repetitions per experimental group were performed.

2.6. Statistical analysis

Data from the brood-rearing experiment was analyzed by using the chi square test of contingency tables as described by Sachs (1997). The data of the longevity experiment was tested for significant differences in survival utilizing the univariate Kaplan–Meier analysis (Sachs, 1997). With respect to the gene expression analyses PCR failures were identified by calculating the difference between both PCR repetitions of the same RNA extract. If the difference was higher than 1.5 these values were considered as technically caused outliers, eliminated and replaced by values that were gained from a repetition of the respective PCRs. The resulting data set was checked with Grubbs' test for outliers ($\alpha = 0.05$) (Burns et al., 2005). After that procedure mean-Cts were calculated from the duplicate PCR. The resulting Ct-values, representing biological replicates were log-transformed, mean centred and autoscaled according to the procedure described by Willems et al. (2008). The relative expression of genes was calculated by the comparative C_T method (Livak and Schmittgen, 2001). Data of the gene expression and of the IZA were subjected to a two-factorial analysis of variance (ANOVA) to test for any significant difference. The two factors examined were diet (maize, mixed pollen) and treatment (injection of immune elicitors).

3. Results

3.1. Measurement of nutritional factors

The pollen substitute is made up of less protein (15% per fresh weight) than the pellets of mixed pollen (23% per fresh weight) and maize pollen (26% per fresh weight).

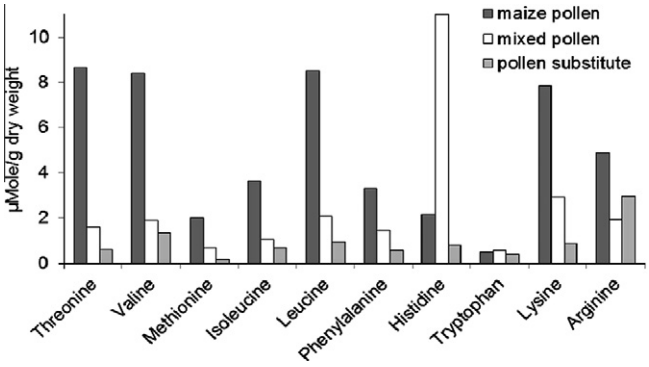

Fig. 1. Content of water soluble bee's essential amino acids of mixed and maize pollen and of the pollen substitute fed to the bees in the experiments. The content of every essential amino acid in maize pollen was twice as high as in the mixed pollen, the only exception being histidine. The pollen substitute showed a lower content of almost all essential amino acids. Presented is the content per dry weight [μM/g] analyzed by cation exchanger chromatography.

The content of each essential amino acid in maize pollen was twice as great as in the mixed pollen, with the exception of histidine. Mixed pollen contained five times as much histidine as maize pollen (see Fig. 1). The pollen substitute showed a lower content of almost all essential amino acids. The only exception was arginine, which was found more in the pollen substitute than in mixed pollen.

3.2. Brood-rearing

Brood-rearing of each test colony was documented by acetate sheets and the Liebefelder method for population estimation.

The colonies fed with pollen substitute reared fewer total bees (170) compared to the colonies fed with mixed pollen (1300 bees) or maize pollen (900 bees). Additionally, the colonies consumed more than twice as much maize pollen paste (862 mg) as mixed pollen paste (373 mg) per reared bee. Bees fed only marginally on the pollen substitute (275 mg).

Based on data recorded by acetate sheet transcripts, there was an emergence-rate of 39% for mixed pollen fed colonies, 25% for maize pollen fed colonies and 7% for the pollen substitute fed colonies. The differences in brood rearing between the nutrition forms were significant (see Fig. 2; chi square test of contingency tables, $\chi^2 \geqslant 20$, $p \leqslant 0.001$).

3.3. Longevity experiment

The longevity was measured by observing 650 newly emerged bees per diet (mixed and maize pollen) for 45 days. Fifteen days after emergence, the difference between mixed and maize pollen fed bees was obvious (see Fig. 3; Kaplan–Meier, $n = 650$, $\chi^2 = 131$, df = 1, $p \leqslant 0.001$). Bees fed maize pollen had a shorter life expectancy compared to bees fed mixed pollen. Half the honeybees fed mixed pollen were still alive at day 31 of the experiment, whereas on day 25 half of the maize pollen fed bees were alive.

During the experiment, the maize pollen fed bees fed in total much more pollen paste (70.41 g) than the mixed pollen fed bees (44.24 g). Randomized to one bee, Fig. 4 shows that the uptake of maize pollen paste increased enormously in the first 3 days after emergence and then from day nine till the end of the experiment (Mann–Whitney-*U*-Test: $U \leqslant 20.0$, $p \leqslant 0.01$).

3.4. Immunocompetence

Because inappropriate diet was suspected to compromise immune function, immunocompetence was evaluated by measuring

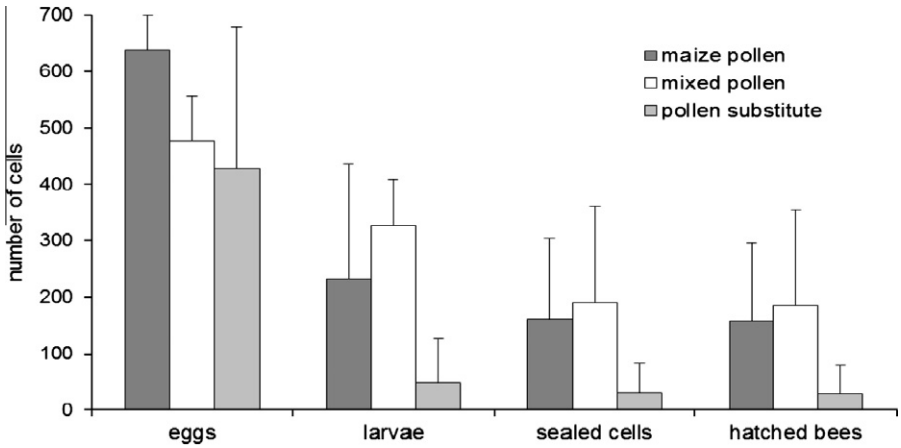

Fig. 2. Development of individual marked brood cells. The colonies fed with pollen substitute reared fewer total bees (170) compared to the colonies fed with mixed pollen (1300 bees) or maize pollen (900 bees). Emergence-rates: mixed pollen 39%, maize pollen 25% and pollen substitute 7%. The differences in brood rearing between the nutrition forms were significant (chi square test of contingency tables, $\chi^2 \geqslant 20$, $p \leqslant 0.001$). Analysis was based on the number of eggs. Presented are the means ± sd of three colonies per diet.

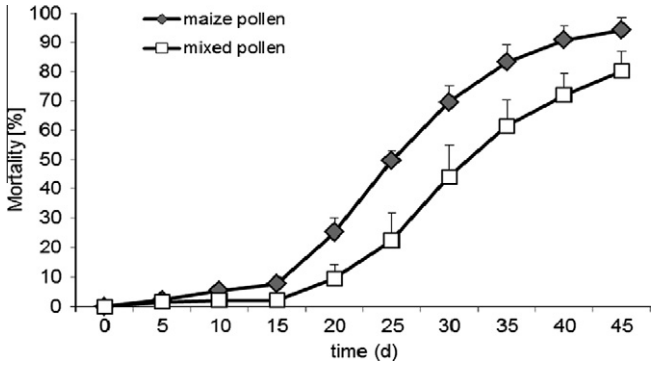

Fig. 3. Mortality of bees reared with maize or mixed pollen diet and fed with these diets during the whole lifespan (larvae + adult). Thirteen cages per diet and 50 bees per cage were observed for 45 days. There is a significant difference in mortality between the diets (Kaplan–Meier, $n = 650$, $\chi^2 = 131$, df = 1, $p \leqslant 0.001$). On day 25, half of the maize pollen fed bees and on day 31, half of the mixed pollen fed bees is still alive. The means ± sd of mortality during the observation are presented.

the transcriptional activity of the hymenoptaecin gene, the antibacterial activity, and the protein pattern of hemolymph samples.

The hymenoptaecin gene was highly activated by the challenging experiments ($p < 0.001$ two-factorial ANOVA), especially by the treatment with *P. larvae*. The upregulation was approximately 150-fold, and was clearly distinguishable from the control groups (see Fig. 5). However, the diet had no effect on the transcriptional level ($p = 0.368$, ANOVA). The hymenoptaecin gene expression was neither suppressed nor potentiated by a pure maize diet in comparison to the mixed pollen diet.

Comparable findings were observed with respect to the antibacterial activity of the hemolymph. The IZA revealed that dietary restriction to a pure maize diet did not impair the bees' capacity to produce antimicrobial peptides ($p = 0.272$ ANOVA, see Fig. 6).

Analyzing the protein spectrum of the hemolymph by SDS–PAGE, the stimulatory effect of the immune provocation was also confirmed. Intensity and number of bands increased from naive bees before the tests to mechanically wounded and saline injected bees. Bees provoked by *P. larvae* showed the highest amount and the most complex pattern of storage and defence related proteins (see Fig. 7). Bands of the defence related proteins hymenoptaecin and defensin were present independent of the type of the diet. However the pattern of the immune stimulated bees fed with mixed pollen was more pronounced than the corresponding pattern of bees fed with maize pollen.

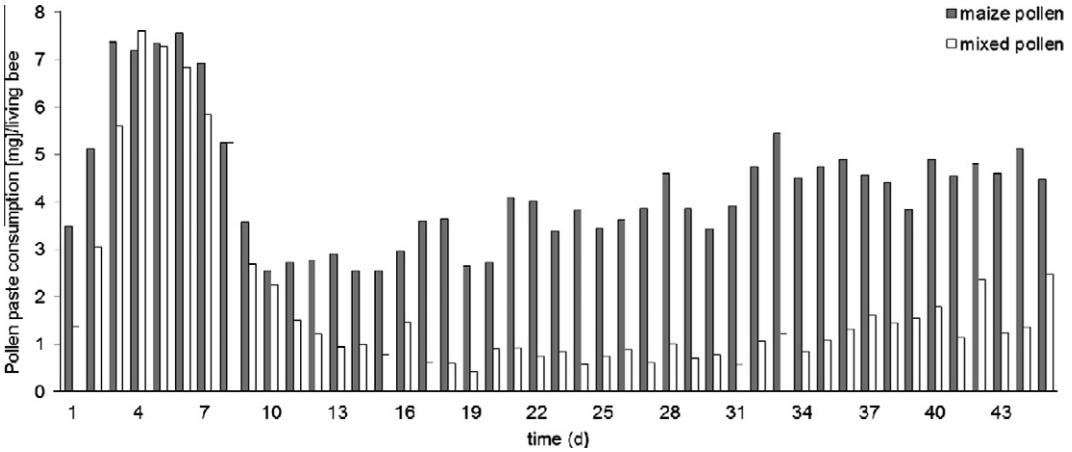

Fig. 4. Pollen consumption [mg] per day and living bee during the longevity experiment. More maize pollen than mixed pollen was consumed at almost all days of the experiment; significant differences occurred during day 1–3 and from day 11 on (Mann–Whitney-*U*-Test: $U \leqslant 20.0$, $p \leqslant 0.01$).

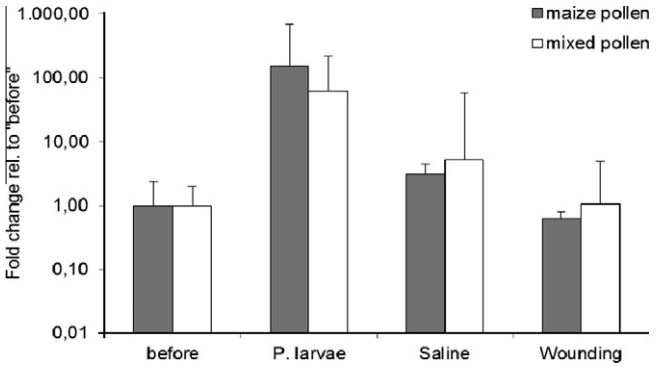

Fig. 5. Relative expression of the hymenoptaecin gene in bees fed with maize pollen and with mixed pollen. Error bars: −95% CI and +95% CI; *P. larvae*: bees injected with *P. larvae* suspension, saline: bees were injected with 0.9% NaCl, wounding: bees were anaesthetized and wounded with the injection needle, but not injected. Before: gene expression level at the beginning of the experiment. Data were log-transformed, mean centered and autoscaled. $N = 5$ independent biological replicates. There was a significant effect of challenging ($p < 0.001$) but no significant effect of the diet ($p = 0.368$).

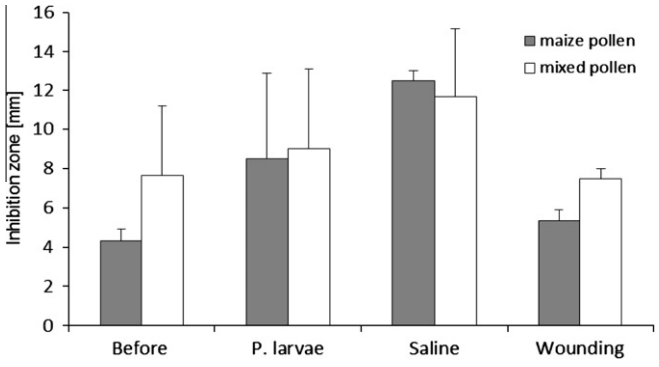

Fig. 6. Diameters of inhibition zones against *Micrococcus flavus* produced by hemolymph of bees at the beginning of the assay (“before”), after injection of *P. larvae* or saline. Wounding: anaesthetized and wounded with the injection needle, but not injected. Shown are means of two technical repetitions and three biological replicates. Diet effects were not significant ($p = 0.272$), but challenging injections had significant results ($p = 0.006$). Error bars: ±1 SD.

4. Discussion

In areas of Central and North Europe, the acreage of maize increases because of recent progress in breeding cold-tolerant maize lines and due to demand for farming high energy crops for livestock as well as for fueling bioenergy-stations. This paper broaches the issue of potentially harmful effects of maize pollen on bees. To address this topic, bees living on a pure maize pollen diet were compared to two control groups, one fed with polyfloral pollen and one fed a pollen substitute. Performances were recorded for brood-rearing, longevity and immunological markers. All three diets were analyzed for protein and amino acid contents, with the goal of identifying causes for differences in performance.

The brood-rearing success depended on diet: bees living on mixed pollen showed the highest productivity, rearing more brood than bees fed maize pollen or bees fed the artificial diet. The dietary quality is again reflected in the longevity. Maize bees had a shorter life expectancy than bees nourished with mixed pollen. No differences were found with respect to immunological fitness.

It was hypothesized that poor productivity and reduced longevity was linked to insufficient protein supply. The protein content of pollen is believed to be one of the best indicators for nutritive quality as it is closely linked to the performance of the consumers (Roulston and Cane, 2000). Previous studies classified the nutritive value of maize according to the protein content (Standifer, 1967; Somerville and Nicol, 2006). This study found a high concentration of protein in maize pollen (26% of fresh weight) which is congruent to the data reported by Goss (1968). Lower concentrations varying from approx. 14% to over 26% were found by others, which reflect differences in maize varieties, method of gathering pollen, use of fertilizers, or other environmental conditions (Maurizio, 1954; Stanley and Linskens, 1974; Stace, 1996; Pernal and Currie, 2001; Lundgren and Wiedenmann, 2004; Somerville and Nicol, 2006). The method of gathering pollen results in a bias in the protein determination of mixed pollen as foragers add important amounts of reducing sugars to the pollen pellets. Therefore measurements of protein relative to fresh weight underestimate the content in bee collected pollen (Roulston and Cane, 2000). Considering this constraint, the comparison of protein and amino acid contents has to be interpreted carefully. Nevertheless, it is unlikely to attribute the poor life expectancy and productivity of the present study to the crude protein content, because maize pollen contained more protein than mixed pollen. Next, a deficit of essential amino acids was supposed to be the limiting factor. Research on dandelion pollen supports the idea of the relevance of amino acids for the brood rearing capability (Herbert et al., 1970; Loper and Cohen, 1987). Honeybees are not able to synthesize arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine. They rely on the external supply of these essential amino acids (De Groot, 1952). Contrary to expectation, the chemical analysis of maize pollen showed that it was not deficient in essential amino acids. The amounts were superior to mixed pollen, with the exception of histidine. Mixed pollen was five times richer in histidine than maize pollen (2.15 versus 10.98 μM/g dry weight). The proportions between the individual essential amino acids were similar to reports in the literature (Goss, 1968; Lundgren and Wiedenmann, 2004).

The pollen substitute was included in the study as it was claimed to be a well-defined nutritive resource (Van der Steen, 2007). The protein content (15%) was within the stipulated range of at least 10% and at most 50% (Herbert et al., 1977), but it contained much less essential amino acids than both natural diets. The extremely limited capacity of the substitute-bees to rear brood might be a consequence of the low amino acid content.

With respect to the maize fed bees, it is again unlikely that their poor performance was linked to a general deficiency of essential amino acids. Either the scarcity of histidine is problematic for bees or maize pollen must lack something else, for example vitamins, minerals or sterols. Further studies are required to elucidate the reasons why a pure maize pollen diet does not support the bees sufficiently.

We believe that the findings of the study are meaningful, as the experimental set up in flight tents ensured that the test animals were exclusively limited to the special diets during the bees’ entire lifespans, including the larval and pupal stages. However, the collection of maize pollen by hand could be a controversial point. A study from Maurizio (1954) suggests that hand collected pollen is less effective than pollen collected by bees. During the process of pelleting the pollen, bees add mainly carbohydrates in the form of honey and saliva. Experimental studies showed that the process of pelleting is not connected with predigestion, which could be suspected to improve the availability of the nutrients (Peng et al., 1985). However, collecting pollen by hand adds the risk of obtaining unripe pollen of poor quality. To avoid crude maize pollen in this study, we gathered it from inflorescences in the field and offered it to bees after mixing with honey, as bees do during the process of pelleting.

Additionally, Maurizio (1954) mentioned that pollen of maize does not shorten the lifespan of bees. In Maurizio’s study the bees

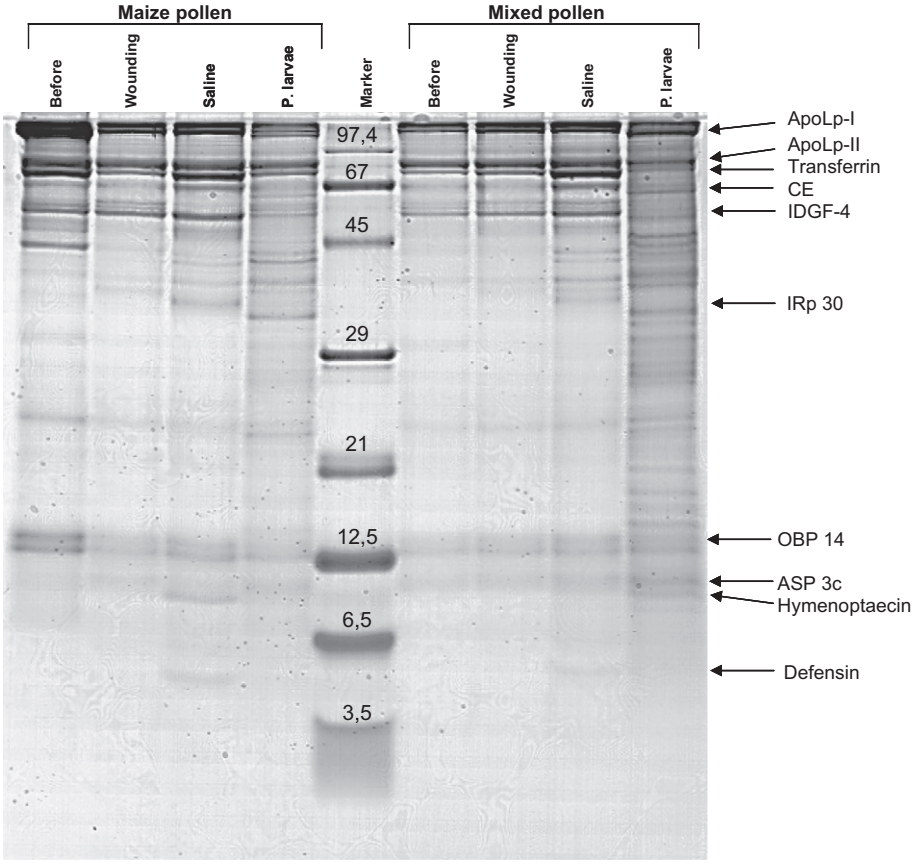

Fig. 7. Characterization of induced antimicrobial peptides (AMPs) in the hemolymph of honeybees fed with a single maize diet (left side) and a polyfloral diet (right side) untreated, wounded, mock infected with buffer (saline) or challenged with *P. larvae*. SDS–PAGE of hemolymph samples 15%; laemmlibuffer; 8.5 cm; Coomassie Blue G250; ApoLp = apolipophorin; CE = carboxylesterase; IDGF = imaginal disc growth factor; IRp30 = immune-responsive protein 30; OBP 14 = odorant binding protein 14; ASP 3c = antennal-specific protein 3c.

were only fed with a pure pollen diet after emerging. Therefore Maurizio's results are not really comparable with our findings, as the bees in our study were subjected to the maize for a long time. The bees were reared with the pure maize pollen diet and the nutritional form continued during the adult phase of the bees.

Bees adopted two behavioral reactions to the dietary stress: on the one hand they adapted their broodcare behavior and cannibalized brood (Newton and Michl, 1974; Weiss, 1984; Schmickl and Crailsheim, 2001). Brood cannibalism was practiced by all colonies, as the numbers of sealed cells were less than the numbers of larvae. More striking was the difference between the number of eggs and larvae for the maize and substitute bees. The initial phases of broodcare were not adequately supported by maize and the synthetic diet. Obviously the eggs did not hatch or were immediately removed and cannibalized by the nurses.

A second behavioral adaptation was observed. The amount of pollen taken up by the bees increased strongly. The bees of the maize group consumed much more pollen than the bees of the mixed pollen group. The quantity of uptake probably increased to compensate for lack of nutrients.

The immunological parameters were not affected by dietary factors. Immunocompetence depends on the genetic architecture of organisms and on environmental factors (Rolff and Siva-Jothy, 2003). Food supply can substantially modulate immunity as shown for humans (Chandra, 1992, 1997, 2002), birds (Latshaw, 1991), cattle (Galyean et al., 1999) and rodents (Fernandes, 2008). Malnutrition compromises immunity of vertebrates (Latshaw, 1991; Chandra, 1992; Lochmiller and Deerenberg, 2000; Cunha et al., 2003) and invertebrates (Azambuja et al., 1997; Butt et al., 2007; Akoda et al., 2009). Availability and quality of food are crucial. Caloric and proteinous restriction, and the ratio between both regulates immune functions (Lochmiller et al., 1993; Pal and Poddar, 2008; Behmer, 2009). Despite this considerable body of knowledge from vertebrates and some invertebrates, little is known about honeybees. There are only a few reports on interactions between nutrition and immunity in bees (Szymas and Jedruszuk, 2003; Alaux et al., 2010). Both studies found significant immune deficits in malnourished bees. Nevertheless, the nutritional environment in our study did not affect the transcription of hymenoptaecin and the production of AMPs. Thus there seems to be a primacy of the immune system in bees over productivity. Bees facing nutritive shortages are more prepared to economize resources by reducing productivity than by reducing a metabolically costly immune response. That was exactly the reaction of the test bees: they reallocated resources to the cost of colony size. Similar observations are reported from bumblebees. Bumblebees facing hostile environments reduce the number of progeny but not the intensity of immune defence (Schmid-Hempel and Schmid-Hempel, 1998). Starved animals activated an immune response even at the risk of reduced life expectancy (Moret and Schmid-Hempel, 2000).

5. Conclusions

The findings of this study do not support our hypothesis that pollen of maize directly harms bees. Compared with extremely scarce situations, bees which have access to maize pollen are in a more favorable situation. But extensive cultivation of maize results in an excess supply of maize pollen and could entrap foragers to

use primarily maize pollen instead of more nutritive floral pollen, which implicates the risk of low productivity.

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