Journal of Insect Physiology 58 (2012) 278–285

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Evaluation of the nutritive value of maize for honey bees

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ARTICLE INFO

Article history:
Received 23 September 2011
Received in revised form 1 December 2011
Accepted 2 December 2011
Available online 7 December 2011

Keywords:
Maize
Longevity
Brood-rearing
Immunocompetence
Essential amino acids

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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0022-1910/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2011.12.001

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