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Digestibility and nutritional value of fresh and stored pollen for honey bees (*Apis mellifera scutellata*)

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ABSTRACT

Pollen, the main protein source for honey bees, is mixed with regurgitated nectar or honey during collection and then stored as 'bee bread' before its consumption, mainly by young nurse workers. It has been suggested that storage of pollen improves its nutritional value and digestibility, but there is little evidence for such changes. We fed two fresh pollen types of different protein content (aloe and sunflower), and two stored pollen types (sunflower and a mixed pollen), to young caged worker bees. We measured daily consumption of pollen and sucrose solution, and survival after 14 days. At day 14 we recorded ovarian activation and extraction efficiency, by counting empty pollen grains in the rectal contents. Extraction efficiency is a measure of pollen digestibility. Contrary to our predictions, bees did not consume more fresh sunflower pollen than fresh aloe pollen to compensate for the lower protein content of sunflower pollen. In addition, they did not consume less sucrose solution when fed stored pollen diets that are already enriched in sugar. Consumption of stored sunflower pollen resulted in a low protein to carbohydrate (P:C) intake. Survival and ovarian activation were higher on diets giving higher P:C intakes. Extraction efficiency was high (up to 99%) for all pollen diets, and comparison of fresh and stored sunflower pollen showed that storage did not make it easier to digest. Changes to pollen during storage do not confer obvious benefits to honey bees.

1. Introduction

Pollen is essential for the development of honey bees (*Apis mellifera* L.), providing nutrients such as proteins, lipids, minerals and vitamins (Brodschneider and Crailsheim, 2010; Wright et al., 2018). In particular, high pollen consumption by adult worker bees in the first few days after emergence (Crailsheim et al., 1992) enables the development of their mandibular and hypopharyngeal glands which produce jelly for feeding brood and other colony members (Crailsheim, 1992; Haydak, 1970; Hrassnigg and Crailsheim, 1998; Lass and Crailsheim, 1996; Winston, 1987). It is well established that the quality of pollen diets, which is frequently equated to their protein content, and the quantity of pollen ingested affects the performance of honey bees, both in cage experiments and under field conditions. In these studies diets are evaluated by measuring fitness parameters such as lifespan, hypopharyngeal gland and ovarian activation, haemolymph protein content, or colony growth and susceptibility to disease (DeGrandi-Hoffman et al., 2016; Di Pasquale et al., 2016; Frias et al., 2016; Hoover et al., 2006; Human et al., 2007; Pernal and Currie, 2000). Most studies of this nature have used diets based on bee-collected pollen pellets. Stored pollen packed into the comb, known as bee bread, has seldom been tested for its effects on performance of honey bees. DeGrandi-Hoffman et al. (2013) compared haemolymph protein concentrations in bees fed bee bread from colonies of Africanized and European honey bees. Carroll et al. (2017) showed that bees prefer to consume freshly-stored pollen, but found no differences in body mass or hypopharyngeal gland protein levels when bees were fed fresh or aged diets. However, bees fed aged stored pollen show deleterious changes in the gut microbiome (Maes et al., 2016). It is not clear whether storage of pollen leads to any improvement in its nutritional value or digestibility.

Analyses of pollen chemistry have demonstrated great variation between plant species in nutritional content, such as protein varying between 2.5% and 61% dry mass (Roulston and Cane, 2000; Roulston et al., 2000). This variation was shown to be related to plant phylogeny in the hand-collected pollens analysed by Roulston et al. (2000). However, analyses of pollen composition are usually done on bee-collected pollen rather than fresh pollen (Serra Bonvehí and Escolà Jordà, 1997; Somerville and Nicol, 2006; Vanderplanck et al., 2014). Foragers moisten pollen grains with regurgitated nectar or honey for transport back to the hive (Harano et al., 2013; Thorp, 1979). The amount of sugar added during collection can be substantial – up to 50% dry mass, but usually unknown - and this alters the macronutrient content of the

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pollen (Nicolson, 2011; Roulston et al., 2000). This addition of sugar to pollen also occurs in stingless bees (Leonhardt et al., 2007). In two plant species we have compared nutrients in fresh pollen, honey bee-collected pollen and stored pollen removed from the comb: *Aloe greatheadii* var *davyana* (aloe) and *Helianthus annuus* (sunflower) (Human and Nicolson, 2006; Nicolson and Human, 2013). Fresh aloe pollen has a much higher percentage of crude protein than fresh sunflower pollen (51% vs 26% dry mass), but for both species this percentage is greatly reduced in stored pollen (28% and 13% respectively). Most of the decrease in protein and increase in carbohydrate occurs during collection, with little difference between bee-collected and stored pollen (Human and Nicolson, 2006; Nicolson and Human, 2013). Protein to carbohydrate (P:C) ratios are important in bee diets, and previous application of the geometric framework approach to macronutrient regulation of caged worker bees has shown that intake targets are strongly biased towards low P:C ratios (Altaye et al., 2010; Archer et al., 2014b; Paoli et al., 2014). This contrasts with the widespread assessment of pollens by beekeepers according to their protein content (e.g. Schmidt et al., 1987; Johannsmeier, 2001; Somerville and Nicol, 2006). The low protein content of sunflower pollen has labelled it as a poor resource for honeybees (Schmidt et al., 1987; Somerville and Nicol, 2006) while aloe pollen is favoured as a winter resource by South African beekeepers (Johannsmeier, 2001).

The nutrients in pollen are not easily obtained. The walls of pollen grains are made up of three main layers, the pollenkitt, exine and intine, with the exine being the main barrier to digestion for honey bees and other pollen feeding animals (Roulston and Cane, 2000; Stanley and Linskens, 1974). In honey bees, it has been suggested that osmotic shock may occur when pollen grains move from a high osmotic concentration in the crop to a low one in the midgut, so that the pores open and release the cytoplasm (Kroon et al., 1974). However, Human and Nicolson (2003) found that maize pollen grains remained intact in the honey bee midgut, demonstrating that osmotic shock is not involved in digestion of these thin-walled pollen grains. Penetration of digestive enzymes such as proteases through germination pores in the pollen grain wall is probably the most important mechanism for pollen digestion by honey bees (Simpson and Neff, 1983). These enzymes process the contents which then leak out through the pores, the walls remaining intact (Peng et al., 1986; Roulston and Cane, 2000). The durability of the pollen grain wall enables measurement of extraction efficiency by counting the numbers of full and empty pollen grains in the faeces of pollen consumers and comparing with fresh pollen (Brice et al., 1989). From data on pollen grain condition in Crailsheim et al. (1992), Roulston and Cane (2000) calculated extraction efficiencies of between 50% and 98% in honey bees, with nurse bees utilising pollen better than foragers. This corresponds with age-related variation in protease activity in the honey bee midgut (Moritz and Crailsheim, 1987).

The experiment of Crailsheim et al. (1992) was carried out with newly emerged workers that were marked and returned to the colony. They were thus consuming comb-stored pollen. It has been assumed that stored pollen undergoes fermentation and nutrient conversion by microbes (Gilliam, 1997), making it more nutritious and more easily digestible than the fresh pollen collected by bees. This has been called the 'beebread maturation hypothesis' (Carroll et al., 2017). However, Herbert and Shimanuki (1978) found only minor changes in nutrient composition, other than a breakdown of starch, and Anderson et al. (2014) demonstrated an absence of microbes in stored pollen. Our analyses of aloe and sunflower pollen (Human and Nicolson, 2006; Nicolson and Human, 2013) show little change in nutrients with storage; however, it is possible that the pollen becomes easier to digest (Kwong and Moran, 2016; Lee et al., 2015). The hydration state of pollen may also be important for digestion: when nectar is added during pollen collection, pollen grains rehydrate and swell, so that previously sunken pores become exposed (Human and Nicolson, 2006; Nepi et al., 2005).

The aim of this study was to compare consumption and digestion of fresh and stored pollen of aloe and sunflower by caged worker bees. For bees on the same diets we also measured consumption of sucrose solution, survival over 14 days and ovarian activation. We made the following predictions. Bees would consume more sunflower than aloe pollen to compensate for its lower protein content, and similarly would consume more stored than fresh sunflower pollen due to the dilution of protein with added sugars. Bees would consume more sucrose solution when fed fresh pollen diets because half of the stored pollen is already sugar. We predicted that survival and ovarian activation would reflect dietary protein content in opposite ways, with survival being reduced by higher dietary protein, and ovarian activation being enhanced. Finally, pollen extraction efficiency would be higher for stored pollen because of possible microbial action, but would differ between aloe and sunflower pollens because of differences in pollen morphology.

2. Materials and methods

2.1. Pollen collection and study sites

Helianthus annuus flowers were collected in March 2015 from a commercial sunflower farm in the Mookgophong area (24°40′S 29°0′E), formerly known as Naboomspruit, Limpopo. Fresh pollen was harvested daily by brushing flowers from multiple plants with a paint brush. Stored *H. annuus* pollen was collected from hives used for sunflower pollination on the same farm. *Aloe greatheadii* var *davyana* pollen was collected from plants in Roodeplaat Nature Reserve, 25 km NE of Pretoria (25°66′S 28°39′E), Gauteng, during the months of July and August 2015. Permission was granted by the Department of Agriculture and Rural Development of Gauteng to set up several hives in Roodeplaat Nature Reserve during the *A. g.* var. *davyana* flowering season. Hives were placed in July and removed in early September. Stored pollen was collected from these hives before their removal. Fresh and stored pollen was kept frozen at −20 °C before use in feeding experiments.

Upon examination of the gut contents of the honeybees fed 'stored aloe pollen', we observed that the pollen was not that of *A. g.* var *davyana* but instead a mixture of five pollen types, with a monocot pollen, probably belonging to one of the liliaceous families, occurring at high frequency (74.5% of 1000 grains examined). We refer to this below as mixed stored pollen. For comparison with our previous analyses of aloe and sunflower pollen, its protein content was obtained by determining total nitrogen using an elemental analyser at the Southern African Grain Laboratory in Pretoria. Nitrogen values were multiplied by a conversion factor of 6.25 (Roulston et al., 2000) to give crude protein as 20.3% dry mass.

2.2. Consumption, survival and ovarian activation

Capped worker brood was removed from five *A. mellifera scutellata* colonies and placed in an incubator at 35 °C and ~50% RH in complete darkness. Within 24 h groups of 100 newly emerged workers from the same colony were collected and placed into standard hoarding cages (11 × 8.5 × 7 cm; Köhler et al., 2013) in a second incubator under the same conditions. Twenty cages were prepared, five for each pollen type, namely: sunflower stored, sunflower fresh, mixed stored and aloe fresh. Each cage was supplied with a piece of comb hanging from the top and three feeding tubes; these were 15 ml plastic tubes with screw on lids and feeding holes cut into the tubes (1 × 0.3 cm). Each cage received a tube with one of the pollen types, a tube with 50% w/w sucrose solution and a tube of fresh water. The tubes containing the pollen and sucrose solution were weighed daily in order to measure consumption and the contents were replaced. Consumption of sucrose was obtained by halving the consumption of 50% sucrose solution. The water was replaced as needed. Dead bees were removed every day and mortality was recorded. Measures of consumption were adjusted for the number