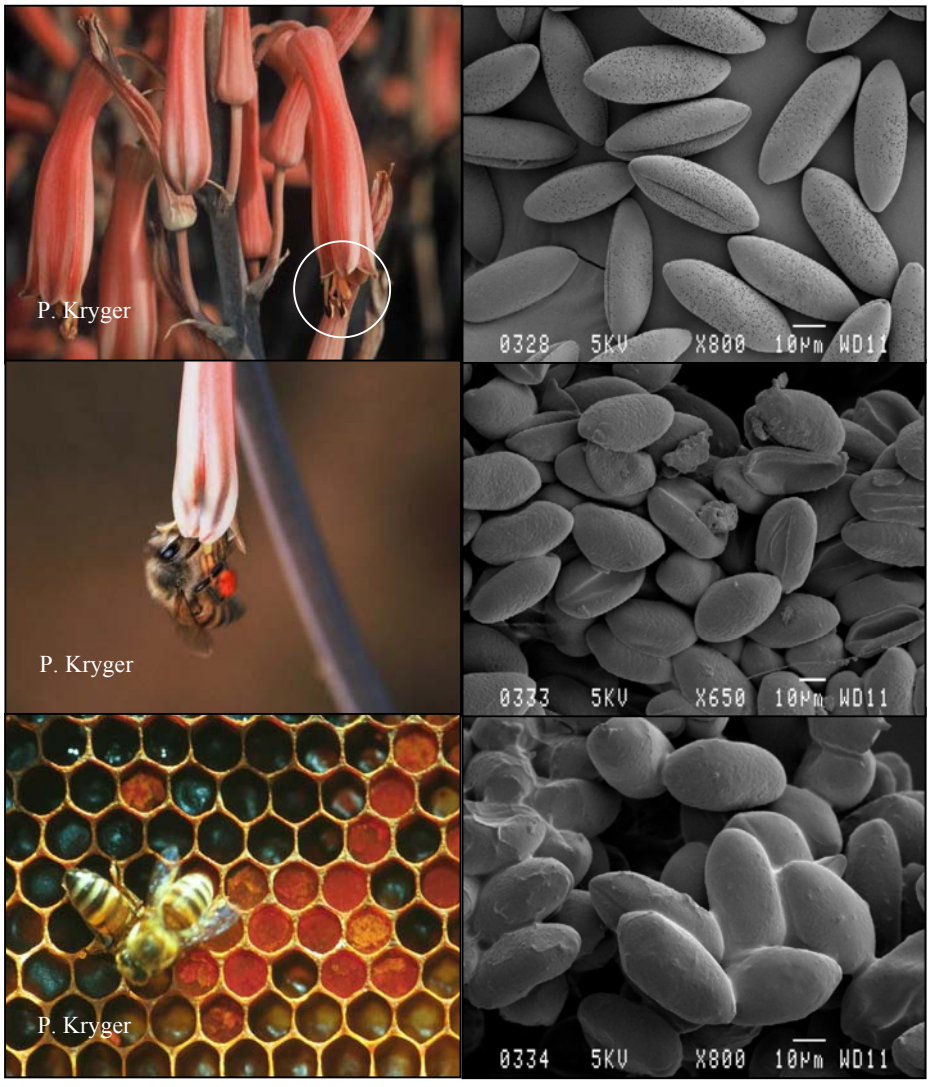
CHAPTER 1

Nutritional content of fresh, bee-collected and stored pollen of *Aloe greatheadii* var *davyana* (Asphodelaceae)

H. Human and S. W. Nicolson

Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa



Paper published in *Phytochemistry* **67**: 1486-1492 (2006)

Abstract

Aloe greatheadii var *davyana* is the most important indigenous South African bee plant. Fresh, bee-collected and stored pollen of this aloe was collected and analysed for its nutritional content, including amino acid and fatty acid composition. Highly significant differences were found between the three types of pollen. Collection and storage by the bees resulted in increased water (13 to 21% wet wt) and carbohydrate content (35 to 61% dry wt), with a resultant decrease in crude protein (51 to 28% dry wt) and lipid content (10 to 8% dry wt). Essential amino acids were present in equal or higher amounts than the known required minimum levels for honeybee development, with the exception of tryptophan. Fatty acids comprised a higher proportion of total lipid in fresh pollen than in bee-collected and stored pollen. This study is the first to compare the changes that occur in pollen of a single plant species after collection by honeybees.

Introduction

In South Africa, the winter flowering *Aloe greatheadii* var *davyana* (Schönland) Glen & D.S. Hardy is the most important indigenous plant utilised by migratory beekeepers (Johannsmeier, 2001). This species belongs to the largest section of the succulent genus *Aloe*, the Pictae or spotted aloes (Glen & Hardy, 2000). It has a widespread distribution across the northern summer rainfall area of South Africa, being very common in the bushveld and on the Witwatersrand (Short, 1962; Smith & Crouch, 2001). Migratory beekeepers commonly move their bees (*Apis mellifera scutellata*) to the aloe fields north of Pretoria in winter, when no other food source is available, in order to make use of the strong pollen and nectar flow of *A. greatheadii* var *davyana* (Fletcher & Johannsmeier, 1978). Apart from honey production, beekeepers use the pollen flow to build up colonies, rear queens and increase colony numbers. Honeybees become particularly aggressive and unmanageable during this period (Doull, 1976; Fletcher & Johannsmeier, 1978). This behaviour might be caused by some properties of the pollen, or it may be natural behaviour patterns of bees in a situation of abundant food and a large amount of sealed brood (Doull, 1976).

The quantity and quality of pollen collected by honeybees affects reproduction, brood rearing and longevity, thus ultimately the productivity of the colony (Kleinschmidt & Kondos, 1978). Apart from small quantities in nectar (Baker & Baker, 1983), honeybees obtain all the proteins, lipids, minerals and vitamins they need for brood rearing, adult growth and development from pollen (Day et al., 1990; Loidl & Crailsheim, 2001). The proportions of these nutrients can vary widely among pollens of different plant species (Todd & Bretherick, 1942; Stanley & Linskens, 1974; Roulston & Cane, 2000), but few complete analyses are available for the chemical composition of pollens.

Pollen analyses are generally carried out on bee-collected pollens because of the ease of collection (Stanley & Linskens, 1974). Bees do not consume fresh pollen. During collection and storage the pollen composition is changed through the addition of mainly nectar, but also glandular secretions (Winston, 1987; Roulston, 2005) and together with a specific bacterial flora associated with stored pollen, this increases the digestibility and nutritive value of pollen for honeybees (Herbert & Shimanuki, 1978). Addition of nectar to fresh pollen will affect all values obtained by chemical analysis, as illustrated

by the data of Louw and Nicolson (1983), who compared the chemical composition of *Virgilia divaricata* (Fabaceae) pollen with the pollen paste used by the carpenter bee, *Xylocopa capitata,* to provision larval cells.

Even though *A. greatheadii var davyana* is regarded as a very important bee plant, no analysis of its pollen is available, except for a single protein measurement (33.8% dry mass) (Johannsmeier, 2001). The only other nutritional information available for *Aloe* pollen is that *A. ferox* pollen has a crude protein content of 47% (Roulston et al., 2000) and that *Aloe* pollen in general, is starchless (Franchi et al., 1996). Pollen analysis is used here to determine the nutritional value of *A. greatheadii* var *davyana* pollen for honeybees, and to investigate whether there is anything special about the pollen that causes the rapid buildup of colonies. We have compared pollen from the flower, the bee corbiculae and the hive with each other and with *Eucalyptus* pollen. *Eucalyptus* species in South Africa provide more than 50% of the country's annual honey crop (Johannsmeier, 2001) and the abundant pollen and nectar also leads to strong build up of honeybee colonies (Fletcher & Johannsmeier, 1978).

Methods

Study site and plant species

Six beehives were moved in June 2005 to Roodeplaat Nature Reserve, 25km NE of Pretoria. Fresh pollen was collected from about 30000 *A. greatheadii* var *davyana* flowers by gently brushing the anthers with a small paintbrush. Bee-collected pollen was obtained directly from bees returning to the hives through a bottom fitting pollen trap. Stored pollen was removed from 10-15 adjacent cells in the brood frames of each of the six hives. These three types of pollen were subjected to scanning electron microscopy, and samples were frozen at -20°C for chemical analyses.

Scanning electron microscopy

Pollen was mounted on SEM stubs and sputter coated with gold, using a Polaron E5200 sputter coater (Watford, UK). Specimens were examined and pictures obtained with a JEOL 840 Scanning Electron Microscope (Tokyo, Japan) at the Laboratory for Microscopy and Microanalysis at the University of Pretoria.

Water content

Samples of the three pollen types (0.3 g of fresh pollen, 1 g of bee-collected and stored pollen) were dried to constant weight at 65ºC to obtain water content as a percentage of fresh weight (AOAC, 2000).

Protein analysis

Crude protein content was determined, in duplicate, according to the Dumas method (AOAC, 2000). Total nitrogen content was determined using an elemental analyser (model FP-428; Leco instruments, Mississauga, Canada), calibrated against known standards. Pollen samples (0.2 g) were weighed into a combustion boat, and combusted at 950ºC. To determine total crude protein, nitrogen values were multiplied by a conversion factor of 6.25 (Roulston et al., 2000).

Amino acid analysis

Pollen samples (10-20 mg) were analysed in duplicate for free amino acids as well as tryptophan in the Department of Biochemistry at the University of Pretoria. The samples were analysed by the Pico. Tag Column®method (3.9 mm x 15 cm) using a Waters HPLC amino acid analyser (Waters, Millipore Corp., Milford, MA). Samples were hydrolysed with 6N HCl, and then derivatised with phenylisothiocyanate (PITC) to produce Phenyltiocarbamyl (PTC) amino acids. These amino acids were analysed by reverse phase HPLC. Buffers used were 0.14M sodium acetate trihydrate and water-acetonitrile (60:40). Absorbance was detected at 254 nm using a UV spectrophotometer. The column operated at 46°C with a flow rate of 1ml/min (Bidlingmeyer et al., 1984).

Lipid content

Pollen grains were ground with a mortar and pestle to release all internal pollen lipids. Rupturing of the pollen grains was verified microscopically. Total lipid content was obtained by chloroform-methanol extraction, in duplicate, of the dried pollen using the method described by Folch et al. (1957), and the lipid fraction was estimated from the difference in weight.

Fatty acids

Standard procedures were used for methylation of lipids, using 0.7 g pollen per analysis, prior to determination of fatty acid composition (Genet et al., 2004). Fatty acids were

identified using a Varian (Varian Ass Inc 1985, USA) 3300 FID chromatograph, with WCOT fused silica capillary columns, CPSIL 88 (100 m, 0.25 mm). Column temperature was 140-240ºC while the injector port and FID were maintained at 250ºC. Helium was used as the carrier gas at an airflow rate of 50 ml/min. The fatty acids in samples were identified through a comparison with the relative retention times of fatty acid methyl ester peaks in standards obtained from Sigma (Taufkirchen, Germany).

Ash

Samples of the three pollen types (0.2 g each) were weighed into porcelain crucibles and placed in a temperature-controlled furnace that was preheated to 600ºC for 2 h. Crucibles were transferred to a desiccator, cooled and weighed immediately thereafter (AOAC, 2000).

Statistical analysis

Data for nutritional content of fresh, bee-collected and stored *A. greatheadii* var *davyana* pollen (water content, crude protein, lipid, ash and carbohydrates) did not meet the assumptions for parametric statistics; variances were not homogeneous and data did not conform to a normal distribution. Statistical comparisons were therefore made using Kruskal-Wallis ANOVA by ranks and the Mann-Whitney U test (Zar, 1984). Analyses were performed with the program Statistica 6.0 (1984-2004). We compared the proportions of essential and non-essential amino acids and the proportions of saturated and non-saturated fatty acids of the different pollen types, based on the absolute amounts in each pollen type, using the Fischer-Exact test. Values are given throughout as means ± SD.

Results

Fresh pollen grains vary in size from 44-50 µm, are bilaterally symmetrical and have an elliptical shape with a deep furrow (Fig. 1A). The surface is perforated-reticulate and exine ornamentations are less visible on the tips of pollen grains where pollenkitt is more abundant (Fig. 1A). The low relative humidity during the flowering season contributes to the dehydrated status of fresh pollen grains. Bee-collected pollen grains are hydrated and swollen compared to fresh pollen. An increase in volume, especially in

the furrow area, is evident in both bee-collected (Fig. 1B) and stored pollen of *A. greatheadii* var *davyana* (Fig. 1C).

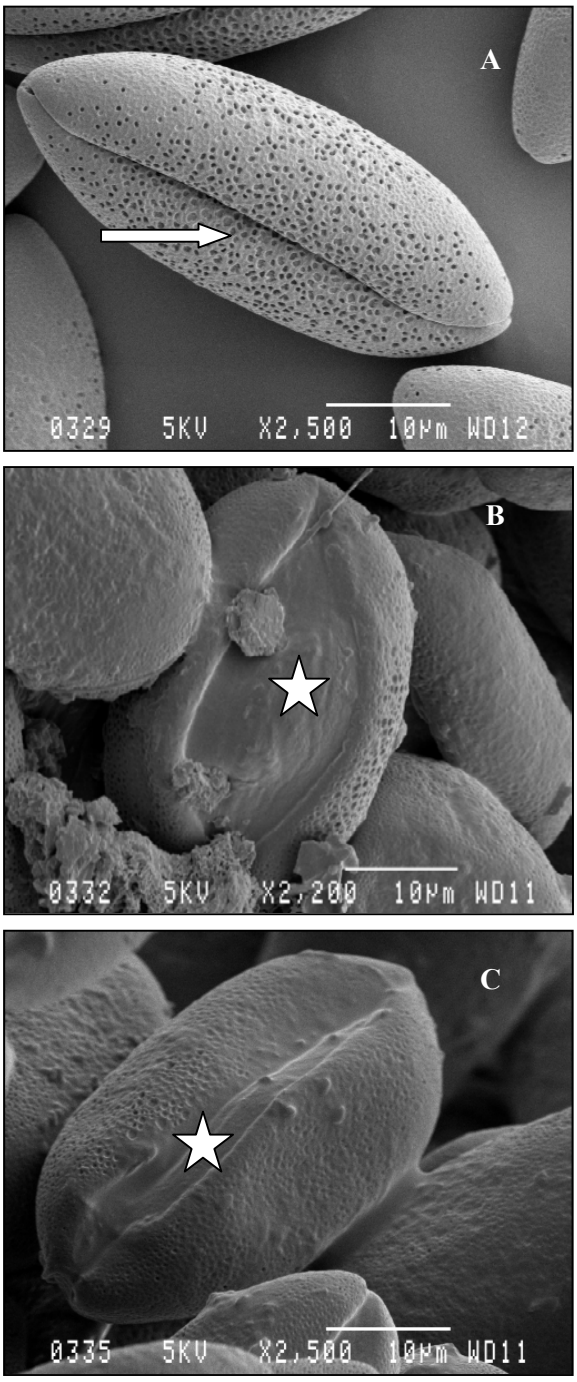


Figure 1. Scanning electron microscopy pictures of **(A)** fresh, **(B)** bee-collected and **(C)** stored *A. greatheadii* var *davyana* pollen. Arrows indicate deep furrow in fresh pollen. Bee-collected and stored pollen is swollen, especially in the furrow area (*) where the intine is exposed.

A summary of the nutritional content of the three types of *A. greatheadii* var *davyana* pollen is presented in Table 1. There are highly significant differences in chemical composition between fresh, bee-collected and stored *A. greatheadii* var *davyana* pollen: for water content ($H_{2, 36} = 25.297$, $P < 0.001$), protein ($H_{2, 36} = 31.207$, $P < 0.0001$), lipid ($H_{2, 36} = 31.195$, $P < 0.001$) ash ($H_{2, 36} = 23.457$, $P < 0.001$) and carbohydrate ($H_{2, 36} = 24.532$, $P < 0.001$). The water content in fresh pollen is significantly lower than that of bee-collected and stored pollen (for both $z = -4.157$, $P < 0.001$). Similarly bee-collected pollen has significantly lower water content than stored pollen ($z = -2.078$, $P = 0.038$).

Table 1. Comparison of the nutritional content of fresh, bee-collected and stored pollen of *A. greatheadii* var *davyana*. Values are means ± SD of 6 samples. Carbohydrate content was obtained by difference.

	Fresh pollen	**Bee collected pollen**	**Stored pollen**
	ξ ± SD	ξ ± SD	ξ ± SD
Water content (% wet mass)	13.1 ± 1.4	18.8 ± 3.3	21.0 ± 2.4
Crude protein (% dry mass)	50.8 ± 2.7	31.4 ± 1.0	28.1 ± 1.6
Lipids (% dry mass)	10.0 ± 1.4	5.5 ± 1.0	7.6 ± 0.2
Ash (% dry mass)	4.5 ± 0.4	3.6 ± 0.2	3.6 ± 0.2
Carbohydrate (% dry mass)	34.7 ± 3.1	59.5 ± 1.3	60.7 ± 1.5

Crude protein content decreases significantly from 51% dry mass in fresh *A. greatheadii* var *davyana* pollen to 31% in bee-collected and 28% in stored pollen ($z = 4.157$, $P < 0.001$, for all paired comparisons). At least 18 amino acids are present in *A. greatheadii* var *davyana* pollen, including the 10 essential amino acids for honeybees (Table 2). The proportions of essential and non-essential amino acids did not differ significantly between fresh, bee-collected and stored pollen ($P = 1.0$).

Table 2. Amino acids in *A. greatheadii* var *davyana* pollen. Quantities are given as g/100g protein and compared with the minimal levels of essential amino acids necessary for honeybees and with royal jelly (De Groot, 1953).

	***Aloe greatheadii* var *davyana* pollen**				
	Fresh	**Bee collected**	**Bee stored**	**Min levels required***	**Royal jelly***
Essential amino acids					
Arginine	5.09	5.13	5.29	3.0	5.10
Histidine	1.89	2.12	2.25	1.50	2.20
Isoleucine	4.08	4.05	4.35	4.00	5.30
Leusine	6.96	7.04	7.48	4.50	7.70
Lysine	6.06	6.35	6.50	3.00	6.70
Methionine	1.53	2.17	2.25	1.50	1.90
Phenylalanine	4.15	4.13	4.44	1.50	4.10
Threonine	4.39	4.71	5.03	3.00	4.00
Tryptophan	0.14	0.16	0.16	1.00	1.30
Valine	4.82	4.89	5.16	4.00	6.70
Non-essential amino acids					
Alanine	5.02	6.01	5.85		
Aspartic acid	8.58	9.05	9.90		
Glutamic acid	9.75	9.37	10.78		
Glycine	3.97	4.26	4.51		
Hydroxyproline	0.92	0.90	0.92		
Proline	6.21	6.90	7.32		
Serine	5.25	5.40	5.78		
Tyrosine	2.75	2.75	2.81		

(*taken from De Groot, 1953)

Table 2 compares the quantities of essential and non-essential amino acids in the three pollen samples with those in royal jelly and the minimum requirements of honeybees (De Groot, 1953). Essential amino acids in *A. greatheadii* var *davyana* pollen are present in equal or higher amounts than the minimum requirements, with the exception of tryptophan. The levels of essential amino acids in all three types of *A. greatheadii* var *davyana* pollen are similar to those in royal jelly.

Lipid content is significantly higher ($z = 4.157$, $P < 0.001$) in fresh pollen than in bee-collected and stored pollen. At the same time bee-collected pollen contains significantly less lipid than stored pollen ($z = -4.157$, $P < 0.001$). The lipid fraction of *A. greatheadii* var *davyana* pollen includes 18 fatty acids and a number of minor unidentified peaks (Table 3). The four dominant fatty acids present in fresh pollen are palmitic acid, stearic acid (C-18), oleic acid and gadoleic acid (C-20:1). These four fatty acids compose 76% (fresh), 72% (bee-collected) and 65% (stored) of the total lipid content found in *A. greatheadii* var *davyana* pollen. The percentage of stearic acid decreases in bee-collected and stored pollen while the percentage of gadoleic acid increases, especially in bee-collected pollen. However, the proportions of saturated, monounsaturated and polyunsaturated fatty acids do not differ significantly between fresh, bee-collected and stored pollen of *A. greatheadii* var *davyana* ($P = 0.2$).

Carbohydrate is significantly lower in fresh than in stored pollen ($z = -4.157$, $P < 0.0001$). There are however no significant differences in carbohydrate content between bee-collected and stored pollen ($z = -1.617$, $P = 0.106$).

In both bee-collected and stored pollen ash content was significantly lower than in fresh pollen ($z = 4.157$, $P < 0.0001$) but did not differ significantly between bee-collected and stored pollen ($z = 0.346$, $P = 0.729$).

Table 3. Fatty acid composition of total lipid fractions extracted from *A. greatheadii* var *davyana* pollen. Data for individual fatty acids are given both as mg/g dry mass of pollen and as a percentage of the total fatty acids. A missing value indicates that the fatty acid was not detected.

Lo Long Chain Fatty Acids		**Fresh pollen**		**Bee collected pollen**		**Bee stored pollen**	
		mg/g	%FA	mg/g	%FA	mg/g	%FA
Myristic	C14:0	2.64	4.37	0.27	0.86	1.47	2.54
Palmitic	C16:0	14.37	23.76	4.39	14.03	12.85	22.19
Stearic	C18:0	10.27	16.99	1.52	4.85	3.51	6.07
Arachidic	C20:0	1.16	1.93	0.3	0.97	0.75	1.30
Behenic	C22:0	0.19	0.32	0.51	1.64	1.34	2.31
Lignoceric	C24:0			0.21	0.68	1.47	2.53
Total saturated		**28.63**	**47.37**	**7.20**	**23.03**	**21.39**	**36.93**
Palmitoleic	C16:1	1.25	2.06				
Oleic	C18:1 n-9	14.17	23.44	3.66	11.71	7.21	12.45
Gadoleic	C20:1	7.00	11.58	12.99	41.53	13.99	24.16
Total monounsaturated		**22.42**	**37.08**	**16.65**	**53.24**	**21.20**	**36.61**
Ricinoleic	C18:2 n-6			0.86	2.75	3.34	5.76
Linoleic	C18:2	1.26	2.08	2.55	8.15	2.16	3.73
Alphalinolenic	C18:3 n-3			0.05	0.17	0.12	0.21
Gamalinolenic	C18:3 n-6	2.18	3.61	2.38	7.61	3.91	6.74
Eicosadienoic	C20:2	0.54	0.90	0.12	0.40	0.50	0.87
Homo-g-linolenic	C20:3 n-6			0.02	0.06	0.04	0.07
Timnodonic	C20:5 n-3	1.31	2.16	0.13	0.42	0.62	1.06
Brassic	C22:2			0.16	0.52	0.20	0.34
DHA	C22:6 n-3	2.31	3.83				
Total polyunsaturated		**7.60**	**12.58**	**6.27**	**20.08**	**10.89**	**18.78**
Unidentified peak a		1.79	2.97	0.11	0.34	0.50	0.86
Unidentified peak b				0.57	1.82	0.46	0.79
Unidentified peak c				0.14	0.44	1.95	3.37
Unidentified peak d				0.16	0.51	0.74	1.27
Unidentified peak e				0.18	0.56	0.22	0.37
Unidentified peak f						0.59	1.01
Total unidentified peaks		**1.79**	**2.97**	**1.05**	**3.68**	**4.46**	**7.67**
Total fatty acids		**60.44**	100%	**31.28**	100%	**57.94**	100%

Discussion

The morphology of *A. greatheadii* var *davyana* pollen is similar to that described for other *Aloe* species (Steyn et al., 1998). Pollen shape changes during development, dispersal and arrival on the stigma due to loss and then uptake of water (Nepi et al., 2001). The increase in moisture content reflects the addition of nectar and glandular secretions. During rehydration, the intine and protoplasm absorb water and increase in volume while the exine stretches (Pacini, 1986), thereby enhancing the availability of nutrients for digestion, because exposure of the intine (Fig. 1B) presents a region for enzymatic penetration and subsequent processing of the pollen grain contents (Human & Nicolson, 2003, Nepi et al., 2005). Thus pollen handling by bees probably prepares the grains for efficient digestion.

The protein content of pollen is considered a direct and reliable measure of its nutritional value (Pernal & Currie, 2000; Cook et al., 2003). Roulston et al. (2000) compiled a database of the crude protein concentrations in hand-collected pollen of 377 plant species, either through their own analyses or from the literature. Contrary to expectation, pollen of animal-pollinated plants was not richer in protein than that of wind-pollinated plants. In spite of the importance of pollen protein to bees, honeybees collect pollens with protein contents ranging widely from 12 to 61% across all plant taxa (Roulston et al., 2000). The crude protein content of fresh pollen lies at the high end of the range of values in the literature and is comparable with that of buzz-pollinated plants (Roulston et al., 2000). The protein content in bee-collected *A. greatheadii* var *davyana* pollen is higher than that of most bee-collected pollens (20-33%) of *Eucalyptus* species (Kleinschmidt & Kondos, 1978; Rayner & Langridge, 1985; Somerville, 2001). Even though the summer flowering aloe *A. zebrina,* belonging to the same *Aloe* Section*,* is not considered an important bee plant, its fresh pollen also has a very high protein content (54.9% dry mass) (Human & Nicolson, unpublished data).

Amino acid composition, however, may define the nutritional value of pollen more accurately than protein content, since the nutritional value is reduced when inadequate amounts of the essential amino acids (De Groot, 1953; Cook et al., 2003) are present. Generally pollen contains all the essential amino acids but the amounts may vary

between plant species (Roulston et al., 2000). The most frequently lacking amino acid in many *Eucalyptus* species is isoleucine, while others have borderline concentrations (Somerville, 2001). Some *Eucalyptus* species are also reported to be limiting in tryptophan (Bell et al., 1983; Rayner & Langridge, 1985). The predominant amino acids in pollen of 62 species, including 20 *Eucalyptus* species reported by Somerville (2001) are glutamic acid, aspartic acid and proline, all non-essential amino acids.

Pollen lipids consist of internal cytoplasmic lipids and external lipids of the pollenkitt, but lipid content reported in the literature is mostly that of lipids derived from the pollenkitt of pollen and may comprise only a small fraction of total lipids (Roulston & Cane, 2000; Manning, 2001). Evans et al. (1987) demonstrated dramatic increases in lipid content after mechanically fracturing pollen grains of *Brassica napus.* Although we used ground pollen for lipid extractions, the pollen of *A. greatheadii* var *davyana* does not have a very high lipid content compared to the range of 0.8% to 31.7% reported in the literature (Farag et al., 1978; Evans et al., 1987; Roulston & Cane, 2000). The latter study recorded lipid content higher than 5% for at least 60% of the plant species. The lipid content of *A. greatheadii* var *davyana* pollen was much higher than average values reported for *Eucalyptus* species (< 2%, Somerville 2001; < 1.42%, Manning & Harvey 2002). For honeybees the lipids, including fatty acids and sterols, are important sources of energy, are used for the synthesis of reserve fat and glycogen, and contribute to the production of royal jelly (Singh et al., 1999; Loidl & Crailsheim, 2001; Manning, 2001; Manning & Harvey, 2002). According to a study by Singh et al. (1999) bees preferred pollens with the highest amount of lipids.

In addition to variation in lipid content, pollen also varies in the relative proportions of fatty acids and in their diversity (Manning, 2001; Markowicz Bastos et al., 2004). Fatty acids are important in the reproduction, development, and nutrition of honeybees (Manning, 2001; Farag et al., 1978). Certain fatty acids, such as linoleic, linolenic, myristic and lauric acids, have bactericidal and antifungal properties that are important for colony hygiene (Manning, 2001; Manning & Harvey, 2002). From the literature it seems that, in general, the dominant fatty acids present in pollens are palmitic (C-16), oleic (C-18:1), linoleic (C-18:2) and linolenic (C-18:3) acids (Manning, 2001). It is known that once pollen is stored, its fatty acid composition changes (Van der Vorst, 1982). The concentrations of individual fatty acids in *A. greatheadii* var *davyana* pollen

are close to values reported for pollens from 46 plant species by Manning (2001), the major difference being the high gadoleic acid content. Gadoleic acid is not listed as being present in other pollens.

Carbohydrate content varies widely in pollen: Todd and Bretherick (1942) recorded values from 1-37% of total dry mass in hand-collected pollen, and from 21–48% in bee-collected pollen. Particularly because of the added nectar, carbohydrate constitutes a large fraction of the nutritional content of *A. greatheadii* var *davyana* pollen. Not all carbohydrates are nutritionally useful, e.g. pectin is an important structural component of the cell wall and essential in plant growth and development but has no known nutritional value for bees (Aouali et al., 2001; De Halac et al., 2003). Pectin content in *A. greatheadii* var *davyana* is 7.1% in fresh pollen, and 8.5% and 8.3% in bee-collected and stored pollen respectively (Human & Nicolson, unpublished data). This increase in pectin content can be due to a response of pollen to hydration: water gain by pollen may initiate the mechanism of pollen germination, where the synthesis of pectins is required for the new wall construction (Shivanna, 2003).

According to Roulston and Buchman (2000), starch content in pollen ranges from 0-22%. Most pollen contain less than 5% starch: sunflower pollen, for example, has a starch content of 0.4% while *Aloe ferox* pollen is starchless (Roulston & Buchmann, 2000). Similarly, no starch is present in *A. greatheadii* var *davyana* pollen (Human & Nicolson, unpublished data). According to Todd and Bretherick (1942) and Herbert and Shimanuki (1978), ash content of pollen ranges between 0.9 and 6.4%. This is contrary to the study of Todd and Bretherick (1942), who determined no differences between the ash content of fresh and bee-collected pollen.

In general, studies on the nutritional content of pollen have focussed only on single aspects, with some exceptions (Roulston & Cane, 2000; Roulston et al., 2000; Manning, 2001; Somerville, 2001). Most of the analyses used bee-collected pollen. To our knowledge, this study is the first to compare the nutritional content of fresh, bee-collected and stored pollen in a single plant species and thus highlight the changes that occur in pollen after collection. The overall nutritional content of *A. greatheadii* var *davyana* pollen appears to be much better than that of *Eucalyptus* species. However,

except for the very high protein content and high concentration of gadoleic acid, there is no specific nutritional aspect of the pollen of *A. greatheadii* var *davyana* that can explain the aggression observed in bees on the aloe fields. The extremely high protein level and overall excellent nutritional content of *A. greatheadii* var *davyana* pollen, together with the movement of apiaries in winter to aloe fields north of Pretoria, contributes to the productivity of the migratory beekeeping industry. Further work needs to examine pollen digestion efficiency and the effect of this high protein content on ovarian and hypopharyngeal gland development.

Acknowledgements

We are grateful to Roodeplaat Nature Reserve for permission to move bees onto the Reserve, to Prof Robin Crewe for his bees and to Dr Per Kryger and Dr Vincent Dietemann for their assistance in working with the bees. Arnold and Danelle Human helped to collect the fresh pollen. The South African National Research Foundation, the Italian Ministry of Foreign Affairs (General Direction for Cultural Promotion and Cooperation) in the framework of the Italy/South Africa Science and Technology Agreement (2001-2004) and the University of Pretoria are thanked for funding this project. We thank Massimo Nepi, Department of Environmental Sciences, University of Siena, Italy, for guidance, the Laboratory for Microscopy and Microanalysis and UP Nutrilab, (Department of Animal and Wildlife Sciences), both at the University of Pretoria.

References

AOAC (2000) *Official Methods of Analysis*. Arlington, Virginia, USA.

Aouali, N., Laporte, P. & Clement, C. (2001) Pectin secretion and distribution in the anther during pollen development in *Lilium*. *Planta* **213**: 71-79.

Baker, H.G. & Baker, I. (1983) Floral nectar sugar and constituents in relation to pollinator type. In Jones, C.E., Little, R.J. (Eds.), *Handbook of experimental pollination biology.* Van Nostrand Reinhold, New York, USA.

Bell, R.R., Thornber, E.J., Seet, J.L.L., Groves, M.T., Ho, N.P. & Bell, D.T. (1983) Composition and protein quality of honeybee-collected pollen of *Eucalyptus marginata* and *Eucalyptus calophylla*. *Journal of Nutrition* **113**: 2479-2484.

Bidlingmeyer, B.A., Cohen, A.C. & Tarvin, T.L. (1984) Rapid analysis of amino acids using pre-column derivatization. *Journal of Chromatography and Biomedical Applications* **336**: 93-104.

Cook, S.M., Awmack, C.S., Murray, D.A. & Williams, I.H. (2003) Are honeybees' foraging preferences affected by pollen amino acid composition? *Ecological Entomology* **28**: 622-627.

Day, S., Beyer, R., Mercer, A. & Ogden, S. (1990) The nutrient composition of honeybee-collected pollen in Otago, New Zealand. *Journal of Apicultural Research* **29**: 138-146.

De Groot, A.P. (1953) Protein and amino acid requirements of the honeybee (*Apis mellifica* L.). *Physiologia Comparata et Oecologia* **3**: 1-83.

De Halac, N., Cismondi, I.A., Rodriguez, M.I. & Fama, G. (2003) Distribution of pectins in the pollen apertures of *Oenothera hookeri.velans* ster/+ster. *Biocell* **27**: 11-18.

Doull, K.M. (1976) The "secret" of the aloes. *South African Bee Journal* **48**: 18-21.

Evans, D.E., Rothnie, N.E., Palmer, M.V., Burke, D.G., Sang, J.P., Knox, R.B., Williams, E.G., Hilliard, E.P. & Salisbury, P.A. (1987) Comparative analysis of fatty acids in pollen and seed of rapeseed. *Phytochemistry* **26**: 1895-1897.

Farag, R.S., Youseff, A.M., Ewies, M.A. & Hallabo, S.A.S. (1978) Long-chain fatty acids of six pollens collected by honeybees in Egypt. *Journal of Apicultural Research* **17**: 100-104.

Fletcher, D.J.C. & Johannsmeier, M.F. (1978) The status of beekeeping in South Africa. *South African Bee Journal* **50**: 5-20.

Folch, J., Lees, M. & Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**: 497-509.

Franchi, G.G., Bellani, L., Nepi, M. & Pacini, E. (1996) Types of carbohydrate reserves in pollen: localization, systematic distribution and ecophysiological significance. *Flora* **191**: 143-159.

Genet, T., Labuschagne, M. & Hugo, A. (2004) Capillary gas chromatography analysis of Ethiopian mustard to determine variability of fatty acid composition. *Journal of Science and Food Agriculture* **84**: 1663-1670.

Glen, H.F. & Hardy, D.S. (2000) Fascicle 1: Aloaceae (First Part): *Aloe*. In Germishuizen, G. (Ed), *Flora of Southern Africa*. National Botanical Institute, South Africa.

Herbert, E.W. & Shimanuki, H. (1978) Chemical composition and nutritive value of bee-collected and bee-stored pollen. *Apidologie* **9**: 33-40.

Human, H. & Nicolson, S.W. (2003) Digestion of maize and sunflower pollen by the spotted maize beetle *Astylus atromaculatus* (Melyridae): is there a role for osmotic shock? *Journal of Insect Physiology* **49**: 633-643.

Johannsmeier, M.F. (2001) *Beekeeping in South Africa*. Agricultural Research Council, Pretoria.

Kleinschmidt, G.J. & Kondos, A.C. (1978) The effect of dietary protein on colony performance. *Australian Beekeeper* **80**: 251-257.

Loidl, A. & Crailsheim, K. (2001) Free fatty acids digested from pollen and triolein in the honeybee (*Apis mellifera carnica* Pollmann) midgut. *Journal of Comparative Physiology B* **171**: 313-319.

Louw, G.N. & Nicolson, S.W. (1983) Thermal, energetic and nutritional considerations in the foraging and reproduction of the carpenter bee *Xylocopa capitata*. *Journal of the Entomological Society of Southern Africa* **46**: 227-240.

Manning, R. (2001) Fatty acids in pollen: a review of their importance for honeybees. *Bee World* **82**: 60-75.

Manning, R. & Harvey, M. (2002) Fatty acids in honeybee-collected pollens from six endemic Western Australian eucalypts and the possible significance to the

Western Australian beekeeping industry. *Australian Journal of Experimental Agriculture* **42**: 217-223.

Markowicz Bastos, D. H., Barth, O.M., Rocha, C.I., Da Silva, Cunha, I.B., De Oliveira Carvalho, P., Torres, E.A. & Michelan, M. (2004) Fatty acid composition and palynological analysis of bee (*Apis*) pollen loads in the states of Sao Paulo and Minas Gerais, Brazil. *Journal of Apicultural Research* **43**: 35-39.

Nepi, M., Franchi, G.G. & Pacini, E. (2001) Pollen hydration status at dispersal; cytophysiological features and strategies. *Protoplasma* **216**: 171-180.

Nepi, M., Cresti L., Maccagnani,B., Ladurner, E. & Pacini, E. (2005) From the anther to the proctodeum: pear (*Pyrus communis*) pollen digestion in *Osmia cornuta* larvae. *Journal of Insect Physiology* **51**: 749-757.

Pacini, E. (1986) An approach to harmomegathy. In Cresti, M, Dallai, R (Eds.), *Biology of Reproduction and Cell Motility in Plants and Animal*. pp. 126-133, University of Siena Press, Italy.

Pernal, S.F. & Currie, R.W. (2000). Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). *Apidologie* **31**: 387-409.

Rayner, C.J. & Langridge, D.F. (1985) Amino acids in bee-collected pollens from Australian indigenous and exotic plants. *Australian Journal of Experimental Agriculture* **25**: 722-726.

Roulston, T.H. & Buchmann, S.L. (2000) A phylogenetic reconsideration of the pollen starch-pollination correlation. *Evolutionary Ecological Research* **2**: 627-643.

Roulston, T.H. & Cane, J.H. (2000) Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution* **222**: 187-209.

Roulston, T.H., Cane, J.H. & Buchmann, S.L. (2000) What governs protein content of pollen: pollinator preferences, pollen pistil interactions, or phylogeny? *Ecological Monographs* **70**: 617-643.

Roulston, T.H. (2005) Pollen as a reward. In Dafni, A., Kevan, P.G., Husband, B.C. (Eds.), *Practical pollination biology*. pp. 234-260, Enviroquest, Cambridge.

Shivanna, K.R. (2003) *Pollen biology and biotechnology*. Science Publishers Inc., Enfield.

Short, F.W.J. (1962) Bees at the aloes: a wonderful winter resource. *South African Bee Journal* **34**: 1-2.

Singh, S., Saini, K. & Jain, K.L. (1999) Quantitative comparison of lipids in some pollens and their phagostimulatory effects in honeybees. *Journal of Apicultural Research* **38**: 87-92.

Smith, G.F. & Crouch, N. (2001) A broader taxonomic concept of *Aloe greatheadii* var *davyana*, a widespread and common maculate from southern Africa. *Bradleya* **19**: 55-58.

Somerville, D.C. (2001) *Nutritional value of bee collected pollens*, Report DAN 134A, A report for the Rural Industries Research and Development Corporation, May 2001.

Stanley, R.G. & Linskens, H.F. (1974) *Pollen: biology, biochemistry, management*. Springer-Verlag, New York.

Statistica (1984-2004) *StatSoft Inc*., Tulsa, USA.

Steyn, E.M.A., Smith, G.F., Nilsson, S. & Grafstrom, E. (1998) Pollen morphology in Aloe (Aloaceae). *Grana* **37**: 23-27.

Todd, F.E. & Bretherick, O. (1942) The compositions of pollens. *Journal of Economical Entomology* **35**: 312-317.

Van der Vorst, E., Mattys, J. & De Jacobs, F.J. (1982) Comparative lipid composition of colony- and laboratory-stored pollen. *Journal of Apicultural Research* **21**: 174-177.

Winston, M. (1987) *The biology of the honey bee*. Harvard University Press, Cambridge.

Zar, J. H. (1984) *Biostatistical analysis*. Prentice-Hall, New Jersey.