Isolation and Identification of Feeding Stimulants in Honey Bee Pollens

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Introduction

Primarily bee pollinated crops contribute to the production of 33% of food worldwide [1]. This could be as much as €153bn worth of agricultural produce annually [2]. In order to meet pollination needs, industrial scale commercial beekeeping and pollinating is becoming increasingly vital. In areas of vast crop monocultures, such as parts of the US, commercial beekeepers may choose to feed high protein supplemental diets to colonies to increase nutrient diversity and stimulate brood production. Studies have shown that the addition of natural pollen, or pollen extract, to diets can increase their uptake [3]; thus suggesting pollens contain naturally occurring phagostimulants (feeding stimulants) which increase honey bee feeding response.

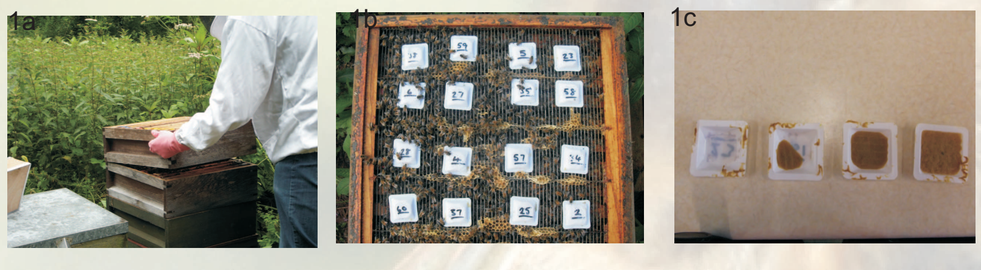
Bees don't feed on pollen in its freshly foraged form [4]. Pollen is mixed with glandular secretions, packed into comb cells, sealed with honey, and converted into a substance known as bee bread prior to consumption. This resultant bee bread has a lower pH, thus aiding storage. It is also partially pre-digested, and has been suggested to be more palatable to bees than fresh pollen [5]. An observed increase in the palatability of bee bread may suggest that any phagostimulants are in greater abundance after pollen processing, and that future work aimed at identifying them should perhaps focus on this base material. Research aimed at assessing the relative palatability of the two pollen forms, followed by attempted isolation and identification of any phagostimulatory compounds is underway.

Chemical analysis of hexane, ethyl acetate, and methanol pollen extracts, conducted in parallel with bioassay feeding trials, are being utilised to attempt to isolate specific pollen extract fractions that increase feeding response. Identification of potential stimulants is the ultimate aim. If successful, it is hoped this work may aid the production of more palatable supplemental diets, that could assist in improving honey bee health through better nutrition.

Bioassay methodology

Palatability bioassays were conducted using managed honey bee colonies on the Keele University campus. Control test diets were formed using soya flour, vegetable fat, sugar and water, with active diets either having a percentage (by mass) of soya flour substituted for pollen, or additional pollen extract added to otherwise control diet formulation.

During each choice trial, four patties of each test diet in weighing boats were inversely inserted into hives above the queen excluders in a regular manner. See Figures 1a, b & c below for illustrations of both the experimental set up, and examples of diets exhibiting different degrees of consumption following a trial.



Figures 1a, b & c: Digital images of a) The placement of test diets in hive, b) diet arrangement, and c) Post trial diets

Trials were conducted over four days, or until the first completely consumed patties were observed. Palatability, and so bee preference, was assessed on the basis of mean diet consumption (mass loss) over the trial. Consumption masses for each test diet in each hive (sixteen values per diet in total) were pooled prior to data processing.

Results and discussion

Figures 2, and 3below show the results from two trials.

Trial one (Figure 2) tested 10% trapped against 10% hive-stored (bee bread) pollen and a control diet.

Trial two (Figure 3) tested pooled hexane, ethyl acetate, and methanol solvent extracts of trapped pollen, added to blank diet, against the 10% extracted pollen, and a control diet. See right hand column for extraction methodology.
Consumption data was analysed using ANOVA following log transformation. Multiple comparisons were carried out using Tukey's test

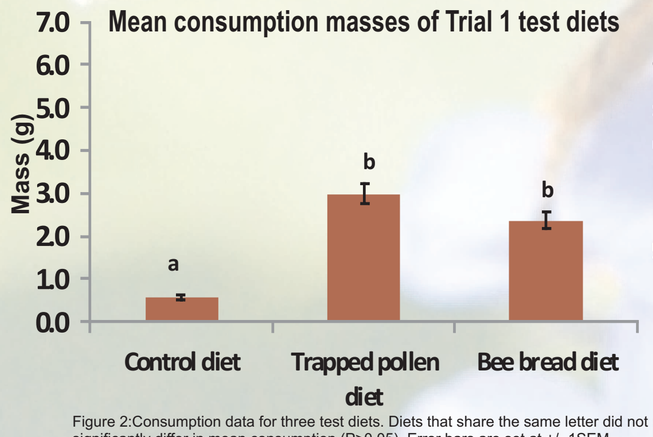


Figure 2:Consumption data for three test diets. Diets that share the same letter did not significantly differ in mean consumption (P>0.05). Error bars are set at +/- 1SEM.

The results displayed in Figure 2 show that there was no significant difference in the consumption levels between either pollen form, with both being consumed in significantly greater amounts than the control.

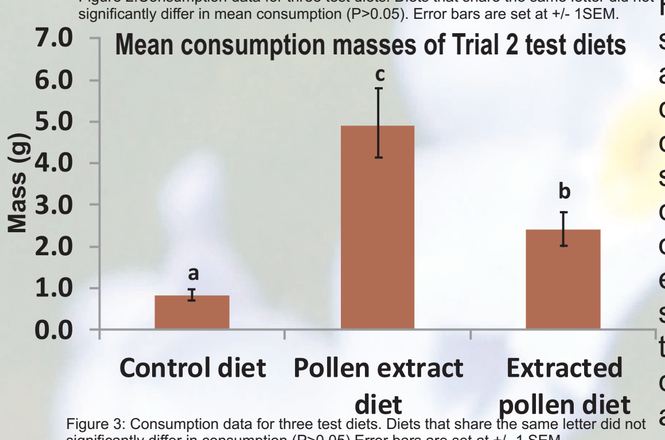


Figure 3: Consumption data for three test diets. Diets that share the same letter did not significantly differ in consumption (P>0.05).Error bars are set at +/- 1 SEM.

Figure 3 shows that when the three solvent extracts of pollen were pooled and added to a blank diet, bees consumed much more. The mean consumption of this diet was significantly more than both the control diet, and a diet containing the originally extracted pollen.The extracted pollen diet was also significantly more greatly consumed than the control diet, implying that complete stimulant extraction is not achieved.

Chemical analysis methodology

A series of solvent extractions performed on samples of freshly trapped pollen and bee bread using hexane, ethyl acetate, and methanol have been obtained and analysed using GC-MS (Figure 5). Such extracts are utilised in palatability bioassays.

- Pollen sample homogenised
- 7mL of extraction solvent added for every 1g of pollen
- Vortexed for 1 minute prior to Ultrasonic assisted solvent extraction
- Centrifuged as required prior the removal of supernatant for derivatisation and GC-MS analysis

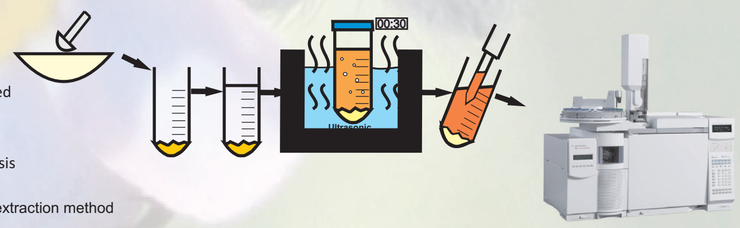
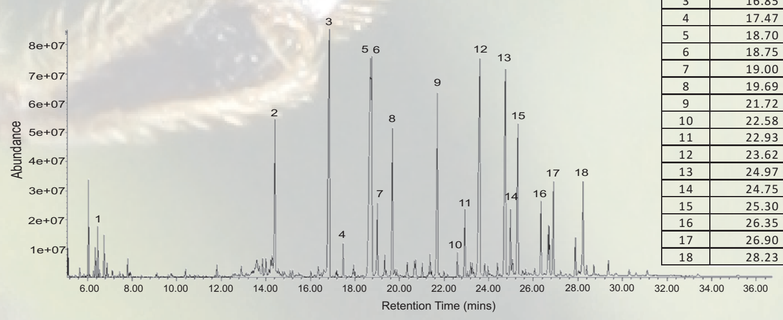


Figure 4 : Diagram illustrating the utilised solvent extraction method

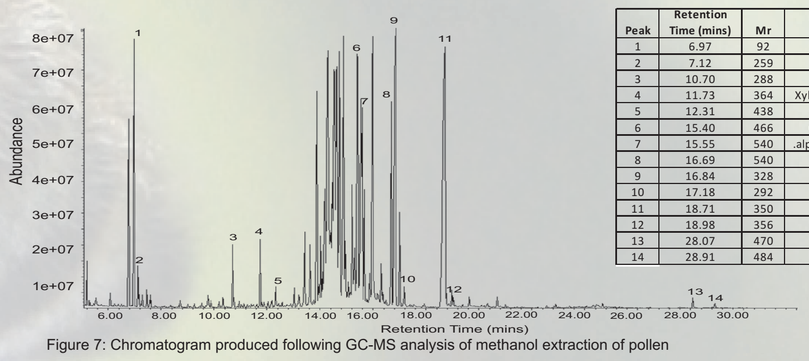
Results and discussion

Utilizing the NIST 08 mass spectral database, a range of compounds have been identified in the solvent extracts. Figure 6 shows a chromatogram of a typical hexane pollen extract, while Figure 7 shows that of a typical methanol pollen extract.



Peak	Retention Time (mins)	Mr	Compound
1	6.46	190	Benzene, 1,3-bis(1,1-dimethylethyl)-
2	14.42	300	Tetradecanoic acid, trimethylsilyl ester
3	16.85	328	Hexadecanoic acid, trimethylsilyl ester
4	17.47	296	Heneicosane
5	18.70	350	.alpha.-Linolenic acid, trimethylsilyl ester
6	18.75	354	Oleic acid, trimethylsilyl ester
7	19.00	356	Octadecanoic acid, trimethylsilyl ester
8	19.69	324	Tricosane
9	21.72	352	Pentacosane
10	22.58	366	Hexacosane
11	22.93	412	Docosanoic acid, trimethylsilyl ester
12	23.62	380	Heptacosane
13	24.97	393	2-methyl, Octacosane
14	24.75	440	Tetracosanoic acid, trimethylsilyl ester
15	25.30	408	Nonacosane
16	26.35	468	Hexacosanoic acid, trimethylsilyl ester
17	26.90	436	Hentriacontane
18	28.23	464	Tritriacontene

Figure 6: Chromatogram produced following GC-MS analysis of hexane extraction of pollen



Peak	Retention Time (mins)	Mr	Compound
1	6.97	92	Trimethylsilyl ether of glycerol
2	7.12	259	L-Proline, 1-(trimethylsilyl)-, trimethylsilyl ester
3	10.70	288	Unknown Pyranone
4	11.73	364	Xylonic acid, 2,3,5-tris-O-(trimethylsilyl)-, .gamma.-lactone, D-
5	12.31	438	3,4,5-Trihydroxypentanoic acid, tetrakis(trimethylsilyl)-
6	15.40	466	Gluconic acid, 2,3,5,6-tetrakis-O-(trimethylsilyl)-, lactone
7	15.55	540	.alpha.-D-Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-
8	16.69	540	D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-
9	16.84	328	Hexadecanoic acid, trimethylsilyl ester
10	17.18	292	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
11	18.71	350	.alpha.-Linolenic acid, trimethylsilyl ester
12	18.98	356	Octadecanoic acid, trimethylsilyl ester
13	28.07	470	Unknown sterol
14	28.91	484	Unknown sterol

Figure 7: Chromatogram produced following GC-MS analysis of methanol extraction of pollen

Extract analysis reveals that the non-polar hexane extracts contain numerous prominent hydrocarbons and fatty acids, in addition to other less abundant unidentified compounds. The polar methanol extracts contain a significant number of unidentified sugar compounds (or their derivatives), pyranones, amino acids, sterols and alcohols. Some fatty acids present in the hexane extract are again observable. Like methanol, the ethyl acetate extracts contain a number of sugar compounds and fatty acids, but appear to lack the other compound classes.

Conclusions

Bioassay data shows that bees exhibited no preference for either freshly trapped pollen, or bee bread. In this instance these results do not support previous assertions that bees find bee bread more palatable. Adding 10% by mass of either pollen type increased diet uptake. Therefore, it seems reasonable to use trapped pollen, which is easier to gather and manipulate than bee bread, when trying to identify potential phagostimulants.

The ultrasonic extraction method extracts sufficient stimulants from pollen to initiate significantly greater consumption of artificial diet, within free-flying colonies, during the summer months. Future work will concentrate on combining further chemical analysis, with bioassay work utilising pollen extracts, to attempt to isolate potential phagostimulants. This displayed bioassay work is discussed in greater detail within a research paper recently submitted for review to the Journal of Apicultural Research

Acknowledgements and References

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