PROJECT SUMMARY

RURAL INDUSTRIES
Research & Development Corporation

Development of a pollen substitute meeting the nutritional needs of honey bees

Robert Manning and John Black

The issue

Beekeepers are at the forefront of observing their bees in the environment and cues that affect them such as dearth periods between nectar flows which readily affects their main income from honey production, pollen production, queen bee production or pollination. Artificial supplements or substitutes for pollen are the only product that could help alleviate the reversal in bee population during periods of drought or constant wet weather and provide the solution (other than relocation) to the problem.

Commercial beekeepers are interested in artificial feedstuffs for bees and that interest exists Australia-wide. The importance of strong colonies is critical for pollination and any improvement in colony population would cause a large and beneficial change because of honey bees' considerable worth to the Australian food industry via pollination.

The project

Development of artificial diets/feedstuffs for honey bees has been an ongoing research investigation consuming the research lives of scientists since the 1880s. Much progress has been made over the years towards the goal of pollen replacement (substitution), however there still remains further discovery in this field. For the first time many oils and protein isolates were tested in a typical way an animal nutritionalist would approach feedstuff development and that included intensive feedstuff tests, diet development, cage and field trials with biochemical analyses. This project developed a pollen substitute that was not as good as pollen, but in field trials did assist colonies to survive.

Background

The aim of the project was to identify the current status of knowledge on the nutrition of honey bees and how this knowledge could be applied to improve the focus of research in the area of honey bee nutrition.

Methods used

Ingredients of feedstuffs and diets were tested using methods employed by many other researchers and involved the use of testing individual products in hives, testing developed diets in cage experiments inside environmental laboratory chambers and field trials under natural nutritional stress.

The research program was in three parts, the first was a feedstuff evaluation from November 2006 to May 2010 where the components of feedstuffs were tested for palatability and preference. The first of these experiments used cellulose as a base component. However, cellulose proved to be too unpalatable and was replaced by a low-fat pollen as a base to screen 27 different oils that could be purchased commercially and rum. (continues overleaf)

Following these experiments, a range of proteins and protein isolates from a variety of sources such as pea, whey, milk, egg, microalgae, lupin and soya bean were screened in a series of experiments using the oil preference and palatability data to formulate a diet. For the first time, protein isolates and a microalgal source were tested in feedstuffs for honey bees.

Following the testing and evaluation of the final diets, cage experiments were used to test the diets on honey bees using longevity, head weight as an indirect measure of hypopharyngeal gland development and nutritional status as indicators of a diet's effectiveness. The cage trials ran from October 2010 to March 2011 and encompassed a repeat trial. The final diets from the cage experiments were then used in a field trial which ran from March to August 2011 where the developed feedstuffs were trialled in nucleus hives over a tail-end redgum (*Corymbia calophylla*) honey flow in late autumn to the end of winter — a time where artificial diets are expected to be used by beekeepers. The period also coincides with Australia's largest pollination program for the almond industry where the supply of populous beehives is required.

Results/key findings

Evaluation

Of the 27 oils tested, six were found to be useful in increasing consumption as did rum. Linseed oil, which contains high levels of linolenic acid, and coconut oil, which is dominant in lauric acid, were consumed in significantly greater quantities than the base pollen. Other oils that stimulated notably high consumption rates were grapeseed oil and evening primrose oil, which are dominant in linoleic acid, plus almond oil and apricot kernel oil. Of all the oils with high preference, almond oil was not considered for feeding alone in further experiments because of its low linolenic and linoleic acid contents giving it a low antimicrobial activity for preservation of the diet. Internationally, the dominant oil used in bee feedstuffs has been 'vegetable' oil, but specifically canola, soya bean or peanut oils, which were found to have low or medium preference. Australian beekeepers who make their own feedstuffs have imitated the use of these relatively lower preference oils.

It was the first time protein isolates have been tested as bee feed. Of the proteins, the protein isolate (PI) from soya bean showed the most promise to be developed into a diet following screening tests. In initial experiments, the consumption of soya bean protein isolate diet was statistically not different to pollen diets (pollen was consumed at 4.1–9.9 g/week compared with soya bean PI with 5% oil that was consumed at 7.2 g/week), but not in subsequent experiments after a new batch of soya bean protein was obtained. Our belief is that the new batch was sodium-extracted rather than water-extracted. When the feedstuffs were analysed after the final experiments, high sodium levels (2500 ppm) were found rather than a value of around 300 ppm, which was calculated to be optimum for honey bee diets.

Cage trials

The developed soya bean protein isolate diet with 5% oil (an equal mix of almond, linseed and evening primrose) in cage experiments had the best longevity of the protein isolates tested, at 26 days. However, it gave significantly less life-span than bees fed pollen and was not significantly different to Feedbee™ - a commercially available artificial bee feed. Feedbee™ fed bees also attained heavier headweights, implying a greater hypopharyngeal gland development. The soya bean protein isolate was from a second batch later found to have a high level of sodium.

Successful methodology was developed in a cheap and effective cage design to house about 1000 honey bees for testing the effectiveness of artificial diets. Water and sugar syrup consumption (14.2–21.3 mg/bee/day) was measured alongside protein based diet consumption data. Water consumption (0.28–0.53 mg/bee/day) was found to be closely linked to diet consumption. Average pollen consumption was 0.71 mg/bee/day and the best soya bean protein isolate was consumed at only 0.26 mg/bee/day. (continues overleaf)

Consumption of the diet containing soya bean protein isolate is expected to be higher for a low sodium, water-extracted product. The information on pollen and syrup consumption is useful to beekeepers for estimating how much diet to feed and how much sugar syrup to supply a beehive without being wasteful.

Field trial

The same feedstuffs used in the cage trial were used in the field trial. The trial was conducted under extremely harsh conditions over 149 days by shaking a 1kg package of bees into an empty hive (no stored food supply), by reducing pollen supply (by pollen trap), by the natural flow of nectar and pollen waning (end of flow) and late autumn-winter conditions (cold and wet). This harsh treatment was necessary to give bees the conditions where supplementary feeding would be appropriate. All hives at the end of the experiment had brood: the soya bean PI with 5% oil (an equal mixture of linseed, evening primrose and almond) diet had a range of 55– 532 cm^2 brood; pollen fed hives had a range of 205–382.5 cm^2 and the Feedbee™ diet fed bees had 0–92.5 cm^2 of brood. All the fed beehives had pollen traps fitted on their hives to restrict protein intake. The amount of pollen trapped per day was in the range of 3.3–6.3 g for the bee colonies fed pollen, whilst the bees offered soya bean PI with 5% oil diet trapped 4.5 9.3 g/day and the Feedbee™ diet fed hives had 3.4–6.2 g/day trapped.

Example of preference tests.
Bees were most attracted to the pollen control, centre right

The harsh conditions led to 50% of the control hives (n=6, where three were with pollen traps and three without traps), 66% of defatted soya bean flour fed hives and one hive fed the soya bean protein isolate (PI) with 10% oil diet to die out. The replicate number chosen for the trial was three hives per treatment. In hindsight, because of the harshness of the conditions the number of replicates should have been elevated to enhance the chances of obtaining statistical significance between treatments. However, the developed diet did allow the bees to survive by consuming 0.15–0.39 g/day (pollen diet was consumed at 2.61–6.53 g/day) compared to the Feedbee™ diet at 0.42–1.61 g/day. The only hive in the trial to have more bees than it started with was one of the control hives that did not have a pollen trap fitted. Besides replicate number being too small (i.e. three) to compensate for deaths arising from the harsh conditions, the large variation in the data for the rest of the trial meant that statistical analysis could not be carried out with any significance.

Feedstuffs

From the literature review, it was concluded that a pollen substitute should have the following specifications: 25–30% protein meeting essential amino acid requirement; 5% lipid meeting essential fatty acid requirements and 1% sterol supplied as cholesterol; 1–1.5% minerals and vitamins meeting the requirement; < 2% starch; 10–20% fibre and 40–60% sugar and/or honey. Using that as a guide, and from a series of experiments, the final mix of ingredients of the 'best' feedstuff developed in the project (which was the soya bean protein isolate) that was statistically not different to feeding Feedbee™ contained the following components: 30% protein; 12.5% water; 10% fibre; 41.7% sugar; 4.4% oil (5% in dry mix); 1% mineral & vitamin and 0.1% cholesterol. However, the soya bean protein isolate used in the cage and field experiments contained sodium concentrations eight times greater than recommended from analysis of the literature.

Dry feeding of the pollen substitute during cage trials was problematic because of excess spillage and time required to calculate consumption rates. Pastes are easier to deal with when measuring consumption, but may not be as readily consumed by bees as a powdered diet more closely represents the parameters of pollen. One thousand bees required access to approximately 50 mL of sugar syrup (50:50) per day.

Implications

A pollen substitute that is as good as pollen was not developed, but this result may have been due to the inadvertent use of a salt-extracted soya bean meal in the cage and field experiments, which resulted in the sodium content of the diet being eight time greater than the recommended concentration.

Pollen substitutes were partially successful in that most colonies that were fed survived the harsh conditions during the field trial than unfed colonies.

Honey bees showed a preference for linseed, coconut, grapeseed, almond, apricot or evening primrose oils or mixtures of each when mixed in artificial diets at 5% or less. The bees showed a relatively low preference for the common vegetable oils from canola, sunflower and soya bean seeds, which suggest these oils are not ideal for use in pollen substitutes. Coconut oil solidifies at less than 24°C, which makes it difficult to be used without heating (liquefying) especially during cooler times of the year.

Soya bean protein isolate is recommended to be used in artificial diets. However, samples should be checked for sodium concentration as the commercial protein extraction method could use water or aqueous solutions of sodium chloride. We believe a second batch of protein used in the later half of the project was extracted by the sodium chloride method but we did not know that until after experimentation. However this proposition needs to be confirmed.

Despite (4), feeding artificial diets when harsh conditions prevail in the environment appears to promote survival of hives in the field. However, the field results were not statistically analysed because of the variation in the data due to the small number of hives used to test each feedstuff.

The soya bean diet made from protein isolate high in sodium gave bees a life-span no different to that obtained by feeding Feedbee™ — a commercially available artificial bee feed. Feedbee™ fed bees attained heavier head weights suggesting greater hypopharyngeal gland development, but smaller brood area.

Recommendations

1. It is recommended that a cage trial be conducted to test the hypothesis that low consumption of artificial diets containing soya bean protein isolate in the latter experiments was due to its excess sodium content. The consumption of artificial pollen diets made from soya bean protein isolate extracted by water or salt water should be compared to the consumption of red gum pollen.

2. It is recommended that the field experiment be repeated over a similar season but ensuring that the soya bean protein is water extracted, the added oil content is not more than 5% and there are at least six replicates for each diet. This is largely because of data variation and a protein source that is suspect to have been sodium-chloride extracted and not water-extracted. Sugar syrup should also be supplied to field hives as occurred in the project.

3. It is recommended that until the issues relating to the sodium content of the artificial diets is resolved, feeding irradiated pollen to bees (in our case WA red gum) is one course of action beekeepers could take to promote longer lived bees when natural food supply is limiting.

For more information

Dr Rob Manning, Department of Agriculture and Food, Western Australia, rob.manning@agric.wa.gov.au;

Dr John Black (John L Black Consulting), jblack@pnc.com.au.

The authors have also published and prepared the following scientific journal articles.

1. Manning R, Speijers J, Harvey M & Black JL. (2010) Added vegetable and fish oils to low-fat pollen diets: effect on honey bee (Apis mellifera L.) consumption. *Australian Journal of Entomology* 49, 182-189

2. Manning R, Vlaskovsky P, Eaton L & Black JL. 2012a. Longevity of honey bees (*Apis mellifera*) fed a protein isolate. In preparation.

3. Manning R, Haskard K, Eaton L, Major T & Black JL. 2012b. Effect of diet on head weight of emerged bees (*Apis mellifera*) fed a protein isolate. In preparation.

4. Manning R, Haskard K, Eaton L & Black JL. 2012c. Effect of protein isolate diets on the nutritional status of caged honey bees (*Apis mellifera*). In preparation.

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