

Can the reduction of cell size reduce the impact of varroa?

M.A. Taylor and R.M. Goodwin
December, 2001

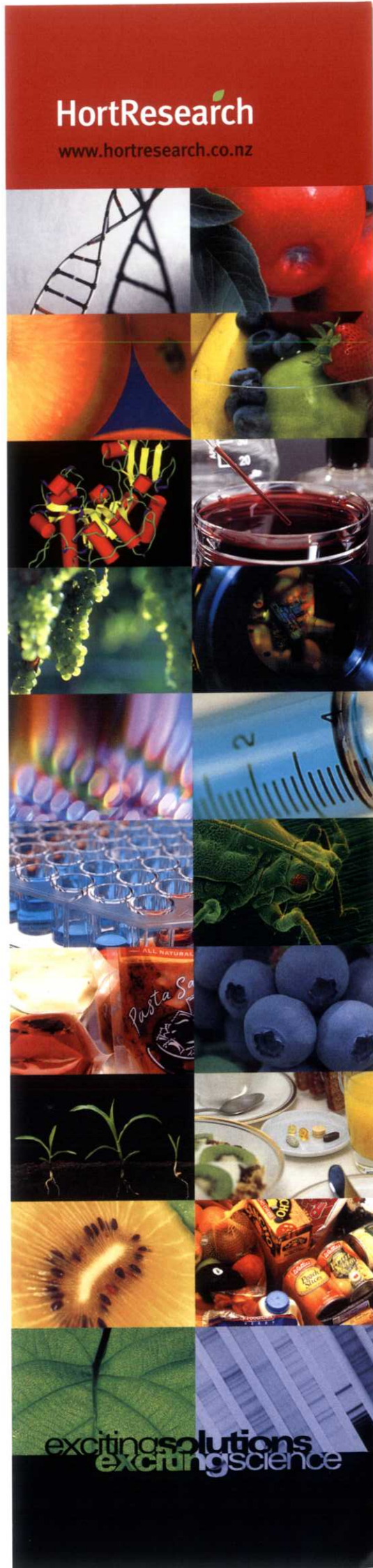
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Report to South Canterbury Branch, National Beekeepers Association

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Can the reduction of cell size reduce the impact of varroa?

Sustainable Farming Fund Report

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Part A: Effect of cell size on the reproductive success of *Varroa destructor*

Executive Summary

The objective of this trial was to determine whether a reduction in the cell size of honey bee (*Apis mellifera*) combs reduces either the reproductive success or the rate with which cells are infested with *Varroa destructor* in New Zealand. Ten nucleus colonies were established with mosaic frames containing five different cell sizes and sister queens. When the brood was 18-20 days old the number of adult and deutonymph mites in each cell were recorded along with the size of each cell. The size of the cells imprinted on the foundation caused no significant difference to varroa reproduction although there was a higher proportion of infestation by adult female mites in 4.8mm sized cells (Chi-square <0.001). "Actual" cell size had no impact on varroa reproductive success or infestation. Because the smaller sized cells were drawn out unevenly, with the base of the cell sometimes larger than the top, the trial should be repeated using 4.8mm foundation that has been drawn out in hives that have undergone a step-down process from the standard 5.4mm sized cells.

Introduction

Varroa destructor is a phoretic external parasite of honey bees (*Apis mellifera*) (Plate 1). When varroa mites remain untreated the beekeeping industry experiences serious losses (De Jong *et al.* 1982). Currently, synthetic miticides are used to control varroa world-wide. However, in some countries varroa mites have been recorded as becoming resistant to these controls (Milani 1999). It is therefore important to find control methods that will either reduce hive damage, or at best enable them to live in equilibrium with honey bees.



Plate 1. An adult worker honey bee with a family of varroa mites on the ventral surface of her abdomen.

In Brazil varroa infestation is relatively low with no reports of colony loss associated with varroosis. Most of Brazil's beekeeping industry is based on Africanised honey bees that build small worker cells (4.5 – 4.6mm) (Message and Goncalves 1995), compared with larger worker cells (4.9 – 5.4mm) built by European races, as found in New Zealand. Message and Goncalves (1995) have shown that these smaller cells contribute to the greater resistance of these bees to varroa, by reducing the frequency of mite infestation and the mites' reproductive rate.

The aim of this trial was to determine whether a reduction in honey bee comb cell size reduces either infestation or the reproductive success of *Varroa destructor* in New Zealand. This varroa mite is a different species to those found in Brazil and it is unknown whether a reduction in cell size will have the same impact on them. The first step of this two part process was to determine whether the size of a honey bee cell had any effect on the reproductive rate of *Varroa destructor*. If a decrease in cell size significantly reduced the reproductive rate of varroa then the second step was to identify whether the selected cell size had any effect on either the size of the bees or honey production. If no significant differences were observed then the second step of the trial would not be conducted.

Methods

This trial was conducted in an apiary run by HortResearch at the Ruakura Research Centre, Ruakura Campus, Hamilton. The South Canterbury Branch of the National Beekeepers Association (NBA) selected five different types of wax foundation to be analysed. The foundation had cells imprinted on them that were 4.8mm, two sources of 4.9mm, one 5.0mm, and one standard 5.4mm size in diameter. The 5.0 and 5.4mm foundations were supplied by the South Canterbury Branch in late January 2001. The 4.8mm foundation from South Africa and the two 4.9mm foundations from United States of America were supplied in late March 2001. All three international shipments were sent directly to Schering-Plough in Upper Hutt for irradiation treatment and then forwarded to HortResearch. The diameter of the ordered foundation size was measured with digital callipers to two decimal places, across ten cells and averaged. It was found to vary slightly from the actual foundation size (Table 1). The actual foundation size is therefore used throughout this study to explain the size of cells that are expected, to one decimal place.

Table 1. Actual versus ordered cell size of foundation measured using digital callipers to two decimal places.

Ordered Foundation size (mm)	Actual Foundation size (mm) (Expected cell size)	Country of origin
4.8	4.71	South Africa
4.9	4.80	United States of America
4.9	5.08	United States of America
5.0	5.11	New Zealand
5.4	5.40	New Zealand

For the cell walls to be drawn out six sheets of each foundation size were embedded in full depth frames and placed in hives with large bee populations. Twenty frames of standard 5.4mm foundation were also drawn out.

The late arrival of foundation meant that the autumn weather patterns stifled the drawing out of the cells. After an attempt to stimulate honey bee cell building behaviour by feeding sugar syrup to the colonies every two days, the undrawn foundation was shifted to hives in an area undergoing a strong flow of rata nectar. As each of the frames of foundation were drawn out they were removed from the colonies, frozen at -21°C for 2 days to kill wax moth, and then cool stored at 3°C .

Once all the frames were drawn they were removed from storage and cut into 5cm x 8cm sections. One section of each of the five cell sizes, plus a second control section (standard cell size of 5.4mm), were randomly inserted into the centre of each of the 20 newly drawn, standard cell-size frames. This method followed that used by Message and Goncalves (1995). These sections replaced the standard cells in a 2 x 3 mosaic grid (Plate 2). Liquid wax was used to hold the sections in place until the bees had drawn out the seams.

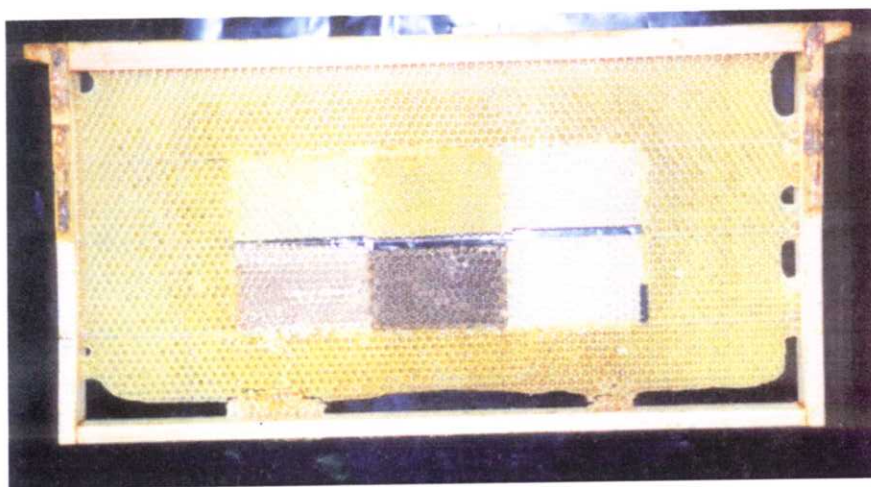


Plate 2. Mosaic frame containing six sections of comb drawn from different sized foundation before being placed into a beehive.

The 4.7 and 4.8mm foundations were not fully drawn out by the bees, so sections with cells that appeared the most consistent were selected for insertion into the centre of the standard frames. Two of these mosaic frames, plus a frame of varroa infected brood, a frame of honey, varroa infested bees and mated sister queens were used to establish ten varroa infected nucleus colonies. Before the cell analysis was begun the colonies were followed through three brood cycles to ensure that the frames were fully drawn out (Plates 3 and 4) and that the queens were laying in each of the sections in the ten colonies.

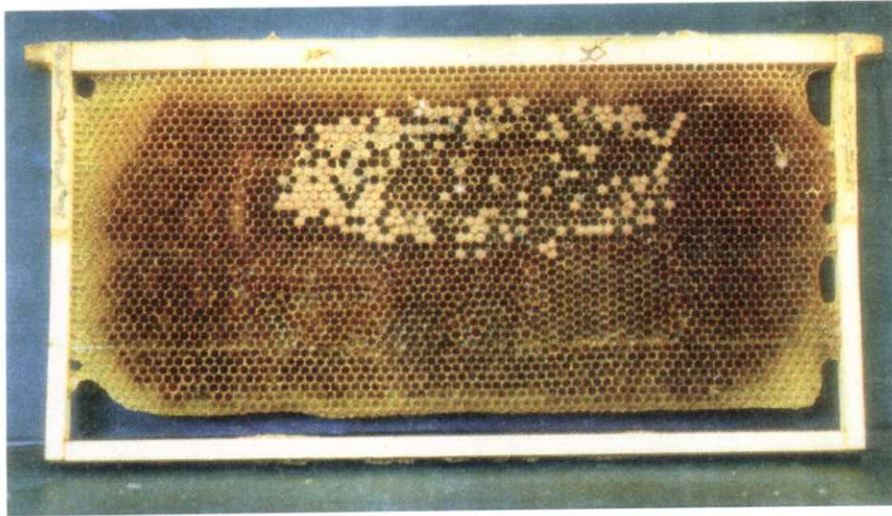


Plate 3. A mosaic frame containing six sections of different sized cells of drawn comb in which the honey bees have drawn out the gaps.

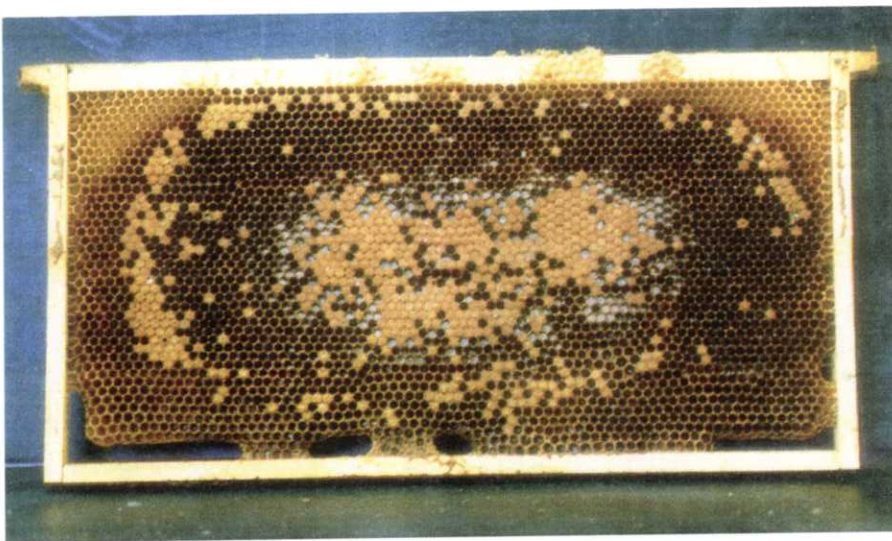


Plate 4. A mosaic frame containing six sections of different sized cells of drawn comb in which the queen has laid eggs and the bees have begun to cap the brood.

Once the queen had laid eggs in all of the sections and the pupae had reached 19-20 days old (Plate 5) the frames were removed from the colonies. The cells were individually uncapped and the age of the bee, the number of adult females and the number of deutonymphs was recorded. The diameter of each uncapped cell ("actual" size) was measured to 2 decimal places using digital callipers.



Plate 5. Head of an 18-day-old honey bee pupa. Identified by the dark purple eyes.

The relationship between “actual” cell size and both mite reproduction and mite preference was analysed using residual maximum likelihood (REML) in GenStat for Windows (5th Edition), and generalised linear models (glms).

Deutonymphs per adult female and the proportion of cells with adult females were analysed using the imposed treatment, “expected” cell size. This data was analysed using generalised linear mixed models (glmmms) to include the different sources of variation and the appropriate error distribution (Poisson for deutonymphs per adult and Binomial for the proportion of adult mites in each cell size). This meant the means and standard errors were given on a transformed scale and were used to investigate treatment effects. Means were also Backtransformed but without standard errors.

Results

The bees easily drew out the cells on the “actual” 5.0, 5.1 and 5.4mm foundation but the development of the 4.7 and 4.8mm foundations proved largely unsuccessful. The cells were drawn out in pockets and were inconsistent in size both between cells and within each cell (Plates 5 and 6). Enough sections were found to use, which when put into the mosaic frames with the other sections and placed into colonies, were drawn out fully by the bees. However, the width of the cells still varied from the base to the top.



Plate 5. Frame of 4.7mm cells inconsistently drawn out by honey bees.

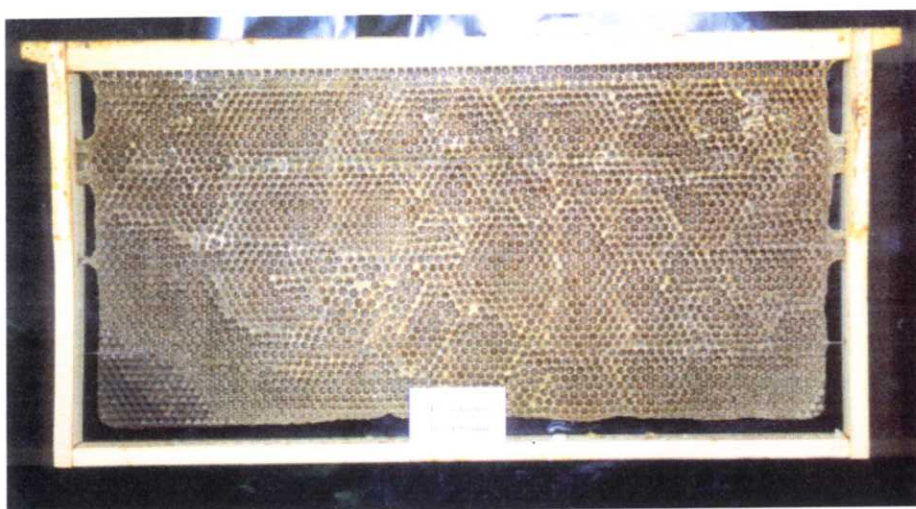


Plate 6. Frame of 4.8mm cells inconsistently drawn out by honey bees.

Not all of the hives had a complete data set (1636 data points were collected instead of the suggested 2500 data points), due to environmental conditions. This meant the treatments confounded any observable hive differences so differences between hives was not assessed. The number of infested cells was between 28% and 47% (Table 2). This was higher than the expected 10% infestation rate, which the 2500 data points accounted for in the proposal.

The “actual” cell size data was analysed to ensure a significant trend existed so that further analysis could be completed if required using “actual” cell size data. As expected the trend was highly significant (Chi-Square <0.001).

One “actual” data point of 6.37mm was omitted from analyses because it was so different from all other points that it had undue influence.

The number of deutonymphs per adult female was not significantly different between cell sizes when analysed using either “expected” cell size (Chi-Square = 0.262) or “actual” cell size (Chi-Square = 0.944) (Figure 1 and Table 2).

There was a significant difference (Chi-square <0.001) between “expected” cell size and the proportion of cells containing adult females. The 4.8mm cells had the highest mite infestation of 46.6% (CI = 40.94 – 52.49). 4.8mm cells also had the highest proportion of adult mites per cell (Table 2). There was no significant relationship with “actual” cell size (Chi-square = 0.883).

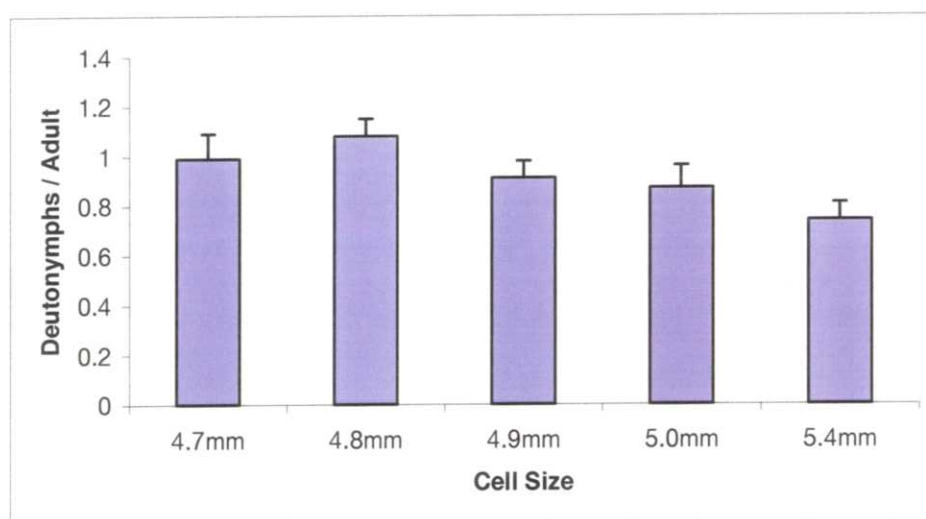


Figure 1. Mean number of deutonymphs per adult mite associated with the five different “expected” cell sizes.

Table 2. The percentage and 95% confidence intervals of varroa infestation rate, and the mean and standard error (SE) of varroa reproduction, in 4.7 to 5.4mm *A. mellifera* worker brood cells.

	4.7mm	4.8mm	4.9mm	5.0mm	5.4mm
Total cells analysed	330	328	304	234	440
Total cells infested (%) & Confidence Intervals	108 (32.7) 28.04 – 38.99	153 (46.6) 40.94 – 52.49	106 (34.9) 28.04 – 38.99	77 (32.9) 26.29 – 38.18	122 (27.7) 23.19 – 32.16)
Total adult varroa	163	294	172	132	226
Total female deutonymphs	156	280	151	114	155
Adults per infested cell	1.509	1.922	1.623	1.481	1.270
Mean Deutonymphs per adult (SE)	0.94 (0.08)	1.08 (0.07)	0.91 (0.07)	0.87 (0.09)	0.74 (0.07)
Number of infested cells without offspring (%)	32 (29.6)	30 (19.6)	22 (20.8)	22 (28.6)	41 (33.6)

Discussion

“Actual” honey bee cell size had no significant effect on the reproduction or infestation of *Varroa destructor*.

“Expected” cell size also had no significant effect on the reproductive rate of *Varroa destructor*. However, “expected” cell size did have a significant effect on the infestation of adult mites in cells. This was observed as a higher proportion of adult mites in the 4.8mm cells, and suggests varroa are slightly more attracted to smaller sized cells.

These results contrast research by Message and Goncalves (1995) who showed that small cells, that had been built by Africanised bees, had lower *Varroa jacosoni* infestation and reproduction in comparison to large cells, that had been built by European honey bees, and fitted into the same frame. However, despite conducting trials using both an Italian queen bee and an Africanised queen bee, both had been open mated in an Africanised bee region. This means that factors relating to Africanised honey bees, such as their shorter development period, may attribute to the reduction in both reproductive and infestation rates, and may explain why different results were obtained from this trial, which used Italian honey bees.

In this trial the frames of 4.7mm and 4.8mm foundations were not successfully drawn out by the honey bees. The cells in these frames were unevenly drawn so that the cell bases were predominantly a different size to the top of the cell. This may have confounded the observed results, which suggest that instead of smaller cells reducing varroa infestation in Italian honey bees, it may be larger cells that cause a reduction. Caution needs to be taken, as correlation does not imply causation. The uneven cells may also have confounded any significant effect of cell size on reproduction. Therefore, the effect of evenly drawn out cells on both reproduction and infestation needs to be assessed to substantiate the results. It is thought that the step down from standard foundation size (5.4mm) to 4.7mm and 4.8mm may have been too large for the bees to adjust too. This may be overcome by using a two-step process whereby the cells in the brood nest are stepped down to 5.0mm and then to 4.8mm.

We suggest that evenly drawn 4.8mm sized cells be drawn out using the two-step process mentioned above and then analysed against the standard (5.4mm) sized comb. This would determine whether the higher infestation rate observed in the 4.8mm cells is attributed to the

variation in cell shape, and also whether this variation confounded any significant effect on varroa reproduction that should have been observed.

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