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The Effects of Hive Size, Feeding and *Nosema ceranae* on the Size of Winter Clusters of Russian Honey Bee Colonies

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Summary

In a first experiment, from August 2007 to February 2008, colonies of Russian honey bees were housed in eight- or 10-frame hives and fed or not fed sugar syrup and protein supplement patties. From August to November when a natural nectar and pollen flow occurred, the colonies were not fed. From August to November colonies in 8-frame hives grew significantly more than colonies housed in 10-frame hives. Colonies that were in eight-frame hives or were fed three pounds (1.36 Kg) of protein supplement for three weeks in November were larger in late January in comparison to their size in November.

In a second experiment, colonies were housed in eight- or 10-frame hives and fed or not fed sugar syrup and protein supplement patties from November 2008 to early February 2009. Colonies in eight-frame hives grew more than colonies in 10-frame hives. Colonies that were fed grew significantly more than colonies that were not fed.

In the second experiment, smaller colonies grew significantly more than larger colonies. Nearly all colonies were tracheal mite-free with the exception of two colonies that had high tracheal mite infestations. Although most colonies did have *Nosema ceranae* infestations, about 80% of the colonies had spore count averages below the commonly accepted treatment level of 1×10^6 spores/bee. Nevertheless, differences among colonies in numbers of *N. ceranae* spores were not associated with different hive sizes, different feeding treatments or different colony growths.

Key Words: *Apis mellifera*, tracheal mites, eight-frame hive, 10-frame hive, protein substitute, almond pollination

Introduction

The importation and subsequent selective breeding of Russian honey bees from far-eastern Russia has resulted in a stock of honey bees which has strong resistance to the parasitic mites *Varroa*

destructor and *Acarapis woodi*, good honey production and strong overwintering abilities (de Guzman *et al.* 2001, 2002, Rinderer *et al.* 2001a, 2001b, 2001c). These characteristics were the breeding goals of the selection program which was begun in 1998 and remain as the breeding goals in the continuing selective breeding of the Russian honey bee stock (Brachman 2009).

Since 1998, renting honey bee colonies for pollination has provided an increasingly larger share of the income of many commercial beekeepers in the United States. A large portion of this increase has come from the pollination of almonds in California. Colonies rented for pollination must meet size standards established in rental contracts with growers. Hence, many beekeepers who intend to rent colonies for pollination use colony management to produce large colonies for mid-February (Traynor 1993).

Russian honey bee colonies are known to build large colonies in the spring after reliable natural pollen becomes available (Tubbs *et al.* 2003). Until then, the colonies are generally small and exhibit traits that favor winter survival such as using food frugally and producing a restricted winter brood nest. That is, the colonies do not tend to grow in late winter and produce large colonies that are often in danger of starvation in early spring. These traits are desirable for high rates of winter survival and general beekeeping practices to produce honey or pollinate crops that bloom in April or later. Indeed, it may be that restricted brood rearing in late winter contributes to overall resistance to *V. destructor* by favoring a winter reduction in the numbers of mites infesting colonies. However, these characteristics of Russian honey bees, which are strengths for most beekeeping, may be viewed as weaknesses in regard to the special goals for almond pollination of producing large colonies by mid-February (Danka *et al.* 2006).

Typically, most beekeepers rely on special management procedures to build large colonies by mid-February. Italian honey bee stocks usually respond favorably to these techniques and large proportions of them become or stay large enough to be rented for almond pollination. Mostly these management techniques involve feeding individual colonies both a liquid sugar feed and a protein substitute, usually in patty form. Protein feeding (Danka and Beaman 2009, Degrandi-Hoffman *et al.* 2008, Mattila and Otis 2007, Nabors 2000, Peng *et al.* 1984 Standifer *et al.* 1973) is known to stimulate brood rearing. The timing, frequency and duration of feeding to prepare colonies for almond pollination rental are less well studied. In one study (Degrandi-Hoffman *et al.* 2008) intermittent feeding of protein and carbohydrate syrup resulted in colonies that dwindled slightly while unfed controls dwindled by half.

Many beekeepers also provide bees with treatments for parasitic mites (*V. destructor* and *A. woodi*), American foulbrood and the two species of *Nosema*, *N. apis* and *N. ceranae*. If uncontrolled, these parasites and diseases can kill colonies in a longer term. In the shorter term, sub-lethal effects of infections and infestations may debilitate colonies, reducing the value of stimulative feeding.

A pioneer in American beekeeping, C. C. Miller, called for research to test the assertion of R. L. Taylor that colonies in eight-frame hives produced larger colonies earlier in the season than colonies in 10-frame hives (Taylor, Miller 1894). Taylor asserted that less space required less effort to heat which resulted in larger and earlier brood nests. Although the debate continued (Taylor *et al.* 1894), no research was reported. However, a complex interaction of multiple opinions, marketing, and a need for an industry standard resulted in most beekeepers having 10-frame hives. Currently, modern advocates of 8-frame hives are challenging the 10-frame convention (Flottum 2005, Forrest 2008). While various advantages

and disadvantages of different hive sizes guide preferences, no published data seem to be available concerning the hypothesis that eight-frame hives produce larger colonies earlier.

One of our research goals is to identify management procedures which will improve the ability of Russian honey bees to meet the needs of pollination and especially almond pollination. The experiments reported here were undertaken to determine: 1) the effects of feeding both sugar and protein to Russian honey bee colonies in late fall and in late fall and winter on colony size in early to mid-February, 2) the effects of using eight-frame hives on the size of Russian colonies in early to mid-February, 3) the relationship between colony growth in fall and winter to the size of colonies in autumn, and 4) the association of February rates of *Nosema* spp. and tracheal mite infestations in colonies to colony growth rates.

Materials and Methods

Experiment 1

Experiment 1 was begun in August 2007 and ended in February 2008. Four apiaries each having 8 colonies with pure-mated Russian queens were established. In each apiary, four colonies were placed in two 8-frame hive bodies with 16 Langstroth "deep" (9 5/8 in) frames of comb. Four other colonies were placed in two 10-frame hive bodies with 20 Langstroth "deep" frames.

Within a week of being established, the colonies were evaluated for the presence of the queen and colony size. The numbers of bees on each side of each frame were estimated as tenths of the frame side covered with bees. Since commercial inspections of colony size for almond pollination consider 3/4 of a frame covered by bees to be one commercial frame of bees (Traynor, 1993), we calculated frames of bees by multiplying our estimate of full frames of bees by 1.25 to estimate commercial frames of bees. Although efforts were made to begin all colonies with about the same numbers of bees, the average colony size was 8.5 ± 3.1 frames.

The colonies encountered an autumn pollen and nectar flow principally from goldenrod (*Solidago* spp.). When the flow was mostly ended in October, colony size data were collected with the same procedures used in August. We then began feeding two randomly selected colonies in 10-frame hives and two randomly selected colonies in eight-frame hives in each apiary. Colonies were fed a 1.1 pound (500 g) patty of Megabee® feed made from dry feed according to manufacturers recommendations and a one gallon (3.8 liter) pail of syrup [60% (W:W) sucrose in water]. Feed was given on October 29, November 8 and November 19. Each time, any remaining food from prior feedings was removed. Generally, the bees consumed over 90% of the patties and removed all the syrup from the pails. This feeding was intended to test the effect of attempting to extend the autumn brood rearing period using supplemental feeding.

Final colony evaluations were made on February 5, 2008. Once again colonies were inspected frame by frame and the numbers of bees were estimated using the same procedures.

Colony size data were converted to changes in size (growth = October size - August size / August size) for the period from August to October. Since no artificial feeding occurred during this period, only the effect of hive size was analyzed by *t*-test. Feeding began in October so the changes in the period from October to February could be effected by both hive size and feeding. Changes in colony size were analyzed by a two-factor analysis of variance (SAS 8.2, SAS Institute 2001).

Experiment 2

By dividing, moving colonies and transferring colonies to new hives, four apiaries were established in October 2008. Each apiary had 32 colonies, 16 in eight-frame hives (16 frames in two hive bodies) and 16 in 10-frame hives (20 frames in two hive bodies). All colonies had Russian queens that were either pure-mated, mated in areas having only apiaries with Russian queens or purchased from commercial sources. Different types of queens were distributed randomly among colonies.

Within a week of being established, the colonies were evaluated for the presence of the queen and colony size. The numbers of bees were estimated by the same methods used in experiment 1. Efforts were made to begin all colonies with about the same numbers of bees; the average colony size was 6.72 ± 0.31 frames.

In the first week of November 2008, eight randomly selected colonies in eight-frame hives and eight randomly selected colonies in 10-frame hives in each apiary were each fed one commercially prepared Megabee® patty (1 pound (454 g)) and a one gallon (3.8 L) pail of syrup [60% (W:W) sucrose in water]. The other colonies were not fed any protein food but some were fed syrup as needed to assure enough carbohydrate food to survive winter. The apiaries were visited weekly from November 2008 to early February 2009. Colonies being fed were given a continual supply of both Megabee® patties and syrup. This method of feeding was intended to test the effects of providing a continuous supply of food as is done in at least one large commercial beekeeping enterprise which specializes in almond and other crop pollination (Card 2008).

Final colony evaluations were made from February 11 to February 13, 2008. Once again colonies were inspected frame by frame and the numbers of bees were estimated. A sample of worker bees was taken from each colony, frozen and later analyzed for the presence of *Nosema* spp. and tracheal mites. The colonies were never treated to reduce the numbers of these parasites.

For *Nosema* spp. evaluations, 50 bees from each colony were pooled and processed as described by Bourgeois *et al.* (In Preparation). Briefly, abdomens were homogenized, filtered, and DNA was subsequently extracted. DNA fragments unique to *N. ceranae* and *N. apis* were simultaneously amplified and quantified using real-time PCR. The data were converted to "spore equivalents" per bee using a calibration factor derived from direct spore counts using a compound microscope.

For tracheal mite evaluations, 30 bees from each colony were dissected as described by Lorenzen and Gary (1986). All tracheae were pulled and placed on a glass slide with double-sided tape. The tracheae were then dissected and examined microscopically for the presence of mites. The proportion of infested bees (prevalence) was calculated for each colony.

Data were standardized to correct for apiary differences. Size data were converted to changes in size (growth = February size - October size / October size). Changes in size could be affected by hive size and feeding, so the data were analyzed by a randomized block, two-factor analysis of variance. Data from 128 colonies were analyzed. Sixteen colonies were lost owing to queen losses and storm related mishaps. A linear regression was used to determine the relationship between initial colony size and colony growth (SAS 8.2, SAS Institute 2001).

Results

Experiment 1

During the natural nectar and pollen flow from August to October colonies generally grew. Overall, colonies gained an

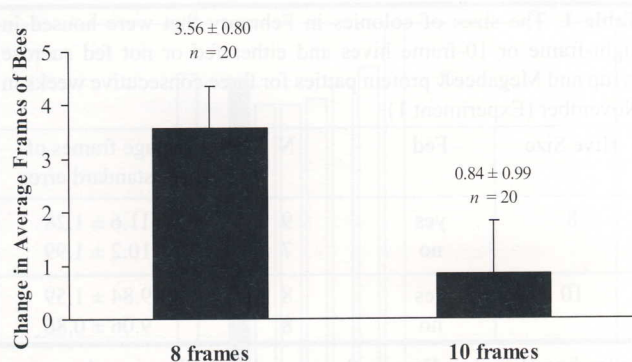


Figure 1a. Growth measured in frames of bees (mean \pm SE) for colonies in 8-frame and 10-frame hives from August to October ($P = 0.05$) (Experiment 1).

average of 2.2 ± 0.80 (mean \pm SE) frames of bees. Colonies in eight-frame hives gained about four-fold more frames of bees than colonies in 10-frame hives (Fig. 1a). Although the variance was high, statistical analysis detected that colonies in the eight-frame hive grew more ($P = 0.05$) during the natural nectar flow.

In the period during which colonies were fed (October to February), colony sizes generally declined. However, colonies in eight-frame hives that were fed lost six-fold fewer frames of bees than colonies in 8-frame hives that were not fed. Colonies in 10-frame hives that were fed lost two-fold fewer frames of bees than colonies in 10-frame hives that were not fed (Fig. 1b). Owing to the large variation in the growth of colonies and small sample sizes, differences are not statistically different between either hive type

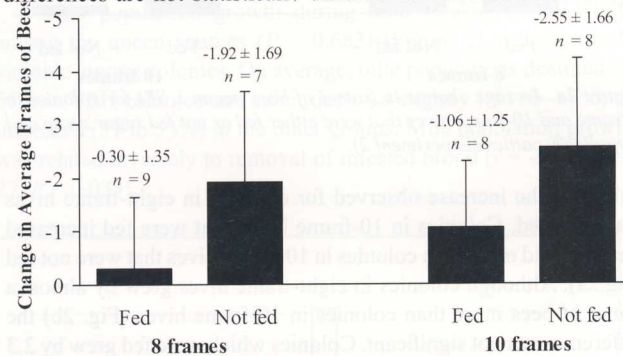


Figure 1b. Dwindling between October and February measured in frames of bees (mean \pm SE) for colonies in 8-frame and 10-frame hives that were either fed or not fed sugar syrup and MegaBee® patties (Experiment 1).

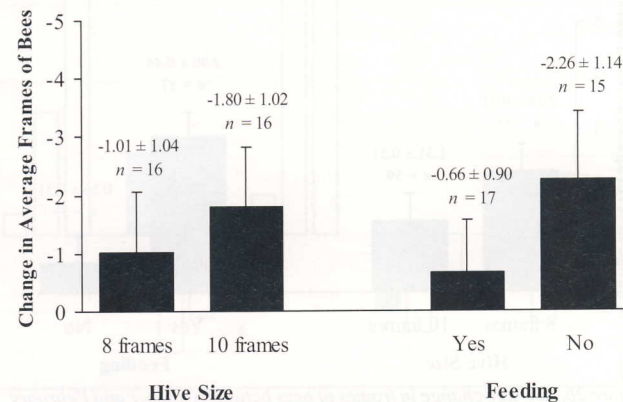


Figure 1c. Dwindling between October and February measured in frames of bees (mean \pm SE) for colonies in 8-frame or 10-frame hives and colonies fed or not fed sugar syrup and MegaBee® patties (Experiment 1).

Table 1. The sizes of colonies in February that were housed in eight-frame or 10-frame hives and either fed or not fed sucrose syrup and Megabee® protein patties for three consecutive weeks in November (Experiment 1).

Hive Size	Fed	N	Average frames of bees ± standard error
8	yes	9	11.6 ± 1.28
	no	7	10.2 ± 1.99
10	yes	8	9.84 ± 1.59
	no	8	9.06 ± 0.86

or feeding (Fig. 1c). Despite the general dwindling in colony size, the colonies were still large in February and suitable for rental for almond pollination (Table 1).

Experiment 2

All colonies grew during the course of experiment 2. Colonies in eight-frame hives that were not fed increased in size by only

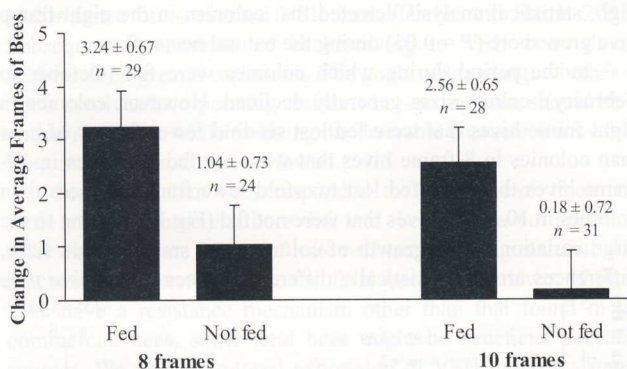


Figure 2a. Average change in frames of bees (mean ± SE) for colonies in 8-frame and 10-frame hives that were either fed or not fed sugar syrup and MegaBee® patties (Experiment 2).

a third of the increase observed for colonies in eight-frame hives that were fed. Colonies in 10-frame hives that were fed increased fourteen fold more than colonies in 10 frame-hives that were not fed (Fig.2a). Although colonies in eight-frame hives grew by almost a frame of bees more than colonies in 10-frame hives (Fig. 2b) the difference was not significant. Colonies which were fed grew by 2.3 more frames of bees than colonies which were not fed ($P = 0.05$) (Fig. 2b). Although the initial average colony size in October was somewhat small (6.72 ± 0.31 frames of bees) the overall average

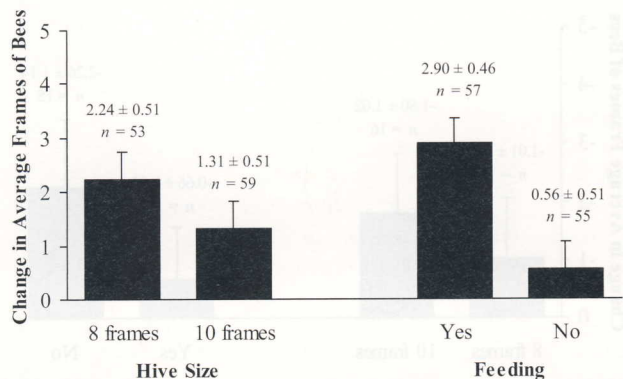


Figure 2b. Average change in frames of bees between October and February in frames of bees (mean ± SE) for colonies in eight-frame or 10-frame hives (ns) and colonies fed or not fed sugar syrup and MegaBee® patties ($P = 0.05$) (Experiment 2).

Table 2. The sizes of colonies in February that were housed in eight-frame or 10-frame hives and either fed or not fed sucrose syrup and Megabee® protein patties continuously from November to February (Experiment 2).

Hive Size	Fed	N	Average frames of bees ± standard error
8	yes	29	9.64 ± 0.72
	no	24	8.72 ± 0.65
10	yes	28	8.59 ± 0.79
	no	31	7.06 ± 0.58

colony size in February (8.47 ± 0.35 frames of bees) was suitable for rental for almond pollination. Only colonies in 10-frame hives which were not fed both syrup and Megabee® patties had hive sizes averaging less than eight frames of bees (Table 2).

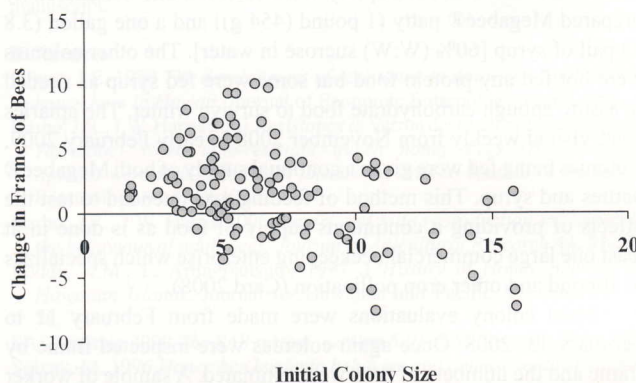


Figure 3. Relationship between the starting size of colonies and their growth. There was a strongly negative ($r = -0.53$) and significant ($P = 0.0001$) relationship between the two variables. In general, smaller colonies tended to grow more.

The analysis of the relationship between the starting size of colonies and their growth indicated a strongly negative ($r = -0.53$) and significant ($P = 0.0001$) relationship. In general, smaller colonies tended to grow more (Fig 3).

Most colonies had no or very few tracheal mites indicating that the Russian stock retains resistance to tracheal mites. Only two colonies had high rates of infestation (100% and 97%). The colonies had sister queens.

No *N. apis* was found. However, all colonies had *N. ceranae*. The estimated average number of spores per bee in colonies ranged from 8.0×10^4 to 3.1×10^6 and averaged $6.4 \times 10^5 \pm 0.06 \times 10^5$. Twenty percent of the colonies had $>1 \times 10^6$ spores per bee. Analysis of variance indicated that neither hive size ($P = 0.49$) nor feeding ($P = 0.30$) was associated with differences in numbers of spores. Also, regression analysis found no relationship ($R^2 = 0.0009$) between colony growth and average numbers of *N. ceranae* spores present at the end of the test.

Discussion

Smaller hive size had a consistently positive effect through both experiments. This effect was most pronounced when the colonies experienced a strong autumn nectar and pollen flow in experiment 1. During that period, colonies in eight-frame hives grew 4.24 times ($P = 0.05$) more than colonies in 10-frame colonies. During the second period of experiment 1, colonies were fed only for a short period in October and November and not fed from November to

February. During this period, all colonies dwindled with colonies in 10-frame hives losing numerically more bees than colonies in eight-frame hives. In the second experiment, colonies were fed from November to February during which colonies in eight-frame hives grew more than colonies in 10-frame hives. Overall, the consistency of the results as well as a significant difference during a natural nectar and pollen flow suggest that eight-frame hives do support greater colony growth of Russian honey bees than we found in 10-frame hives.

This result should not be extrapolated to be a general recommendation for using eight-frame hives. Their use is beneficial in growing larger colonies from August to February. This observation is helpful for producing colonies suitable for almond pollination and probably is helpful for queen rearing by producing colonies likely to produce drones earlier. However, this study is restricted to the specific goal of producing larger colonies early. Eight-frame hives may not be optimum for all beekeeping goals.

These differences in growth in different sized hives occurred from August to February when temperatures range from hot through cool to cold. Taylor (Taylor *et al.* 1894) asserted that the eight-frame hive size favored better heat regulation in winter and thereby better brood nest conditions which resulted in larger colonies earlier in spring. The temperature regulation hypothesis is still reasonable if it includes more efficient cooling as well as heating. However, no study has been done to test the effect of a smaller hive on temperature regulation. Also, while many beekeepers (pers. communication) offer the opinion that colonies grow more quickly in smaller hives, especially in the spring, this is the first experimental confirmation of this favorable effect of smaller hives.

Feeding colonies also had generally favorable effects. In Experiment 1, colonies were fed only for a brief period in October-November. By February, all colonies had become smaller but colonies that were fed lost the smallest number of bees. In Experiment 2 feeding began in November and continued to February. In this experiment all colonies increased in size with the colonies which were fed becoming significantly larger. Hence, a combination of statistical significance and a consistency of trends in the two experiments support the conclusion that autumn and winter feeding produces larger colonies of Russian honey bees in February.

For Experiment 2, the analysis clearly supported the conclusion that smaller colonies tended to grow the most. Colonies in the range of five to eight frames of bees at the beginning of the experiment in October grew well and attained sizes exceeding eight frames of bees in February. Despite the effects of hive size and feeding, larger colonies grew less or dwindled slightly although most of them also exceeded eight frames of bees in February. This suggests that colonies regulate their size during autumn and winter such that it is very difficult to provide management which will increase the size of Russian colonies from large colonies to very large colonies. It further suggests that summer and autumn colony divisions that are reasonably robust can be managed to increase the numbers of colonies that will achieve a rentable size in February. However, these experiments were conducted in Louisiana where periods of moderate weather during winter permit honey bees to collect small amounts of natural pollen and nectar. Colony divisions are not likely to grow as well in areas with less favorable autumn and winter weather.

The relationship between initial colony size and colony growth suggests additional honey bee management opportunities. Smaller colonies (five to eight frames of bees) in October grew substantially

and typically exceeded eight frames of bees in February. Reducing the size of very large colonies in August or September by splitting and requeening with young queens would take advantage of the tendency of smaller colonies to grow when stimulated to do so through autumn and winter. This would increase the number of colonies suitable for rental for almond pollination in February.

Results of the tracheal mite analysis indicate that Russian honey bees generally retain their resistance to tracheal mites. However, the discovery of two sister queens heading colonies with high tracheal mite infestations indicates that caution still must be maintained in the selection of Russian honey bee stock. Potential breeders must be screened for tracheal mite resistance to assure that the trait continues in high frequency in the stock.

Malone and Stefanovic (1999) found that percentage of *N. apis* infection and longevity of infected bees are not influenced by honey bee race. However, individual colonies apparently vary in resistance to *Nosema* (Rinderer, Sylvester 1978) and genetic parameters suggest that resistance to *Nosema* can be improved with selective breeding (Rinderer *et al.* 1983). The results of our *Nosema* spp. analysis contain no information to evaluate the comparative *Nosema* resistance status of Russian honey bees. Hence, Russian honey bees, like other stocks of honey bees, should be periodically surveyed for the presence of *Nosema* and treated as required. The variation in rates of infestation may suggest the stock has some resistance to *Nosema* that could serve as a starting place for breeding for resistance. However, this remains to be determined through more rigorous experimentation.

Although *N. apis* is reported to cause weakening of colonies during winter months (Farrar 1942), the potential negative effects of *N. ceranae* infestations on Russian honey bees remain unclear. The lack of relationship between estimated spore numbers and colony growth suggests that Russian colonies remained healthy enough to grow, despite a fifth of them having high infestations. This lack of an effect of *Nosema* infestation rates on colony growth is somewhat puzzling. It is reasonable that a higher *Nosema* infestation would retard growth of colonies by debilitating and reducing the longevity of bees. These two consequences of *Nosema* infestation should have resulted in reduced colony growth. However, since 80% of the colonies had spore count averages below the commonly accepted treatment level of 1×10^6 recommended by B. Furgala (Mussen 2009), it may be that the overall infection rates were too low for negative effects on colonies to be apparent. Also, higher spore counts may only have occurred near the end of the test. In contrast to the observations of Eischen and Graham (2008) feeding had no effect on *Nosema* levels. It may be that only a small number of bees in the general colony populations were highly infested. Or perhaps very large numbers of spores, which elevated the estimates of average per bee spore counts, only occurred in bees that had diminished individual resistance since they were near death from another cause. Nonetheless, caution suggests that *N. ceranae* infestations in Russian colonies should be presumed to be harmful. Knowledge concerning the structure of the variation of *N. ceranae* infestation within the colony and studies of the long term effects of *N. ceranae* infestation on Russian honey bees may suggest more appropriate treatment procedures.

These results may expand the range of management options for some beekeepers. The knowledge that Russian honey bee colonies can be caused to be larger in February through management may encourage some beekeepers that pollinate almonds to consider using this mite resistant stock. Both the use of eight-frame hives and feeding encourage the development of larger colonies for almond

pollination. This is likely to be true for all stocks of honey bees. In our experiments both eight-frame hives and feeding, especially prolonged and continual feeding, each produced an additional one to two frames of bees in February. These increases in average colony size probably would result in a higher proportion of colonies being large enough to rent for almond pollination and a larger average size for colonies that are rented.

Conclusions and Recommendations

Russian honey bee colonies can be managed through autumn and winter to produce colonies which average 8 frames or more of bees in February.

Colonies kept in eight-frame hives grow larger than colonies kept in 10 -frame hives through autumn and winter.

Colonies that are fed both sucrose syrup and protein supplement grow larger through autumn and winter with prolonged and continual feeding being especially favorable.

Small colonies (five to eight frames of bees) tend to grow more during autumn and winter than larger colonies.

Tracheal mite infestations in Russian honey bee colonies are of little concern. However, Russian colonies can have high infestations of *N. ceranae*. Surveys of *Nosema* infestation levels to guide treatment decisions are recommended.

Every successful change in beekeeping procedures requires learning and refinements by individual beekeepers. Beekeepers interested in changing their methods to include Russian honey bees, eight-frame hives or different feeding regimes should first attempt to make changes in one or a few apiaries. This will provide both an opportunity to evaluate the usefulness of changes in individual beekeeping enterprises and the experience needed to perfect and adapt the changes to specific environments.

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