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Honey bee colony winter losses and treatments against Varroa destructor in New Jersey, USA, 2010-11

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The number of managed honey bee (*Apis mellifera* L.) colonies in the United States decreased during the period of 1947 to 2008 by 61%, from 5.9 to 2.3 million colonies. Over this time period, a variety of factors including bacterial, fungal and viral diseases, parasites, pesticide usage, climate, genetics, land-use change, and socio-economics have all had measurable effects on managed honey bee populations (vanEngelsdorp and Meixner, 2010). Since 2006 Colony Collapse Disorder (CCD) has been implicated in widespread losses in the United States and has prompted the deployment of surveys aimed at quantifying losses, especially due to CCD (e.g., vanEngelsdorp *et al.* 2008,vanEngelsdorp *et al.* 2011). However, some U.S. states, such as New Jersey, have few reports of the main symptom of CCD (complete absence of bees in dead colonies), yet still report high levels of colony loss. Here we report results from asurvey conducted in New Jersey to quantify the number of colonies lost during the winter of 2010-2011 and to determine the relative importance of managing the mite *Varroa destructor* on winter colony survival.We use this initial survey to

illustrate the applicability of using simple standardized surveys to monitor managed bee colonies and evaluate best management practices.

On April 1, 2011, we distributed a survey by e-mail to the approximately 900 members of the New Jersey Beekeepers Association. We requested respondents to report the number of colonies they managed that were alive on December 1, 2010 and the number which survived until March 15, 2011, the location of their apiary by county, whether or not they treated for mites, if so which treatment was used and in which month they started the treatment. In all, 217 respondents, representing 1,939 colonies in all 21 counties of New Jersey, responded to the survey. Respondents operated an average of nine colonies. Out of 1,939 colonies reported alive in 2010, 1,290 were still alive on April 1, 2011, representing a total mortality rate of 33%(Table 1).We modeled individual hive survival with a generalized linear mixed model (glmm) by using a binomial error distribution as implemented in package *lme4* ofthe statistical environment R (**http://www.r-project.org/**). The mite treatment was included as a fixed factor with the operator as a random factor in order to take into account the non-independency of the data. Colonies receiving no mite treatment had an overallmortality rate of 65%. The best performers were ApiGuard® (thymol gel), formic acid, andApiLifeVar® (74.08% thymol, 16.00% eucalyptus oil, and 3.70% L-menthol),with mortality rates of 18%, 23% and 25%, respectively (Table 1). Coumaphos treated colonies averaged lower mortality rates than untreated colonies but the difference was not significant, probably because of the low number of operators that used this treatment (two operators representing 23 colonies). Both Apistan® (synthetic pyrethroid tau-fluvalinate) and powdered sugar treatments were not statistically distinguishable from untreated colonies(Table 1).Because a large number of operators treated with ApiGuard®(72 operators representing 1,207 colonies) we were also able to examine the relationships between colony mortality and ApiGuard® treatment timing. Most operators using ApiGuard® began treatment in August (84.3% of colonies treated) with the remainder beginning treatment in July, September or October. Sixteen percent of colonies treated with ApiGuard® in July did not survive compared with 17% for treatment beginning in August, 24% in September, and 47% in October. Only the October treatment was significantly different from the other ApiGuard® treatment timings, although we must acknowledge that the uneven distribution of treatment timings (i.e., that the vast majority of operators began treatment in August) makes determining statistical significance problematic.

Interestingly, overall losses reported in our survey were similar to those of the United States in recent years, 35.8% in the winter of 2007-2008 and 34.4% in 2009-2010, but were larger than the 15.1% and 10.4% losses reportedfor those years in New Jersey in national surveys (vanEngelsdrop *et al.*2008, vanEngelsdorp *et al.* 2011). While the greater losses captured in our survey could be the result of year to year variation or differences in various components of survey methodology, we believe that they represent differences based on which operators participated in each survey. vanEngelsdorp *et al.* (2008, 2011) employed a surveying strategy which, for New Jersey, captured predominantly large operations. They surveyed fifteen operators representing 23,532 colonies in 2007-2008 and 31 operators representing 3,966 colonies in 2009-2010. That is an average of 1,569 and 128 colonies per operator compared with our average of nine colonies per operator. Therefore we believe we are capturing a sample of smaller scale operators in our current survey. The fact that our survey records over twice the winter loss in New Jersey than vanEngelsdrop *et al.* (2008, 2011) may indicate that smaller scale operators are employing less successful hive management strategies than larger, and in many cases migratory, operators. Since vanEngelsdrop *et al.* (2008, 2011) do not record any specific management practices related to disease and pest control, we cannot directly compare management strategies of small and large scale operators. In any case, small operators constitute an important fraction of the managed colonies in New Jersey and warrant study. We recorded 65% colony mortality when no *V. destructor* treatment was employed; hence, we hypothesize that mite pressure is the single greatest challenge to colony winter survival in New Jersey. This hypothesis is strengthened by recent research indicating that failure to control *V. destructor* may be the main factor explaining winter colony losses in Canada (Currie *et al.* 2010) and specifically in Ontario (Guzmán-Novoa*et al.* 2010).

In conclusion, our survey suggests that *V. destructor* is sometimes being improperly managed in New Jersey, at least by smaller operators. ApiGuard®, formic acid, and ApiLifeVar® are clearly superior treatment options. Coumaphos and Apistan® are not significantly more effective than no treatment. Though the number of respondents using these two chemistries precludes any strong statements, it is possible that their observed lack of efficacy could result from evolved resistance of *V. destructor* which has been noted in both coumaphos and Apistan® (Pettis 2004) or could be a result of improper usage. In our single most successful cohort, operators beginning ApiGuard® treatments in July and August, the colony mortality rate was only around 18%. These results indicate that with proper management strategies, even smaller scale beekeepers in New Jersey should be capable of achieving winter losses below 20%. Clearly, there are limitations in interpreting survey results from a single year. We plan to repeat this survey

	Summary Statistics			**Generalized linear mixed model estimates compared against a baseline of no treatment**			
Treatment	No. or colonies	No. or operators	Colony mortality (%)	Estimate	Std. Error	z value	Pr(>|z|)
ApiGuard®	1,207	72	18%	1.76	0.36	4.93	<0.001
Formic Acid	91	19	23%	1.96	0.59	3.33	<0.001
ApiLifeVar®	28	8	25%	2.10	0.88	2.38	0.017
Coumaphos®	23	2	48%	1.48	1.42	1.04	0.300
No treatment	453	97	65%	0.00	0.00	-	-
Apistan®	16	4	69%	-0.35	1.12	-0.32	0.751
Powdered Sugar	121	15	72%	0.94	0.63	1.50	0.134
All Treatments	1,939	217	33%				

Table 1. Results of winter colony losses and *Varroaa destructor* treatment deployed by 217 operators (beekeepers), representing 1,939 colonies, from New Jersey (2010-2011). Percent mortality is calculated blind to operator for ease of interpretation.

next year from a larger and more diverse subset of New Jersey beekeepers. Furthermore, we believe that this survey illustrates a simple and cost effective strategy for elucidating management factors which may be contributing to mortality rates among managed bee populations. Adding basic questions to beekeeper surveys regarding use and timing of acaricides, other pesticides, fungicides, antibiotics, feeding, and other cultural practices has the potential to inform management recommendations and research throughout the beekeeping community.

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A Note on Introducing Four-Day-Old Virgin Queens into Nucleus Colonies Using Artificial Queen Cells in Alberta, Canada

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Short Title: Introducing Virgin Queens into Nucleus Colonies

Beekeepers on the Canadian prairies frequently produce large numbers of queens for the production of nucleus colonies in the spring (Nelson *et al.* 1992). These nucleus colonies are typically initiated with a single frame of sealed brood, covered with queenless adult worker bees and a single 14-day-old queen cell. We investigated the possibility of modifying this procedure by introducing virgin queens rather than queen cells. This would offer a number of advantages over cells, including decreasing the time needed to produce a mated queen, eliminating queen losses prior to emergence and facilitating queen phenotyping or genotyping prior to introduction (Perez-Sato and Ratnieks 2006).

Virgin queen introduction, however, is highly inconsistent compared with the introduction of virgins following their natural emergence from a queen cell (reviewed by Perez- Sato *et al.* 2007). One method that appears to overcome this variability is to introduce incubator-emerged virgin queens using an artificial queen cell consisting of a 4-d-old virgin placed in a plastic queen cell protector (JZs BZs, Menlo Park, CA) covered with paper and masking tape. The hole at the top of the artificial cell is closed with a plastic queen cup (JZs BZs) and at the tip with a thin wax-honey plug. This method has previously yielded over 90% acceptance (Perez-Sato *et al.* 2007). The objective of this study was to confirm the success of introducing virgins via this method and evaluate it against the use of mini queen cages or natural queen cells.

On 1 July 2010, 40 nucleus colonies were established at the AAFC Research Farm near Beaverlodge, Alberta, Canada (55° 12′ 34″ N, 119° 25′ 45″ W). Colonies were randomly allocated into four treatment groups and received one of the following: 1) 14-day-old queen cell, 2) 4-day-old virgin introduced using an artificial queen cell sealed with a wax-honey plug, 3) 4-day-old virgin introduced using a California mini queen cage (C.F. Koehnen & Sons, Inc, Glenn, CA) sealed with soft candy made from four parts liquid glucose syrup (# 11 Nulomoline, Grandma Food Products St. John, NB) and one part Drivert® (**Industrial Commodities, Inc.** Glen Allen, VA), or 4) 4-day-old virgin introduced using a California mini queen cage sealed with a wax-honey plug. All queens were daughters of an instrumentally inseminated hybrid of Minnesota Hygienic (Spivak *et al.* 2009) and a line selected for high Varroa Sensitive Hygiene (Danka *et al.* 2008) and were reared using the standard Doolittle method (Laidlaw and Page 1997). Queen cells were transferred from cell finisher colonies when queen cells were 14-day-old and either installed into colonies, or incubated at 34°C until emergence into glass vials containing soft candy. The successful release of virgins from cells or cages was inspected four days after introduction. If the queens had not been successfully released by this date they were manually released. Colonies were inspected for the presence of the virgins on day 10 and the presence of newly-laid worker eggs on day 21.

We did not observe the high levels of acceptance using artificial queen cells reported by Perez-Sato *et al.* (2007) as less than a quarter of virgins introduced using this method survived to egg-laying (Table 1). Although not significantly different from other treatments, the use of natural queen cells had the highest level of survival (Table 1).

The poor performance of the artificial queen cells may be attributed to the composition of the wax-honey plugs used in our study. Perez-Sato *et al.* (2007) used a wax-honey plug derived from the colony in which the virgin was to be introduced. Our wax and honey, in contrast, was of mixed origin. We hypothesize that the colonies familiarity with volatiles in the wax may be a significant variable in determining acceptance.

Although all but one of the virgins in the artificial cells was released by day four, virgins in cages with wax-honey plugs largely remained in their cages. In the cages we observed that while