Original paper

Comparison of three methods to multiply honey bee (*Apis mellifera*) colonies

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Abstract – In Canada, there is a growing need for additional honey bee colonies to satisfy the demand for pollination services and compensate for high winter colony mortality. The objective of our study was to compare methodologies that would be both better structured and adapted to producing new colonies in today's beekeeping industry. Efficacy of three colony production techniques was compared at the Deschambault Research Center for Animal Sciences, Québec: package bees, one brood frame, two brood frames. Experimental colonies were monitored from July 2014 to June 2015, and several parameters were measured to evaluate their strength and the presence of pathogens. Results showed no statistical difference in colony strength between methods. However, making nuclei from one brood frame offered the greatest multiplication potential and the most advantageous economically. This study also confirmed that nuclei production reduces swarming and varroa infestation levels in mother colonies.

Apis mellifera / honey bees / nuclei / package bees / population dynamic

1. INTRODUCTION

In recent years, the Canadian beekeeping industry has experienced strong growth, in part, due to the increase of pollination services associated with lowbush blueberry and cranberry production (Belzile and Li 2014). These two crops represent 90% of the 2015 colony rentals in the Canadian province of Québec (39,714 colonies; ISQ, 2015).

At the same time, factors affecting the health of honey bee colonies in Canada including diseases, parasitism by *Varroa destructor* and *Nosema spp* . shortage of flower resources, pesticide exposure, and stresses associated with pollination services are causing important winter losses (Kevan et al. 2007; Currie et al. 2010; Nars et al. 2016). In Canada, the average rate of winter losses for the past 10 years has been 25% (Nars et al. 2016),

while the rate considered acceptable by many beekeeping professionals is 10 to 15% (Furgala and McCutcheon 2008). These severe winter losses and the greater demand for pollination services have pushed beekeepers to multiply colony numbers by producing more nuclei during the productive season. Multiplying colonies is achieved by creating a new colony with a young mated queen and either just bees ("package bees") or brood and bees ("nucleus bees") (Ambrose 2008). Despite the efforts of beekeepers, the available bee colonies in Canada still do not meet the demand for pollination services (Chapleau 2012). As a result, beekeepers must import package bees from New Zealand and Australia, and queens from California, Australia, Chile, Hawaii, or neighboring Canadian provinces (Vickery and Levac 2005; Rousseau et al. 2015).

Bee imports are an acceptable but unsustainable short-term solution, since they are costly and pose important health risks to Canadian colonies (Chapleau 2012). Although imported bees are regulated by heath authorities, they may

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nonetheless bring added risk of disease or parasites (Canadian food inspection agency 2013). Furthermore, bee imports introduce foreign honey bee stock poorly adapted to the country's climate and beekeeping industry, and thus limit efforts to establish an entirely local honey bee genetic profile through domestic breeding programs and local queen producers (Chapleau 2005).

Developing a rigorous methodology for honey bee colony multiplication will assist beekeepers in all these areas (Desjardins et al. 2006). To our knowledge, little information is available in either technical or scientific apiculture literature on efficient methods for producing new colonies. It appears that relevant technical knowledge is passed on from generation to generation among beekeepers, and individual beekeepers may have their own, more or less effective method (Jean-Prost and Le Conte 2005; Ambrose 2008). The aim of this study was to compare the efficacy of three methods of colony multiplication to help develop a methodology that beekeepers can follow. At the same time, this approach may help beekeepers replace their losses, meet pollination demand, prevent the introduction of parasites or diseases, and contribute to maintaining locally adapted and selected honey bee populations. Our general hypothesis was that colony multiplication method influences the development of both the new and mother colonies. Specific hypotheses were (A) new colonies that are started with more brood frames will develop faster; (B) new colonies started with package bees (no brood) will have reduced pathogen loads; and (C) mother colonies from which most brood is taken to produce new colonies, will show reduced development, lower swarming behavior and higher *V. destructor* infestation, and *Nosema spp.* infection rates.

2. MATERIAL AND METHODS

2.1. Biological material

This study was conducted at the Deschambault Research Center for Animal Sciences (CRSAD, N 46° 40.27′, W 10° 71.50′), Québec, Canada in 2014–2015. Mother colonies ($n = 15$) used to make nuclei, and package honey bee colonies were composed of two brood boxes and had sister queens (European-derived) that had been introduced the year before. Prior to the beginning of our study, colonies were evaluated for strength (weight and brood amount), as well as for *V. destructor* infestation and *Nosema spp.* infection rates.

2.2. Experimental design

Three methods of colony multiplication were compared: package of bees (PB), nuclei with one brood frame (1BF), and nuclei with two brood frames (2BF). Fifteen mother colonies were classified into three groups of five colonies with equal strength (weight and brood amount); bees and brood from them were used to make new colonies. Each group of mother colonies was used to make only one nuclei method: five mother colonies used to make eight PB, five mother colonies were used to make 15 1BF nuclei, and another five mother colonies were used to make 15 2BF nuclei (Figure 1). Mother colonies were kept for comparison purposes with control colonies (no used for making nuclei) which were in the same apiary than mother colonies.

2.3. Colony multiplication: nuclei and package bee assembly

Before assembling nuclei, all queens from the mother colonies were found and temporarily retained in queen cages for the duration of the process. Nuclei and package bees were prepared on June 25, 2014 and were started in standard Langstroth hives with the brood chambers composed of eight, seven, or six frames with drawn comb (depending on the method) and one frame feeder (Propolis-etc..., Saint-Pie, QC, Canada; FE-1300).

Bee packages were made with adult bees from frames of the mother colonies. Frames were shaken above a funnel placed over a brood chamber and a platform scale (CAS-USA, East-Rutherford, New-York, USA; CAS CI-2001 BS) until a weight of 1 kg of worker bees was reached (Ambrose 2008; Laidlaw 2008). Nuclei (1BF and 2BF) were made with brood frames from mother colonies, three brood frames used from each mother colony to produce 1BF and six brood frames used from each mother colony to produce
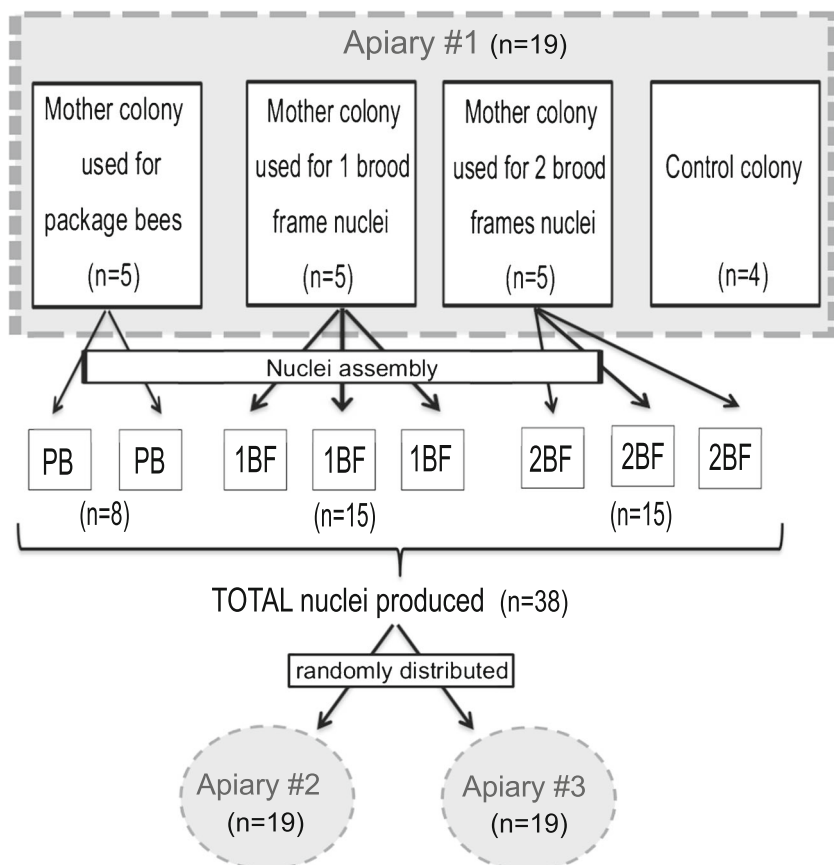
Figure 1. Diagrammatic representation of different nuclei assembly methods. Fifteen mother colonies produced: eight packages of bees (PB), 15 nuclei with one brood frame (1BF), and 15 nuclei with two brood frames (2BF). An additional four mother colonies were used for control. The new colonies were randomly and equally distributed between the two apiaries. A third apiary received mother colonies.

2BF. Each of these new colonies was installed in a nine-frame Langstroth hive body mounted on with a screened bottom board (Dadant & Sons Inc., Hamilton, Illinois, USA; #M01650).

All newly formed colonies were transported and placed into an environmentally controlled room (15 ± 1 °C) for 24 h to insure colony cohesion, and then, the following day, moved to two apiaries (separated by 2.6 km). The hives from each experimental group were randomly and equally distributed between the two apiaries. They were immediately fed with 2 L of sucrose-water solution (1:1) and 500 g of protein supplement (Global Patties Inc., Airdrie, Alberta, Canada; standard 15% pollen patty). Sister queens from hybrid local stock were introduced in each colony.

Entrance reducers were installed in the hives to help honey bees regulate internal colony temperature (Vickery and Levac 2005). From then on, colonies were managed for honey production, and honey supers were added as needed. At the end of August 2014, honey supers were removed and colonies were reduced to one brood box. Each colony was then fed 24 L of sucrose-water solution (2:1) using a top feeder (Propolis-etc…, Saint-Pie, QC, Canada; FE-1100). On September 15, 2014, all colonies received a mite treatment consisting of two Thymovar® strips placed on the top bars of the brood box. Young colonies were overwintered from November 15, 2014 to April 25, 2015 in an environmentally controlled room (4 ± 1 °C and 40% RH).

2.4. Measurement of dependent variables

The following variables were monitored from June 25, 2014 until May 25, 2015:

Brood population: the area occupied by immature worker honey bees (eggs + larva + capped brood) in colonies was evaluated by measuring width and length of the brood surface on each sides of every brood frame (Giovenazzo and Dubreuil 2011).

Adult bee population: for each colony, the size of the cluster of bees was measured by opening each hive and counting the number of frames occupied by the bee cluster around the brood as viewed from above.

Colony weight gain: hives were weighed individually throughout the season each time a honey super was added or removed by placing the entire colony (brood chamber and honey supers) on a platform scale (CAS-USA, East-Rutherford, New-York, USA; CAS CI-2001 BS).

Observation of swarming behavior in mother colonies: swarming behavior was verified once every 2 weeks. All frames in each colony were carefully inspected and queen cells were counted and destroyed (Delaplane et al. 2013).

V. destructor infestation levels: infestation was assessed by the natural mite fall method (Imdorf et al. 2003; Dietemann et al. 2013). Mites that had fallen onto sticky boards placed on the bottom boards of all hives were counted. Sticky boards covered the entire bottom surface of hives and were placed in June, July, August, and September 2014 and May 2015 during 7 days.

Nosema spp. infection levels: approximately 60 forager bees were retrieved in each colony and preserved in 70% ethanol. *Nosema spp*. infection levels were assessed by determining spore counts per bee (Fries et al. 2013).

2.5. Statistical analysis

Statistical tests were conducted using SAS software (ver. 9.4, SAS Institute Inc., Cary, NC, USA) at the 0.05 level of significance. All dependent variables were tested for normality using the Shapiro-Wilk test, and a Box-Cox power transformation was used when necessary to meet the normality assumptions of the model. Variables were analyzed by means of an analysis of variance (ANOVA), with repeated measurements for a mixed model using Glimmix and Mixed procedures. When a significant effect was observed ($P < 0.05$), the means of experimental groups were compared using Tukey-Kramer grouping's least significant difference method. Swarming behavior data was analyzed with a binomial mixed model. Mortality was analyzed with a mixed logit model. Only mortality data of nuclei 1BF and 2BF were taken into account for this analysis because package bee colonies were all alive and to add these data into analyses could skew the results.

3. RESULTS

3.1. New colonies

Five of the initial 38 new colonies died or were eliminated during the experiment. Three of these had drone-laying queens from the start, one queen was not accepted and one colony died during wintering. Mortality was similar between the three methods ($F = 1.03$; df = 1, 8; $P = 0.3399$).

For colony strength, Figure 2 shows that after 1 month (in mid-July), the mean number of cells occupied by brood (±SE) was not significantly different between groups PB, 1BF, and 2BF ($F = 1.42$; df = 2, 131; $P = 0.2465$). Furthermore, Figure 2 shows that significantly more inter-frames were occupied by bees in the 2BF group colonies compared to other groups for the same measurement time ($F = 1.71$; df = 16, 256; $P = 0.0454$). Overall, average colony strength (mean number of cells occupied by brood and inter-frames occupied by bees) was similar throughout the 2014 season and during the following spring (means ± SE of number of inter-frames of bees on 20 May 2015 of 2BF nuclei, 1BF nuclei, and PB, respectively: $5.7 ± 0.3$; $5.3 ± 0.4$; $5.8 ± 0.4$; $F = 0.64$; df = 2, 256; $P = 0.5281$).

Figure 2 shows that group 2BF colonies were significantly heavier than those of 1BF and PB groups from July to September ($F = 2.82$; df = 18, 287; $P = 0.0002$). However, in November 2014 and May 2015, the weight of colonies from the three groups was similar ($F = 0$; df = 2, 287; $P = 1.00$; and $F = 0$; df = 2, 287; $P = 0.9961$, respectively).
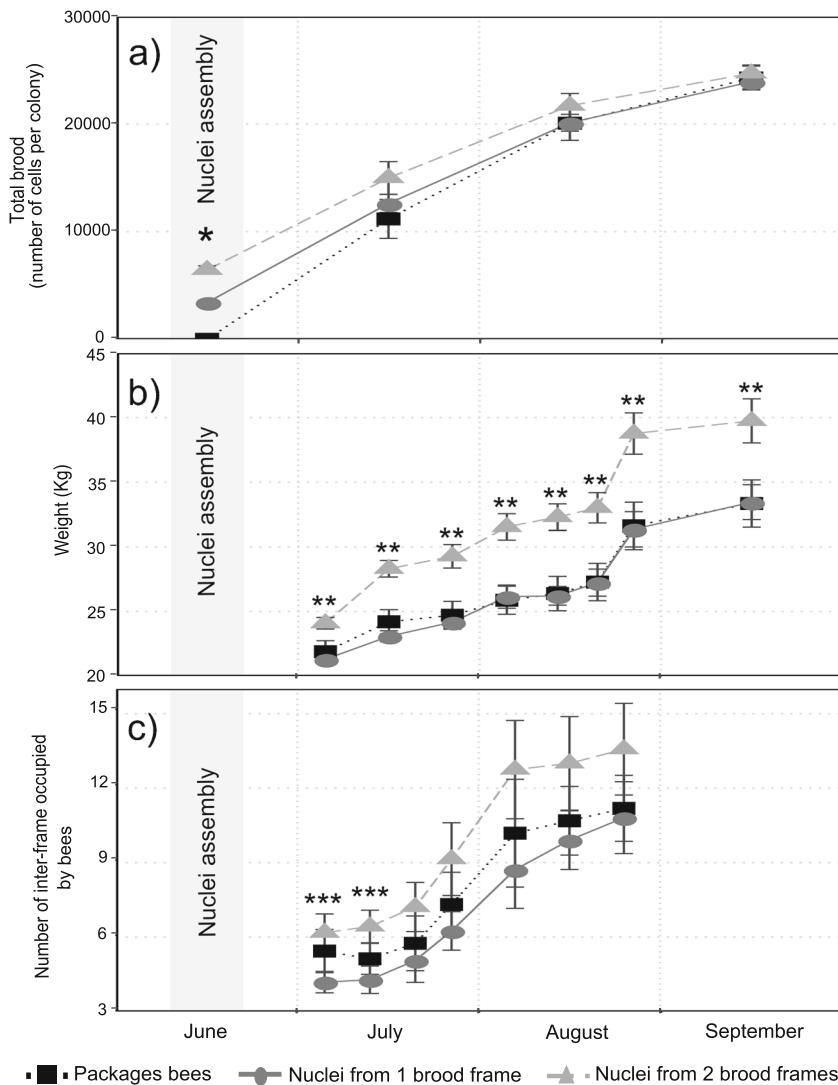
Figure 2. Nuclei strength evaluation in relation to production method. Means (±SE) of brood surface, weight, and number of inter-frame occupied by bees of each method are given throughout the beekeeping season (2014). **a** Nuclei brood surface in relation to the production method. **b** Nuclei weight in relation to the production method. **c** Nuclei inter-frames occupied by bees in relation to the production method. *Represents significant difference between three methods, **Represents significant differences between nuclei from two brood frames and other methods (nuclei from one brood frame and package bee), ***Represents significant differences between nuclei from two brood frames and nuclei from one brood frame (Tukey-Kramer grouping test $P < 0.05$).

V. destructor infestation levels were low (below one mite daily drop) and similar between the three groups throughout the experiment ($F = 0.43$; df = 6, 99; $P = 0.8578$). In July 2014,

Nosema spp. infection levels were significantly higher in the colonies of group PB compared to the colonies of groups 1BF and 2BF (Figure 3, $F = 6.90$; df = 2, 31; $P = 0.0033$). However,
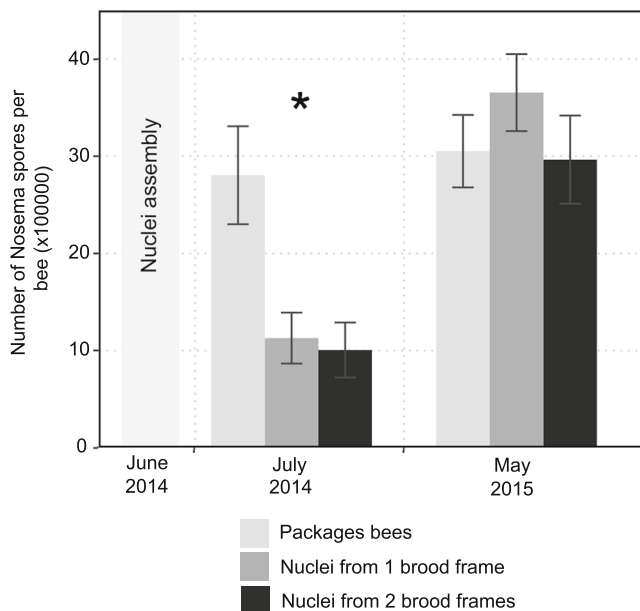
Figure 3. Nuclei *Nosema spp.* infection rate in relation to production method. Means (±SE) of *Nosema spp.* infection levels of each method are given 1 month after nuclei and package bees assembled (July 2014) and in spring 2015. There was a significant difference between package bees and other methods in July 2014.

Nosema spp. infection levels were similar between all groups in May 2015 ($F = 1.05$; df = 2, 30; $P = 0.3610$).

3.2. Mother colonies

Mother colonies were of similar brood surface ($F = 1.65$; df = 3, 11; $P = 0.2354$) and had similar weight ($F = 1.91$; df = 3, 11; $P = 0.1866$) before and after nuclei assembly.

Swarming behavior was absent or low in July for colonies in which we took brood or bees, whereas control colonies produced more queen cells during this month; high variability between colonies was evident as well. At the end of the summer, mother colonies and controls produced fewer queen cells. If data for all mother colonies were pooled, control colonies produced significantly more queen cells ($\chi^2 = 4.2124$; df = 1; $P = 0.0401$).

V. destructor infestation rates were low (below one mite daily drop) and similar between all groups (mother and control colonies) prior to colony multiplication from June 20 to August 4 (Figure 4). However, in September 2014, mite infestation rates were significantly higher in control colonies ($F = 15.69$; df = 3, 32; $P < 0.0001$).

Infection rates of *Nosema spp.* (Figure 4) were similar between all groups prior to colony multiplication ($F = 2.08$; df = 3, 13.1; $P = 0.1519$). One month later, *Nosema spp.* infection rates were significantly lower in all groups but similar among groups ($F = 1.94$; df = 3, 14; $P = 0.1693$).

4. DISCUSSION

4.1. New colonies

Brood population: Regardless of the quantity of brood used to set up a nucleus colony, all nuclei (the three methods confounded) had similar brood strength at the end of the first summer of the experiment. In the spring of the following year, colonies resulting from the three methods met pollination standards because all had more brood (means ± SE of number of brood frames in May 2015 of 2BF nuclei, 1BF nuclei, and PB, respectively: 11.2 ± 0.37; 10 ± 0.5; 10.44 ± 0.48) than the six brood frames required in pollination
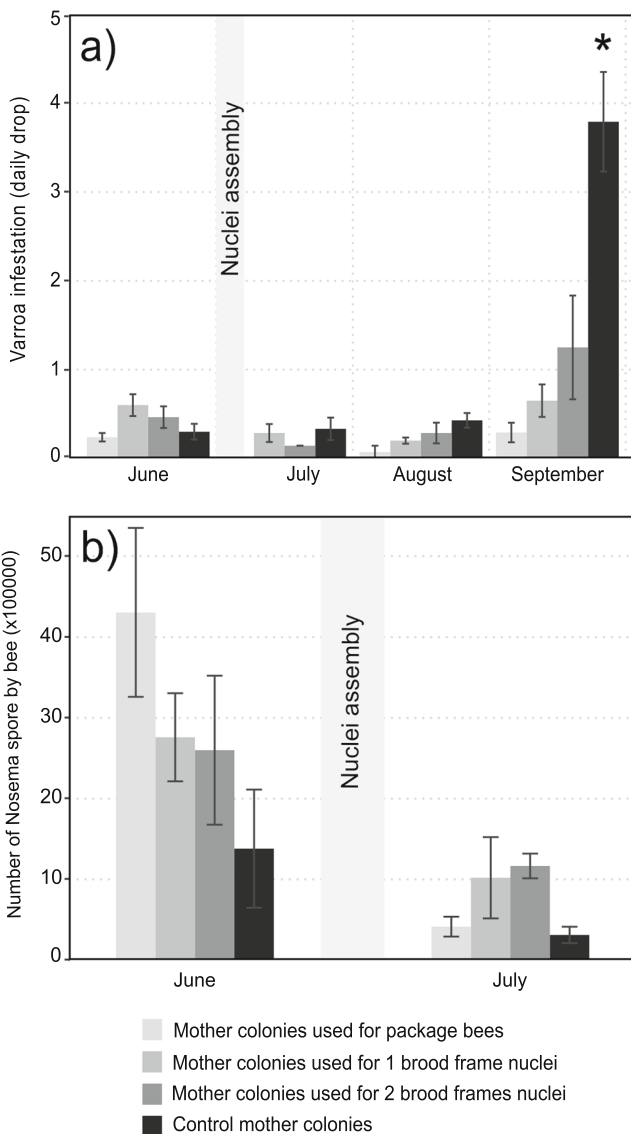
Figure 4. a *V. destructor* infestation in mother colonies in relation to nuclei-making method. Means (±SE) of mite infestation levels of each mother colony group are given throughout the beekeeping season (2014). There was a significant difference between mother colonies and control colonies (Tukey-Kramer grouping test: $P < 0.05$). **b** Nosema infection rate in mother colonies in relation to nuclei-making method. Means (±SE) of *Nosema spp.* infection levels of each mother colony group are given before and after making nuclei.

contracts (FAQ 2015; Hoopingarner and Waller 2008).

Adult bee population: During the first 3 weeks following nuclei creation, 2BF nuclei had more inter-frames occupied by bees than 1BF nuclei, since their larger brood quantity at the start led to

more emerging bees in the early weeks of the experiment, and hence resulted in a larger honey bee population. This observation regarding the surplus of honey bees confirmed our hypothesis concerning the difference in weight of 2BF nuclei compared to the other methods (1BF and PB). At

the end of the summer 2014, no significant differences in bee populations were found between 2BF and 1BF nuclei, confirming findings by Liebig (1998). We can add, based on the findings of this study, that PB follows the same population development as 1BF and 2BF nuclei by the end of the season. However, two factors may have skewed our results. First, our inter-frame-based bee-counting system certainly amplified measurements of adult bee population for PB nuclei because the reduced amount of brood in these nuclei during the initial weeks following their creation did not require that bees clustered around the brood area to ensure its thermoregulation and care as occurred with the other two methods (Le Conte et al. 1990). A second factor that may have skewed our results is the measuring procedure, which focused only on the top of the inter-frames. This may have introduced bias to our data, because bees may have been concentrated on the top of the frames but not on the bottom, and the inter-frames may nonetheless have been counted as fully occupied by bees. Consequently, overestimation of the population of these nuclei is probable.

The 2BF nuclei were heavier than all others throughout the summer of 2014. We suppose that their bee population grew faster, therefore having more bees available for foraging following their establishment (Hoopingarner and Waller 2008). This surplus of provisions explains the difference in weight between 2BF nuclei and the others (PB and 1BF). In fact, this weight difference persisted all summer, exclusively due to the surplus of provisions harvested shortly after colony establishment. No honey supers were installed on the brood chambers of the nuclei; therefore, honey bees stored pollen and nectar in the brood chambers. Thus, we could not dissociate colony weight and honey weight. However, the weight gain in 2BF nuclei (about 5 kg) seemed too large for it to represent only brood and honey bee weight gain. Moreover, the weight curves of three nuclei-making methods plotted on the graph seem identical; the curve representing 2BF nuclei is merely shifted, compared to the two others. In the autumn of 2014, all nuclei had a similar weight, because during this period the honey frames had been harvested from the nuclei, all colonies had been

reduced to one brood chamber and were fed with sucrose syrup to prepare them for the winter.

The true mortality rate of our nuclei was the one winter loss (a single nucleus). In this experiment, we considered the status of nuclei with a drone-laying queen or with queen acceptation problems as equivalent to mortality. However, a beekeeper can overcome such difficulties by introducing new queens into these colonies in order to keep them alive. In our case, introduction of new queens in these nuclei would have slowed their development compared to properly functioning nuclei. Consequently, we eliminated these nuclei from our experiment to avoid distorting our results.

While no significant difference in *V. destructor* infestation level was evident among the three methods, low levels measured may explain our results. If mite infestation levels had been higher when assembling various nuclei groups, 1BF and 2BF nuclei would have been more infested than PB nuclei because 70 to 80% of mites are in the brood during summer months (Colin and Gonzales-Lopez 1986; Büchler et al. 2013; Pernal and Clay 2015; Zemene et al. 2015).

In 2014, *Nosema spp.* infection levels were higher in PB nuclei. We suppose this result may be explained by the fact that nosema disease only affects honey bees at the adult stage (Fries et al. 2013; Pernal and Clay 2015) and that PB had in it more adult bees than nuclei 1BF and 2BF after nuclei making. Making nuclei exclusively with adult bees could thus lead to greater dispersion of *Nosema spp.* among them. In 2015, all nuclei had similar *Nosema spp.* infection levels, because after one beekeeping season the entire nuclei had aged honey bees, and were thus potentially more infected by *Nosema spp* .

4.2. Mother colonies

Retrieving brood from mother colonies to establish nuclei reduced the amount of brood in them, but they recovered to levels similar to those of control colonies after a few weeks. The populous mother colonies used to obtain brood or bees to establish nuclei were found to have population dynamic similar to those of colonies that swarm naturally. Indeed, when a colony swarms

naturally, the amount of brood and adult bee population decline rapidly because the queen and part of the colony's population leave the hive (Winston 1987; Gary 2008). In this situation, brood production is no longer ensured by the queen, and the population will resume growing only after a few weeks, when a new queen will be "ready-to-lay" (Seeley and Visscher 1985; Liebig 1999; Imdorf et al. 2010). Colonies are thus naturally adapted to survive losses of brood or bees. In this experiment, the mother colonies may have rapidly recovered a brood surface similar to that of the control colonies because, contrary to natural swarming, the queens did not leave the hives and continued to lay eggs. Retrieving brood or adult bees thus disturbed the colony's brood development less than natural swarming would have, since there was no break in laying during the season.

No significant difference in weight was found between all mother colonies before and after nuclei making as well as between mother colonies and control colonies after making nuclei during the 2014 season. It is important to clarify that our weight measurements did take honey production into account, because the weight of honey supers was deducted from that of the hive. Brood and bee removal for nuclei making did not influence colony weight.

Removing adult bees and brood from colonies delays and considerably moderates swarming behavior by mother colonies (Jean-Prost and Le Conte 2005; Imdorf et al. 2010). Indeed, natural swarming is a mechanism by which a colony multiplies, allowing honey bees to propagate the species and compensate for losses due to mortality. This behavior is observed in populous colonies in late spring or early summer, when floral resources are abundant (Winston 1987; Gary 2008; Human et al. 2013). Removing adult bees and brood from colonies slows down their development and causes a temporary reduction in population. Thus, we did not observe queen cells in mother colonies in the spring of 2014 compared to control colonies.

No significant differences in *V. destructor* infestation levels were observed in our colonies throughout the summer of 2014. Infestation levels remained below 1 mite dropped/day. It is known that during summer months, majority of mites lurk in the brood (Pernal and Clay 2015), which is why the extent of mite infestation was not observable during the summer with our screening method. In September, however, when the queen's egg-laying rate slows down, mites can no longer lodge in the brood; the degree of infestation in hives becomes evident (Zemene et al. 2015). Thus, *V. destructor* infestation levels were found to be greater than 3.5 parasites dropped/day in control colonies during this period. However, the adult bees or brood taken from mother colonies in early summer reduced the level of infestation found at the end of the summer. Removing brood or bees for the purpose of making nuclei thus helps control mite infestation.

The Nosema spp. infection level was higher in early June 2014 probably due to the presence of a certain number of winter bees still in the mother and control colonies. Those had higher level of Nosema spp. infection because Nosema spores multiplied in the ventriculus of the insects throughout the winter. Subsequently, in July, Nosema spp. infection levels were lower than in June, probably because bee populations were renewed and, young bees had not yet been extensively infected by Nosema spores, but also because foraging conditions improved (Smart and Sheppard 2012; Huang et al. 2015; Pernal and Clay 2015). Removing bees or brood from colonies did not seem to influence Nosema spp. infection levels since there were no significant differences between mother and control colonies for this variable.

4.3. Conclusion

The main purpose of this study was to assess development and parasitic status of new colonies assembled with three methods of colony multiplication (PB, 1BF, 2BF). The mother colonies were also compared with control colonies (colonies from which of brood or adult bees were retrieved). In conclusion, our results showed that the three nuclei-making methods did not significantly differ in terms of development, growth, or health and resulted in high-performing colonies. However, weight differences observed between nuclei methods during experimentation lead us to believe

that the 2BF nuclei method showed a slight advantage compared to other nuclei methods. Furthermore, nuclei-making methods did not affect development and health of mother colonies and also reduced their swarming behavior and *V. destructor* infestation levels. Finally, the most important result of this study is that colonies of group 1BF showed the highest potential for multiplication of the three methods tested. Indeed, from an economic point of view, we could make six 1BF nuclei with one mother colony instead of making three 2BF nuclei with one mother colony. This way, a beekeeper could double his nuclei production but could also, for example, double his profits renting these hives for pollination services the following year.

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CONTRIBUTIONS

PG conceived this research and designed experiments; SM performed experiments, analysis, and wrote the paper; all authors participated in the revisions of it. All authors read and approved the final manuscript.

Comparaison de trois méthodes pour multiplier les colonies d'abeilles mellifères (*Apis mellifera*)

***Apis mellifera* / abeilles mellifères / noyaux / paquet d'abeilles / dynamique des populations**

Vergleich dreier Methoden zur Vermehrung von Bienenvölkern (*Apis mellifera*)

***Apis mellifera* / Honigbienen / Ableger / Paketbienen / Populationsdynamik**

REFERENCES

Ambrose, J. T. (2008) Management for honey production, in: Graham J. M. (Eds.), The Hive and the Honey Bee. Hamilton, Illinois: Dadant & Sons, inc., pp.602–654

Belzile, L., Li, J. (2014) IRDA : La croissance de l'industrie apicole québécoise: une fausse joie? [online] https://www.irda.qc.ca/assets/documents/Publications/documents/belzile-li-2014_fiche_croissance_apicole_qc.pdf. Accessed 24 May 17

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Kezic, N. et al. (2013) Standard methods for rearing and selection of *Apis mellifera* queens. J. Api. Res. **52** (1), 1–29

Canadian food inspection agency (2013) Honey bee producer guide to the National Bee Farm-Level Biosecurity Standard [online] http://publications.gc.ca/collections/collection_2014/acia-cfia/A104-107-1-2013-eng.pdf. Accessed 24 May 17

Chapleau J.-P. (2005) Apiculture : Production commerciale de reine abeilles [online] https://www.agrireseau.net/references/7/presentation.pdf. Accessed 24 May 17

Chapleau J.-P. (2012) Table filière apicole: Plan d'action visant à réduire les pertes anormales d'abeilles au Québec [online]. http://www.crsad.qc.ca/fileadmin/fichiers/fichiersCRSAD/Recherche/apicole/plan_d_action_reduction_des_pertes_abeilles.pdf. Accessed 6 September 17

Colin, M. E., Gonzales-Lopez, M. D. (1986) Traitement de la varroatose de l'abeille domestique: chimiothérapie, mesures adjuvantes et perspectives de lutte biologique. Rev. sci. tech. Off. int. Epiz. **5** (3), 677–687

Currie, R. W., Pernal S. F., Guzmán-Novoa E. (2010) Honey bee colony losses in Canada. J. Api. Res. **49** (1): 104–106

Desjardins, F. DMV., Gauvin Y., Houle E., Jacques D., Marceau J., Pettigrew A. (2006) Apiculture: Trousse d'information et de démarrage. Centre de référence en agriculture et agroalimentaire, Québec, Canada.

Delaplane, K. S., Van Der Steen, J., Guzman-Novoa, E. (2013) Standard methods for estimating strength parameters of *Apis mellifera* colonies. J. Api. Res. **52** (1), 1–12

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B. et al. (2013) Standard methods for varroa research. J. Api. Res. **52** (1), 1–54

Fédération des Apiculteurs du Québec (2015) Contrat de pollinisation - Location de ruches.[online] http://www.apiculteursduquebec.com/documents/123/bleuet%202.0.pdf. Accessed 24 May 17

Fries, I. Chauzat M-P., Chen Y-P., Doublet, V., Genersch E. et al. (2013) Standard methods for Nosema research. J. Api. Res. **52** (1), 1–28

Furgala, B., McCutcheon, D. M. (2008) Wintering productive colonies, in: Graham J. M. (Eds.), The Hive and

the Honey Bee. Hamilton, Illinois: Dadant & Sons, inc., pp. 829–864

Gary, N. E. (2008) Activities and behavior of honey bees, in: Graham J. M. (Eds.), The Hive and the Honey Bee. Hamilton, Illinois: Dadant & Sons, inc., pp. 269–361

Giovenazzo, P., Dubreuil, P. (2011) Evaluation of spring organic treatments against *Varroa destructor* (Acari: Varroidae) in honey bee *Apis mellifera* (Hymenoptera: Apidae) colonies in eastern Canada. Exp. Appli. Acarol **55** (1), 65–76

Hoopingarner, R.A., Waller, G. D. (2008) Crop pollination, in: Graham J. M. (Eds.), The Hive and the Honey Bee. Hamilton, Illinois: Dadant & Sons, inc., pp. 1044–1080

Huang, W-F., Solter, L., Aronstein, K., Huang, Z. (2015) Infectivity and virulence of *Nosema ceranae* and *Nosema apis* in commercially available North American honey bees. J. Invertebr. Pathol. **124** , 107–113

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J. D., et al. (2013) Miscellaneous standard methods for *Apis mellifera* research. J. Api. Res. **52** (4), 1–57

Imdorf, A., Charrière, J., Kilchenmann, V., Bogdanov, S., Fluri, P. (2003) Alternative strategy in central Europe for the control of *Varroa destructor* in honey bee colonies. Apiacta **38** (3), 258–278

Imdorf, A., Ruoff, K., Fluri, P. (2010) Agroscope Liebefeld-Posieux forum n° 68 : le développement des colonies chez l'abeille mellifère. Station de recherche Agroscope Liebefeld-Posieux, Berne.

Institut de la statistique du Québec (2015) Statistiques relatives à la location de colonies à des fins de pollinisation selon le type de culture, Québec, 2014 [online] http://www.stat.gouv.qc.ca/statistiques/agriculture/apiculture-miel/h7_2014.htm. Accessed 24 May 17

Jean-Prost, P., Le Conte, Y. (2005) Apiculture : Connaître l'abeille, Conduire le rucher. Tec & Doc, Paris.

Kevan, P., Guzman, E., Skinner A., Van Englesdorp D. (2007) Colony collapse disorder in Canada: Do we have a problem? Hivelights **1** , 14–16

Laidlaw, H. H. (2008) Production of queens and package bees, in: Graham J. M. (Eds.), The Hive and the Honey

Bee. Hamilton, Illinois: Dadant & Sons, inc., pp. 989–1041

Le Conte, Y., Trouiller, J., Masson, C., Chappe, B. (1990) Identification of a Brood Pheromone in Honeybees. Naturwissenschaften **77** , 334–336

Liebig, G. (1998) Mehr bringt nicht unbedingt mehr. Zur Bildung und Entwicklung von Jungvölkern, D. Bienen J. **6** (1), 4–7

Liebig, G. 1999. Wenn aus eins zwei werden. Der Vermehrungsakt und seine Folgen, Bienenpflege **6** (1), 189–191

Nars, M., Jordan, C., Kempers, M., Kozak, P., Lafreniere, R. et al. (2016) Canadian association of professional apiculturists statement on honey bee wintering losses in Canada [online] http://www.capabees.com/shared/2015/07/2016-CAPA-Statement-on-Colony-Losses-July-19.pdf. Accessed 24 May 17

Pernal, S. F., Clay, H. (2015) Maladies et organismes nuisibles de l'abeille domestique, 3e édition. Canada: Association canadienne des professionnels de l'apiculture, Beaverlodge, Alberta.

Rousseau, A., Fournier, V., Giovenazzo, P. (2015) Apis mellifera (Hymenoptera : Apidae) drone sperm quality in relation to age, genetic line, and time of breeding. Can. Entomol. **147** (6), 702–711

Seeley, T. D., Visscher, P. K. (1985) Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. Ecol. Entomol. **10** (1), 81–88

Smart, M. D., Sheppard, W. S. (2012) *Nosema ceranae* in age cohorts of the western honey bee (*Apis mellifera*). J. Invertebr. Pathol. **109** (1), 148–151

Vickery, M. V., Levac, B. (2005) Les paquets d'abeilles. Le Centre de Référence en Agriculture et Agroalimentaire du Québec (CRAAQ).

Winston, M. L. (1987) The biology of the honey bee. Harvard University Press, Cambridge, Massachussetts.

Zemene, M., Bogale, B., Derso, S., Belete, S., Melaku, S., Hailu, H. (2015) A review on varroa mites of honey bees. Acad. J. Entomol. **8** (3), 150–159