

Effects of various fall feeding sugar sources on survival, health, and productivity of honey bee colonies (*Apis mellifera* L.)

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Abstract: In Canada, beekeepers must supplement their colonies with sugar in the fall to ensure survival during winter, when floral resources are absent. Sucrose syrup is the predominant choice due to its high availability, chemical stability, and ease of use. However, upcoming revisions to Canada's organic standards will prohibit the use of conventional sugar syrup, necessitating a shift toward honey or organic sugar syrup as overwintering resources. The implications of this transition on colony survival, development, productivity, and pathogen prevalence remain insufficiently characterized.

This study evaluated honey bee (*Apis mellifera* L.) colonies fed with either conventional sugar syrup, organic sugar syrup, summer honey, or fall honey. Key parameters assessed included winter survival, colony development, honey production, and pathogen development (*Varroa destructor*, *Nosema* spp., and six viruses).

Results indicate that organic sugar syrup, summer honey, and fall honey are viable alternatives to conventional sugar syrup for overwintering. However, precise colony weight management following fall feeding is critical to prevent starvation. In most cases, a minimum of nine honey frames per colony housed in a Langstroth single-brood chamber or four frames supplemented with 12L of organic sugar syrup supported successful overwintering, although adjustments may be required depending on colony size and winter severity.

No statistically significant differences were observed in brood and bee population development, honey production, or pathogen development across feeding treatments. These findings suggest that while organic beekeepers can effectively overwinter colonies using alternative carbohydrate sources honey-based feeding warrant careful consideration.

Keywords: honey, honey bee, organic, overwinter, sugar

INTRODUCTION

Flower nectar is the main source of natural sugar harvested by foraging honey bees (*Apis mellifera* L.). The nectar is transported in the workers' crop to the colony, where it is processed by the bees. They reduce the water content to 16-20% humidity and add enzymes that break down complex sugars into simpler sugars, which are then stored as honey in the cells [7]. Sugar is used for energy production, which supports several metabolic functions, such as flight and thermoregulation, and can also be converted and stored as fat [34]. In temperate climates, like that of Canada, long winters, short blooming periods, and sometimes unfavorable weather conditions for bee activity, mean that colonies often require beekeepers' intervention with supplementary sugar or protein feedings [19]. The energy cost associated with wintering is very high, and the colony must have sufficient sugar reserves to survive until the return of food resources, which can be more than seven months in temperate climates. The thermoregulation cost during the winter months is 0.42 kg/week for colony survival in the absence of brood, and 0.84 kg/week once brood rearing begins in the colony [30]. In preparation for winter and spring, when nectar sources are scarce, beekeepers often supplement

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their colonies with sugar to ensure their survival and development during the months without floral resources, thus preventing starvation. Beekeepers use various sugar sources to feed their colonies, including inverted syrup, fondant, and high-fructose corn syrup [2010]. The choice of sugar source depends on current practices in the country where beekeeping is practiced and the costs associated with different sugar sources. However, sugar syrup (from sugar cane or sugar beet) is the most used and recommended due to its abundance and simple, stable composition [31].

According to the Canadian Organic Standards of the Canadian Federation of Organic Agriculture (OFC), which apply to organic beekeepers, the primary food source for honeybees must be the nectar and pollen harvested by the colony. In cases of regional or seasonal food shortages, or for winter feeding of colonies, it is allowed to use, in order of preference: 1) organic honey from the operation; 2) organic sugar (e.g., inverted syrup, fondant); 3) non-organic honey from a conversion operation; or 4) non-organic sugar that is not genetically modified (COG, 2020). But in December 2019, the Interpretation Committee of the Standards (SIC) determined that the winter period alone cannot justify the annual recurrent feeding of bees with syrup. In 2025, article 7.1.11.1 regarding the feeding of honeybees in the Canadian Organic Standards will be revised. This revision is expected to eliminate non-organic sugar from the options available to organic beekeepers, unless data on the potential negative impacts of wintering bees with honey or organic sugar are provided.

Feeding colonies with honey during the winter, while practiced, is a less common technique in Canada. In fact, honey composition is dependent on floral sources, making it difficult to generalize the feeding method. Several risks associated with wintering bees on honey as the sole carbohydrate source, such as honey crystallization and dysentery in bees, have been identified in the past [2, 14, 17]. Honey crystallization is a natural process related to the glucose content that becomes supersaturated in certain types of honey (e.g., canola honey, *Brassica napus L.*). The winter mortality of colonies fed honey has been linked to insufficient reserves and honey crystallization [14], as crystallized honey in frames becomes unavailable to the bees. Among others, dandelion, maple, sweet clover, and alfalfa honeys have been associated with mortality due to crystallization [33, 16]. However, glucose solubility is influenced by other honey components, and current knowledge is insufficient to predict crystallization [5]. It seems that adding sucrose to honey reduces crystallization up to a total of 34% sucrose in the mixture [32]. Dysentery is also a problem associated with wintering bee colonies on certain honeys with excessively high moisture content [2]. Honeys produced late in the season, which the bees did not have time to properly process, such as aster or goldenrod honey, can also cause dysentery and mortality [33, 16, 24]. Some honeys, however, are beneficial for wintering bees, such as clover and buckwheat honey [33, 16]. It has also been demonstrated that a more complete nutrition of workers compared to a diet of sugar syrup can impact the quantity of *Nosema* spores per bee [4, 37] and may improve bee tolerance to pathogens [28]. Despite these observations, the overall understanding of honey as a winter feed remains incomplete. Few recent studies have investigated the potential of wintering bee colonies with honey, especially in climates similar to that of Canada. As a result, many questions remain unanswered regarding the feasibility and consequences of using honey instead of refined sugar, including its effects on colony survival, bee health, and post-winter productivity.

The main objective of this study is to assess the impact of the type of sugar used for feeding bee colonies on winter survival, bee health, and colony productivity. Different types of honey, organic sugar syrup, and conventional sugar syrup were characterized and administered to bee colonies in the fall to assess winter survival, winter sugar consumption, development of major bee pathogens (*Varroa destructor*, *Nosema spp.*, and six viruses), as well as colony development and honey production.

MATERIALS AND METHODS

To assess the impact of fall feeding types on the honeybee, the first experimental phase took place at the Centre de recherche en sciences animales de Deschambault (CRSAD). The objective of this phase was to compare traditional sugar syrup feeding methods (both conventional and organic) with honey feeding methods (summer honey and fall honey). This phase allowed for complete control of the experimental setup. The second experimental phase was conducted with two Quebec-based certified organic beekeepers. This phase aimed to evaluate the impact of feeding type in a commercial and organic context.

FIRST EXPERIMENTAL PHASE 2022-2023

Queen rearing and preparation of experimental colonies

The 2022-2023 experimental phase took place at CRSAD. In June 2022, 50 sister queens from the CRSAD-UL breeding program [22] were produced. These laying queens were introduced into 50 nuclei that had been pre-prepared with two frames of brood and one frame of honey/pollen. The nuclei were then randomly distributed across two apiaries located in the city of Pont-Rouge (Picard and 365 apiaries). On August 24, 2022, the colony strength was assessed to ensure the colonies were evenly distributed across the experimental groups. The number of developing worker bees (eggs, larvae, and pupae) was estimated by measuring the brood area (width x length) on both sides of the 10 frames in the brood chamber. The resulting rectangular area was multiplied by 0.8 to account for the elliptical shape of the brood pattern. A factor of 25 worker cells per 6.25 cm^2 was used to convert the area into the number of immature worker bees [12]. The adult bee population in each colony was also estimated visually [6]. On September

1, 2022, a sample of bees was collected for pathogen analysis (viruses and Nosema spp.), as well as an alcohol wash to assess the varroa infestation rate. The 50 colonies were then evenly distributed across the following five experimental groups.

Summer honey

In mid-July 2022, 80 frames of capped honey were harvested from colonies located at a single site in an agricultural area with meadows in the city of Pont-Rouge. These frames were stored in a cold room until feeding began. On September 15, for the colonies fed with summer honey, the lower brood chamber was rearranged by placing two frames of pollen at the edges and adding eight frames of capped honey in the center. The brood frames removed from the lower chamber were placed into a second super, which was installed above the first, separated by a queen excluder. The queen remained in the lower super. Three weeks later, after the brood had emerged, the second super was harvested to allow for wintering with a single super, as with the other experimental groups.

Fall honey

In early September, 80 frames of honey, partially capped, were harvested from hives located at a single site in an agricultural area with meadows in the city of Pont-Rouge. On September 15, colonies receiving fall honey underwent the same procedure as those receiving summer honey.

Organic syrup

The organic syrup was prepared using organic cane sugar from Brazil in bulk packages (Costco, Washington, USA). On September 15, 2022, the colonies receiving organic syrup were moved to a single super using a bee escape (Propolis etc., BE-1200), and each colony received 15 L of 2:1 sugar syrup in a Miller-type surface feeder. One week later, an additional 8 L of 2:1 sugar syrup were provided to complete the feeding.

Conventional syrup

The conventional sugar syrup was purchased in bulk as a liquid (Saint-Stanislas-de-Kostka, Quebec, Canada). On September 15, 2022, colonies receiving conventional syrup underwent the same procedure as those receiving organic syrup.

Mixture (50% fall honey and 50% organic syrup)

In early September, 40 frames of honey, partially capped, were harvested from hives located at a single site in an agricultural area with meadows in the city of Pont-Rouge. On September 15, for colonies receiving the mixture, four frames without brood or with brood that was about to emerge were removed from the brood chamber of each colony. These frames were replaced by four frames of fall honey. The removed frames were placed in a second super, which was positioned above the first and separated by a queen excluder. One week later (after the capped brood had emerged), the second super was harvested to allow for wintering with a single super, as with the other experimental groups. The colonies were then fed 12 L of organic sugar syrup (2:1 ratio) in a Miller-type surface feeder (Propolis etc., FE-1102).

Honey analysis

The melissopalynological analysis of the honey used for feeding was conducted by the company Bizzbilis (Baie-Saint-Paul, QC, Canada).

Antiparasitic treatments and wintering

Along with feeding, a Hopguard® II treatment was applied to all colonies according to the manufacturer's recommendations. On October 27, 2022, the hives were treated with oxalic acid via dripping. The size of the cluster before winter was determined by estimating the total number of frames fully covered with bees when the temperature was below 10°C. The number of frames covered with bees on the top and bottom of the hive was noted, and the average was calculated for each colony (Büchler et al. 2013). The hives were weighed using a portable scale (capacity of 160 kg, minimum sensitivity of 0.1 kg). The hives were then moved to two wintering apiaries in the city of Pont-Rouge and wrapped with double-bubble reflective Thermofoil insulation, and a rigid R10 insulating panel made of extruded polystyrene was placed on the inner cover of each hive.

Evaluation of survival, colony development, and pathogens

In April 2023, the colony coverings were removed. Then, the cluster size was evaluated in the same way as in the fall, and the hives were weighed to estimate winter sugar consumption. Hives weighing less than 24 kg were fed with four litters of 2:1 syrup to prevent starvation, while all other colonies received 0.5 L of syrup to account for the stimulatory effect of spring feeding [1, 34, 28]. The strength of the colony was assessed by measuring the brood and estimating the population of worker bees (as described previously) in May and June 2023 to determine the spring development of the colonies. Bee samples were collected in May for pathogen analysis (viruses and Nosema spp.) as

well as alcohol washes to determine varroa infestation rates. The honey production of the colonies was estimated by subtracting the weight of the empty honey supers from the weight of the harvested honey supers at the end of August 2023.

Second experimental phase 2023-2024

Queen rearing and preparation of experimental colonies

The experimental phase of 2023-2024 took place at two certified organic beekeeping businesses in Quebec. During the first week of June, each beekeeper prepared 30 nuclei consisting of two frames of brood and one frame of honey/pollen, into which they introduced a young, mated queen of their operation. In mid-August, the CRSAD team visited both beekeepers to assess the colonies (brood and bee population) and collect samples for viral analyses, Nosema infection, varroa infestation rate, and syrup and honey analyses. The 60 colonies (30 hives per business) were then evenly distributed into the following three experimental groups.

Summer honey

In July, the beekeepers set aside partially capped honey frames for fall feeding. These frames were stored in a low relative humidity environment (40-45%) and at room temperature not exceeding 40°C. The beekeepers began feeding the colonies in mid-September 2023. In the summer honey feeding group, each colony's queen was located in the brood box. The brood box was then removed from the hive stand and replaced with a honey super containing nine frames of summer honey. One frame of brood, along with the queen, was then placed in the honey super. A queen excluder was positioned between the two supers. This way, the brood box was placed above the queen excluder and the honey super containing the queen (Figure 1). Three weeks later, when the brood had emerged, the brood box was removed.

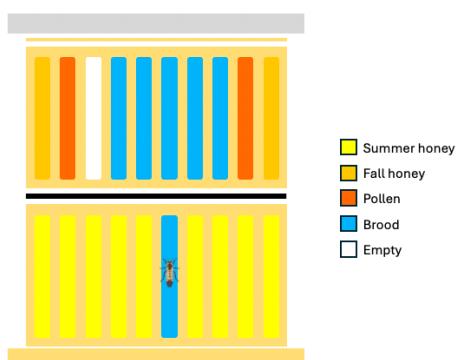


Figure 1. Honey feeding technique for wintering using a single brood box, applied in the second experimental phase (© Laurence Plamondon).

Organic syrup

Beekeeper #1's organic sugar syrup was prepared from organic cane sugar bags from Mexico (IAM, Saint-Hubert, QC, Canada). Beekeeper #2's organic sugar syrup was prepared from organic cane sugar bags from Colombia (Farinex, Boisbriand, QC, Canada). In early September, the colonies receiving syrup were moved to a brood box using a bee escape and were given an initial quantity of 2:1 sugar syrup in an individual feeder. One week later, the colonies received a second dose of 2:1 sugar syrup to complete the feeding. Each colony thus received a total of 24 L of syrup.

Conventional syrup

The conventional sugar syrup was purchased in bulk liquid form by Beekeeper #1 and prepared from sugar bags by Beekeeper #2. In early September, the colonies receiving syrup were moved to a brood box using a bee escape and were given an initial quantity of 2:1 sugar syrup in an individual feeder. One week later, the colonies received a second dose of 2:1 sugar syrup to complete the feeding. Each colony thus received a total of 24 L of syrup.

Syrup and honey analyses

The melissopalynological analysis of the honeys used for feeding during the second experimental phase was conducted by the company Bizzbilis (Baie-Saint-Paul, QC, Canada). The company Environex (Quebec City, QC, Canada) conducted the physicochemical analysis of the honeys and syrups.

Parasite control and wintering

Along with the feeding, the beekeepers applied a Thymovar® treatment. The CRSAD team completed the treatments with an oxalic acid drip treatment in early November 2023. At the same time, samples were collected for viral analysis, Nosema testing, and varroa infestation rates. The colonies were weighed, and the cluster size was

evaluated as previously detailed. The hives were then individually wrapped with double-bubble Thermofoil reflective insulation, and a rigid R10 insulation panel made of extruded polystyrene was placed on the inner cover of each hive.

Evaluation of colony survival, development, and pathogens

In May 2024, the CRSAD team returned to each beekeeper's site to assess the survival of the project colonies and evaluate the spring recovery. In mid-April, the colonies were weighed, and cluster size was assessed as previously described. Colonies weighing less than 17 kg received a frame of honey from the previous fall's brood box, as hive weights differed from the first year due to variations in materials and the absence of a cover during weighing. In mid-May, the colonies were evaluated for brood area as previously described. A sample of bees for pathogen analysis (viruses and *Nosema* spp.) and an alcohol wash for Varroa infestation rates were collected from each colony.

Virus analyses

Six common bee viruses were analyzed: Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Deformed Wing Virus variants A and B (DWV-A, DWV-B), Israeli Acute Paralysis Virus (IAPV), and Kashmir Bee Virus (KBV). The bees were euthanized by placing them on dry ice. All samples were stored at -80°C until analysis. CRSAD carried out the viral analyses following the protocol described by Plamondon et al. (2024).

***Nosema* spp. counts**

The intestines of 60 bees were collected and placed in a mortar for tissue grinding with a pestle. Then, 30 mL of distilled water was added and mixed until the solution became homogeneous. The macerate was transferred into a tube and vortexed. *Nosema* spores were quantified following the method by Fries et al. (2013). Two counts were performed for each sample, and the arithmetic mean of these counts was used to calculate the number of spores per bee: number of spores/bee = average number of spores for 5 squares / 5 squares / hemocytometer volume × dilution factor.

Statistical analyses

Statistical analyses were performed using R (v.4.2) (R Core Team, Vienna, Austria), with a significance level of 0.05. Variations in the ANOVA models, estimated with mixed linear models (nlme::lme [Pinheiro and Bates 2000]; lme4::lmer [Bates et al. 2015]) were conducted according to the experimental design of each variable. Fixed effects included the group and, when applicable, time and their interaction. Random effects included the apiary and the colony. Global tests for fixed effects were performed using the emmeans::joint_tests function (Lenth 2022). When a significant difference was found, pairwise comparisons were made using Tukey's adjusted tests (functions emmeans::emmeans and emmeans::pairs [Lenth 2022]). The normal distribution and homogeneity of variances were validated on the model residuals using histogram, and residual plots versus predicted values. In the presence of heteroscedasticity, heterogeneous variances were modeled based on the problematic factor. The spores and virus data were transformed using a log+1 transformation to meet the normality assumption. For spores data only, p-values come from the model with transformed data, while means and SE are derived from model using untransformed data. To test mortality differences, chi-square (χ^2) tests followed by Fisher's tests were used. The results are presented using ggplot2 (Wickham 2016).

Results

First experimental phase 2022-2023

Analyses of syrups and honeys

The analysis of the floral origin of the pollen grains from the honeys used for feeding the colonies in 2022 shows that they are monofloral honeys. The summer honey is a monofloral honey from the Brassicaceae family, as it exceeds the highest established threshold for this taxon, which is 80%. In this honey, the pollen from Brassicaceae plants were quite similar, which could be explained by a single floral source, likely of agricultural origin, such as canola or mustard (Supplementary file 1). The fall honey is a monofloral honey from the group of Eupatorium, asters, and goldenrods (Supplementary file 2). No crystallization of the honey was observed.

Winter mortality

In the fall of 2022, 46 colonies were prepared for outdoor wintering (Table 1). The following spring, 36 colonies had survived the winter period, resulting in a mortality rate of 21.7%. A significant difference in mortality is observed between the experimental groups ($\chi^2 = 19.877$, $p < 0.0001$), indicating that the type of feeding influences colony survival during winter. No dysentery was observed at the entrances of the hives.

Cluster size and colony weight before and after wintering

Cluster size is significantly affected by the group ($F_{4,32} = 3.704$, $p = 0.0138$), time ($F_{1,32} = 130.241$, $p < 0.0001$), and their interaction ($F_{4,32} = 5.926$, $p = 0.0011$). In the fall, the summer honey group and the fall honey group have a significantly smaller average cluster size compared to the organic syrup group (mean \pm SE; 6.44 ± 0.581 frames,

Experimental groups	Number of wintered colonies	Number of surviving colonies in spring	Winter mortality (%)
Summer honey	9	5	44
Fall honey	9	3	67
Organic syrup	9	9	0
Conventional syrup	9	9	0
Mixture	10	10	0

Table 1. Number of colonies wintered in the fall, number of colonies surviving in the spring, and winter mortality percentage for 2022-2023 in each experimental group.

6.27 ± 0.583 frames, and 8.48 ± 0.581 frames, respectively). The average cluster size of the summer honey group is also significantly smaller than the conventional syrup group (8.24 ± 0.581 frames). In the spring, the summer honey group has a significantly smaller average cluster size compared to the organic syrup and fall honey groups (3.20 ± 0.647 frames, 5.62 ± 0.581 frames, and 6.02 ± 0.817 frames, respectively). Regarding the interaction, the fall honey group maintains a stable average cluster size, while the cluster size of the other groups decreases (Figure 2).

The weight of the hive is significantly affected by the group ($F_{4,32} = 17.194$, $p < 0.0001$) and time ($F_{1,32} = 297.364$, $p < 0.0001$), but is not affected by their interaction ($F_{4,32} = 0.669$, $p = 0.6184$). In the fall, the summer honey group and the fall honey group have a significantly lower average weight compared to the biological syrup and conventional syrup groups (mean \pm SE; 32.5 ± 1.89 kg, 30.6 ± 1.90 kg, 42.3 ± 1.89 kg, and 40.6 ± 1.89 kg, respectively). The average weight of the mixed group is also significantly lower than the biological syrup group (36.2 ± 1.86 kg). In the spring,

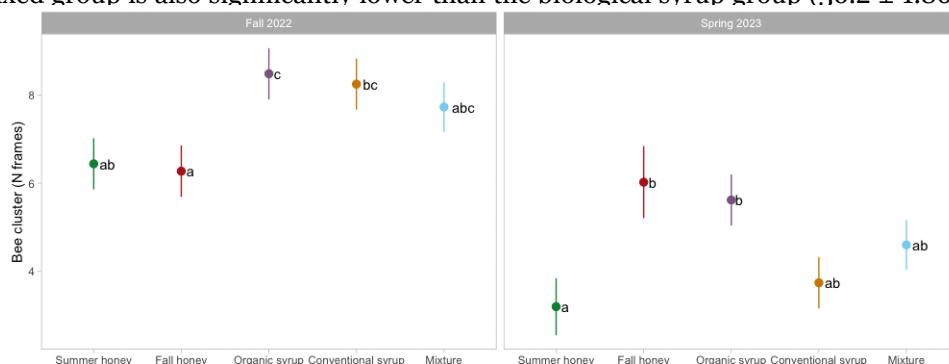


Figure 2. Cluster size (number of frames covered by bees) in fall and spring, based on experimental groups (mean \pm SE). Different letters indicate statistically significant differences between groups. ($p < 0.05$).

the summer honey group and the fall honey group have a significantly lower average weight than the biological syrup and conventional syrup groups (22.9 ± 2.03 kg, 20.2 ± 2.39 kg, 30.1 ± 1.89 kg, and 29.8 ± 1.89 kg, respectively) (Figure 3).

Colony development

Bee population is significantly affected by the group ($F_{4,32} = 5.525$, $p = 0.0017$), time ($F_{1,32} = 114.867$, $p < 0.0001$), and their interaction ($F_{4,32} = 4.592$, $p = 0.0048$). In the spring, during June, the summer honey group and the fall honey group have a significantly lower average bee population than the other groups (mean \pm SE; 7.145 ± 1.74 frames

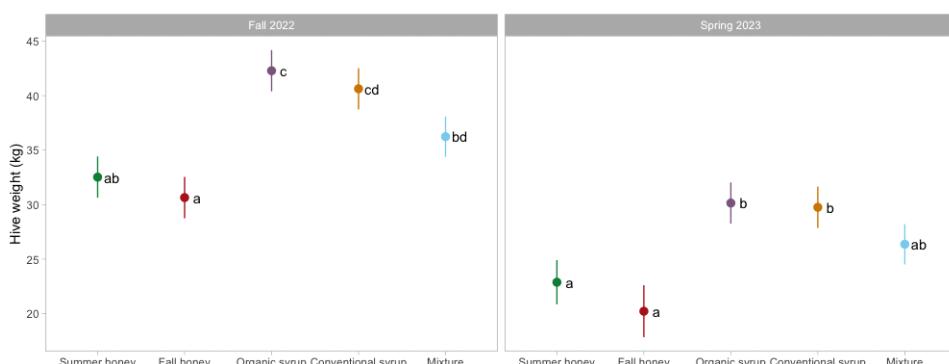


Figure 3. Hive weight (kg) in fall and spring, based on experimental groups (mean \pm SE). Different letters indicate statistically significant differences between groups ($p < 0.05$).

and 5.120 ± 1.75 frames, respectively). Regarding the interaction, the summer honey group and the fall honey group show a lower average growth in bee population compared to the other groups (Figure 4).

Time significantly affects total brood ($F_{4,30} = 114.416$, $p < 0.0001$). The group ($F_{4,30} = 0.788$, $p = 0.5420$) and the interaction between group and time ($F_{4,30} = 1.869$, $p = 0.1419$) do not have a significant effect (Figure 5).

Honey production

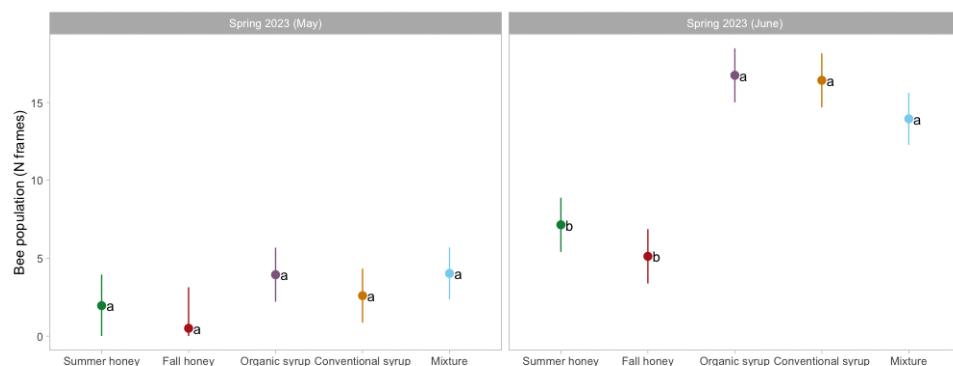


Figure 4. Bee population (number of frames covered with bees) in May and June, based on experimental groups (mean \pm SE). Different letters indicate statistically significant differences between groups ($p < 0.05$).

Honey production from May to August 2023 is not significantly different depending on the type of feeding received in the previous fall ($F_{4,29} = 0.314$, $p = 0.8664$) (Figure 6).

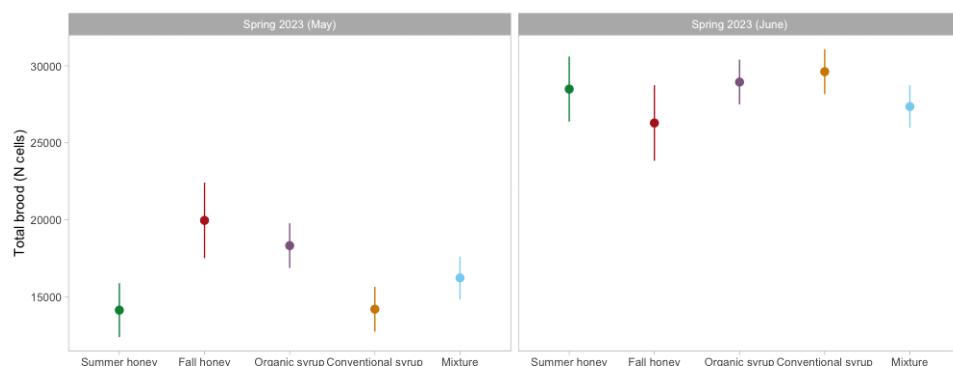


Figure 5. Total brood population (number of cells of eggs, larvae, and pupae) in May and June, based on experimental groups (mean \pm SE).

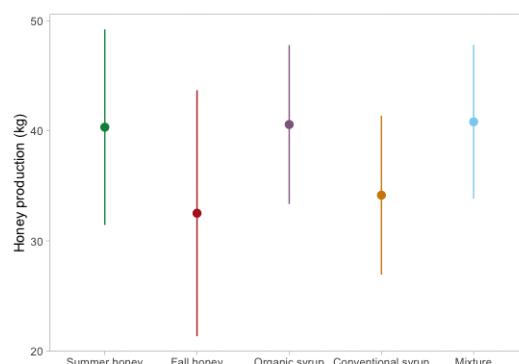


Figure 6. Honey production (kg) from May to August, based on experimental groups (mean \pm SE).

Pathogen development

Varroa destructor

Only five hives, spread across the different experimental groups, had a varroa infestation rate greater than 0% but less than 1% in the fall of 2022 and spring of 2023.

Second experimental phase 2023-2024

Analysis of syrups and honeys

The palyntological analysis of the honey from beekeeper #1, used to feed the colonies in the experimental honey group, reveals that this honey contains several underrepresented species, including *Epilobium* and thistles. According to the Sawyer method, which accounts for the representativity of the pollen, no taxon exceeds the 45% threshold. However, the main nectar source is estimated to come from *Epilobium* at a concentration of 40.9%. This plant produces pollen that is very poorly represented in the honey compared to its nectar contribution. Thus, the honey would be classified as polyfloral since no single species stands out (Supplementary file 3). The sugar quantification shows that the honey from this beekeeper contains 40% fructose and 34% glucose, with sucrose, maltose, and lactose all present at less than 0.1%. No crystallization of the honey was observed (Supplementary file 5).

The analysis of honey from beekeeper #2 shows a significant proportion of *Rubus* species, but it is not sufficient to classify the honey as monofloral. However, it could be considered as a Rosaceae honey since the combined percentage of *Rubus* species and *Geum* species (which also belong to the same family) exceeds the general 45% threshold (Supplementary file 4). The sugar quantification for this honey shows 40% fructose and 32% glucose, with sucrose, maltose, and lactose present at less than 0.1% (Supplementary file 5). No crystallization of the honey was observed.

Winter mortality

Out of the sixty colonies prepared by the two organic beekeepers during the summer of 2023, two colonies became queenless before the fall feeding. In the fall of 2023, a total of 58 colonies were prepared for outdoor wintering. In the following spring, 55 colonies had survived the winter period, resulting in a mortality rate of 5.2%. The three colonies were from organic beekeeper #2 and were part of the honey-fed group. A significant difference in mortality is observed between the experimental groups ($\chi^2 = 7.0303$, $p = 0.02974$), indicating that the type of feeding influences colony survival during winter. Additionally, no dysentery at the colony entrances was identified.

Cluster size and colony weight before and after wintering

Cluster size is significantly affected by time ($F_{1,52} = 12.037$, $p = 0.0011$). The group ($F_{2,52} = 1.205$, $p = 0.3078$) and the interaction between group and time ($F_{2,52} = 0.924$, $p = 0.4034$) have no significant effect (Figure 7).

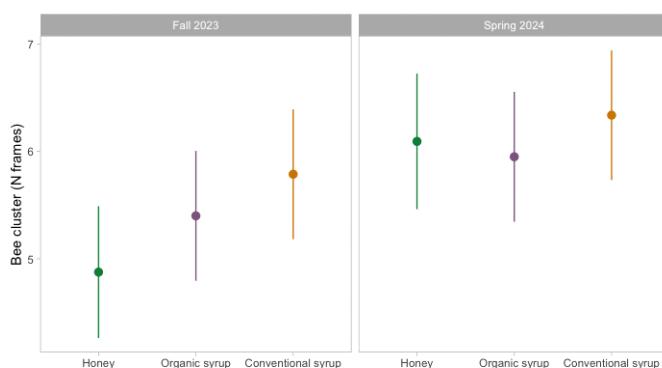


Figure 7. Cluster size (number of frames covered by bees) in fall and spring, based on experimental groups (mean \pm SE).

Hive weight is significantly affected by the group ($F_{2,54} = 34.347$, $p < 0.0001$) and time ($F_{1,54} = 765.134$, $p < 0.0001$), but their interaction is not significant ($F_{2,54} = 0.339$, $p = 0.7139$). The honey group is significantly lighter than the organic syrup group and the conventional syrup group in both fall (mean \pm SE; 26.2 ± 1.49 kg, 30.6 ± 1.47 kg, and 31.1 ± 1.47 kg, respectively) and spring (16.3 ± 1.48 kg, 20.3 ± 1.47 kg, and 20.4 ± 1.47 kg, respectively) (Figure 8).

Colony development

The bee population and brood size are not significantly different based on the type of feeding received the previous fall ($F_{2,50} = 1.872$, $p = 0.1644$; $F_{2,50} = 0.712$, $p = 0.4955$; respectively) (Figure 9).

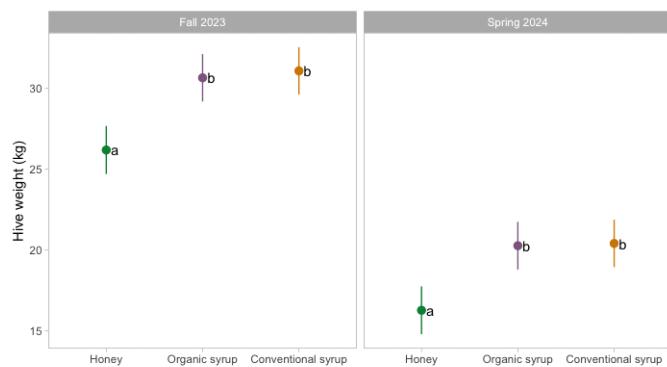


Figure 8. Hive weight (kg) in fall and spring, based on experimental groups (mean \pm SE). Different letters indicate statistically significant differences between groups ($p < 0.05$).

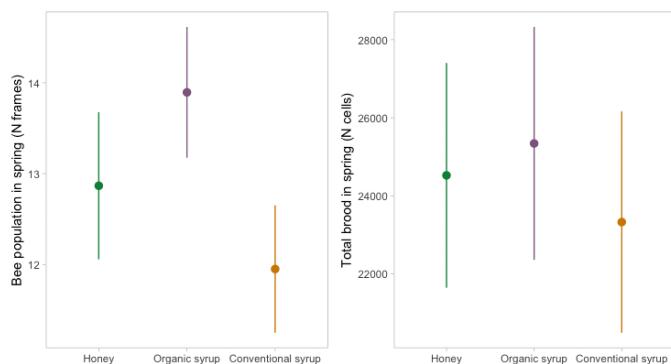


Figure 9. Bee population (number of frames covered with bees) on the left graph and total brood (number of cells with eggs, larvae, and pupae) on the right graph in spring, based on experimental groups (mean \pm SE).

Pathogen development

Varroa destructor

All colonies had a varroa infestation rate of 0% in summer 2023, and only 2 colonies had an infestation rate greater than 0%, but less than 1%, in spring 2024.

Viruses

No colonies tested positive for IAPV and KBV. All tested viruses were unaffected by the group (ABPV : $F_{2,54.06} = 0.534$, $p = 0.5894$; BQCV : $F_{2,54} = 1.271$, $p = 0.2888$; DWV-A : $F_{2,54.21} = 0.950$, $p = 0.3930$; DWV-B : $F_{2,54.01} = 0.971$, $p = 0.3852$) nor by the interaction between group and time (ABPV : $F_{2,55} = 0.574$, $p = 0.5665$; BQCV : $F_{2,55} = 0.997$, $p = 0.3755$; DWV-A : $F_{2,55} = 1.181$, $p = 0.3145$; DWV-B : $F_{2,55} = 0.965$, $p = 0.3874$). However, all tested viruses were affected by the sampling time (ABPV : $F_{1,55} = 52.311$, $p < 0.0001$; BQCV : $F_{1,55} = 11.749$, $p = 0.0012$; DWV-A : $F_{1,55} = 58.309$, $p < 0.0001$; DWV-B : $F_{1,55} = 116.542$, $p < 0.0001$) (Figure 10).

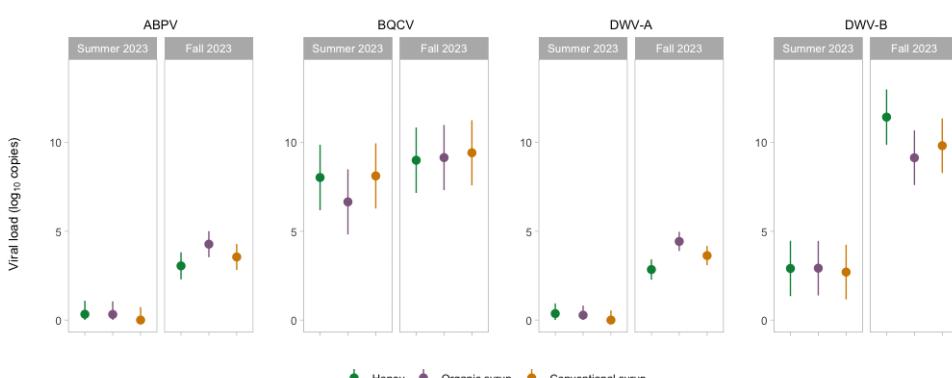


Figure 10. Viral load (\log_{10} copies) for different viruses in summer and fall, based on experimental groups (mean \pm SE).

Nosema spp.

The presence of *Nosema* spores is unaffected by the group ($F_{2,54.59} = 0.019$, $p = 0.9814$) nor by the interaction between group and time ($F_{4,106.42} = 0.895$, $p = 0.4696$). However, the effect of time is significant ($F_{2,107.02} = 16.170$, $p < 0.0001$) (Figure 11).

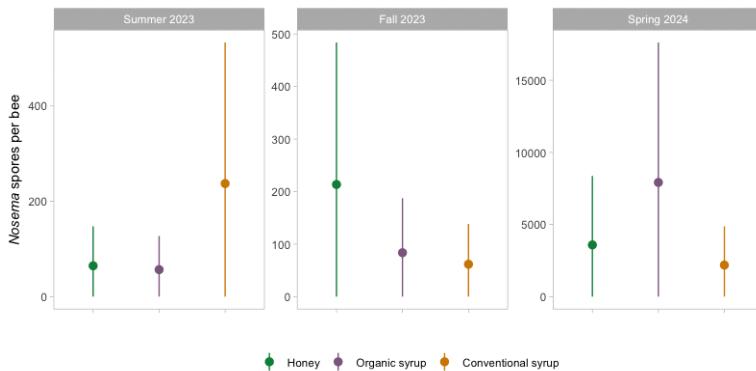


Figure 11. Number of *Nosema* spp. spores per bee in summer, fall and spring, based on experimental groups (mean \pm SE from model using untransformed data).

DISCUSSION

In this project, we tested the impact of the type of feeding sugar provided to bee colonies in the fall on colony survival, health, and productivity. The first phase aimed to develop feeding techniques using honey and syrup, while the second phase focused on implementing and assessing the feasibility of different feeding techniques with two organic beekeepers. Our results show that it is possible to overwinter colonies with honey, but special attention must be given to ensure that colonies have enough honey, which should be at least nine frames of honey. Furthermore, no differences were observed between organic and conventional sugar in terms of survival, colony development, and pathogens.

Colony size, hive weight, and winter mortality

The high winter mortality observed in the groups fed summer honey (44%) and fall honey (67%) during the first phase of the project can be explained by the fact that eight frames of honey are insufficient to sustain the colonies until spring when resources return. Indeed, the weight of colonies fed summer or fall honey was 4.5 kg lower compared to colonies fed conventional or organic syrup immediately after the fall feeding. This weight difference also persisted in the spring, with colonies fed honey weighing on average 3.8 kg less than those fed conventional or organic syrup. It appears that the addition of organic syrup in the mixed group helped achieve a colony weight and size that allowed better survival over the winter compared to the group fed only fall honey.

Winter weight loss is similar across all experimental groups. On average, colonies lost 10.6 kg. This weight loss is comparable to the average weight loss of the last five years for CRSAD colonies overwintered outside, which was 9.3 ± 1.4 kg. The similar weight loss across all groups suggests that the mortality of colonies in the honey-fed groups could potentially have been avoided if the colonies had received more honey in the fall. The second phase of the project suggests that adding a ninth frame of honey improved the winter survival of honey-fed colonies, with 100% survival in the first beekeeper's colonies and 89% survival in the second beekeeper's colonies. The weight of two of the three colonies that died over the winter with the second beekeeper (12.2 and 11.3 kg) suggests that these colonies died from starvation. The weight of the third colony (21.6 kg) indicates that this colony died before winter, likely from an unknown cause, having consumed little to none of its stores. It is possible that overwintering the colonies with two supers, rather than one, could have reduced the risk of food shortages for the colonies during the winter in the honey-fed groups.

In this project, we chose to test overwintering colonies with a single brood box to verify its feasibility, knowing that this technique is recommended in Quebec, while also reducing economic implications for beekeepers. The results show that it is possible to overwinter colonies fed with conventional syrup, organic syrup, summer and fall honey, and a mixture of fall honey and syrup in Quebec. However, special attention should be paid to the weight of the colonies after feeding to ensure that sugar stores are sufficient. It is important to note that in the winter of 2023-2024, the average temperature across the province was 5.2°C higher than the reference average (ECCC 2024). However, winter temperatures vary from year to year, which affects the consumption rates of bees overwintered outside. Further research in different regions of Quebec will need to be conducted to determine the safe target weight for colonies in the fall to ensure winter survival, regardless of weather conditions.

Colony development and honey production

In the first phase of the project, the colonies were evaluated for bee population and brood development in May and then again in June. The average amount of brood was the same across all groups and increased between the two evaluations, regardless of the type of feeding received in the fall. The number of bees also increased for all groups between May and June. These results are typical of the development of a bee colony during the beekeeping season in Quebec, where the bee population doubles between May and June (CRAAQ 2020). The bee population was similar across all groups in May. However, in June, the honey-fed groups had a significantly lower adult bee population compared to the other groups. It would have been interesting to continue evaluations beyond June to determine if this difference persisted over time. It is important to note that the evaluation of the adult bee population in a colony is a visual estimate of the frame coverage by the bees. Additionally, the estimation of bee population can vary with weather conditions, as well as the time of day when evaluations are done, and results may vary considerably (Chabert et al. 2021, Dainat et al. 2020, Hernandez et al. 2020). The amount of brood is a variable that is not affected by weather conditions or the timing of the evaluation. Thus, the similar amount of brood between the groups in June suggests that an evaluation of the adult bee population in July might yield similar results across the groups.

Honey production during the 2022 beekeeping season, with an average of 38 kg per colony, indicates that foraging effort was the same across all colonies throughout the beekeeping season and that the average honey harvest per colony is higher than the Quebec average for 2022, which was 29.8 kg per colony (ISQ 2022). The results from the second phase, with the organic beekeepers, also show no impact of the type of feeding received in the fall on brood and adult bee populations in the spring. The absence of differences between organic and conventional sugar can be explained by the fact that both organic and conventional syrups contain the same amount of sucrose. Indeed, syrup consumption rates are similar when sugar concentrations are the same (Pridal et al. 2023). No difference was observed between summer and fall honey regarding the evaluated colony parameters. We can conclude that the type of feeding in the fall had no impact on colony development and production in the following season.

Pathogen development

Varroa infestation was not influenced by the type of feeding given in the fall and remained below the treatment threshold (MAPAQ 2024) in all groups, for both phases of the project. It was in the second phase of the project that the development of Nosema disease and the presence of viruses were evaluated. The average number of spores per bee remained below the economic damage threshold of one million (Bailey and Ball 1991), and no impact of the type of feeding was detected. Previous studies conducted in cages have demonstrated the impact of more complete nutrition of workers compared to a diet of sugar syrup on the quantity of Nosema spores per bee (Basualdo et al. 2014, Zheng et al. 2014). It is primarily the abundance and diversity of pollen, rather than the sugar source, that may improve bee tolerance to pathogens (Holt and Grozinger 2016). Since the colonies in this project had access to the same environmental pollen sources, this may explain why they did not show differences in infestation levels for varroa, Nosema spores, or viral load.

CONCLUSION AND LIMITATIONS OF THE STUDY

This project aimed to compare the survival, development, productivity, and major pathogens of bee colonies that received different sugar sources in the fall. Few differences were noted between the experimental groups for the evaluated variables. However, it is already known that, compared to feeding with honey, feeding bee colonies with sucrose syrup results in differences in fat content and the expression of over a hundred genes in the fat body of winter bees (Quilan et al. 2023, Wheeler and Robinson 2014). Thus, it appears that at the physiological level, the nutritional status differs between bees fed with syrup and those fed with honey. Our results show that at the colony level, there is little impact of the type of sugar given to bees in the fall on performance and disease resistance in the following season. However, the implications of the physiological differences highlighted in previous studies could be further investigated to clearly establish the ideal nutritional status for the honeybee before winter.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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