

Honeydew honey production in honey bees colonies affected by the River disease in Uruguay

Producción de mielatos en colonias de abejas afectadas por el mal del río en Uruguay

Produção de melada em colônias de abelhas afetadas pelo Mal del Río no Uruguai

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Abstract: In Uruguay, the excretions of the flatidae *Epormenis cestri* when located in sarandí colorado trees (*Sebastiania schottiana*) are the cause of the massive death of young honeybee larvae, a phenomenon that beekeepers call River disease. A strong income of honeydew is frequently observed in these affected colonies despite the continuous loss of bees due to lack of replacement. The aim of this study was to determine a handling of the colonies that allows obtaining honeydew honey. Forty-eight colonies were transported to an apiary affected by River disease and were divided into 5 groups according to the following treatments: Group 1: regular honeydew harvest, adding of brood and sugar syrup supply (N=10); Group 2: regular honeydew harvest and sugar syrup supply (N=10); Group 3: regular honeydew harvest and adding of brood (N=10); Group 4: regular honeydew harvest (N=10); Group 5: only one harvest of honeydew once the study had finished (N=8). The colonies from groups 1 and 3 produced the biggest amount of honeydew honey, averaging 32.0 and 28.6kg, respectively. The physicochemical analysis of honeydew honey showed characteristics of this product such as high electrical conductivity (0.98-1.14 mS/cm), diastase activity (>50%) and color (71-83 mm Pfund). This study provides the basis of a colony handling that allows beekeepers to obtain important harvests of a honeydew honey not yet known in the international market.

Keywords: *Apis mellifera*, disease, honeydew honey, larvae mortality.

Resumen: En Uruguay, las excreciones de los flátidos *Epormenis cestri*, cuando se localizan en árboles de sarandí colorado (*Sebastiania schottiana*), causan mortalidad masiva de larvas jóvenes de abejas melíferas, fenómeno que los apicultores denominan «mal del río». Frecuentemente se observa una fuerte entrada de mielatos en las colonias afectadas a pesar del despoamiento de abejas generado por la falta de reemplazo. El objetivo de este estudio fue determinar un manejo de las colonias que permita obtener mielatos. Se transportaron 48 colonias a un colmenar afectado por el mal del río y se dividieron en 5 grupos de acuerdo con el tratamiento: Grupo 1: cosecha regular de mielatos, adición de cría y suministro de jarabe de azúcar

(N = 10); Grupo 2: cosecha regular de mielatos y suministro de jarabe de azúcar (N = 10); Grupo 3: cosecha regular de mielatos y adición de cría (N = 10); Grupo 4: cosecha regular de mielatos (N = 10); Grupo 5: solo una cosecha de mielatos una vez finalizadas las secreciones de *E. cestri* (N = 8). Las colonias de los grupos 1 y 3 produjeron la mayor cantidad de mielatos, con un promedio de 32,0 y 28,6 kg, respectivamente. El análisis fisicoquímico de los mielatos mostró características de este producto, como alta conductividad eléctrica (0,98-1,14 mS / cm), actividad diastasa (> 50%) y color (71-83 mm Pfund). Este estudio proporciona la base de un manejo de las colonias que permite a los apicultores obtener cosechas importantes de un mielato aún no conocido en el mercado internacional.

Palabras clave: *Apis mellifera*, enfermedad, mielato, mortalidad larvaria.

Resumo: No Uruguai, as secreções dos flatídeos *Epormenis cestri*, quando estão localizados em árvores de Sarandí Colorado (*Sebastiania schottiana*), causam a mortalidade massiva de larvas jovens de abelhas melíferas, fenômeno que os apicultores denominam de Mal do Río. Frequentemente é observado um forte influxo de melada nas colônias afetadas, apesar do despovoamento de abelhas gerado pela falta de reposição. O objetivo deste estudo foi determinar um manejo das colônias que permita a obtenção de melada. 48 colônias foram transportadas para um apiário afetado por Mal do Río e foram divididas em cinco grupos de acordo com o tratamento: Grupo 1: colheita regular de melada, adição de cria e fornecimento de xarope de açúcar (N = 10); Grupo 2: colheita regular de melada e fornecimento de xarope de açúcar (N = 10); Grupo 3: colheita regular de melada e adição de cria (N = 10); Grupo 4: colheita regular de melada (N = 10); Grupo 5: apenas uma colheita de melada após o final das secreções de *E. cestri* (N = 8). As colônias dos grupos 1 e 3 produziram a maior quantidade de melada, com média de 32,0 e 28,6 kg, respectivamente. A análise fisicoquímica das meladas mostrou características deste produto como alta condutividade elétrica (0,98-1,14 mS / cm), atividade de diastase (> 50%) e cor (71-83 mm Pfund). Este estudo fornece a base para o manejo de colônias que permite aos apicultores obter colheitas significativas de uma melada ainda não conhecida no mercado internacional.

Palavras-chave: *Apis mellifera*, doença, melada, mortalidade larvária.

1. INTRODUCTION

Honeybees (*Apis mellifera* L.) often collect honeydew, which are sugary solutions of extra floral origin, especially those coming from insect excretions⁽¹⁾. The honeydew supply may be very abundant, what makes it possible to obtain important honeydew honey harvests, like the case of bracatinga (*Mimosa scabrella*) in

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Brazil⁽²⁾, oak honeydew (*Quercus pirenaica*) and evergreen (*Quercus ilex*) in Spain⁽³⁾, and pine (*Pinus brutia*) in Turkey⁽⁴⁾.

Recently, Invernizzi and others⁽⁵⁾ discovered in Uruguay that the excretions of the flatidae *Epormenis cestri*, when fed in the sarandí colorado trees (*Sebastiania schottiana*), are the cause of the massive death of larvae of one day of age, a phenomenon that beekeepers call River disease. This disease, present in some years during the end of spring and beginnings of summer in the colonies located close to rivers and streams with abundant riparian vegetation, causes depopulation and the eventual death of colonies due to lack of replacement of bees⁽⁵⁾.

During the inspection of colonies affected by the River disease, it is normal to perceive an abundant income of honeydew, even though the colonies lack brood. From these observations, the possibility of achieving a handling technique of the colonies to produce honeydew honey was proposed. This way, beekeepers would find a lucrative opportunity in the areas where the River disease appears, whereas historically it was recommended to retire the affected apiaries. Besides, they would have the possibility of obtaining a differentiated product with a more significant value, since honeydew honey is usually sold at a better price than honey in the international market, especially Europe⁽⁵⁾⁽⁶⁾.

To establish the productive handlings of the colonies in the areas affected by the River disease there should be taken into account that the massive mortality of the larvae leads irremediably to the depopulation of the colonies. In order to avoid quick depopulation, handlings that allow reducing the deaths of the larvae and/or provide bees to the colonies must be found. That way, a possible handling is to harvest every few days all the honeydew collected expecting that the decrease of the toxic substances inside the colonies enables a higher survival of the brood. An alternative, considering the possibility of this measure not being enough, is to make a supply of sugar syrup in a low concentration to dilute the toxic effect of the honeydew that gets into the colony. Regarding that, in a previous experiment it was found that the sugar syrup supply at the beginning of the River disease allowed a survival of 64% of the larvae, even though the effect of adding sugar syrup decreases along the period in which the excretions of *E. cestri* are available (Enrique Nogueira, submitted). Finally, another way to avoid the productive impact of depopulation is to transfer colonies prepared with abundant brood and introducing bees regularly during the 40-60 days approximately that the honeydew income lasts⁽⁵⁾, the most accessible way being adding combs with brood.

The aim of this work is to evaluate handlings of the colonies affected by the River disease that allow producing honeydew honey.

2. MATERIALS AND METHODS

2.1 Preparation of the colonies

In December 17th, 2017 at Sarandí Grande, Florida department, the bee population and brood area of 48 colonies with new queens were evaluated. The colonies were in a brood chamber with 9 frames. The population was estimated as the number of combs covered by bees, and the brood area was visually estimated as the percentage of the comb occupied by brood⁽⁷⁾. The brood of all the colonies was leveled in 7 almost full frames and in the extremes of the brood chamber frames with wax foundation were put. At the end of the evaluation one medium-sized honey super with 8 empty combs was added. Two days later (December 19th) the colonies were transported to an apiary in Durazno, located between the Yi river and the Maciel stream. In this apiary, which had had River disease records in the two previous years⁽⁵⁾, there were sentinel colonies that showed absolute loss of larvae since December 2nd.

2.2 Handling of the colonies

The colonies were divided in 5 groups regarding the following treatments: Group 1: regular honeydew harvest, adding of brood and sugar syrup supply (10 colonies); Group 2: regular honeydew harvest and sugar syrup supply (10 colonies); Group 3: regular honeydew harvest and adding of brood (10 colonies); Group 4: regular honeydew harvest (10 colonies); Group 5: only one harvest of honeydew once the season has finished (control group) (8 colonies).

In December 23rd sugar syrup was supplied to the colonies designated for this operation. The honeydew harvest was performed in 5 opportunities every 10 to 13 days, approximately (January 2nd, 12th and 23th, and February 2nd and 15th). In each harvest, all the combs that contained honeydew, regardless of the volume, were removed, marking in each frame the number of the colony. Then the frames were weighted both before and after the honeydew extraction. After the first harvest all the medium-sized honey super were removed and only the brood chamber combs were harvested. In February 15th a sole harvest of the colonies of group 5 was performed. The brood adding (a frame occupied with at least 80% of capped brood) and the sugar syrup supply (2 liters of sugar syrup 1:4 weight:volume through a Boardman feeder) was done in 4 opportunities (January 2nd, 12th and 23rd, and February 2nd).

2.3 Quantification of the larvae death

In order to determinate the percentage of larvae that died intoxicated by the honeydew, a picture was taken in each colony to a comb with eggs, which was once again photographed 10-13 days after. A digital reflex photo camera Nikon D5300 with 50mm lenses was used. The first picture of the combs with eggs was taken in December 23rd, and in the next visits (January 2nd, 12th and 23rd, and February 2nd) the previously photographed panel and a new one with eggs were photographed. This way the larvae mortality was evaluated in 5 occasions throughout the study period. In the computer, a sector of the comb picture was selected and a 195-hexagon grill was overlapped to facilitate the individual identification of the cells. In the picture of the same comb taken days after, a grill was overlapped over the same cells previously inspected. Based on the initial egg number and the number of pupae, the percentage of mortality of the larvae was determined.

2.4 Physiochemical analysis of the honeydew honey

In each one of the 5 harvests, collective samples of the honeydew honey from the colonies that did not receive sugar syrup were taken and sent to be analyzed at the Quality Services International (Bremen, Germany) to determine the following commercial value parameters: humidity, hydroxymethylfurfural concentration, diastase activity, acidity, electric conductivity, glucose/fructose relation and color.

2.5 Evaluation of the colonies at the end of the River disease

In February 10th, no more *E. cestri* specimens were observed on the sarandí Colorado, and in the colonies a bigger number of larvae survivors were found, to which it was considered that no more secretions of the insect were entering. In February 15th the adult population of the colonies was estimated.

2.6 Statistical analyses

Throughout the study, 10 colonies lost their queens (1 in Group 1, 3 in Group 2, 2 in Group 3, 2 in Group 4, and 2 in Group 5). These colonies were included in the analysis for viability of the brood as long as they had a queen and none were included in the honey production analysis.

All the statistical analyses were carried out with the General Linear Model (GLM). The post hoc analysis was performed using the Least Significant Difference test (Fisher's LSD). P values under 0.05 were considered as significant. The analyzes were carried out with the SAS⁽⁸⁾.

3. RESULTS

3.1 Size of the colonies at the beginning of the study

Three days before transporting the colonies to the apiary affected by the River disease, the population of the colonies oscillated between 7 and 8 combs covered by bees (15000 to 18000 bees) with no differences among the 5 groups ($F=0.54$; $P=0.70$). The brood area of the colonies was around 0.950m^2 (approximately 40000 insects in immature stages) with no differences among the 5 groups ($F=2.04$; $P=0.11$).

3.2 Survival of the larvae

The survival of the larvae was estimated 5 times throughout the study period, finding in general extremely low values (Table 1). In the first recording the colonies of groups 1, 2 and 4 showed higher survival rates of the larvae compared to groups 3 and 5 ($F=2.69$; $P=0.03$), even though on average they did not surpass the 25%. In the following 3 recordings the mortality of the larvae was almost of 100% in all groups, increasing slightly in the last registry in the colonies of Group 1, which differentiated from the rest ($F=2.90$; $P<0.0001$) (Table 1).

3.3 Production of honeydew honey

The amount of honeydew collected by the colonies of groups 1 and 3 was higher than the groups 2, 4 and 5 ($F=10.08$; $P<0.0001$), averaging 32.0 and 28.6kg, respectively (Figure 1).

3.4 Physiochemical analysis of the honeydew honey

Summarized in Table 2 are the main physiochemical indicators of the honeydew honey throughout the study period and its commercial qualification as table or industry product.

3.5 Size of the colonies at the end of the study

The colonies of the different groups (without considering the colonies that lost their queen) presented a similar population ($F=2.69$; $P=0.05$), even though there was a trend ($P<0.10$) in the colonies of Group 4 to being more depopulated than the rest (Figure 2).

TABLE 1:
Percentage of larvae survival of the colonies during the honeydew collection period

Larvae survival (%)					
Group	Dec 23rd	Jan 2nd	Jan 12th	Jan 23th	Feb 2nd
1	20.7 (a)	1.0 (a)	0.5 (a)	2.6 (a)	13.1 (a)
2	24.2 (a)	4.7 (a)	0.8 (a)	0.9 (a)	4.1 (b)
3	2.7 (b)	0.0 (a)	0.0 (a)	0.0 (a)	0.3 (b)
4	14.8 (a)	0.0 (a)	0.1 (a)	0.1 (a)	0.1 (b)
5	7.5 (b)	0.0 (a)	0.0 (a)	0.0 (a)	0.0 (b)

Group 1: regular honeydew harvest, adding of brood and sugar syrup supply; Group 2: regular honeydew harvest and sugar syrup supply; Group 3: regular honeydew harvest and adding of brood; Group 4: regular honeydew harvest; Group 5: only one harvest of honeydew once the season has finished. Different letters in a column indicate significant differences ($P < 0.05$) for the Fisher LSD test.

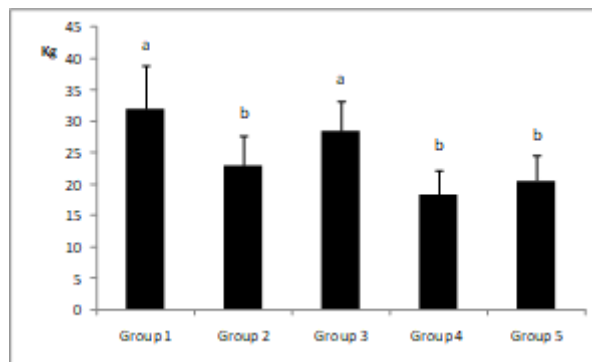


FIGURE 1:
Honeydew honey production of the colonies

Group 1: regular honeydew harvest, adding of brood and sugar syrup supply; Group 2: regular honeydew harvest and sugar syrup supply; Group 3: regular honeydew harvest and adding of brood; Group 4: regular honeydew harvest; Group 5: only one harvest of honeydew once the season has finished. Different letters indicate significant differences ($P < 0.05$) for the Fisher LSD test.

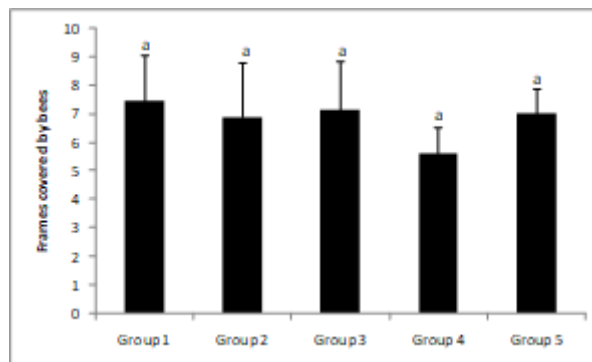


FIGURE 2:
Population of the colonies at the end of the River disease period

Group 1: regular honeydew harvest, adding of brood and sugar syrup supply; Group 2: regular honeydew harvest and sugar syrup supply; Group 3: regular honeydew harvest and adding of brood; Group 4: regular honeydew harvest; Group 5: only one harvest of honeydew once the season has finished. Different letters indicate significant differences ($P < 0.05$) for the Fisher LSD test.

TABLE 2:
Physiochemical indicators of honeydew honey samples obtained in different moments of the period in which colonies were affected by the River disease

Parameters	Jan 2nd	Jan 12th	Jan 23th	Feb 2nd	Feb 15th
Moisture content (%)	18.4	19.3	17.9	20.7	20.8
HMF (mg/kg)	1.7	1.2	1.3	1.2	2.0
Diastase activity (Schade)	>50%	>50%	>50%	>50%	>50%
Ph	4.9	4.6	5.0	4.6	4.4
Electric conductivity (mS/cm)	1.13	0.98	1.14	1.08	0.98
Fructose/Glucose	1.14	1.14	1.12	1.07	1.10
Colour (mm Pfund)	71	71	71	75	83
Table/Industry	Table	Table	Table	Industry	Industry

4. DISCUSSION

The measures to handle the colonies performed in this study with the purpose of reducing the death of honeybee larvae in the colonies affected by the River disease did not have any effect. The extraction of all the honeydew from the colonies (Groups 1, 2, 3 and 4) every 10 to 13 days did not prevent the larvae mortality from being almost total. Not even adding diluted sugar syrup to the colonies (Group 2) stopped larvae death. Only during the first days, sugar syrup prevented almost 20% of larvae death, possibly because bees had recently discovered *E. cestrus* excretions and the amount of honeydew that entered the colonies was very low, so the syrup worked as a diluent. In the rest of the period in which the bees had access to honeydew, the almost total loss of the brood was not prevented. It is possible that the daily income of honeydew was enough to intoxicate practically all larvae, even when almost all the reserves were removed from the combs.

Even though the performed handlings did not attenuate larvae death, they did have an effect on honeydew honey production. The colonies that received a brood comb regularly (Groups 1 and 3) were the ones that produced more honeydew honey, adding up to 30kg on average. The effect of adding bees (around 16000 to 20000 bees emerged throughout the study period) probably derived in an increase in the number of forager bees and thus in the capability to collect honeydew. Another positive effect that a viable brood comb might have had in the colonies infected by the River disease was the stimulation of the foraging⁽⁹⁾⁽¹⁰⁾, which may not be caused solely by the presence of eggs. It should be noted that the honeydew honey production could have been higher, since the colonies arrived to the apiary 17 days after noting the massive death of larvae, so they only took advantage of the honeydew offer for approximately 40 days. Nevertheless, not in all the places in which the disease is present harvests will be the same, whether it is for the size of the *E. cestrus* population and/or the amount of sarandí colorado present. This study was performed in an area located between two watercourses with abundant riverside vegetation.

The chemical analysis of the honeydew honey showed characteristics typical of this product, such as an elevated electrical conductivity and a high diastase activity⁽¹¹⁾, so it fulfills with the requirements by the Honey Directive from the European Community to be called this way. Invernizzi and others⁽⁵⁾ performed a palynological analysis of the honeydew honey of the colonies infected by the River disease finding a low content of pollen and a great amount of fungus and conidia. This is a common characteristic of honeydew honey⁽¹²⁾⁽¹³⁾.

Humidity of honeydew honey in the last two harvests was above 20%, which leads to categorize it as an industrial use product. The humidity of the honeydew honey can be reduced noticeably by harvesting only the combs that are full (over 2 kg), and leaving for the next harvest those combs that are less charged, which most probably have a higher humidity content. It should be noted that in this study total extraction of honeydew combs was performed with the aim of decreasing the massive death of larvae.

After the colonies remained 59 days affected by the River disease, most of the time with practically no brood, population showed a decrease of about 15% with respect to the initial number. Strikingly, the colonies of groups 1 and 3, which received a brood comb in 4 opportunities, did not present a higher population than the rest of the colonies. The decrease in population recorded, less than expected, might be underestimated, since a high level of disorganization was noticed in the ill colonies, with the bees occupying a big part of the combs, but with less individuals in the surface than the ones seen in healthy colonies. This tendency of the bees to expand around the nest had already been observed in other opportunities in colonies affected by the River disease. On the other hand, the colonies arrived to the apiary with a brood area artificially established that contained around 40000 brood forms, higher than what corresponded to the adult population, and even higher than what bigger colonies usually maintain. This number of bees, which emerged in the 3 weeks following the arrival of the colonies, could have mitigated the depopulation of the colonies.

At the end of the study the colonies were 'packaged' and the bees were put in a brood chamber over empty combs, since there was an evident income of nectar. The brood developed since then without a problem and most of the colonies made it to March with almost all the brood chamber covered by bees, and an abundance of reserves to begin wintering (unregistered data). This way, the risk of weakening or loss of the colonies due to depopulation that the beekeepers fear is overcome.

5. CONCLUSIONS

This study showed that an easy handling of the colonies based on harvest and adding brood on a daily basis allows obtaining great harvests of honeydew honey generated by the excretions of the *E. cestri* in the sarandí colorado. The handling performed in this study must be taken as a basis to be improved taking into account the characteristics of the beekeeper and the area where the River disease appears in order to maximize honeydew honey production with the minor cost.

The honeydew honey obtained by the *E. cestri* excretions when feeding on the sarandí colorado causes the death of one-day-old larvae, but does not affect larvae of more than two days nor adult bees⁽⁵⁾. When consumed by humans, it does not imply any type of risk, having been consumed for decades in Uruguay without any problem.

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