

## Final Evaluation Report

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Your Details	
<b>Full Name</b>	Lívia Maria Negrini Ferreira
<b>Project Title</b>	Attractiveness and combined toxicity of pesticides to wild bees: subsidies for pollinator conservation
<b>Application ID</b>	30578-1
<b>Grant Amount</b>	£5.904
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<b>Date of this Report</b>	01 November 2021

**1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.**

Objective	Not achieved	Partially achieved	Fully achieved	Comments
To test the hypothesis that stingless bees prefer to forage in pesticide contaminated food rather than in uncontaminated food				There were some changes in the methods used, as compared to those in the project submitted. Nevertheless, this objective was achieved successfully
To test the hypothesis that field contamination with pesticides impairs stingless bee colonies				We are currently doing the experiments to test this hypothesis and fully achieve this objective

**2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.**

My main difficulties were due to the COVID-19 pandemic. In March 2020, my institution had closed a few weeks after I had started to perform the first experiments. Therefore, I did not have access to the laboratory, and I also had difficulties to acquire the materials necessary to conduct the project. When we were able to restart the project, after about 6 months, it was an unfavourable season for bees, and the colonies were weak. Therefore, I could not manipulate them, to avoid experimental biases. In order to perform an appropriate toxicological test, it is necessary to work with health and strong colonies.

Until the colonies had completely recovered from the cold months, I had performed some laboratory experiments to investigate the effects of the ingestion of different concentrations of acephate and glyphosate on the individual foragers.

As soon as the colonies were strong enough, I restarted the main semi-field experiments. That was when a new difficult arose. Again, I had problems with the environmental conditions. On sunny days appropriate for bee foraging and for my trials, the temperature reached up to 47 °C inside the greenhouses. With such a high temperature, the colonies became stressed, and the digital camera did not work properly. So, I had to come up with another structure to keep the colonies isolated, but in conditions close to the field one. That took a few months, because I had to buy new materials and to wait for its delivery, under an unfavourable scenario due to the pandemics. Also, I had to find a new place to build this structure. Finally, I built a tent, consisting of an 8 m<sup>3</sup> (2m x 2m x 2m) cube with edges made with PVC pipe and then covered with a translucent fabric. This tent was placed in a building, in an area that is open but not directly exposed to the sun. Because of that, the inside of the tent wouldn't get too high. Also, I adjusted the methods in a way that I reduced

the number of hours of the experiment, without losing hours of observation. I did that by filming the bees during the whole time they were visiting the feeders. By doing this, I was able to observe all four feeders at the same time, reducing the experiment time by four times, without losing data.

In the meanwhile, I was also having difficulties with the second part of the experiments (WP2), planned to test the hypothesis that field contamination with pesticides impairs stingless bee colonies. In December 2020, after the colonies were strong enough, I transferred them to the observational nests for the experiment. It took months for the bees to adapt to the new nests. Also, while they were in this adaptation period, the cold season in our region started and my main concern was to keep them alive until the temperature would rise again. The cold season ended a few months ago and I have been waiting for the bees to recover again. Some colonies are still too weak to be part of the experiment. I have started a pilot with a colony that is strong enough and it went well. Now I hope to perform this experiment as soon as possible.

### **3. Briefly describe the three most important outcomes of your project.**

1 - In laboratory trials, the ingestion of the pesticides, mostly acephate, decreased the lifespan and increased the mortality of the foragers. Also, sub-lethal concentrations of the tested pesticides impaired the foragers' flight ability.

2- The semi-field choice experiments showed that these bees do not avoid food contaminated with the tested pesticides. This result indicates that there is a high probability that *P. lucii* foragers ingest contaminated pollen and nectar in the field, increasing their exposure risk.

3- The methods I used for WP1 and the ones that I intend to use for the WP2 were innovative and successful. They are pioneer methods that our team developed and should help me and other scientists to perform field-simulated experiments with stingless bees in the future, aiming their conservation.

### **4. Briefly describe the involvement of local communities and how they have benefited from the project.**

I have been in contact with the Association of Meliponine Beekeepers of Minas Gerais (AME-MG) since the beginning of my research. In November 2020 I participated of a seminar about stingless bees, where I gave a lecture about the effects of pesticides to them. I also wrote a book chapter about it for the seminar, which should be published in Portuguese. In December 2020, I participated in an AME-MG meeting, where I talked about the risks of the pesticides to stingless bees. During the event, I had the opportunity to answer questions of the meliponine beekeepers about this issue. I'm not a member of AME-MG because I'm not a beekeeper. Nevertheless, I'm still in contact and available to assist them.

**5. Are there any plans to continue this work?**

Yes, there are. One of the challenges faced by us was the establishment of the methods to study stingless bees in a protected environment that could partially simulate the field conditions. Now that we have established these methods, we intend to perform more studies like this. We have plans to perform field-simulated experiments to evaluate, besides pesticides studies, the effects of the electromagnetic field on stingless bees. My advisor, Dr. Maria Augusta Lima Siqueira, is co-author of a pioneering study that have shown detrimental effects of the electromagnetic field to *Apis mellifera* (<https://doi.org/10.1038/s41598-018-26185-y>). Using the structure that we built for this project, the equipment we have now, and the methods we developed during this project, we want to evaluate these effects in stingless bees under semi-field conditions.

**6. How do you plan to share the results of your work with others?**

I am writing a manuscript to submit to a scientific journal. Probably, in the near future, I will prepare another paper, including the results about the WP2. In addition, I expect to publicise via popular means of communication our main results. In part, this objective was already achieved, because I wrote a book chapter in partnership with a beekeeper association.

**7. Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?**

The anticipated length of the project was from March 2020 to March 2021. Nevertheless, due to the COVID-19 pandemics, the project was extended until October 2021. I used the grant from July 2020 to March 2021.

**8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.**

Item	Budgeted Amount	Actual Amount	Difference	Comments
Continuously Adjustable Repeater-Pipette	£379	£372	-£7	
High accuracy positive displacement pipette, volume range from 0,5 to 10 µL, and low retention pipet tips	£59		-£59	The price of the positive displacement pipette was much higher than the price we found when we made the budget, so we had to buy a simpler pipette

High accuracy positive displacement pipette, volume range from 10 to 100 µL, and low retention pipet tips	£342		-£342	These resources were reallocated to buy several other materials
External Hard Drive	£117	£154	+£37	
Nobreak	£117	£112	-£5	
Expenses with scientific events	£84		-£55	These resources were reallocated, since the presential events were cancelled due to the COVID-19 pandemics
Fuel	£130		-£130	These resources were reallocated to buy several other materials
Inverter Air Conditioner	£1167	£812	-£645	
Pollen	£50		-£50	These resources were reallocated to buy several other materials
Feeders	£300	£361	+£61	
Precision balance	£1334	£1501	+£167	
Language editing	£167	£155	-£12	
Chemicals (Alcohol& Glyphosate)	£9	£6	-£3	
Taxes and operational expenses	£1181	£1180	-£1	
Two airplane tickets	£334		-£334	
Accommodation	£134		-£134	
<b>Subtotal</b>	<b>£5904</b>	<b>£4653</b>	<b>-£1251</b>	
Single channel pipette, with adjustable volume from 10 - 100 ul and high precision		£84	+£84	Bought with the pipette resources
Macro lens (Nikon AF-S DX Micro NIKKOR 40mm f/2.8G)		£657	+£657	Bought with the scientific events resources
Nitrile gloves		£210	+£210	Bought with the pipette, fuel and pollen resources
Surgical masks		£16	+£16	
SD Memory cards		£52	+£52	
Backup battery for the camera		£46	+£46	
Alcohol meters		£25	+£25	
Polyester voil fabric		£61	+£61	
Graduated cylinders		£19	+£19	
Duct tapes		£45	+£45	
Acetone		£13	+£13	

Isopropyl alcohol		£18	+£18	
<b>TOAL</b>	<b>5904</b>	<b>5899</b>	<b>-£5</b>	The entire budget was exchanged from British Pound Sterling to Brazilian Real in July 2020, using the exchange rate of 1 GBP = 5,78 BRL.

**9. Looking ahead, what do you feel are the important next steps?**

First of all, my next step is to finish the experiments of the WP2. I believe we are doing something really innovative, and we cannot give up because of the issues we have had. After finishing all the experiments, they must be publicised not only in scientific papers, but also in the media. I also intend to use the pioneering methods here developed to conduct new experiments about the effects of different types of stressors on wild bees. Our team will continue to study the anthropogenic impacts on bees and finding ways to raise attention to pollinators conservation.

**10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?**

Yes, I used the logo in all my presentations (seminars, lectures, dissertation defence). When I finish the paper, I will certainly acknowledge The Rufford Foundation for the funds I received, as I did in my dissertation. I also posted my webpage in the site of The Rufford Foundation on my social media and on my CV.

**11. Please provide a full list of all the members of your team and briefly what was their role in the project.**

**MSc. Lívia Maria Negrini Ferreira:** principal investigator, designed the experiments, executed the experiments, performed the data analysis.

**Dr. Maria Augusta Lima Siqueira:** advisor, designed the experiments.

**Dr. Michael Hrcir:** co-advisor, designed the experiments.

**Dr. Lucio Antonio de Oliveira Campos:** co-advisor, designed the experiments.

**Danilo Vieira de Almeida:** assisted in the execution of the experiments.

**Dr. José Henrique Schoeder:** assisted in the data analysis.

**12. Any other comments?**

We would like to thank very much The Rufford Foundation for the financial support of this project. Without these resources we would not be able to conduct this study. I appreciate the thoughtfulness of the members of RF, who were very understanding regarding the pandemic's situation, and the issues that we had along this year and

a half. Special thanks to Jane Raymond and Simon Mickleburgh, who were directly in contact with me via e-mail and assisted me on many occasions.