NOTA

Using a sentinel colony of *Apis mellifera* (Hymenoptera: Apidae) to assess pesticides and food sources

Uso de una colmena centinela de *Apis mellifera* (Hymenoptera: Apidae) para evaluar presencia de plaguicidas y sus fuentes de alimentación

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ABSTRACT

Honey bee populations are declining as occurs with other pollinators. One suggested cause of this decline is the impact of pesticides. To improve bees' health, pesticides and food sources may be monitored using sentinel hives, given that bees forage in a 2.5 km radius around the hive. We extracted 20 (twenty) bees, as well as samples of wax, honey and pollen from a sentinel hive. Six pesticides were detected in the samples, except for the honey. All detected pesticides in the sentinel hive are prohibited in Argentina. Eight different plant families and genera were detected in the honey and pollen samples. Our work suggests that monitoring pesticides with sentinel beehives will be useful to improve agricultural practices in the region.

Keywords — Coumaphos, foraging behavior, honey bee management, pollen, spiridiclophen.

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RESUMEN

Las poblaciones de abejas melíferas están disminuyendo, como ocurre con otros polinizadores. Una de las causas sugeridas de este declive es el impacto de los pesticidas. Para mejorar la salud de las abejas, se pueden monitorear los pesticidas y las fuentes de alimentación mediante colmenas centinela, dado que las abejas pecorean en un radio de 2,5 km alrededor de la colmena. Extrajimos 20 (veinte) abejas, así como muestras de cera, miel y polen de una colmena centinela. Se detectaron seis plaguicidas en las muestras, excepto en la miel. Todos los plaguicidas detectados en la colmena centinela están prohibidos en Argentina. Se detectaron ocho familias y géneros de plantas diferentes en las muestras de miel y polen. Nuestro trabajo sugiere que el monitoreo de plaguicidas con colmenas centinela será útil para mejorar las prácticas agrícolas en la región.

Palabras clave — Coumafos, comportamiento de pecoreo, manejo de abejas melíferas, polen, espiridiclofeno.

Populations of *Apis mellifera* L. and other pollinators have diminished in the last years as a result of a combination of factors (IPBES, 2016, Requier *et al*., 2018), including the pervasive use of pesticides in agricultural fields (Porrini *et al*., 2016). Honeybee colonies depend on floral resources in the surroundings, where bees forage for pollen and nectar in a radius of 1-2 km up to 10 km (Couvillon *et al*., 2015). When foraging, workers are exposed to xenobiotic substances (e.g. pesticides) that may have arrived at the crop and non-crop flowers (Poquet *et al*., 2016); non-lethal effects of this exposure include alterations in development, orientation, mobility, sleep patterns, and circadian rhythm (Vázquez *et al*., 2020). Sentinel honeybee hives, which are colonies that have been isolated from the apiary and left unmanaged, have been used to study these pesticides' effects on bees. In addition, individual (Traynor *et al*., 2016) or multiple (Van der Steen *et al*., 2015) sentinel hives have been used to assess the surrounding environment in terms of available food resources, climatic effects, and land use changes. In addition, these hives have been used to detect pests' geographical expansion (e.g., Chalup *et al*., 2018), and are especially useful to study aspects of bees' behavior without the effect of the high densities observed in apiaries.

Monitoring the implementation and use of pesticides and their consequences on bees' health is a valuable tool to improve the sustainability of agriculture. At the same time, monitoring the plant species that bees use enables the design of optimal landscapes for bees' productivity. Our objectives were to survey pesticides that bees incorporated into a sentinel hive and to identify the main plant species that bees use for adult and larvae food in a subtropical agroforestry-natural environment.

This experiment was conducted in an agroforestry-natural border, surrounded by crop fields, within the Yungas biome in Argentina (26°47'28.6"S, 65°19'33.9"W; Fig. 1), which has a subtropical climate with seasonal rains during the summer. One healthy hive was isolated, before the beginning of the experiment, from other hives

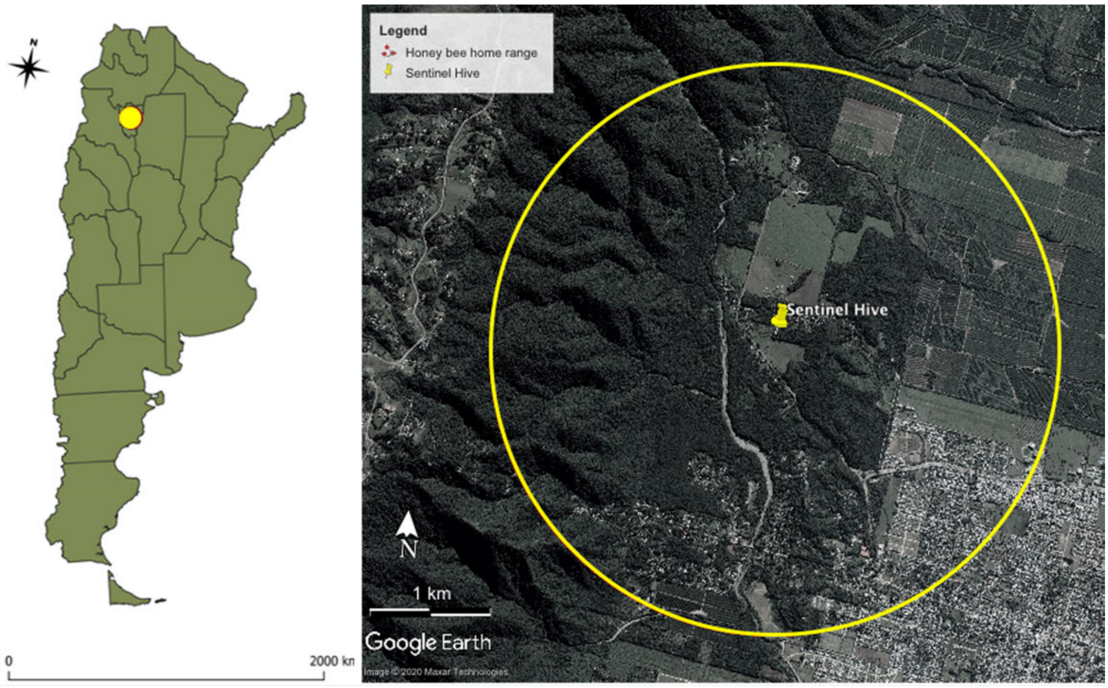

Figure 1. Left image: map of Argentina and study area shown with the yellow circle. Right image: home range of forager honey bees at our field station in the Escuela de Agricultura y Sacarotecnia, Argentina.

Figura 1. Imagen de la izquierda: mapa de Argentina y zona de estudio indicada con el círculo amarillo. Imagen de la derecha: rango de hogar de las abejas melíferas pecoreadoras en nuestro apiario experimental en la Escuela de Agricultura y Sacarotecnia, Argentina.

for at least 16 days, and then used as sentinel hive. We monitored the hive for one month in the middle of the 2019 summer season.

For the pesticides assessment we collected one 50 ml sample from each of four matrices within the hive: virgin's beeswax, adult bees (20 individuals), honey, and stored pollen. For pesticides analysis we used the multi-residue analysis of pesticides (developed and validated by Herrera *et al.*, 2016, Niell *et al.*, 2017, and García *et al.*, 2017). High Performance Liquid Chromatography analysis (HPLC) was used to determine the presence/absence of 90 pesticides on each sampled and to quantify other 37 pesticides (Appendix). This process consists of two stages, in the first stage the pesticides are extracted using acetonitrile and other salts and on the second stage the pesticide analyses take place, using mass spectrometry and liquid chromatography.

Pesticides were classified in classes according to toxicity and target, in accordance to the Ministry of Agriculture and Fisheries of Argentina (Gallo, 2006). To characterize the floral environment visited by bees, we have also conducted a palynological analysis in honey and pollen collected from the hive.

Six xenobiotics compounds were found in three of the four analyzed matrices (Table 1). Bees presented three pesticides on their bodies, suggesting that they could have been affected while foraging in the surrounding crops. Coumaphos, ametryn, and carbendazim were found in the beeswax samples. No pesticides were detected in the honey matrix. The pesticides concentrations were below the lethal levels, but all detected pesticides are prohibited or restricted under Argentinian regulations.

Table 1. Concentration of the pesticides detected on each of the sampled matrices in this study and classification by toxicity in Argentina (Gallo 2006, Imtrade Australia 2014, IUPAC 2021, Minnesota Department of Agriculture 2021, Universal crop protection 2015, Gregorc *et al.,* 2018, Interoc 2015).

Tabla 1. Concentración de los plaguicidas detectados en cada una de las matrices muestreadas en este estudio y clasificación por toxicidad en Argentina (Gallo 2006, Imtrade Australia 2014, IUPAC 2021, Minnesota Department of Agriculture 2021, Universal crop protection 2015, Gregorc *et al.,* 2018, Interoc 2015).

Pesticides	Wax (ug/kg)	Pollen (ug/kg)	Bees (ug/kg)	Honey (ug/kg)	Class	Type	Comment	Oral LD50 (ug/bee)
Coumaphos	82	0	0	0	No Class.	Acaricide	Toxic for bees	3–6
Spirodiclophen	0	8	10	0	4	Acaricide	Toxic for bees	>100
Carbendazym	2	0	14	0	4	Fungicide	Extremely toxic	50
Triphloxystrobin	0	0	3	0	4	Fungicide	Extremely toxic	>208.8
Pyraclostrobin	0	0	4	0	2	Fungicide	Moderately toxic for bees	> 110
Ametryn	2	0	0	0	3	Herbicide	Moderately toxic for bees	> 100

The pollen analysis showed that bees collected nectar from eight different plant families (pollen present in the honey sample) and pollen from three plant families (samples from stored pollen). The Myrtaceae family and eight plant genera were found both in honey and pollen. Other plant families found only in honey samples were: Rutaceae (Citrus sp.); Sapindaceae (*Allophyllus edulis* Camb.); Boraginaceae (Heliotropium sp.); Piperaceae (Piper sp.); Junglandaceae (Juglans sp.), and Oleaceae (*Ligustrum lucidum* Ait, Fraxinus sp.). Taking these results into consideration, one could speculate that a bee has a bigger chance at getting exposed to pesticides by collecting pollen since the greater the diversity of food sources the risk of exposure to pesticides decreases (Smart *et al*., 2016).

The acaricide coumaphos was the most concentrated pesticide detected in our study. At sublethal doses, coumaphos produces erratic movements in bees (Williamson and Wright, 2013), and a concentration of 10 mg/kg in beeswax, which is higher than the one found in this study, can contaminate honey (Kochansky *et al*., 2001). Detoxification from this acaricide by bees has been observed due to the action of three groups of enzymes (Mao *et al*., 2013); but given that multiple pesticides where present in our sentinel hive, the efficiency of these metabolic pathways might be decreased. Though we can't assert this hypothesis, because we didn't carry out an enzyme analyses in our sentinel hive. Accordingly, we might expect that the bees in our study could have behavioral alterations, including disorientation, which could be studied using microchips in bees (Ayup *et al*. 2021).

Our study shows how the use of sentinel hives for the monitoring of both, pesticides and food resources within beehives, might shed valuable information not only to design sustainable agri-environments, but also it could be used by beekeepers and regulatory organisms.

Appendix.

Pesticidas	Abeja	Miel	Cera	Polen
3H Carbofuran	ND	ND	ND	ND
Acetamiprid	ND	ND	ND	ND
Ametryn	ND	ND	2	ND
Azoxystrobin	ND	ND	ND	ND
Benalaxyl	ND	ND	ND	ND
Benzoximate	ND	ND	ND	ND
Boscalid	ND	ND	ND	ND
Carbaryl	ND	ND	ND	ND
Carbendazim	14	ND	2	ND
Carbofuran	ND	ND	ND	ND
Chlorpyrifos	ND	ND	ND	ND
Coumaphos	ND	ND	82	ND
Cyprodinil	ND	ND	ND	ND
Difenoconazole	ND	ND	ND	ND
Dimethoate	ND	ND	ND	ND
Dimoxystrobin	ND	ND	ND	ND
Fipronil	ND	ND	ND	ND
Fludioxonil	ND	ND	ND	ND
Flufenoxuron	ND	ND	ND	ND
Fluoxastrobin	ND	ND	ND	ND
Fluxapyroxad	ND	ND	ND	ND
Imazalil	ND	ND	ND	ND
Imidacloprid	ND	ND	ND	ND
Malathion	ND	ND	ND	ND
Methiocarb	ND	ND	ND	ND
Picoxystrobin	ND	ND	ND	ND
Prochloraz	ND	ND	ND	ND
Propoxur	ND	ND	ND	ND
Pyracarbolid	ND	ND	ND	ND
Pyraclostrobin	4	ND	ND	ND
Pyrimethanil	ND	ND	ND	ND
Quinalphos	ND	ND	ND	ND
Quinoxyfen	ND	ND	ND	ND
Spirodiclofen	10	ND	ND	8
Tebuconazole	ND	ND	ND	ND
Thiabendazole	ND	ND	ND	ND
Thiophanathe-methyl	ND	ND	ND	ND
Trifloxystrobin	3	ND	ND	ND

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PARTICIPATION

MG and AGC participated in all research processes. NC participated in the experimental design and article writing. AS analyzed the samples for pesticides.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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