Oxidative stress detection as a promising tool for biomonitoring the honeybee expositions to pesticides

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Introduction

French Guyana is the only French department to experience an increase in the number of farms and the utilized agricultural area (UAA). The agricultural area will increase further, leading to increasing pressures on environment in general, especially pollinators.

In order to anticipate this evolution, significant changes in practices are to be initiated. The **Ecophyto Plan** has been launched at the national level to meet the objectives of the « Grenelle de l'Environnement », which aims to reduce pesticides by 50 % by 2018. In parallel, the Ecophyto plan focuses on the need to monitor and to understand the impact of the use of plants protection products on the environment by promoting innovative solutions.

In this context, **new environmental monitoring methods** are essential. Indeed, even if technological methods are the only ones to bring very precise quantitative information; **"biological" methods** can provide additional information (e.g. spatio-temporal qualitative information).

Here, we used **honeybees *Apis mellifera*** (Hymenoptera: Apidae) as a bioindicator species in order to monitor three beehives unders different pesticides exposition. The aims of this study were to compare the **bioaccumulation of pesticides** in beeswax and the impact on bee health through the **level of protein oxidation**.

Methodology

Apiaries choice

The study area is located around the village of **Cacao** (French Guyana), the most important agricultural area of French Guyana for fruits and vegetables. In this area, 3 apiaries of *A. mellifera* were selected (**Fig.1**) :

1 **Conventional crop**, located in the village of Cacao;

2 **Sustainable crop**, located at the crossroads of the national N2 road and the departmental road of Cacao, at the "Boulanger" Creek;

3 **Control non-agricultural crop**, without impact, located on the Nancibo trail (30km after Cayenne).

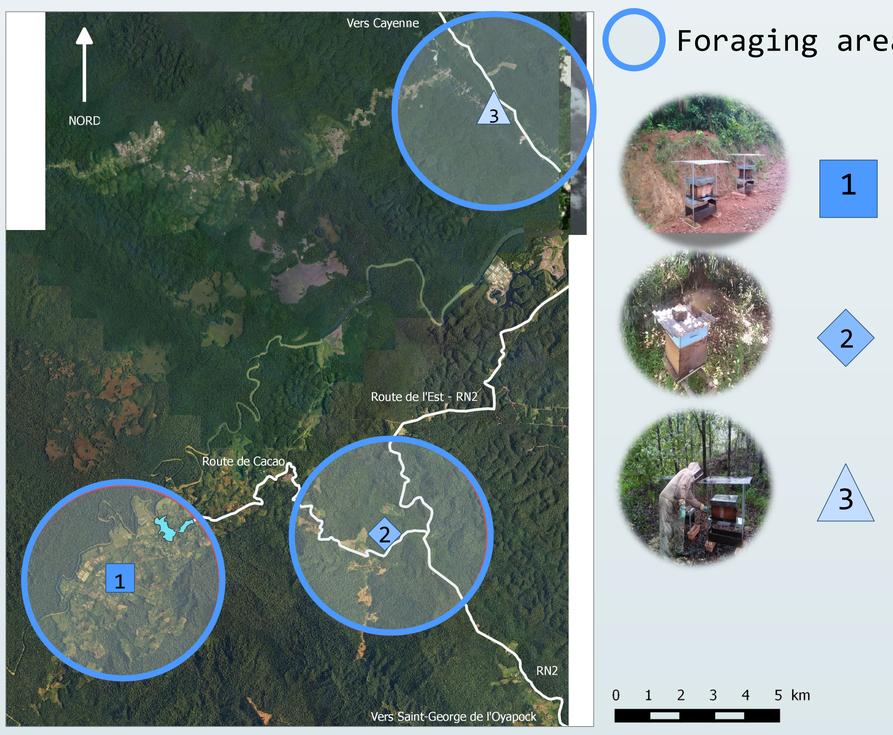

Figure 1: Apiaries localisation and foraging area

Sampling

Two samples were taken during the study, following the AFNOR X43-909 normalization :

- One in the **rainy season** (08/2017),
- The other in the **dry season** (11/ 2017)

At each sampling, weather data (wind, pluviometry and temperature), bees and wax were collected for pesticides and oxidative stress analysis.

Pesticides analysis

A broad screening of **500 pesticides** was performed by gas or liquid chromatography (as required) coupled to a mass spectrophotometer (GC/MS-MS or LC/MS-MS).

Oxidative stress analysis

Analyses were carried out in quadruplicate (repeated 4 times for each sample). Total proteins were extracted and quantified using Bradford's method. Carbonylated proteins were then labeled with fluorescent probes and separated by electrophoresis (SDS-PAGE)(Fig.3).

A densitometric analysis was performed to quantify the fluorescent signal of the carbonylated proteins (fluorescence units) in relation to the signal obtained with the total proteins, thus determining the **Carbonyl Score** (Fig.4).

Results

All of the weather data collected at both times of the year was consistent with regular bee outflow and foraging. The sampling results were therefore **representative of a normal exposure of bees to their environment.**

Pesticides analysis

Among the 500 pesticides screened, analyses showed the **presence of 13 molecules** detected, 9 of which were measured at concentrations above the limit of quantification. Fig.2 shows results without pesticides used in beekeeping (suposed without deleterious effect on bees).The higher 2,4-D concentration in the crop 2 does not appear to reflect its toxicity in bees since it is not proportionnal to the level of protein oxidation (Fig.3).

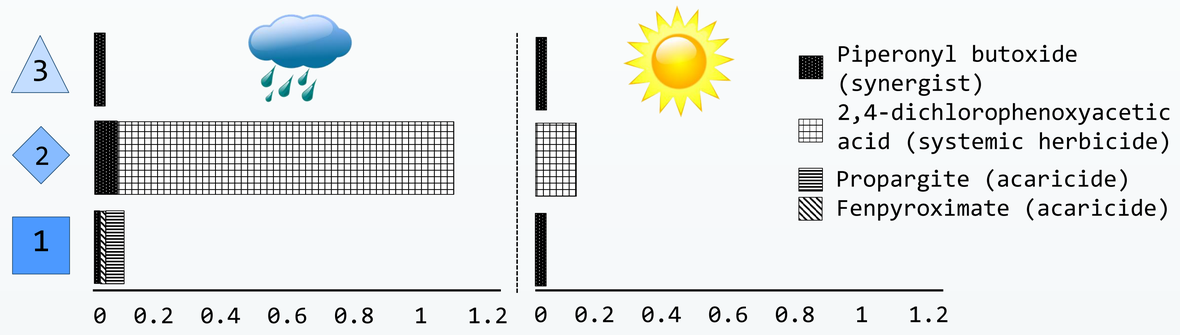

Figure 2 : Pesticides concentration by sampling season for each crop

Oxidative stress analysis

1) Regardless of the season of sampling, the **level of protein oxidation significantly increased** between the non-agricultural control crop, the sustainable crop and the conventional crop ($F_{2,18}$ = 623.66, $p < 0.001$; Fig.3 and 4).

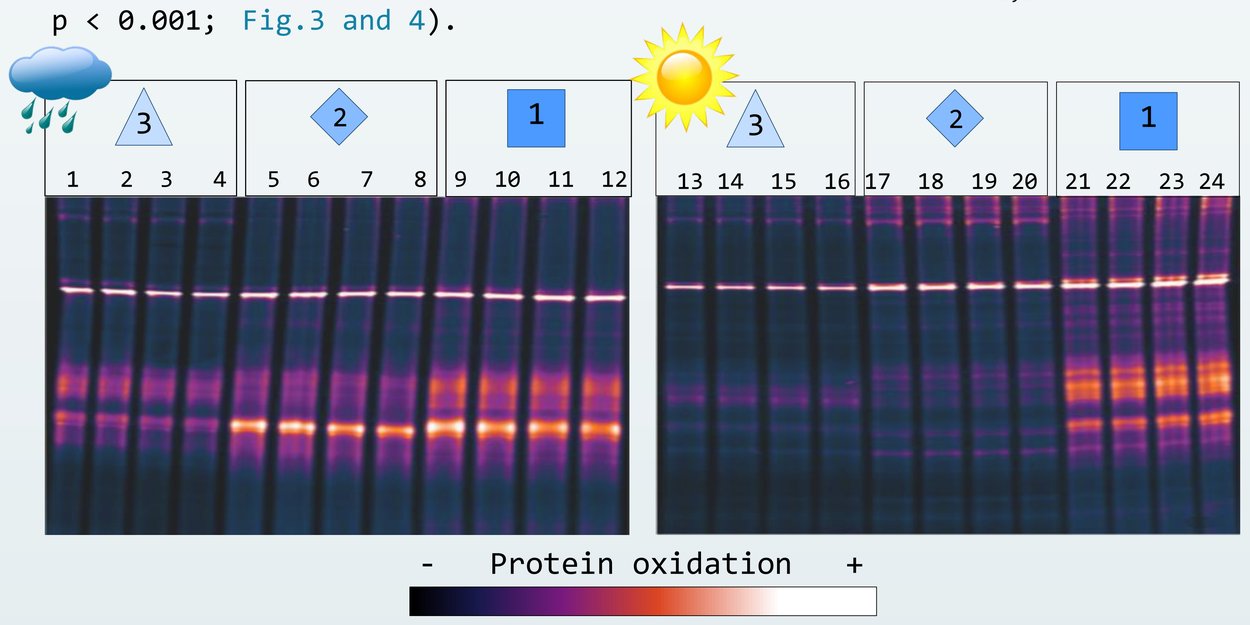

Figure 3 : Carbonylated protein intensity for each sample

2) Regardless of the crop, the **level of protein carbonylation was significantly higher** in the rainy season sample than in the dry season sample (~ x3, $F_{1,18}$ = 5696.3, $p < 0.001$)(Fig.4).

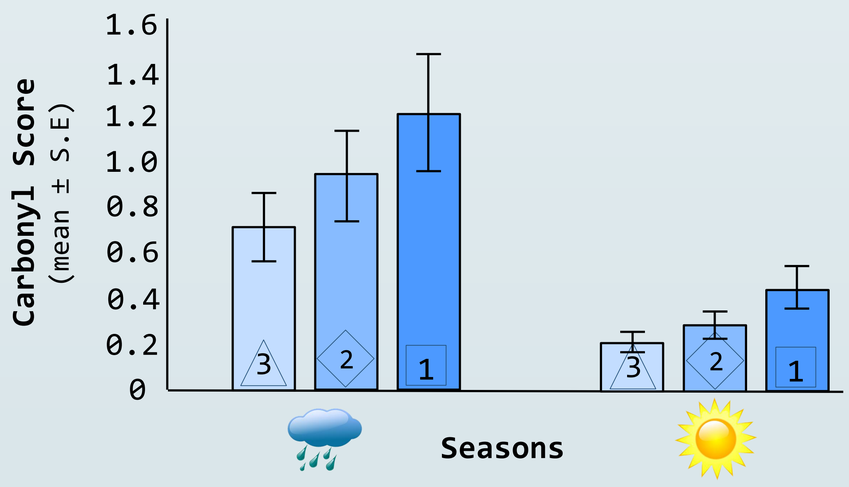

Figure 4 : Carbonyl Score for the 2 samples at each crop

Conclusion Current methods for evaluating bee health risks of pesticides (a single active ingredient at a time) raise questions about greater hazards of pesticide products due to possible synergistic effects when combined with other pesticides. Here, we have shown that the simple detection of pesticides is not enough to determine their toxic effects on bee health. This study is the first to describe **oxidative stress detection as a potential tool for bee health survey**. For future studies, it would be interesting to carry out pesticide analyses on bee breads to avoid the history of the hive and to have a representative image of the treatments emitted into the environment only during the month preceding the sampling.

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