https://doi.org/10.35662/unine-master-biology-002

Université de Neuchâtel
Faculty of Science
Laboratory of Soil Biodiversity

Pesticides distribution and chronic honeybee colony exposure in Swiss agricultural landscapes

MSc THESIS

to obtain the title of

Master of Science in Biology

of the University of Neuchâtel

presented by

Esteban Paul Chèvre

On June 30, 2023

Supervisors:

Prof. Alexandre Aebi (Director)

Dr. Julie Hernandez

Dr. Yann-David Varennes

Summary

Abstract

Due to their foraging behavior, honeybees interact with the environment. In agricultural landscapes, honeybees play a crucial role as pollinators collecting pollen and nectar from mass-flowering crops, orchard and vineyards which provide important resources for honeybees. Therefore, pesticide contamination of bee products is a widespread phenomenon. In the agricultural landscapes of western Switzerland, an ultra-trace level determination of neonicotinoid analysis was performed. A total of 200 honey and pollen samples from 30 apiaries over three years were analyzed based on efficient modified quick, easy, cheap, effective, rugged, and safe extractions (QuEChERS) followed by a UHPLC-MS/MS quantification. Furthermore, brood size and pollen stocks area were evaluated using ColEval method to assess the sublethal chronic honeybee colonies exposure. 94% of our samples contained at least one of the targeted neonicotinoids, 63% of the samples were contaminated by doses greater or equal to 0.1ng/g and 62% of the samples contained two or more neonicotinoid. Thiacloprid and acetamiprid were the most frequently detected molecules. Also, this study found traces of banned or highly regulated neonicotinoids, suggesting their persistence in the environment. The incidence of contamination was higher in honey than in pollen samples, indicating a more frequent occurrence. The prevalence was higher in pollen samples, suggesting higher levels of contamination per samples. The fact that pollen samples were grouped by period ensured greater consistency than honey samples for long-term analysis of pesticide residues. Samples from apiaries located in crop-dominated landscapes were found to be more contaminated than samples from grassland-dominated landscapes. Finally, this study showed that a cumulated concentration of neonicotinoid found in spring pollen samples reduced the surface area of further pollen stocks and brood size. These results may argue in favor of the deleterious effects of larval exposure to a mixture of neonicotinoid and other pesticides on the foraging capacity of newly emerged bees. Moreover, these results might indicate a deleterious indirect effect of contaminated pollen on nurse bees' foraging. Further efforts should focus on monitoring honeybee colonies exposure to a mixture of pesticides in agricultural landscapes and could integrate farmers' practical management as a reference for sampling.

Keywords: pesticides; neonicotinoid; *Apis mellifera*; honeybee colony; colony size; brood size; pollen stocks area; newly emerged bees; agricultural landscape; field surveys.

Acknowledgment

I would like to express my deep gratitude to Alex Aebi for sharing his knowledge and guiding me through the beauty of interdisciplinarity in applied agricultural research. I feel armed and envious to be able to bring agroecosystems and agri-environmental governance into dialogue with the help of biology and socio-anthropology. All this, with the firm aim of seeing bioethnology evolve and assert itself as a fully-fledged (and necessary) field of research.

Thanks to Sylvie Guinchard and Gaëtan Glauser for their explanations and unfailing support during the laboratory analyses. I would also like to thank all the people who made my year in the laboratory of soil biodiversity so pleasant and convenient.

Thanks to Jan Hattendorf for his statistical analysis. His time and interest in this thesis were much appreciated.

This study would not have been possible without the support of the "Agriculture and Pollinators" project funded by the Federal Office for Agriculture (OFAG) and the cantons of Jura, Bern, and Vaud.

I would particularly like to thank Julie Hernandez and Yann-David Varennes for their invaluable help and advice as well as their friendliness from the beginning to the end of this master's thesis.

Finally, I am grateful to the University of Neuchâtel for the five remarkable and fruitful years I have spent there.

Love on my family and my friends

1. Introduction

1.1 Agriculture and beekeeping: a relationship

Agriculture and beekeeping have an ancient close relationship. In the mid-nineteenth century in France, hardly any farm or agricultural holding, in the broadest sense of the term, existed without the presence of an apiary (Fortier, Dupre & Alphandery, 2020). Beekeeping was part of the mixed livestock farming system that prevailed at the time, combining self-sufficiency and the marketing of bee products (Fortier, Dupre & Alphandery, 2020). The decline of "peasant" beekeeping and the separation of beekeeping from agriculture began in the second half of the 19th century, under the combined effects of industrialization and the gradual abandonment of honey and wax as basic supplies (Sutherland et al., 2020). In addition, the technical and economic modernization of agriculture in many Western countries has pushed farmers to become ever more productive by adopting an agronomic approach (Sutherland et al., 2020). As a result, a gap has opened between agriculture and beekeeping. The main form of beekeeping in the West today is the result of a break with the agricultural activity with which it was long associated (Fortier, Dupre & Alphandery, 2020).

The increase in agricultural productivity can be attributed to two key factors. First, the process of mechanization and rural exodus has led to significant transformations in agricultural landscapes. Second, the utilization of synthetic agropharamceutical products has contributed to improved performance in both plants and animals (Cornu et al., 2018; Chouquer, 2003). However, these changes in agricultural landscapes resulted in a substantial loss of habitats for bees (Cornu et al., 2018). During the 20th century, conflicts started between beekeepers and farmers regarding the use of pesticides on mass flowering crops such as oil seed rape, sunflowers, and maize (Chouquer, 2003). From the 1990s, the honeybee colony numbers in Europe were observed with a steep decline (vanEngelsdorp & Meixner, 2010). Additionally, in 1994 French beekeepers started to report alarming, peculiar foraging behavior and a significant increase in honeybees' winter mortality (Maxim et al., 2013). This abnormal phenomenon was named Colony Collapse Disorder (CCD) in the absence of a known cause (Underwood & vanEngelsdorp, 2008; vanEngelsdorp, 2009; USDA, 2012). Usually, there are plenty of food stores in these colonies abandoned by worker bees (Farooqui, 2016) suggesting colony mismanagement, nutrient deficiencies, and chemicals in the environment (Underwood & vanEngelsdorp, 2008; FAO, 2019).

However, the dependence of crops on an efficient pollination service persists (Klein et al., 2007). In this vein, the Millenium Ecosystem Assessment (MEA) report established the idea that pollination can be seen as a "supply and support" ecosystem service (for food security and for biological diversity), contributing to "human well-being” (MEA, 2005). Currently, reports indicate that the overall state of insects, including honeybees, is currently below the optimal threshold for effective pollination of flowering plants in arable land and grassland (FAO, 2019). For instance, a study based on a country-wide dataset of 54 major crops in France, found that intensification fails to enhance the yield of pollinator-dependent crops and even reduces the stability of their yields over time (Deguines et al., 2014). This suggests that the advantages of agricultural intensification can be counteracted by declines in pollination services.

1.2 Our study

The increased awareness of pollinators declines (Potts et al., 2010; Goulson et al., 2015; FAO, 2019), reports showing global scale pesticides contamination in beehive products (Mitchell et al., 2017; Murcia-Morales et al., 2022) and the below optimal threshold pollination service (Gallai et al., 2009; FAO, 2019) highlights the need for a new and sustainable dialogue between agriculture and beekeeping. Our study involves itself in this dynamic by highlighting the distribution of pesticides in Western Switzerland and the exposure of honeybee colony to create a new dialogue between farmers, beekeepers, researchers, and policy makers (Sutter et al., 2019). This, while following Switzerland recent efforts to reduce the risks associated with the use of pesticides.

In 2017, the Swiss government implemented an action plan intending to reduce pesticide risks by 50% before 2027. In addition, the Swiss government is also promoting floral resources in agricultural ecosystems and supporting alternatives to synthetic pesticides (Swiss Government, 2017; Swiss Government, AS 2022.263, 2021). More precisely, a “national plan of measures for bee health” has been established to prioritize research and actions that protect the health of wild and domestic bees in a sustainable way (Swiss Government, 2014). To achieve this, several projects have been funded in compliance with articles 77a and 77b of the Federal Law in Switzerland (Swiss Government, RS 910.1). These projects, such as “Agriculture and Pollinators”, are designed to support the protection and enhancement of pollinators, ensuring their vital role in maintaining healthy ecosystems and sustaining agricultural productivity.

1.2.1 “Agriculture and Pollinators” project

The present study carried out at the University of Neuchâtel, was part of the “Agriculture and Pollinators” (AGRIPOL) project initiated by “ProConseil” and the “Federation Rurale Interjurassienne” (FRI) which received support from the Federal Office for Agriculture (OFAG) as well as the Jura, Bern, and Vaud Canton. The “Agriculture and Pollinators” project has put forward a series of agroecological measures. These measures such as “avoiding the use of neonicotinoids in seed treatments”, “avoid using insecticides on the farm's flowering crops” or “delayed mowing of temporary grassland” aimed to support the growth and well-being of honeybee colonies and wild bees. These measures are intended to be implemented by farmers with the objective of identifying practices that effectively enhance honeybee colonies’ health by monitoring colony size parameters (Sutter et al., 2019). Consequently, the scientific methodology of the project was designed to assess the significance of all these agroecological measures.

1.2.2 Pesticides distribution and chronic honeybee colony exposure to neonicotinoids

In an agricultural environment, honeybees play a crucial role as pollinators, collecting pollen and nectar from mass-flowering crops such as oilseed rape, sunflowers, and maize (Requier et al., 2015). These crops, as well as orchard and vineyards with weed flowers, provide abundant floral resources for bees, particularly during the spring season. However, the use of pesticides in resource-rich areas poses a significant (direct) risk to foraging bees (Yang et al., 2008) and to the entire colony as pesticides can be found in apicultural matrices (e.g., pollen, honey, and beebread) by showing indirect (delayed) effects (Daniele et al., 2018; Murcia-Morales et al., 2022, Wu-Smart & Spivak, 2016). In fact, oilseed rape, sunflower and maize are frequently treated with pesticides, including neonicotinoids, making them the focus of extensive research on the impacts of agricultural practices on bee health (Lundin et al., 2015).

Neonicotinoids are a class of systemic insecticides widely used in agriculture and known for their neurotoxic effects on bees (Tsvetkov et al., 2017; Moffat et al., 2016). While acute toxicity is well-documented, there is evidence suggesting that sub-lethal doses of neonicotinoids can have significant delayed impacts on honeybee colony development (Potts et al., 2010; Wu et al., 2011; Wood & Goulson, 2017). In fact, study found that hygienic and foraging behavior were altered when larvae were exposed to sublethal doses of clothianidin for 25-36 days (Morfin et al., 2019). Their systemic nature as well as their prophylactic mode of action have made them "the most widely used insecticides in the world" (FAO & WHO, 2019).

1.2.3 Objectives

First, we aimed to investigate the presence of pesticide residues in pollen and honey samples collected from apiaries in agricultural landscapes. Specifically, we sought to examine the prevalence and types of molecules that can be detected in these samples. By analyzing the levels of contaminants in honey and pollen, we aimed to shed light on the extent of pesticide exposure in the agricultural environment in western Switzerland.

Second, our study aimed to examine the potential relationship between contaminants and honeybee colony size, specifically focusing on the sub-lethal and delayed effects of neonicotinoids. To address this aspect, we sought to evaluate whether the presence of sub-lethal realistic traces of neonicotinoids and other contaminants in spring pollen and honey samples collected in agricultural landscapes of Switzerland could influence further honeybee colonies size. More precisely, we used brood size and pollen stocks area which are important indicators of honeybee colony development (Wu-Smart & Spivak, 2016).

To achieve these objectives, we formulated the following questions:

- What types of molecules can be detected in honey and pollen samples collected from apiaries in agricultural landscapes of western Switzerland?
- Do the quantities of contaminants differ between agricultural landscapes dominated by grassland and those dominated by annual crops?
- Can the sub-lethal delayed effects of neonicotinoids be assessed using data on brood size and pollen stocks area collected as part of the "Agriculture and Pollinators" field project?

To our knowledge, there is a paucity of comprehensive studies focusing on the presence and distribution of pesticide residues in spring pollen and honey samples from agricultural landscapes. Therefore, our study will contribute valuable insights into the potential risks posed by realistic pesticide exposure to honeybees in agricultural landscape. In addition, our results can contribute to the development of effective strategies for monitoring the threats posed by pesticides to pollinators and for maintaining the sustainability of agricultural systems.

Our main goal was to add value to the data collected under the "Agriculture and Pollinators" project with an ultra-trace level determination of neonicotinoid residues in 2019, 2020 and 2021 honey and pollen samples. Second, we measured the potential impacts of neonicotinoids on honeybee colonies. Furthermore, we aimed to identify any limitations in the experimental design of the project, specifically regarding the monitoring of pesticide-related measures’ effects on colony size. This research is significant as it addressed the long-term consequences of sub-lethal neonicotinoid exposure on honeybee colony development *in vivo* conditions. Understanding the potential effects of these pesticides as well as their interaction with other contaminants on colony size can provide valuable insights for developing sustainable agricultural practices that minimize the negative impacts on pollinators and ensure the long-term viability of bee populations.

2. Materials and Methods

2.1 Scientific monitoring

The study was conducted for three years in western Switzerland, covering 30 apiaries located in the cantons of Jura, Bern, and Vaud. The Swiss beekeepers' associations relayed calls for participation in the project to eligible beekeepers between the ages of 18 and 70. The volunteer beekeepers followed the Swiss national recommendations for beekeeping practices and varroa management, as provided by the SSA ApiService - Apicultural Health Service[1]. A total of 300 colonies of *Apis mellifera* were kept in Dadant or Swiss Bürki beehive systems, and each apiary was monitored four times per year in April, June, the end of July/beginning of August, and October. At each visit, the colony size i.e., the adult worker bees, the number of capped brood cells, and the area (dm^2) of pollen and honey/nectar was determined using ColEval method described further (See §2.1.1). These variables enabled us to evaluate the colony's development throughout the beekeeping season.

The 30 apiaries hosted 10 colonies each, and a circle of two kilometers radius (Gallot et al., 2019) around each apiary has been defined and monitored to assess the landscape around each apiary (Fig. 1). These two kilometers correspond to an average estimate of the distance travelled by the foraging honeybees from the hive to the resource collection defined by Gallot et al. (2019) and Steffen-Dewenter & Kuhn (2003). Each monitored sector was separated by nine km to avoid any interference and ensure the independency of each apiary.

All the data used in this master thesis have been collected under the “Agriculture and Pollinators” project financed by the Swiss Federal Office for Agriculture (OFAG). The project proposed a series of agroecological practices that farmers could choose to apply in their fields. The project aimed to determine which measures could enhance the availability of floral

---

[1] https://abeilles.ch/ (Accessed 15.04.23).

resources or include a change in agricultural management in favor of honeybees, thereby promoting their health (Sutter et al., 2019).

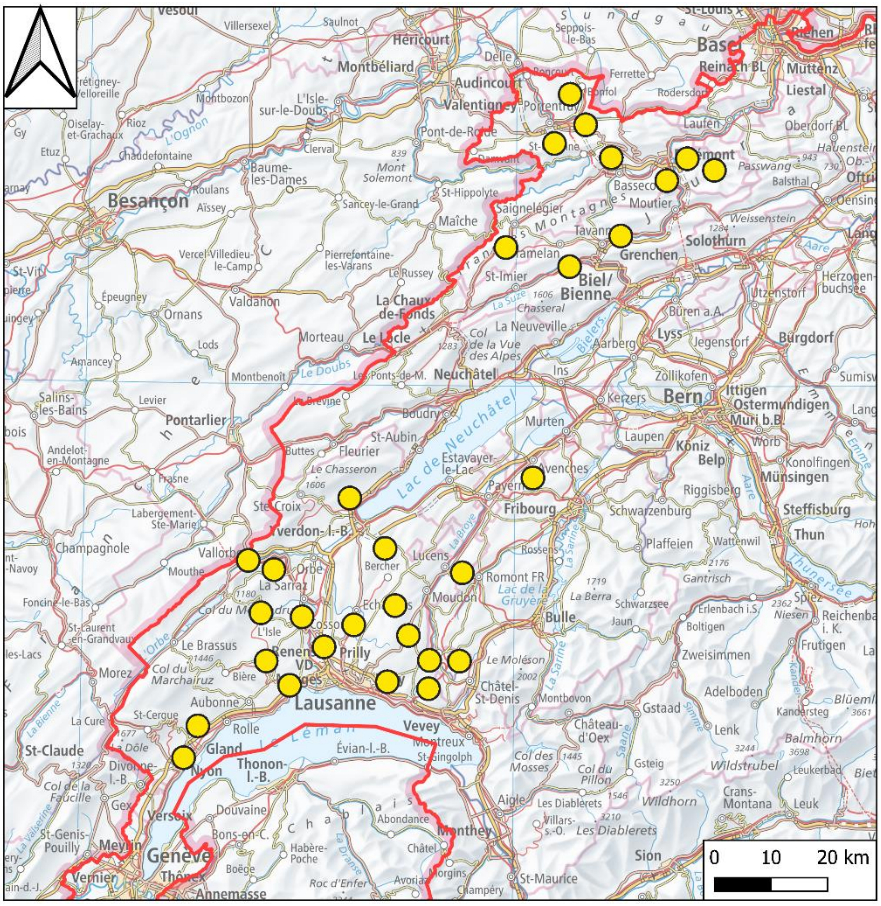


Figure 1: The 30 apiaries in the cantons of Jura, Bern, and Vaud. Few changes occurred in the partner apiaries during the project (see chapter 4.1).

2.1.1 Evaluation of honeybee colony size

The honeybee colony size was evaluated by the ColEval method (Hernandez et al., 2020). This method is based on a calibrated estimation of the percentage of the comb surface area occupied by adult honeybees, by capped brood cells, by the honey and pollen, and subsequently converted into a number of adult individuals and brood cells, and into an area (dm2) of honey and pollen. Those estimates took place four times during the beekeeping season (Fig. 2). In this thesis, we use data from the first (min. 7th April to max. 4th May), second (min. 2nd June to max. 8th July) and third (min. 20th July to max. 31st August) report.

2.1.2 Honey and pollen sample collection

Honey

Honey samples were collected by beekeepers twice a year (Fig. 2). The first harvest corresponds to the spring honey harvest (end of May/beginning of June) and the second to the summer honey harvest (end of July/beginning of August). After extracting the honey, the partner beekeepers filled a 5 mm falcon tube with as much honey as possible. Samples were stored in freezers and collected by field assistants of the “Agriculture and Pollinators” project during the apiary visits.

Pollen

Pollen samples were collected using pollen traps. A pollen trap is a device used to collect pollen brought into the hive by foraging bees (Dimou et al., 2006). They were placed at the entrance of three hives at each apiary and worked by forcing income bees to pass over a filter that wrests pollen from the bees (Dubois et al., 2016). Beekeepers were asked to set pollen traps on three colonies next to each other to avoid drifting effects. The traps were set in place at the end of April. However, at that time the filter used to collect the pollen was removed for the bees to adapt to the trap. Those periods occurred every three weeks from early May to the beginning of October (Fig. 2). Pollen traps were activated from 24h to 72h to ensure enough pollen for the analyses. The pollen collected was directly frozen and dated. Pesticide residue levels were assessed at the apiary level after homogenization of pollen samples divided into three periods (spring with May samples, earlier summer with June samples and end of season with July-October samples). For this thesis, we analyzed pollen samples from the 1st-3rd May and 27th-29th May collection which corresponds to pool one (spring) (Fig. 2). This methodological choice was made to focus on the period when mass-flowering crops are the most vulnerable to pests and the demand for plant protection products is the highest. The time and cost of laboratory analysis were also parameters that influenced the design of the study.

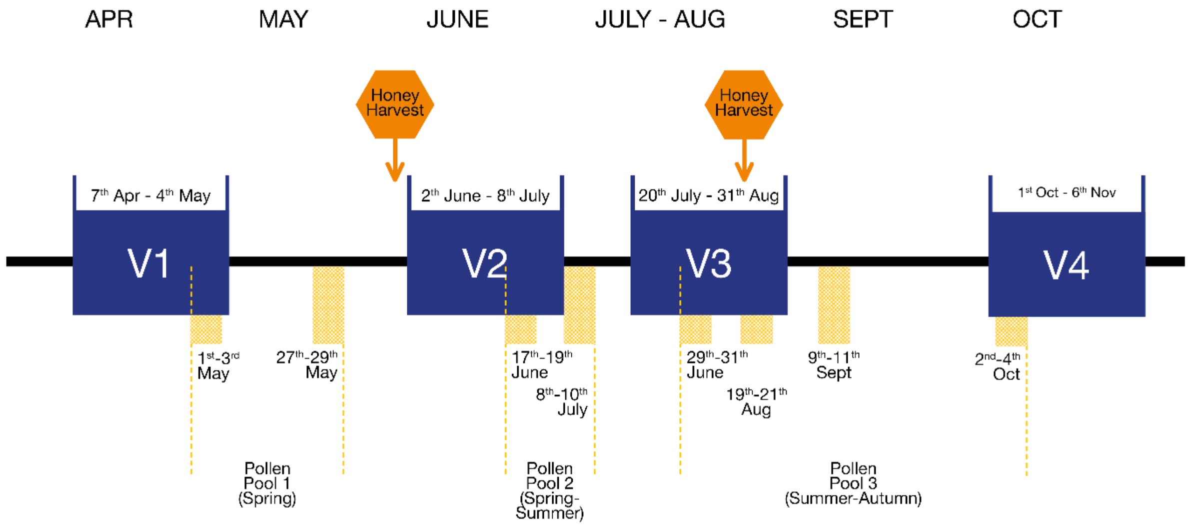


Figure 2: Chronological chart of the maximal time range for ColEval visits (V1, V2, V3 and V4), honey (honey harvest), and pollen samples collection.

2.2 Neonicotinoids residues analysis in honey and pollen matrices

2.2.1 Pesticides extraction using QuEChERS

The aim of the following protocol was to isolate neonicotinoids from matrices for analysis by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), while avoiding interference from other components present in the sample. The approach employed for the extraction of neonicotinoids is known as the QuEChERS methodology, an acronym that stands for Quick, Easy, Cheap, Effective, Rugged and Safe. The QuEChERS procedure comprises two phases of extraction, the first being a salting-out assisted liquid-liquid extraction (SALLE) and the second being a dispersive solid phase extraction (d-SPE) (Anastassiades et al., 2003; Kammoun et al., 2019).

Honey

Honey samples were weighed into 15 mL Falcon (50 mg) after being defrosted and warmed at 30°C using a water bath. To the 15 mL falcon tube, 9 mL of H2O:Acetonitrile (50:50, v/v) and 20 µl of internal standard solution (125 ng/mL of Thiamethoxam, Clothianidin, Acetamiprid and Thiacloprid in ACN except for Imidacloprid at 25 ng/mL) were added and dissolved by handshaking and ultrasonication. The sample was transferred to a "salt" tube (15 mL Falcon tube) filled with 3.25 g of extraction salts. The extraction salt tube is composed of 2 g MgSO4 anhydrous, 0.5 g NaCl, 0.5 g trisodium citrate dihydrate, and 0.25 g trisodium citrate sesquihydrate. The tube was shaken vigorously by hand to detach the pellet of salt from the bottom. Next, 1 mL of H2O:Acetonitrile (50:50, v/v) was added to the first tube containing the honey pellet, shaken by hand for 10-20 s, and transferred to the "salt" tube. The "salt" tube was then shaken vigorously by hand for 1-2 min. The "salt" tube was centrifuged (4000 rpm) and the supernatant (4.5-4.8 mL) was collected using a 1000 µl pipette and transferred to a "purification" tube containing 100 mg PSA, 100 mg of C18 and 150 mg MgSO4. The tube was shaken vigorously for 30 s, centrifuged (8000-10000 rpm) and the supernatant (4-4.5 mL) was collected using a 1000 µl pipette into a 13x100 mm glass tube. The samples were evaporated under vacuum using Speedvac (LABCONCO CentriVap Cold Trap), and 500 µl of H2O:MeOH (75:25, v/v) was added to each tube. The tubes were vortexed for 10-20 s and placed in the ultrasonic bath for 1 min. Finally, the samples were filtered using a 13 mm hydrophilic PTFE filter into an HPLC vial containing a 250 µl conical insert for analysis of neonicotinoids.

Pollen

Pollen samples were defrosted at ambient temperature and then placed in a drying oven at 35°C overnight. Samples were then grounded for optimal sample homogeneity (Valverde et al., 2016). Next, approximately 25 mg of pollen were weighed and placed in a 2mL Safe-Lock Eppendorf tube with three to five small glass beads. Then, 1 mL of acetonitrile and 25 µL of internal standard solution (40 ng/mL in ACN) were added to the tube, which was shaken for four minutes using the Retsch 400 MM tissue lyser at 30 Hz. The tube was then centrifuged for five minutes at 12,000 xg, and as much supernatant as possible was pipetted into a 5 mL Eppendorf tube containing 1000 mg of extraction salts. The mixture was vortexed until the salt pellet detaches. The first tube was then refilled with 700µL of acetonitrile, shaken for four minutes, and centrifuged again. The supernatant was added to the "salt" tube. Then, 1.6 mL of water was added to the "salt" tube and vigorously shaken by hand for one to two minutes until the salt pellet detached from the tube. The tube was then centrifuged for five minutes at 6000 rpm, and the supernatant was collected and placed in a 2 mL (Safe-Lock Eppendorf) "purification" tube containing 45 mg MgSO4, 35 mg PSA, and 35 mg C18. After vigorous shaking for 30 seconds, the tube was centrifuged for three minutes at 12,000 xg, and the supernatant was transferred to a new 2 mL Eppendorf tube. The sample was then evaporated in a Speedvac (LABCONCO CentriVap Cold Trap) at 35°C overnight. Finally, the sample was resuspended in 200 µL of H2O:MeOH (75:25, v/v), vortexed for 20 seconds, ultrasonicated for one minute and filtered through 13 mm hydrophilic PTFE filters into an HPLC vial fitted with an insert for the analysis.

2.2.2 UHPLC-MS/MS analysis

The UHPLC-MS/MS analysis was performed at the Neuchâtel Platform of Analytical Chemistry (NPAC). In the study, neonicotinoids were analyzed according to Mitchell et al., 2017 and Kammoun et al., 2019) using an Acquity UPLC I-Class coupled to a TQ-XS mass spectrometer (Waters). The separation of the five neonicotinoids was achieved on a Cortecs UPLC C18+ column (2.1x100mm, 1.6 µm) with specific parameters, including a temperature of 25°C and a flow rate of 0.4 ml/min. Mobile phase A consisted of H2O+0.05% FA+1mM NH4FA and mobile phase B was ACN+0.05% FA. The gradient program used was 2-30.5% B in eight minutes, 30-100% B in five minutes, holding at 100% B for two minutes and returning to initial conditions at 2% B for four minutes. The injection volume was 2.5 µl. The analysis was carried out in positive electrospray mode, and data acquisition was performed in the multiple reaction monitoring (MRM) mode using optimized precursor-to-fragment transitions for each compound and their corresponding labeled forms. The capillary voltage was set at +1 kV, and the desolvation temperature was 600°C. The data was processed using Masslynx 4.2 (Waters) (Kammoun et al., 2019).

Limits of quantification (Table 1) for honey were set according to Kammoun et al., 2019. For pollen, the validation method has not been published yet but LOQs have been defined by signal-to-noise ratios equal to 10 in real samples.

	Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid	Thiacloprid
Honey (500 mg)	5	10	10	2	2
Pollen (25 mg)	10	25	10	10	10

Table 1: Limit of quantification for neonicotinoids in honey (500 mg) and pollen (25 mg) in pg/g.

2.3 Broad-spectrum analysis of pesticides in honey and pollen matrices

The broad-spectrum analysis of pesticides was carried out by the French laboratory GIRPA according to their protocol. The limits of quantification varied from 10 to 50 ng/g (1000 times higher than NPAC) depending on the molecules and matrices. We refer to those contaminants as “toxic” in the following chapters. The “toxic” category includes highly toxic and moderately toxic compounds for bees interpreted by the Pesticides Properties Database (PPDB)[2]. If contact and oral toxicity were not interpreted similarly by PPDB, we chose the most toxic mention. For the broad-spectrum analysis, a compromise between the number of contaminants analyzed and the limit of quantification was made. By accepting a reduction in sensitivity in favor of increased diversity, we were able to evaluate the cocktails of molecules contaminating our samples, thus the honeybee colonies.

2.4 Statistical analysis

The first aim of this master thesis was to see the distribution of the neonicotinoids in honey and pollen samples among the apiary locations. We used principal component analysis (PCA) to classify the landscapes around the apiaries as either grassland-dominated or crop-dominated as implemented in R's 'ade4' package. The landscape data used in this study originated from various public sources. Forest, urban, and water area information were obtained from the Cantonal Services of Geographic Information, namely the Geoportal SIT-Jura, Geoportal Canton de Berne, and ASIT-VD catalogue. The Cantonal Services of Agriculture (Jura, Bern, Vaud, Fribourg) provided data on all agricultural land-use types, including field location, crop type, surface area, and agroecological measures implementation. These datasets were used to

---

[2] https://sitem.herts.ac.uk/aeru/ppdb/ (Accessed 08.02.23)

calculate the total surface area per land use within 2 km-radius sectors around the monitored apiaries. In total 36 variables were available including 19 variables for annual and perennial crops, nine variables for cultivated grassland, five variables for seminatural habitats and finally forests, settlements, and water bodies. Because all variables are measured in the same unit (m2), we have centered but did not scale the variables. Afterwards, visual inspection resulted in two landscape types which were clearly separated by PCA (See appendix A.3). One category was mainly characterized by grassland.

To analyse the effect of neonicotinoids on honeybee colonies we used linear regression models to investigate the relationship of variables measured at the apiary level. Each time, three different models were created to assess the outcome variables' relationship with neonicotinoid, non-neonicotinoid, and their interaction. Violations of regression assumptions were visually assessed via QQ plots, residual vs fitted plots and leverage plots. Although all models were pre-specified, we log-transformed the outcome variable when appropriate (e.g., to normalize the distribution of the residuals). If any zero values were present, a constant of one was added to all values before the log transformation. Model fit was assessed using R2. For outcome variables measured on the hive level, linear mixed models were used to account for potential correlation within apiaries. In this case, apiary ID was included in the models as a random effect. All mixed effect models were analyzed using R's 'lme4' and 'LmerTest' packages.

3. Results

3.1 Data overview of residues in pollen and honey samples

3.1.1 Neonicotinoids

The neonicotinoid analysis and the broad-spectrum analysis of pesticides performed, stratified by apiary (RX), seasons, and years showed a considerable number of missing data (Fig. 3 & 4), especially for spring honey in 2019 and 2021. Results show higher concentrations in spring pollen than in other seasons and matrices for all the analyzed years (2019, 2020, 2021). 2020 showed less contamination than 2019 and 2021, as well as more consistency in terms of sample collection. Several apiaries showed a neonicotinoid cumulated concentration above the minimal concentration (0.1 ng/g) for which a significant effect on bees is documented (Mitchell et al., 2017). Excluding inconsistencies linked to change in certain apiary location (eg. R12 → R12b; n=6) and the missing samples (n=46), 63% of the contaminated samples overpass 0.1 ng/g. Seven samples have neonicotinoids cumulated concentration values above 10 ng/g with a maximum value of 53 ng/g in the 2019 honey spring (R25). Only six apiaries (R01, R02, R16, R19, R20 and R25) possessed a complete set of data. Therefore, the consistency required to process strong statistical tests will be difficult to achieve for the following parts.

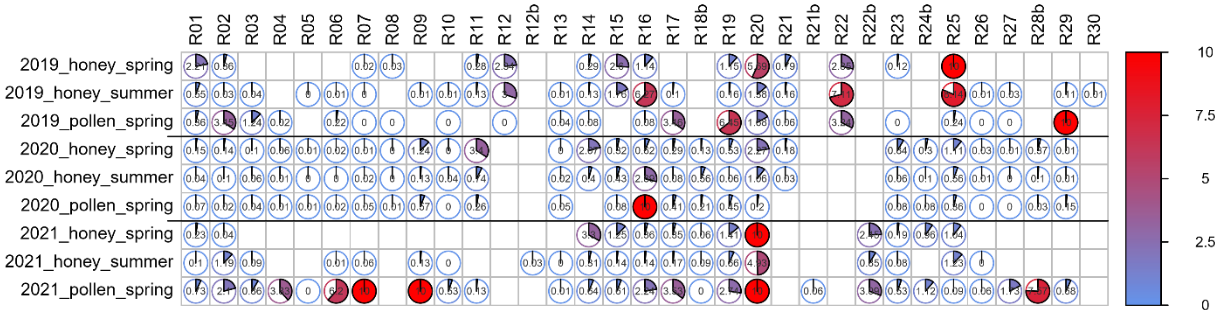


Figure 3: Neonicotinoid in honey and pollen stratified by apiary, seasons, and year. Values are given in ng/g and truncated at 10 ng/g. Neonicotinoids concentration below limit of quantification level (0.002 – 0.01 ng/g) correspond to zero values.

3.1.2 Other contaminants

Most of the samples showed no traces of contaminants. Some samples show strong contamination by different molecule types (herbicides, fungicides, and pesticides) around some apiaries (e.g., R15, R16, R17). The 2019 and 2021 samples are more contaminated and sample collection was less consistent than for 2018 and 2020.

As for the neonicotinoid analysis, spring pollen samples showed the highest concentration of contaminants. Several apiaries, such as R16 and R17, have been simultaneously contaminated by fungicides, herbicides, neonicotinoids, and other insecticides. Summer pollen samples showed for both 2019, 2020 and 2021 a similar or higher contamination level compared to spring pollen samples.

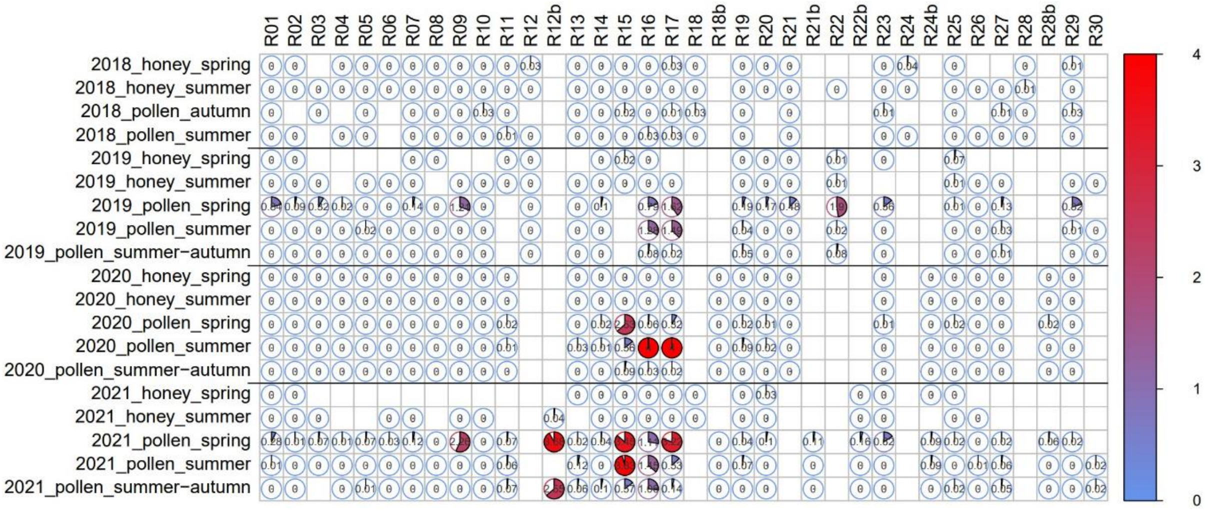


Figure 4: Sum of other detected molecule types (fungicide, herbicide, and insecticides) in honey and pollen stratified by apiary, seasons, and years. Values are given in mg/kg and truncated at 4mg/kg. LOQ= 10ng/g.

3.2 Descriptive analysis of neonicotinoids contamination

Honey matrix

Neonicotinoids concentration analysis of honey samples in the spring and summer of 2019, 2020, and 2021 showed a general downward trend between 2019 and 2021. The percentage of contaminated samples was 54% in 2019, 43% in 2020 and 49% in 2021 (See appendix A.1.1). Thiamethoxam, clothianidin and imidacloprid concentration decreased linearly between 2019 and 2021. On the contrary, thiacloprid – representing the most frequently detected molecule for all years – and acetamiprid showed an increase in concentration from 2020 to 2021. This significant increase reaches eight times for thiacloprid and four times for acetamiprid from 2020 to 2021 (Fig. 5; See appendix A.1.3). Finally, our results showed that 78% of the total honey samples (n=126) contained two or more different molecules (Fig. 6).

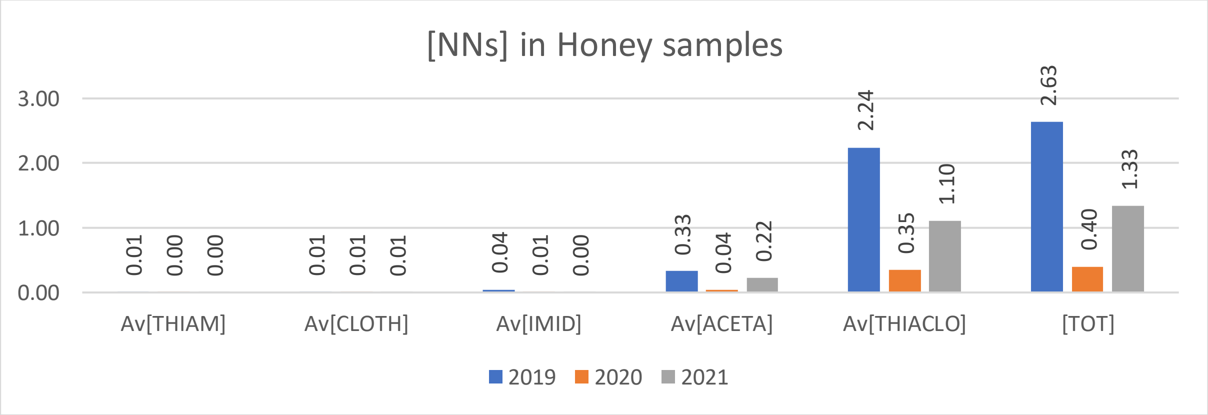


Figure 5: Average neonicotinoids concentration in honey per year in ng/g. THIAM = thiamethoxam, CLOTH = clothianidin, IMID = imidacloprid, ACETA = acetamiprid, THIACLO = thiacloprid, and TOT = neonicotinoid cumulated concentration.

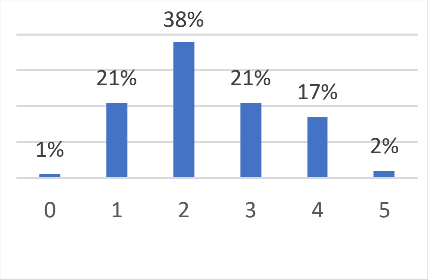


Figure 6: Number of different neonicotinoids found per honey samples.

Pollen matrix

The neonicotinoid concentration of pollen samples in spring 2019, 2020 and 2021 showed an upward trend between 2019 and 2021. In 2019, 22% of samples were above the limit of quantification which is less than in 2020 (28%) and 2021 (32%) (See appendix A.1.2). Pollen samples showed no traces of contamination with thiamethoxam. Only one sample was contaminated with clothianidin in 2019 (0.0013ng/g) and the concentration of imidacloprid decreased linearly between 2019 and 2021.

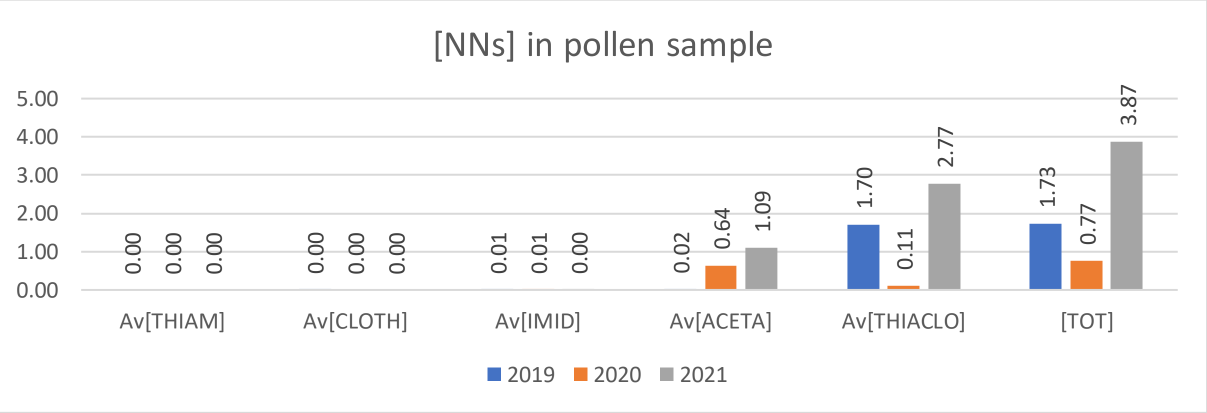


Figure 7: Average neonicotinoids concentration in pollen per year in ng/g. THIAM = thiamethoxam, CLOTH = clothianidin, IMID = imidacloprid, ACETA = acetamiprid, THIACLO = thiacloprid, and TOT = total neonicotinoid concentration.

As for honey samples, thiacloprid is the most detected molecule of all years (12% in 2019, 17% in 2020 and 18% in 2021). Acetamiprid represented 4% of the detected molecules in 2019, 6% in 2020 and 11% in 2021 (Fig. 7; See appendix A.1.4). Finally, our results showed that 45% of the total pollen samples (n=74) contained two or more molecules. 16 samples were below the quantification level and 39% contained just one molecule (Fig. 8).

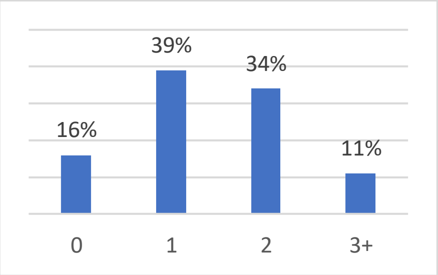


Figure 8: Number of different neonicotinoids found per pollen samples.

3.2 Spatial distribution of neonicotinoid contamination

Neonicotinoids cumulated concentration in pollen and honey samples showed differences according to apiary locations highlighting the diversity of landscapes and management types surrounding the apiaries (Fig. 9). Of the five landscape types tested (See appendix A.4) only meadow was found to be significantly associated with lower neonicotinoid concentration in pollen (difference in log(ng/g) per km2 increase: estimate=-0.43, t-value=-2.4, p-value=0.02) (Fig. 10 &11). This indicates that sites with higher meadow coverage had lower neonicotinoid concentrations. In our model, the year of the sample collection was found to be a significant predictor of neonicotinoid concentration too, with 2021 having a positive association with neonicotinoid concentration (estimate=0.9, t-value=2, p-value=0.02), while 2020 was not significantly associated with neonicotinoid concentration (estimate=-0.6, t-value=-1, p-value=0.1).

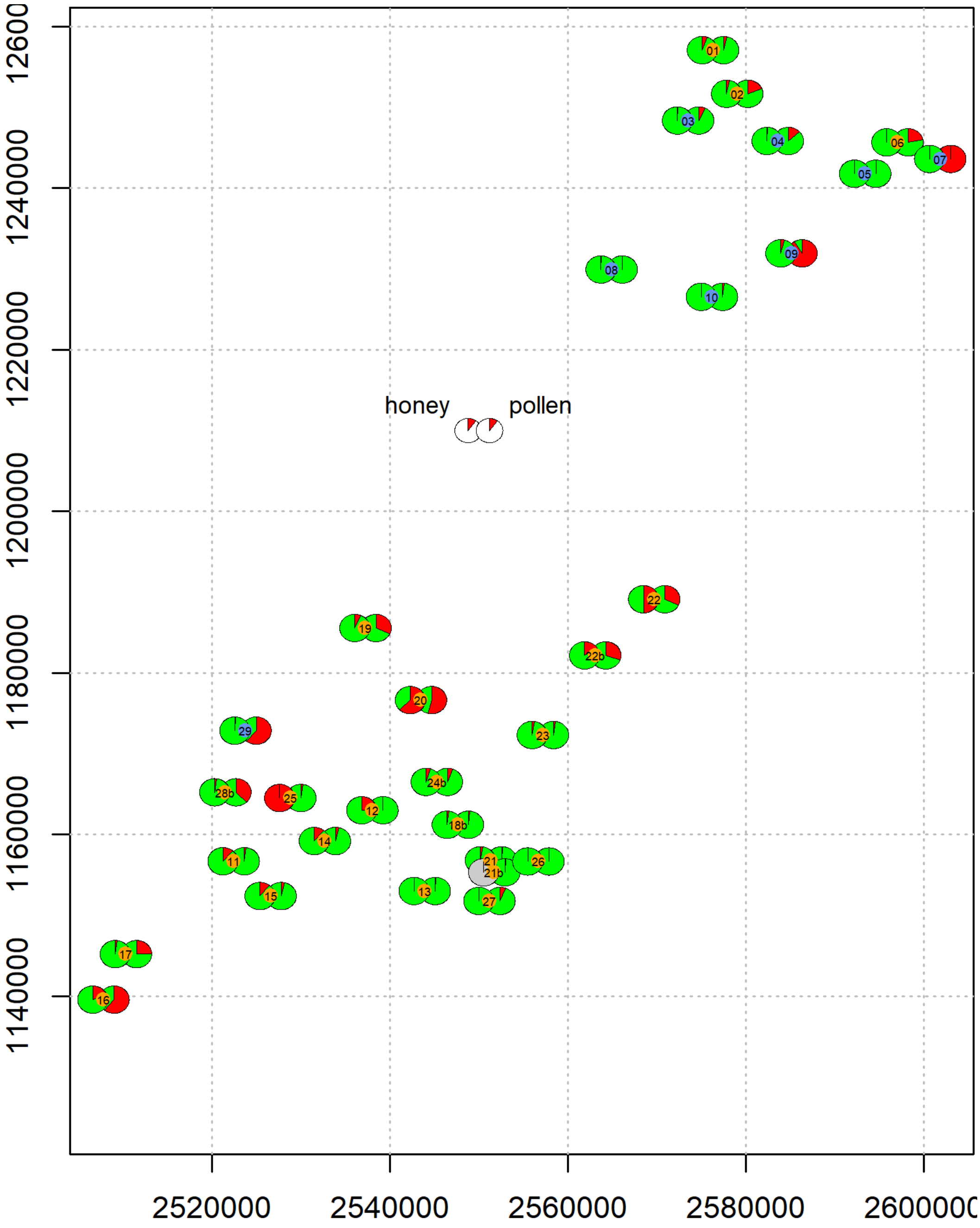


Figure 9: Spatial distribution of total neonicotinoid concentration according to apiary coordinates. Values are truncated at 10 ng/g (full red circle) and numbers in the middle correspond to apiaries. Blue = grassland/meadow and orange = crops. (Circles under 1200000 correspond to apiaries located in Vaud and others from Jura and Bern canton).

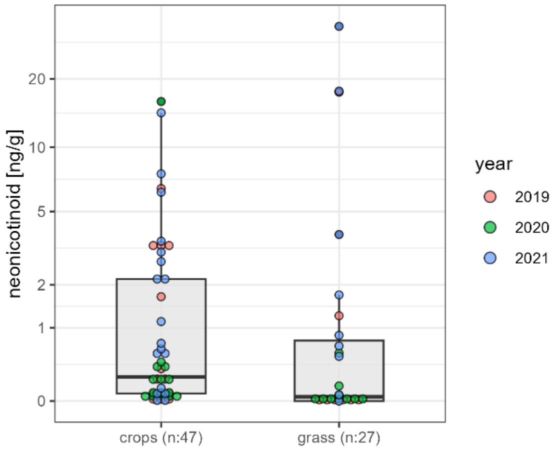


Figure 10: Comparison of the total neonicotinoid concentration between crops and grassland.

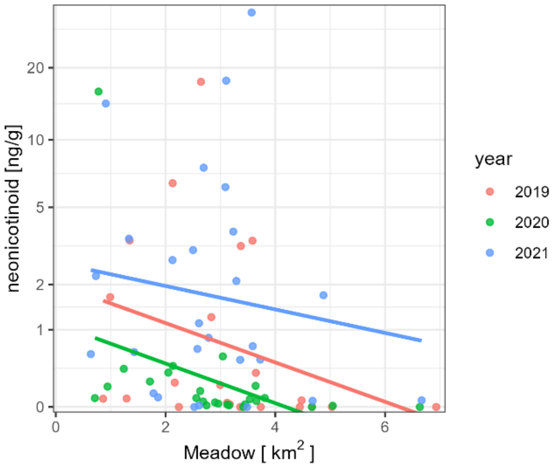


Figure 11: Association between total neonicotinoid concentration (ng/g) and size of meadow (km^2).

3.3 Effect of contaminants on selected colony size parameters

3.4.1 Spring and summer brood size

We fitted a linear mixed-effects model to test if brood size in spring (visit 2) and summer (visit 3) was influenced by and several predictor variables, including neonicotinoid total concentration, non-neonicotinoid pesticides total concentration, year, and their interactions. A total of 707 observations from 31 colonies were included in the analysis, after excluding cases with the absence of capped brood cells. No significant effects of neonicotinoid and other pesticides on brood size in spring (Fig. 13) were found (p-value>0.05). Surprisingly, the predicted values of our model suggested a positive (but not significant) correlation between the levels of neonicotinoid and other contaminants, and the predicted values of brood size in spring. Specifically, the greater the concentration of neonicotinoid and other contaminants, the higher the predicted brood size, as illustrated in Figure 12. At visit 3, the interaction model revealed a significant negative effect of non-neonicotinoid pesticides on brood size (estimate=-3305, t-value=-2.7, p-value=0.008). In addition, we observed a significant negative effect of neonicotinoid contaminated concentration on brood size (estimate=-159, t-value=-3.8, p-value=0.0001), as well as a significant positive effect of the year 2021 (estimate=1688, t-value=3.6, p-value=0.0002) compared to the 2019 (Fig. 14; See appendix A.5.1).

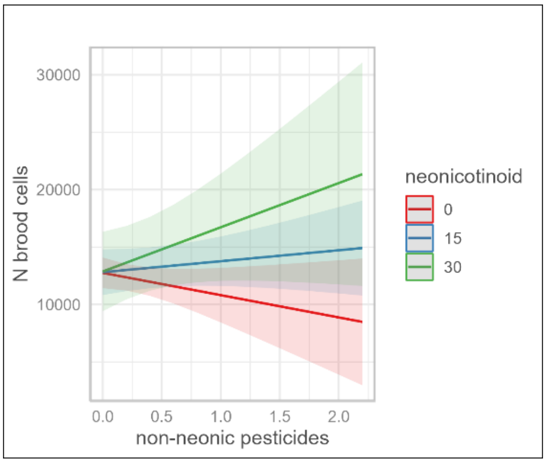


Figure 12: Predicted values of brood size according to neonicotinoids and other contaminants (toxic).

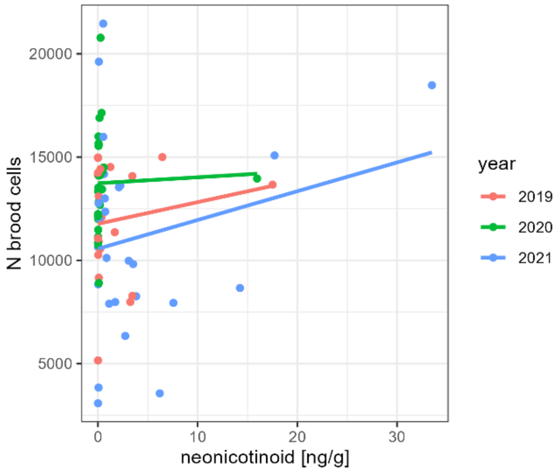


Figure 13: Association between total neonicotinoid concentration (ng/g) and brood size (N) at visit 2 (spring).

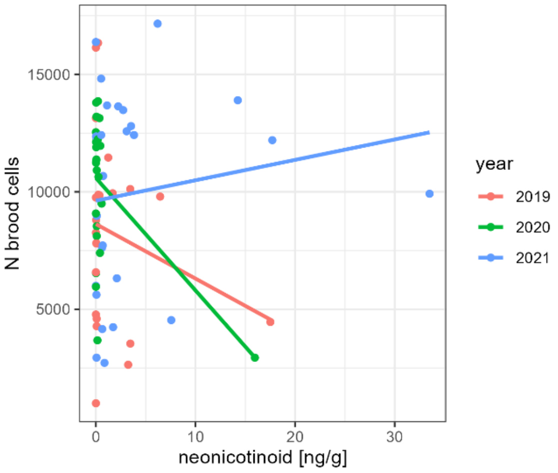


Figure 14: Association between total neonicotinoid concentration (ng/g) and brood size (N) at visit 3 (summer).

3.4.2 Pollen stocks area

A linear mixed-effects model was fit to assess the interaction between the quantity of pollen in the honeybee colony in spring (visit 2) and summer (visit 3), neonicotinoid cumulated concentration and year. A minimum of 667 observations and 31 colonies were included in the analysis after excluding cases with no pollen stock. At visit 2, the estimate for neonicotinoid cumulated concentration (estimate=-0.32, t-value=-3.7, p-value=0.0002) was negative, indicating that higher neonicotinoid cumulated concentration and the year 2020 were associated with lower pollen stocks (Fig. 15). However, the estimate for the year 2021 was positive but not statistically significant (See appendix A.5.2). At visit 3, the results suggest that neonicotinoid cumulated concentration had a significant negative effect on pollen stocks area (estimate=-0.42, t-value=-3.1, p-value=0.002), indicating that higher neonicotinoid concentrations are associated with lower pollen stocks in summer (Fig. 16). The year 2021 also has a significant negative effect on pollen stocks area (estimate=-4.50, t-value=-3 p-value=0.002), indicating that pollen stocks were lower in 2021 compared to other years in the dataset. The year 2020 has a positive effect on the pollen stock area, but this effect is not significant (estimate=1.91, t-value=1.3, p-value=0.1) (See appendix A.5.2).

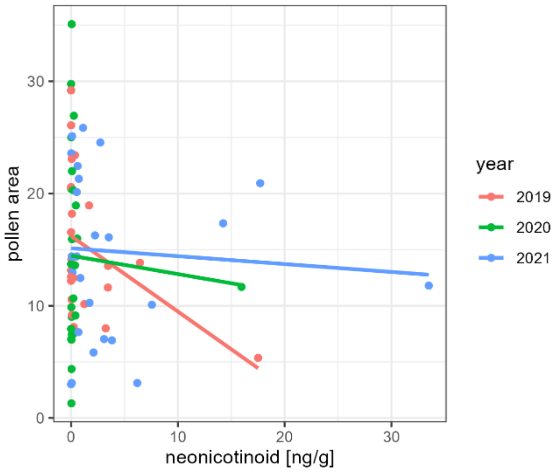


Figure 15: Association between total neonicotinoid concentration (ng/g) in spring pollen and pollen stock area (dm^2) at visit 2 (spring).

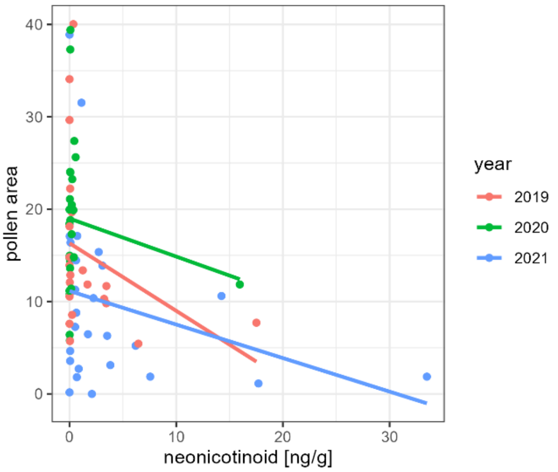


Figure 16: Association between total neonicotinoid concentration (ng/g) in spring pollen and pollen stock area (dm^2) at visit 3 (summer).

4. Discussion

4.1 Overall contaminant analysis and missing values

Missing values were explained by a withdrawal from the project of partner beekeepers as well as low honey harvest driven by weather conditions and apicultural practices. It was therefore difficult to ensure sampling regularity over the three years for colony monitoring with honey and pollen samples. Honey is the matrix where consistency has been the most difficult to achieve, with the average honey harvested in 2019 and 2021 being below normal. Indeed, those two years were presented by our partner beekeepers as particularly challenging from a meteorological point of view. Beekeepers related an early start of the flowering season with warm weather followed by a quick drop in temperature and rain which stopped the bees in their foraging activity. Consequently, we suggest that the amount of pollen and nectar brought back to the colony by foragers was sufficient to meet the demand for beebread, but poor (or no) honey stocks remained. As beebread is used to feed the larvae, the colony could be further affected, and the apiary's productivity (seen as honey from the beekeeper's point of view) has been impacted with some apiaries being exempt of honey. In addition, each pollen pool (spring, summer, and autumn) was sampled at least twice whereas honey was sampled only once during spring and summer harvests (See chapter 2.1.2, Fig. 2). Hence, the use of pooled pollen as the main matrix for analyzing contaminant residues seems to be more suitable for a long-term study as it is not an object of economic value, not limited by the beekeeper's harvest and collected more frequently.

Finally, the overview figures of neonicotinoid and other contaminants showed the importance of considering the potential "cocktail effect" of those molecules on honeybee's colony size (See chapter 3.1, Fig. 3 & 4). Even though herbicides and fungicides were considered non-toxic to *Apidea* for a long time, Almasri et al. (2020) demonstrated that a cocktail of imidacloprid and glyphosate (herbicide), imidacloprid and difenoconazole (fungicide), and the combination of three molecules decreased the survival rate of honeybees after a 20-days chronic exposure at low-realistic concentration. Thus, the pesticide registration procedure's approach - primarily relying on individual acute toxicity - has notable shortcomings in detecting bee-toxic pesticides.

In our case, a more complete analysis of the spring-summer and summer-autumn pollen pools could help to better evaluate the direct and indirect (delayed) neonicotinoid effect on honeybee colony health parameters. To thoroughly investigate direct contamination and its effects, a highly effective approach would involve closely monitoring beehives to gather and analyze freshly collected nectar at very short intervals.

4.2 Neonicotinoids contamination overview

As seen in the previous discussion part, sampling the pollen matrix seems more reliable than sampling the honey matrix for long-term analysis. However, the analysis of honey samples allowed us to compare years and molecule residues with a stronger detection rate and a larger set of samples. In addition, it permitted us to highlight differences between matrices. We observed that pollen sample contamination rates were higher than in honey in 2020 and 2021. Yet, the year 2019 showed a lower concentration of each molecule in pollen compared to honey samples. This may be the result of better conservation of neonicotinoid molecules in honey than in pollen. However, no comparative study has been found in the literature. Hence, a proper degradation study of these molecules in pollen and honey would be needed to test this assumption. Another explanation could be linked to the methodology. The analyzed pollen pool (spring) was the result of three pollen traps activated six to nine days at the beginning and at the end of May (See Chapter 2.1.2, Fig. 2). Therefore, the chances of non-synchronicity between neonicotinoid application and pollen trap activation were higher than for honey which is the result of several months of nectar foraging. Moreover, not all fields around an apiary were treated with neonicotinoids or contained residues of systemic molecules used in the past. Hence, the concentration found in matrices also depends on diverse flowering times, foragers' choices, and weather, as well as neonicotinoid persistence in soils and uptake by following crop or non-crop plants. Finally, the implemented experimental design necessitated the data processing to be conducted on an apiary basis, as the pesticide residue analysis was limited to only three out of the ten colonies that constitute an apiary. This ruled out potential contamination from the feeding of other colonies. Therefore, the obtained contaminant results for each apiary represent only a small portion of the potential exposure that an entire apiary could have experienced.

4.2.1 Thiamethoxam, clothianidin and imidacloprid

Low concentrations or absence of thiamethoxam, clothianidin and imidacloprid in both honey and pollen samples suggest a positive consequence of the European ban on neonicotinoid pesticides adopted in 2018 (EUR-Lex, Commission Implementing Regulation, 29.05.2018). The ban aimed at the three mentioned molecules and their outdoor seed-coated prophylactic application (OPPh., RS 916.161, Art. 86f, status on 01.04.2023). In Switzerland, the suppression of thiamethoxam and clothianidin from the market has come into force in July 2020, and in July 2021 for the products containing imidacloprid (OPPh., RS 916.161, Art. 86f, status on 01.07.2022). However, documentation shows that time limits for sales and use have been granted for both thiamethoxam and Imidacloprid (See appendix A.2). A product containing thiamethoxam for seed treatment application could be used until July 2022. Four Imidacloprid-based products could be used until June 2022 including two for seed treatments, but no time limit for the disposal of product stocks has been granted for clothianidin[3] which is a metabolite of thiamethoxam (Daniele et al., 2017). Other hypotheses may also explain these systemic molecules' contamination of honey and pollen samples.

First, thiamethoxam, clothianidin and imidacloprid neonicotinoids could have been uptaken from the soil by following crops which meet the current body of evidence regarding the persistence of the molecules in the environment (Bonmatin et al., 2015). The most used seed treatments containing thiamethoxam, clothianidin or imidacloprid have half-lives in the soil generally between 200 and 1000 days (Goulson et al., 2013) and would depend on the type of soil, UV radiation, moisture content, temperature, and soil pH (Bonmatin, et al., 2015; Wood & Goulson, 2017). Undeniably, agricultural soils contain detectable amounts of neonicotinoids over a year after planting treated seeds, which suggests that neonicotinoids persist for a longer duration than the typical annual agricultural cycle, leading to frequent uptake by wild plants surrounding agricultural fields (Wood & Goulson, 2017). Also, an analysis done by Botía et al., (2015) demonstrated that all three molecules were detected in a 54-pollen wildflower sample, showing non-targeted plant contamination. In this vein, a study designed to reduce the transport of neonicotinoid from agricultural fields through edge-of-field buffers sequestration highlighted that crimson clover (*Trifolium incarnatum*) could accumulate up to 50% of the applied neonicotinoid in the soil, suggesting a further risk to pollinators (Morrison et al., 2023). Finally, imidacloprid has a longer half-life than thiamethoxam and clothianidin (Goulson et al., 2013; Jones et al., 2014) which, combined with the expanded time limit granted to imidacloprid, could explain its higher contamination rate in our samples compared to thiamethoxam and clothianidin.

Second, contamination of honey and pollen samples by three highly regulated neonicotinoids might indicate a drift phenomenon either by leaching or by aerial pathways (Wood & Goulson, 2017). Solubility of thiamethoxam, clothianidin and imidacloprid in water varies regarding

---

[3] List - « Produits phytosanitaires retirés avec délais d'écoulement de stocks et d'utilisation », status on 18.08.2022, OSAV. Available at : www.blv.admin.ch (accessed 01.05.23)

molecule and local conditions such as ambient temperature, water pH and the form in which the neonicotinoids are applied (granules, spray, seed coating, etc.) (Bonmatin et al., 2015; Wood & Goulson, 2017). Moreover, neonicotinoids have been identified entering waterways via direct leaching into groundwater, decay of treated plant material, and dust from treated seeds or spray drift (Krupke et al., 2012; Nuyttens et al, 2013). Therefore, even though the use of products containing thiamethoxam, clothianidin, and imidacloprid are strongly regulated, their systemic nature as well as their ability to drift from targeted crops to non-targeted vegetation through various pathways, combined with stock clearance time could explain their presence in honey and pollen samples.

4.2.2 Acetamiprid and thiacloprid

The contamination levels of acetamiprid and thiacloprid in honey and pollen samples illustrated the regular use in 2019, 2020 and 2021 of these two molecules which were registered for outdoor and indoor use on a plethora of crops. According to the Swiss phytosanitary index[4], 23 products containing acetamiprid and 18 containing thiacloprid were registered at the time our samples were taken. The increase of both acetamiprid and thiacloprid in our analysis between 2020 and 2021 could be interpreted as a consequence of meteorological conditions. Indeed, monthly and summer reports showed more precipitation and sunshine duration in 2021 compared to 2020[5] (See appendix A.6.1 and A.6.2). As a result, pests thrived and more pesticides are needed to treat crops (Dewar & Aiming, 2020; Mills et al., 2020). Another explication can be that the honeybees' foragers flying time - influenced by temperature and solar radiation (Clarke & Robert, 2018) - could be linked with the time when professionals or non-professional actors could apply phytosanitary treatment as each behavior is weather dependent. This could be particularly true for pollen analysis, as sampling took place during short and specific times, also influenced by weather patterns.

4.2.3 Contaminants distribution according to landscape type

Our statistical models suggested that landscape factors and the year of sample collection played a role in neonicotinoid concentration, grassland, and the year 2021 being significant predictive factors. Extensive arable fields are inherently less subject to phytosanitary treatments. In fact, Swiss Phytosanitary Index[6] indicates that only three molecules (fungicides or insecticides) are homologated to treat grassland pests. However, our results suggested that

---

[4] https://www.psm.admin.ch/fr/wirkstoffe (accessed 28.04.23)

[5] https://www.meteosuisse.admin.ch/ (accessed 28.04.23)

[6] https://www.psm.admin.ch/fr/wirkstoffe (accessed 10.05.23)

several pollens sampled at apiaries located near grasslands such as R07 (53.6ng/g), R09 (17.7ng/g), and R29 (17.5ng/g) showed strong contaminations. This highlights a limitation of the Principal Component Analysis (See appendix A.3) carried out to discriminate between landscapes dominated by grassland and annual crops, as well as a limitation of the experimental design to address ecotoxicological questions. Indeed, samples collected at the apiaries R07, R09 and R29 could have been highly contaminated by only a small intensive colza field in a grassland-dominated landscape. Palynological analysis tends to support this hypothesis as corresponding pollen samples were all composed of a majority of *Brassica napus* (Oil seed rape) or *Prunus sp.* (data not shown). In addition, a study conducted by Schaad et al. (2023) in an apiary located in an agricultural region of Switzerland revealed that the presence of thiacloprid in beebread coincided with the flowering of oil seed rape in spring. Our results, therefore, enhance the body of evidence relating to hives matrices contamination by neonicotinoids in agricultural areas. However, several other samples are strongly contaminated by neonicotinoids although their composition is dominated by non-crop species. This supports the hypothesis of diffuse contamination from conventional management type or a conventional arable past to actual grassland as demonstrated in Switzerland by Humann-Guilleminot et al. (2019) and Riedo et al. (2022).

4.3 Influence of contaminants on brood size and pollen stocks area

4.3.1 Brood size

Our results showed a significant effect of pesticides and neonicotinoid-cumulated concentration on brood size in summer, which may suggest a delayed deleterious effect of spring neonicotinoid-contaminated pollen. Our results are consistent with the findings of Wu-Smart & Spivak (2017) who found that colonies fed with imidacloprid in syrup (10, 20, 50 and 100ng/g) showed long-term negative effects on brood production (as well as food stores[7]), after a 23-days chronic exposure. Regarding food stores, Wu-Smart & Spivak (2017) findings indicated that “significant differences among treatment levels became more prominent with colony size”, indicating that smaller colonies showing less resilience might not be able to buffer agrochemical exposure. In fact, honeybees appear to be less susceptible to stressors compared to other species due to so called “superorganism resilience” (Straub et al., 2015). Hence, we suggest that colony size may be integrated as another predictive variable in further multifactorial analysis inside the “Agriculture and Pollinators” project.

---

[7] In Wu-Smart & Sivak (2017), food stores are the number of cells completely or partially filled with nectar or honey and pollen.

Lastly, our models suggested a potential link between neonicotinoids and toxicity with an increase in brood size, which should be carefully considered. It's important to note that higher crop density indicates a greater availability of floral resources, which in turn leads to an increase in brood size (Alaux et al., 2017; Decourtye et al., 2010; Hernandez et al., 2022). However, as previously discussed (See paragraph 4.2.3), higher crop density may be linked with a greater use of phytosanitary products (Lundin et al., 2015), making it difficult to distinguish between the effect of more floral resources and toxicity. Ultimately, our results underscore the challenge of interpreting data in such scenarios and discriminating the effect of floral resources availability from toxicity on brood size.

We acknowledge that individual control of these factors in a field condition like “Agriculture and Pollinators” project was practically impossible due to the plurality of landscapes surrounding the apiaries and the lack of information concerning pesticides treatment applied within the 2 km-radius sectors around the monitored apiaries (See chapter 2.1). To overcome this challenge, we propose to compare samples from different apiaries that share strong similarities in terms of landscape type and biogeography. However, these apiaries would be in proximity to farms that employ different cropping practices. In theory, a Principal Component Analysis (PCA) would be needed to group apiaries according to landscape type and biogeographical parameters. This method would help us identify clusters or similarities among the apiaries. Next, the apiaries' agricultural environment should be classified according to crop type and cultivation practices. This step will ensure that the only significant difference between the apiaries lies in the cropping practices of the surrounding fields, such as organic or conventional farming. In doing so, we would aim to isolate the influence of different pesticides treatment on the observed outcomes (e.g., brood size), while minimizing the effects of floral resource availability. Conversely, Humann-Guilleminot et al. (2019) found diffuse contamination by neonicotinoid in organic soil and traces on organic seeds which highlights the difficulty to attain a negative control in field studies. Nevertheless, the average neonicotinoids concentration in organic soil (0.6ng/g) and plants (0.2ng/g) were ten times lower than in conventional farming soils (6.36ng/g) and plants (2.11ng/g) (Humann-Guilleminot et al., 2019). We therefore support that it would be possible to sense a difference between cropping practices on colony size parameters.

4.3.2 Pollen stocks area

Our results indicate that neonicotinoid-contaminated spring pollen had a significant reductive and delayed impact on the summer pollen stocks area. It is known that sub-lethal neonicotinoids' neurotoxic action can disorientate bees and impair their foraging ability (Desneux et al., 2007; Friol et al., 2017; Yang et al., 2008; Woodcock et al., 2017). However, pollen isn't directly consumed by foragers but mixed with nectar by worker bees and stocked as beebread after lactic acid fermentation (Vásquez & Olofsson, 2009). The beebread is then consumed by the colony members and processed by the nurse bee cohort to produce royal jelly, for larval feeding (Böhme et al., 2018; Roessink & van der Steen, 2021). The uptake of multiple pesticides by nurse bees from a pollen source resulted solely in minor contamination of royal jelly (Böhme et al., 2018; Végh et al., 2023). Yet, nurse bees may be more frequently exposed to pesticides in pollen than the rest of the colony (Roessink & van der Steen, 2021) and play a key role in detoxification (Vannette et al., 2015). Hence, neonicotinoid-contaminated pollen may impact nurse bees at early stages of life (6 to 12 days) and lead to neurologically damaged workers and foragers at further stages (12 to 35 days) because of sub-lethal chronic exposure. In contrast with royal jelly which showed only minor contamination according to Böhme et al (2018), beebread - which is typically used to feed worker bees and developing brood - accumulates more pesticides than bees and wax (Daniele et al., 2017). Hence, pollen stored in the form of beebread can have deleterious effects on the brood. As a result, this may impact further adult foraging capacity which translates into a decrease of pollen stocks area in our case. In this vein, our results are consistent with those of Morfin et al., (2019) who showed that larvae exposed to clothianidin (0.67 and 1.33ng in water) significantly reduced their foraging activity when evaluated 25-36 days later as adult bees, which may compromise pollen collection. Evidence from several *in vitro* experiments documented that newly emerged bees fed with neonicotinoid at larval stages showed several altered traits in organs analyzed by transmission electron microscopy. These morphological changes may reduce foraging ability as documented by Friol et al., (2017) who found that when exposed to thiamethoxam (1ng/g) at larval stages, newly emerged bees showed alterations in the mushroom bodies (MBs). Moreover, almost the same group of searchers found a decrease in the synapsin level in the MBs of pupae previously exposed to 1 and 1440ng/g thiamethoxam, as well as a decrease in MBs and antennal lobes (ALs) in newly emerged bees previously exposed to 1440ng/g[8] (thiamethoxam) (Tavares et al., 2019). As the MBs are brain structures involved in stimuli reception and are associated with both the process of learning and memory (Sandoz, 2013), the damage caused in such structures as well as a decrease of synapsin level - an important hormone for the regulation of

---

[8] 1440ng/g represents 1/10 of LC_{50} (Lethal Concentration 50%) for the Africanized honeybees (Tavares et al., 2019).

neurotransmitter release - could lead to disorientation of the bee and harm their foraging ability (Friol et al., 2017; Tavares et al., 2019). Another study by Tavares et al. (2017), found that a significant decrease of the adult emergence rate was observed with thiamethoxam exposure at a concentration of 1ng/g because of the increased mortality of larvae. It is important to note that none of our contaminated samples contained as much thiamethoxam. Nevertheless, nothing is known about the effect of realistic pesticide cocktails on these morphological changes and our results may encourage further research in this direction. The "cocktail effect" refers to the combined impact of multiple pesticides (mixture), which may interact synergistically or additively, potentially resulting in intensified effects compared to individual pesticides alone (Almasari et al., 2020; Kortenkamp & Faust, 2019). Despite the recognized importance of studying this phenomenon, such assessments have not been undertaken in Switzerland. In addition, the pesticide registration procedure’s approach primarily relies on individual acute toxicity which sets aside the accumulation effect. For this reason, a Mixture Assessment (Allocation) Factor (MAF) has been developed and proposed to assess the risks associated with contaminants cocktail when registering phytosanitary products (Kortenkamp & Faust, 2019).

All our samples contaminated by thiamethoxam - which we recall is a prohibited molecule for outdoor use since 2018 in the European Union - had a higher or equal concentration of 0.01ng/g. However, after exposure to this molecule at 0.01ng/g pupae and emerging bees displayed a significant increase in Acetylcholinesterase (AChE), an enzyme associated with the nicotinic acetylcholine receptors (nAChR) to which neonicotinoid strongly binds (Tavares et al., 2017). The increase of AChE might be a biological response to compensate for the permanent activation of cholinergic neurons caused by the neonicotinoid (Tavares et al., 2017), thus emphasizing long-term exposure and adaptation of honeybees to neonicotinoids.

Even though deleterious effects of larval thiamethoxam exposure on newly emerged bees have been demonstrated, adult honeybees exhibit variations in their sensibility to different neonicotinoid insecticides, with a significantly lower sensitivity towards cyano-substituted[9] neonicotinoids like thiacloprid (Alptekins et al., 2016). Our findings therefore suggest that a cumulated concentration of neonicotinoid dominated by acetamiprid and thiacloprid (See Appendix A.1.4) found in spring pollen, decreased further pollen stocks which may suggest a deleterious effect of larval exposure to a “cocktail” of neonicotinoid on the foraging capacity

---

[9] Type of neonicotinoid that contains a cyano group (CN) as a substituent of hydrogen in the chemical structure of the compound. The cyano group alters the chemical properties of the neonicotinoid compound including its stability, solubility, and interaction with target receptors in insects (Iwasa et al., 2004).

of newly emerged bees. Consequently, brood production might be negatively impacted, depending on pollen stock quantity (Requier, 2019). Moreover, contaminated spring pollen could also impact further foragers after being chronically exposed at nurse stages and explain the delayed reduction of pollen stocks. However, while our models showed significant effects of cumulative neonicotinoid concentration on pollen stock area and brood size, the cause of a reduction in pollen stock could mean greater feeding activity, and the sign of an efficient, brood-producing colony. Also, fewer pollen reserves could mean fewer floral resources as well as a perturbed foraging time. In this vein, it is crucial to consider that the year 2021 had a significant impact on nearly all our models suggesting a strong weather influence on both brood size and pollen stocks area (Kretzschmar & Frontero, 2017; Odoux et al., 2014).

Finally, to fully consider the contamination of neonicotinoids in pollen and honey matrices as well as their potential sub-lethal impact on colony size parameters, it might be crucial to integrate an analysis of the metabolites of thiamethoxam, clothianidin, imidacloprid, acetamiprid and clothianidin in further research. Studies found that neonicotinoid metabolites were detected with more frequency and at higher average concentrations in honey and pollen samples than the untransformed molecule (Suchail et al., 2009; Coddling et al., 2016). Also, Coddling et al. (2016) highlight that imidacloprid-olefin - an metabolite - has a greater toxic potency than the untransformed molecule.

5. Conclusion

Our data indicate that the use of pooled pollen as a matrix for contaminant residue analysis has proved well suited and better than honey for long-term studies in an agricultural environment in Switzerland, as it has no economic value, is not dictated by the beekeeper's harvest, and is sampled at least twice to form a pool. Although some years were dominated by unfavorable weather conditions for data collection, our methodology shows that pollen pooling enables us to keep an adequate number of samples for further analysis. Our analysis shows that despite stringent regulation governing the use of thiamethoxam, clothianidin, and imidacloprid their systemic nature as well as their potential drift from targeted crops to non-targeted plants through various pathways may explain their presence in our samples. Also, our findings suggest that neonicotinoids were less present in grassland than in crop-dominated landscapes. However, some samples were strongly contaminated in grassland-dominated landscapes most probably by only small intensive fields or crop rotation. Thus, indicating a limit of our methodology and the strong persistence of neonicotinoids in the environment. Regarding honeybee colony size, our results suggest that a cumulated concentration of neonicotinoid dominated by acetamiprid and thiacloprid found in spring pollen decreased further pollen stocks area and brood size. These results may advocate deleterious effects of larval exposure to a mixture of neonicotinoid and other pesticides on the foraging capacity of newly emerged bees, as well as a deleterious effect on nurse bees' foraging future. In mixture ecotoxicology - a branch of ecotoxicology specialized in assessing the effects of contaminant cocktails - data are consistent: the total risk of a mixture (cocktail of contaminants) exceeds the risk of each individual component (Kortenkamp & Faust, 2019). An allocation factor may be used in further field condition studies such as the "Agriculture and Pollinators" project to evaluate and then integrate the total toxicity of a contaminant's mixture in statistical models. Finally, it is crucial to consider that the observations were conducted across three years spanning from 2019 to 2021. In fact, the colonies' development patterns were impacted by varying climatic conditions during each year, which affected the availability of floral resources, including pollen, thus the colony size. Mitigating the effect of years would require either a longer-time study or an adaptative data collection following bioindicators more than an *a priori* fixed schedule. As our study was conducted in close relationship with the agricultural sector, we think that farmers' practical management, inherently dictated by meteorological conditions, could be used as benchmarks for sampling.

Bibliography

Alaux, C., Allier, F., Decourtye, A., Odoux, J.-F., Tamic, T., Chabirand, M., Delestra, E., Decugis, F., Le Conte, Y., Henry, M., 2017. A "Landscape physiology" approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats OPEN. Scientific Reports 7. https://doi.org/10.1038/srep40568

Almasri, H., Tavares, D.A., Pioz, M., Sené, D., Tchamitchian, S., Cousin, M., Brunet, J.-L., Belzunces, L.P., 2020. Mixtures of an insecticide, a fungicide and a herbicide induce high toxicities and systemic physiological disturbances in winter Apis mellifera honey bees. Ecotoxicology and Environmental Safety 203, 111013. https://doi.org/10.1016/j.ecoenv.2020.111013

Alptekin, S., Bass, C., Nicholls, C., Paine, M.J.I., Clark, S.J., Field, L., Moores, G.D., 2016. Induced thiacloprid insensitivity in honeybees (Apis mellifera L.) is associated with the up-regulation of detoxification genes. Insect Molecular Biology 25, 171–180. https://doi.org/10.1111/imb.12211

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86, 412–431.

Bonmatin, J.-M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A.D., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ Sci Pollut Res 22, 35–67. https://doi.org/10.1007/s11356-014-3332-7

Chouquer, G., 2003. Françoise Burel et Jacques Baudry, Écologie du paysage. Concepts, méthodes et applications. Études rurales 329–333. https://doi.org/10.4000/etudesrurales.2968

Clarke, D., Robert, D., 2018. Predictive modelling of honey bee foraging activity using local weather conditions. Apidologie 49, 386–396. https://doi.org/10.1007/s13592-018-0565-3

Codling, G., Al Naggar, Y., Giesy, J.P., Robertson, A.J., 2016. Concentrations of neonicotinoid insecticides in honey, pollen and honey bees (Apis mellifera L.) in central Saskatchewan, Canada. Chemosphere 144, 2321–2328. https://doi.org/10.1016/j.chemosphere.2015.10.135

Commission Implementing Regulation (EU) 2018/783 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance imidacloprid (Text with EEA relevance.), 2018., OJ L.

Commission Implementing Regulation (EU) 2018/784 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance clothianidin (Text with EEA relevance.), 2018., OJ L.

Commission Implementing Regulation (EU) 2018/785 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance thiamethoxam (Text with EEA relevance.), 2018., OJ L.

Cornu, P., Valceschini, E., Bournay, O.M., Mauguin, P., 2018. L'histoire de l'Inra, entre science et politique. Editions Quae.

Daniele, G., Giroud, B., Jabot, C., Vulliet, E., 2018. Exposure assessment of honeybees through study of hive matrices: analysis of selected pesticide residues in honeybees, beebread, and beeswax from French beehives by LC-MS/MS. Environ Sci Pollut Res 25, 6145–6153. https://doi.org/10.1007/s11356-017-9227-7

Decourtye, A., Mader, E., Desneux, N., 2010. Landscape enhancement of floral resources for honey bees in agro-ecosystems. Apidologie 41, 264–277. https://doi.org/10.1051/apido/2010024

Deguines, N., Jono, C., Baude, M., Henry, M., Julliard, R., Fontaine, C., 2014. Large-scale trade-off between agricultural intensification and crop pollination services. Frontiers in Ecology and the Environment 12, 212–217. https://doi.org/10.1890/130054

Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52, 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Dewar, A.M., Qi, A., 2021. The Virus Yellows Epidemic in Sugar Beet in the UK in 2020 and the Adverse Effect of the EU Ban on Neonicotinoids on Sugar Beet Production. Outlooks on Pest Management 32, 53–59. https://doi.org/10.1564/v32_apr_02

Dimou, M., Thrasyvoulou, A., Tsirakoglou, V., 2006. Efficient use of pollen traps to determine the pollen flora used by honey bees. Journal of Apicultural Research 45, 42–46. https://doi.org/10.1080/00218839.2006.11101312

Dubois, E., Reis, C., Schurr, F., Cougoule, N., Ribière-Chabert, M., 2018. Effect of pollen traps on the relapse of chronic bee paralysis virus in honeybee (Apis mellifera) colonies. Apidologie 49, 235–242. https://doi.org/10.1007/s13592-017-0547-x

FAO., 2019. The State of the World's Biodiversity for Food and Agriculture. Rome, Italy. https://doi.org/10.4060/CA3129EN

FAO and WHO, 2019. Detoxifying agriculture and health from highly hazardous pesticides. Rome, Italy.

Farooqui, T., 2013. A potential link among biogenic amines-based pesticides, learning and memory, and colony collapse disorder: A unique hypothesis. Neurochemistry International 62, 122–136. https://doi.org/10.1016/j.neuint.2012.09.020

Fortier, A., Dupré, L., Alphandéry, P., 2020. Les mondes apicoles entre agriculture et environnement. Études rurales 206, 8–26. https://doi.org/10.4000/etudesrurales.23382

Foucart, S. 2019. Et le monde devint silencieux. Comment l'agrochimie a détruit les insectes. Editions du Seuil.

Friol, P.S., Catae, A.F., Tavares, D.A., Malaspina, O., Roat, T.C., 2017. Can the exposure of Apis mellifera (Hymenoptera, Apiadae) larvae to a field concentration of thiamethoxam affect newly emerged bees? Chemosphere 185, 56–66. https://doi.org/10.1016/j.chemosphere.2017.06.113

Gallai, N., Salles, J.-M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68, 810–821. https://doi.org/10.1016/j.ecolecon.2008.06.014

Gallot, M. et al. 2016. Cultures intermédiaires automnales et développement des colonies d'abeilles mellifères. Recherche Agronomique Suisse. 2016, Vol. 7, 3.

Goulson, D., 2013. REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology 50, 977–987. https://doi.org/10.1111/1365-2664.12111

Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined Stress from parasites, pesticides, and lack of flowers. Science 347. https://doi.org/10.1126/science.1255957

Hernandez, J., Maisonnasse, A., Cousin, M., Beri, C., Le Quintrec, C., Bouetard, A., Castex, D., Decante, D., Servel, E., Buchwalder, G., Brunet, F., Feschet-Destrella, E., de Bellescize, K., Kairo, G., Frontero, L., Pédehontaa-Hiaa, M., Buisson, R., Pouderoux, T., Aebi, A., Kretzschmar, A., 2020. ColEval: Honeybee COLony Structure EVALuation for Field Surveys. Insects 11, 41. https://doi.org/10.3390/insects11010041

Hernandez, J., Varennes, Y.-D., Aebi, A., Dietemann, V., Kretzschmar, A., 2023. Agroecological measures in meadows promote honey bee colony development and winter survival. Ecosphere 14, e4396. https://doi.org/10.1002/ecs2.4396

Humann-Guilleminot, S., Binkowski, Ł.J., Jenni, L., Hilke, G., Glauser, G., Helfenstein, F., 2019. A nation-wide survey of neonicotinoid insecticides in agricultural land with implications for agri-environment schemes. Journal of Applied Ecology 56, 1502–1514. https://doi.org/10.1111/1365-2664.13392

Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, R.M., 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, Apis mellifera. Crop Protection 23, 371–378. https://doi.org/10.1016/j.cropro.2003.08.018

Jones, A., Harrington, P., Turnbull, G., 2014. Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years. Pest Management Science 70, 1780–1784. https://doi.org/10.1002/ps.3836

Kammoun, S., Mulhauser, B., Aebi, A., Mitchell, E.A.D., Glauser, G., 2019. Ultra-trace level determination of neonicotinoids in honey as a tool for assessing environmental contamination. Environmental Pollution 247, 964–972. https://doi.org/10.1016/j.envpol.2019.02.004

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc Biol Sci 274, 303–313. https://doi.org/10.1098/rspb.2006.3721

Kortenkamp, A., Faust, M., 2018. Regulate to reduce chemical mixture risk. Science 361, 224–226. https://doi.org/10.1126/science.aat9219

Kretzschmar, A., Frontero, L., 2017. Factors of honeybee colony performances on sunflower at apiary scale. OCL 24, D604. https://doi.org/10.1051/ocl/2017054

Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7, e29268. https://doi.org/10.1371/journal.pone.0029268

Lundin, O., Rundlöf, M., Smith, H.G., Fries, I., Bommarco, R., 2015. Neonicotinoid Insecticides and Their Impacts on Bees: A Systematic Review of Research Approaches and Identification of Knowledge Gaps. PLOS ONE 10, e0136928. https://doi.org/10.1371/journal.pone.0136928

Maxim, L., van der Sluijs, J.P., Gee, D., Grandjean, P., Hansen, S.F., van den Hove, S., MacGarvin, M., Martin, J., Nielsen, G., Quist, D., Stanners, D., 2013. Chapter 16 - Seed-dressing systemic insecticides and honeybees. https://doi.org/10.2800/70069

Millennium Ecosystem Assessment (Program) (Ed.), 2005. Ecosystems and human well-being: synthesis. Island Press, Washington, DC.

Mills, K.B., Madden, L.V., Paul, P.A., 2020. Quantifying the Effects of Temperature and Relative Humidity on the Development of Wheat Blast Incited by the Lolium Pathotype of Magnaporthe oryzae. Plant Disease 104, 2622–2633. https://doi.org/10.1094/PDIS-12-19-2709-RE

Mitchell, E.A.D., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., Aebi, A., 2017. A worldwide survey of neonicotinoids in honey. Science 358, 109–111. https://doi.org/10.1126/science.aan3684.

Moffat, C., Pacheco, J.G., Sharp, S., Samson, A.J., Bollan, K.A., Huang, J., Buckland, S.T., Connolly, C.N., 2015. Chronic exposure to neonicotinoids increases neuronal vulnerability to mitochondrial dysfunction in the bumblebee (Bombus terrestris). The FASEB Journal 29, 2112–2119. https://doi.org/10.1096/fj.14-267179

Morfin, N., Goodwin, P.H., Correa-Benitez, A., Guzman-Novoa, E., 2019. Sublethal exposure to clothianidin during the larval stage causes long-term impairment of hygienic and foraging behaviours of honey bees. Apidologie 50, 595–605. https://doi.org/10.1007/s13592-019-00672-1

Morrison, B.A., Xia, K., Stewart, R.D., 2023. Evaluating neonicotinoid insecticide uptake by plants used as buffers and cover crops. Chemosphere 322, 138154. https://doi.org/10.1016/j.chemosphere.2023.138154

Murcia-Morales, M., Heinzen, H., Parrilla-Vázquez, P., Gómez-Ramos, M. del M., Fernández-Alba, A.R., 2022. Presence and distribution of pesticides in apicultural products: A critical appraisal. TrAC Trends in Analytical Chemistry 146, 116506. https://doi.org/10.1016/j.trac.2021.116506

Nuyttens, D., Devarrewaere, W., Verboven, P., Foqué, D., 2013. Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review. Pest Manag Sci 69, 564–575. https://doi.org/10.1002/ps.3485

Odoux, J.-F., Aupinel, P., Gateff, S., Requier, F., Henry, M., Bretagnolle, V., 2013. ECOBEE: a tool for long-term bee colony monitoring at the landscape scale in West European intensive agrosystems. Journal of Apicultural Research 53. https://doi.org/10.3896/IBRA.1.53.1.05

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution 25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Requier, F., 2019. Bee colony health indicators: Synthesis and future directions. CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources 14, 1–13. https://doi.org/10.1079/PAVSNNR201914056

Requier, F., Odoux, J.-F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., Bretagnolle, V., 2015. Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. Ecological Applications 25, 881–890. https://doi.org/10.1890/14-1011.1

Riedo, J., Herzog, C., Banerjee, S., Fenner, K., Walder, F., Van Der Heijden, M.G.A., Bucheli, T.D., 2022. Concerted Evaluation of Pesticides in Soils of Extensive Grassland Sites and Organic and Conventional Vegetable Fields Facilitates the Identification of Major Input Processes. Environ. Sci. Technol. 56, 13686–13695. https://doi.org/10.1021/acs.est.2c02413

Roessink, I., van der Steen, J.J.M., 2021. Beebread consumption by honey bees is fast: results of a six-week field study. Journal of Apicultural Research 60, 659–664. https://doi.org/10.1080/00218839.2021.1915612

Roßberg, D., Aeckerle, N., Stockfisch, N., 2017. Erhebungen zur Anwendung von chemischen Pflanzenschutzmitteln in Zuckerrüben. Gesunde Pflanzen 69, 59–66. https://doi.org/10.1007/s10343-017-0389-5

Sandoz, J.-C., 2013. Chapter 30 - Neural Correlates of Olfactory Learning in the Primary Olfactory Center of the Honeybee Brain: The Antennal Lobe, in Menzel, R., Benjamin, P.R. (Eds.), Handbook of Behavioral Neuroscience, Invertebrate Learning and Memory. Elsevier, pp. 416–432. https://doi.org/10.1016/B978-0-12-415823-8.00030-7

Schaad, E., Fracheboud, M., Droz, B., Kast, C., 2023. Quantitation of pesticides in bee bread collected from honey bee colonies in an agricultural environment in Switzerland. Environ Sci Pollut Res 30, 56353–56367. https://doi.org/10.1007/s11356-023-26268-y

Steffan-Dewenter, I., Kuhn, A., 2003. Honeybee foraging in differentially structured landscapes. Proc Biol Sci 270, 569–575. https://doi.org/10.1098/rspb.2002.2292

Straub, L., Williams, G.R., Pettis, J., Fries, I., Neumann, P., 2015. Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors. Current Opinion in Insect Science, Neuroscience * Special Section: Insect conservation 12, 109–112. https://doi.org/10.1016/j.cois.2015.10.010

Suchail, S., Guez, D., Belzunces, L.P., 2009. The discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in Apis mellifera. Environmental Toxicology and Chemistry 20, 2482–2486. https://doi.org/10.1002/etc.5620201113

Sutherland, R.J.F.B., Jérémie Forney, Paul Stock, Lee-Ann, 2020. The Good Farmer: Culture and Identity in Food and Agriculture. Routledge, London. https://doi.org/10.4324/9781315190655

Sutter, L., Aebi, A., Buchwalder, G., Caballé, P., Dietemann, V., Girardin, O., Hernandez, J., Jacopin-Bucher, E., Mayor, P., Ménétrier, V., et al. (2019). Agriculteurs, apiculteurs et chercheurs unis pour la sauvegarde des pollinisateurs. Rech. Agron. Suisse, 10:424–429.

Swiss Government, 2014. Plan national de mesures pour la santé des abeilles. https://www.agroscope.admin.ch/agroscope/fr/home/themes/animaux-rente/abeilles/bienen-gesellschaft/massnahmeplan.html (accessed 08.06.23).

Swiss Government, 2017. Plan d'action visant à la réduction des risques et à l'utilisation durable des produits phytosanitaires. https://www.blw.admin.ch/blw/fr/home/nachhaltige-produktion/pflanzenschutz/aktionsplan.html (accessed 08.06.23).

Swiss Government, 2021. AS 2022 263. Loi fédérale sur la réduction des risques liés à l'utilisation de pesticides (Modification de la loi sur les produits chimiques, de la loi sur la protection des eaux et de la loi sur l'agriculture). https://www.bk.admin.ch/ch/f/pore/rf/cr/2021/20210841.html (accessed 08.06.23).

Swiss Government, RS 916.161 - Ordonnance du 12 mai 2010 sur la mise en circulation des produits phytosanitaires (Ordonnance sur les produits phytosanitaires, OPPh), n.d. URL https://www.fedlex.admin.ch/eli/cc/2010/340/fr (accessed 03.15.23).

Swiss Government. RS 910.1 - Loi fédérale du 29 avril 1998 sur l'agriculture (Loi sur l'agriculture, LAgr), n.d. URL https://www.fedlex.admin.ch/eli/cc/1998/3033_3033_3033/fr (accessed 08.06.23).

Tavares, D.A., Dussaubat, C., Kretzschmar, A., Carvalho, S.M., Silva-Zacarin, E.C.M., Malaspina, O., Bérail, G., Brunet, J.-L., Belzunces, L.P., 2017. Exposure of larvae to thiamethoxam affects the survival and physiology of the honey bee at post-embryonic stages. Environmental Pollution 229, 386–393. https://doi.org/10.1016/j.envpol.2017.05.092

Tavares, D.A., Roat, T.C., Silva-Zacarin, E.C.M., Nocelli, R.C.F., Malaspina, O., 2019. Exposure to thiamethoxam during the larval phase affects synapsin levels in the brain of the honey bee. Ecotoxicology and Environmental Safety 169, 523–528. https://doi.org/10.1016/j.ecoenv.2018.11.048

Tsvetkov, N., Samson-Robert, O., Sood, K., Patel, H.S., Malena, D.A., Gajiwala, P.H., Maciukiewicz, P., Fournier, V., Zayed, A., 2017. Chronic exposure to neonicotinoids reduces honey bee health near corn crops. Science 356, 1395–1397. https://doi.org/10.1126/science.aam7470

Underwood, R., VanEngelsdorp, D., 2007. Colony Collapse Disorder: Have we seen this before? Bee Culture 35, 13–18.

Valverde, S., Bernal, J.L., Martín, M.T., Nozal, M.J., Bernal, J., 2016. Fast determination of neonicotinoid insecticides in bee pollen using QuEChERS and ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. ELECTROPHORESIS 37, 2470–2477. https://doi.org/10.1002/elps.201600146 vanEngelsdorp, D., Evans, J.D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B.K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D.R., Pettis, J.S., 2009. Colony Collapse Disorder: A Descriptive Study. PLOS ONE 4, e6481. https://doi.org/10.1371/journal.pone.0006481

vanEngelsdorp, D., Meixner, M.D., 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology 103, S80–S95. https://doi.org/10.1016/j.jip.2009.06.011

Végh, R., Csóka, M., Mednyánszky, Z., Sipos, L., 2023. Pesticide residues in bee bread, propolis, beeswax and royal jelly – A review of the literature and dietary risk assessment. Food and Chemical Toxicology 176, 113806. https://doi.org/10.1016/j.fct.2023.113806

Vannette, R.L., Mohamed, A., Johnson, B.R., 2015. Forager bees (Apis mellifera) highly express immune and detoxification genes in tissues associated with nectar processing. Sci Rep 5, 16224. https://doi.org/10.1038/srep16224

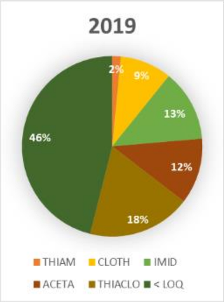
Vásquez, A., Olofsson, T.C., 2009. The lactic acid bacteria involved in the production of bee pollen and bee bread. Journal of Apicultural Research 48, 189–195. https://doi.org/10.3896/IBRA.1.48.3.07

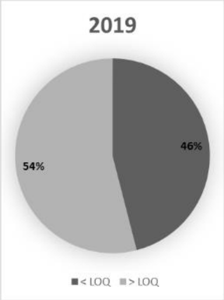
Wood, T.J., Goulson, D., 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. Environ Sci Pollut Res 24, 17285–17325. https://doi.org/10.1007/s11356-017-9240-x

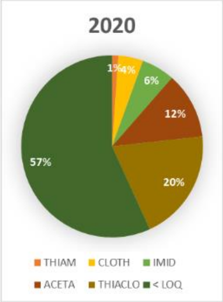
Wu, J.Y., Anelli, C.M., Sheppard, W.S., 2011. Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker Honey Bee (Apis mellifera) Development and Longevity. PLOS ONE 6, e14720. https://doi.org/10.1371/journal.pone.0014720

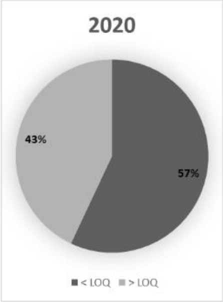
A. Appendix

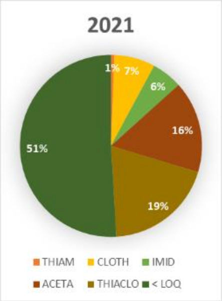
A.1.1 Pie charts - Contamination of honey samples

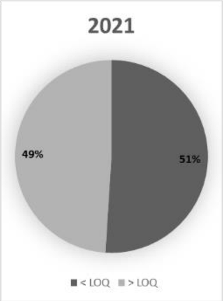




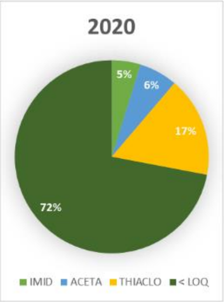


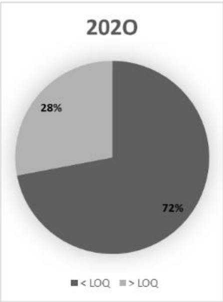


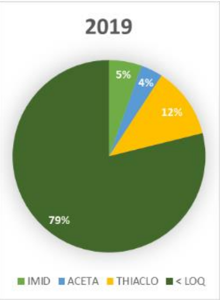


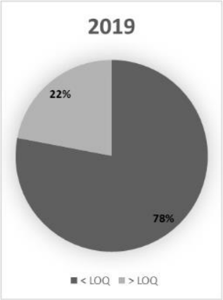


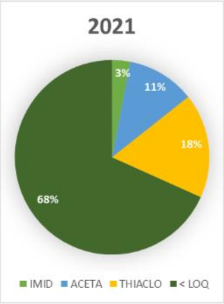
A.1.2 Pie charts – Contamination of pollen samples

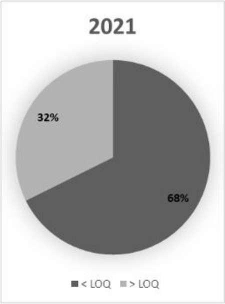












A.1.3 Table - Summary statistics of neonicotinoids measured in a collection of honey from 30 apiaries in Switzerland

	Statistic	Neonicotinoid				
		Acetamiprid	**Clothianidin**	**Imidacloprid**	**Thiacloprid**	**Thiamethoxam**
Total	% samples > LOQ	59.8%	30.9%	41.2%	91.7%	4.1%
	Maximum [ng/g]	6.130	0.720	0.860	53.490	1.94
	Median [ng/g]*	0.038	0.018	16.302	0.013	0.101
	Average [ng/g]*	0.176	0.007	0.016	1.133	0.003

A.1.4 Table - Summary statistics of neonicotinoids measured in a collection of pollen from 30 apiaries in Switzerland

	Statistic	Neonicotinoid				
		Acetamiprid	**Clothianidin**	**Imidacloprid**	**Thiacloprid**	**Thiamethoxam**
Total	% samples > LOQ	37.8%	1.4%	21.6%	78.0%	0.0%
	Maximum [ng/g]	17.711	0.028	0.062	33.498	0.000
	Median [ng/g]*	0.043	0.028	0.033	0.043	0.000
	Average [ng/g]*	0.622	0.000	0.007	1.557	0.000

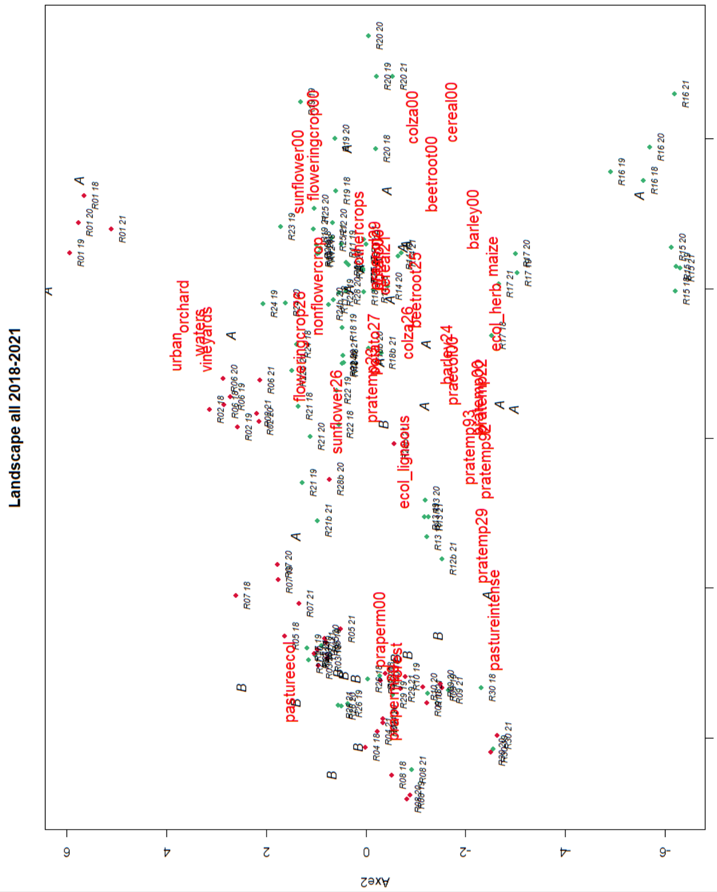
A.2 Table - Summary of Swiss plant protection products index for neonicotinoids

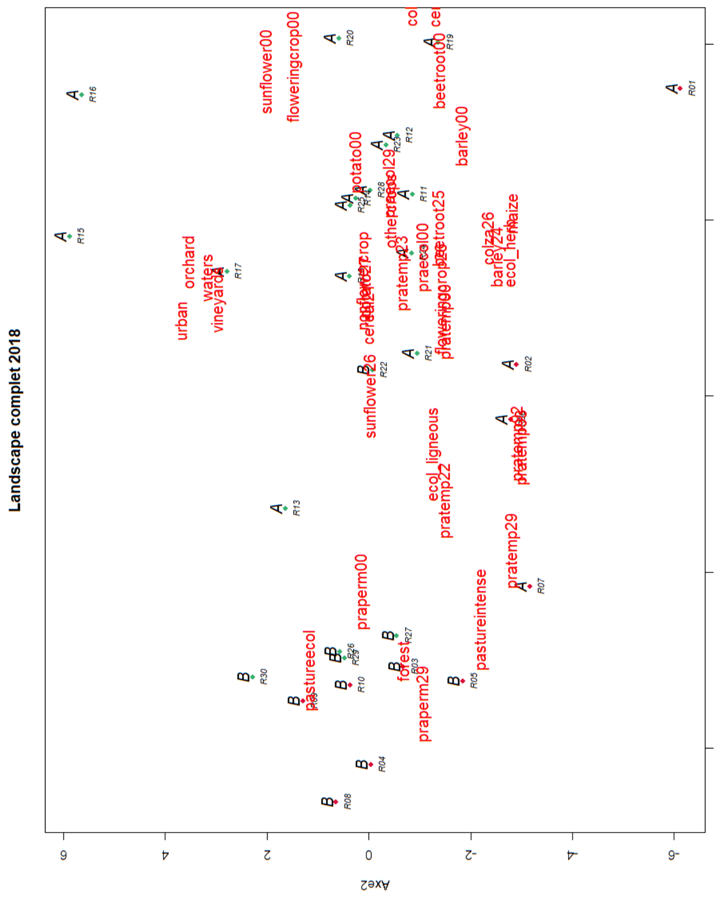
	Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid	Thiacloprid
2011	x	x	x	x	x
2012	x	x	x	x	x
2013	x	x	x	x	x
2015	x	x	x	x	x
2016	x	x	x	x	x,y
2017	x	x	x	x	x,y
2018	x	x	x	x	x,y
2019	x	x	x	x	x,y
2020	x, z	x,z	x	x	x, y
2021	NA	NA	x	x	x
2022	NA	NA	x1 (12.2021), x2 (06.2022)	x	x1 (09.2021), x2 (12.2021)
2023	NA	NA	x1 (12.2021), x2 (06.2022)	x	x1 (09.2021), x2 (12.2021)

References: Phytosanitary Products Ordinance (OPPh) (status on 01.04.2023); Phytosanitary Products withdrawn with delay (status on 18.08.2022).

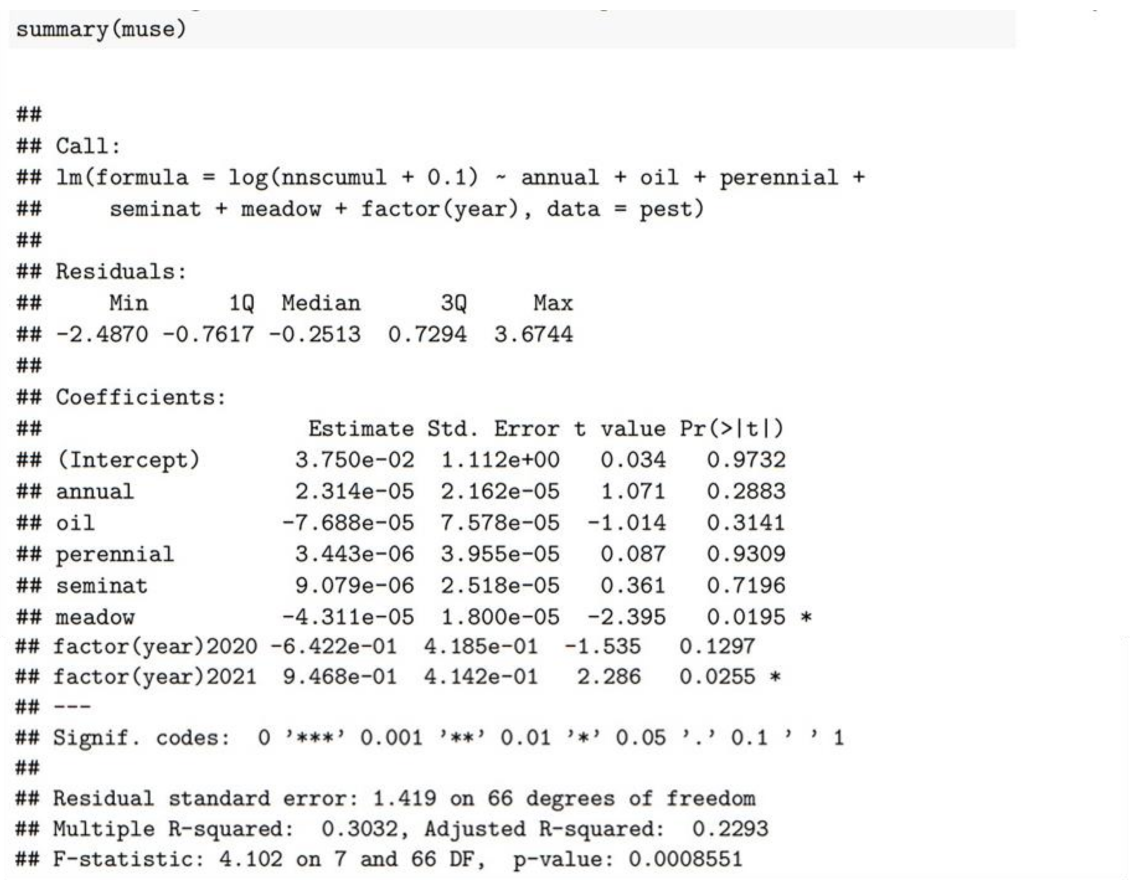
x = approved active substance authorised for use in plant protection products
x0 = approved active substance authorised for use in plant protection products indoor only
x1 = time limit for the sale
x2 = time limit for the use
y = substance being considered for substitution
z = approved active substance to be re-evaluated
NA = do not appears on ordinance

A.3 Principal Component Analysis - Landscape types





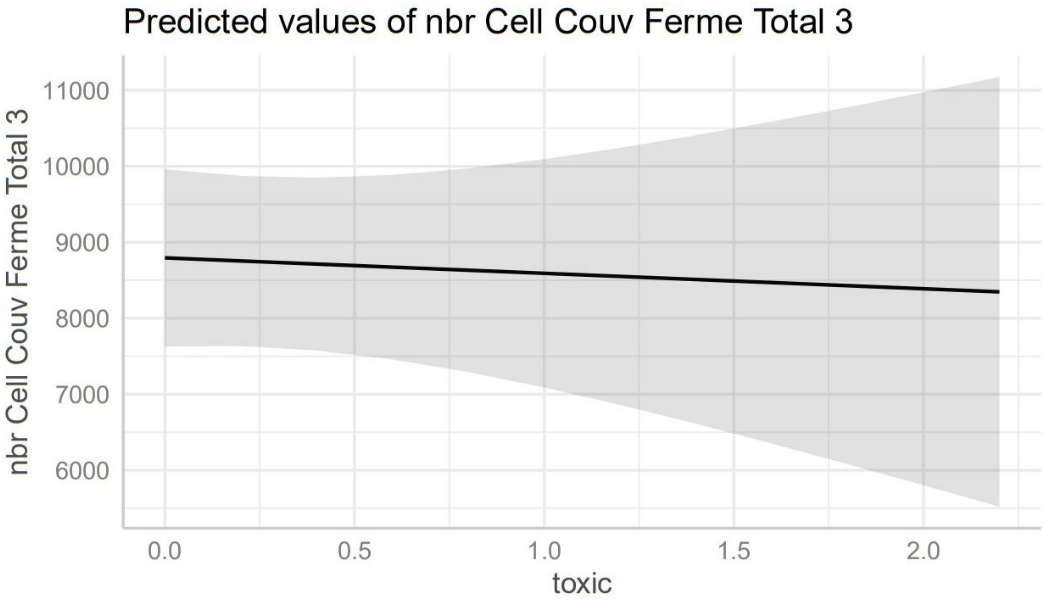
A.4 Model - Association neonicotinoids and land use parameters



```
summary(muse)
```

```
##
## Call:
## lm(formula = log(nnscumul + 0.1) ~ annual + oil + perennial +
##     seminat + meadow + factor(year), data = pest)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.4870 -0.7617 -0.2513  0.7294  3.6744
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)       3.750e-02  1.112e+00   0.034   0.9732
## annual            2.314e-05  2.162e-05   1.071   0.2883
## oil              -7.688e-05  7.578e-05  -1.014   0.3141
## perennial         3.443e-06  3.955e-05   0.087   0.9309
## seminat           9.079e-06  2.518e-05   0.361   0.7196
## meadow           -4.311e-05  1.800e-05  -2.395   0.0195 *
## factor(year)2020 -6.422e-01  4.185e-01  -1.535   0.1297
## factor(year)2021  9.468e-01  4.142e-01   2.286   0.0255 *
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 1.419 on 66 degrees of freedom
## Multiple R-squared:  0.3032, Adjusted R-squared:  0.2293
## F-statistic: 4.102 on 7 and 66 DF,  p-value: 0.0008551
```

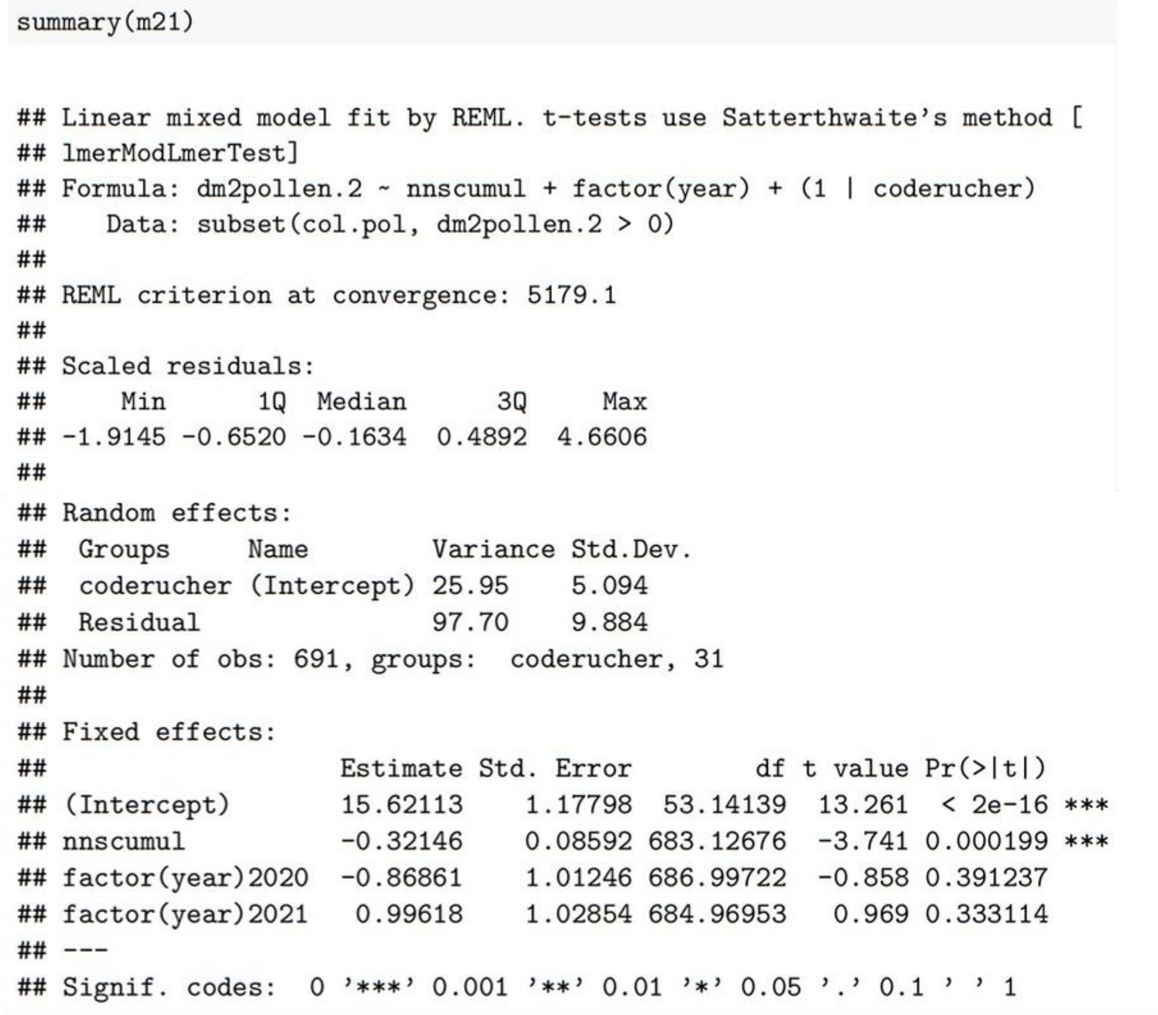
A.5.1 Linear mixed model fit by REML - Brood size in summer (visit 3)



```
summary(m3)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite’s method [
## lmerModLmerTest]
## Formula: nbrCellCouvFermeTotal.3 ~ toxic * nnscumul + factor(year) + (1 |
##     coderucher)
##    Data: subset(col.pol, nbrCellCouvFermeTotal.3 > 0)
##
## REML criterion at convergence: 13793.8
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.9039 -0.5770 -0.0655  0.5902  4.9279
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  coderucher (Intercept)  8083822 2843
##  Residual               17632934 4199
## Number of obs: 707, groups:  coderucher, 31
##
## Fixed effects:
##                   Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)         9201.0      669.1    59.4  13.751  < 2e-16 ***
## toxic              -3305.9     1236.3   240.2  -2.674 0.008010 **
## nnscumul            -159.2       41.7   700.7  -3.818 0.000147 ***
## factor(year)2020    1079.2      488.0   687.6   2.211 0.027340 *
## factor(year)2021    1688.7      463.0   700.9   3.647 0.000285 ***
## toxic:nnscumul       315.0       90.6   395.9   3.477 0.000564 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
```

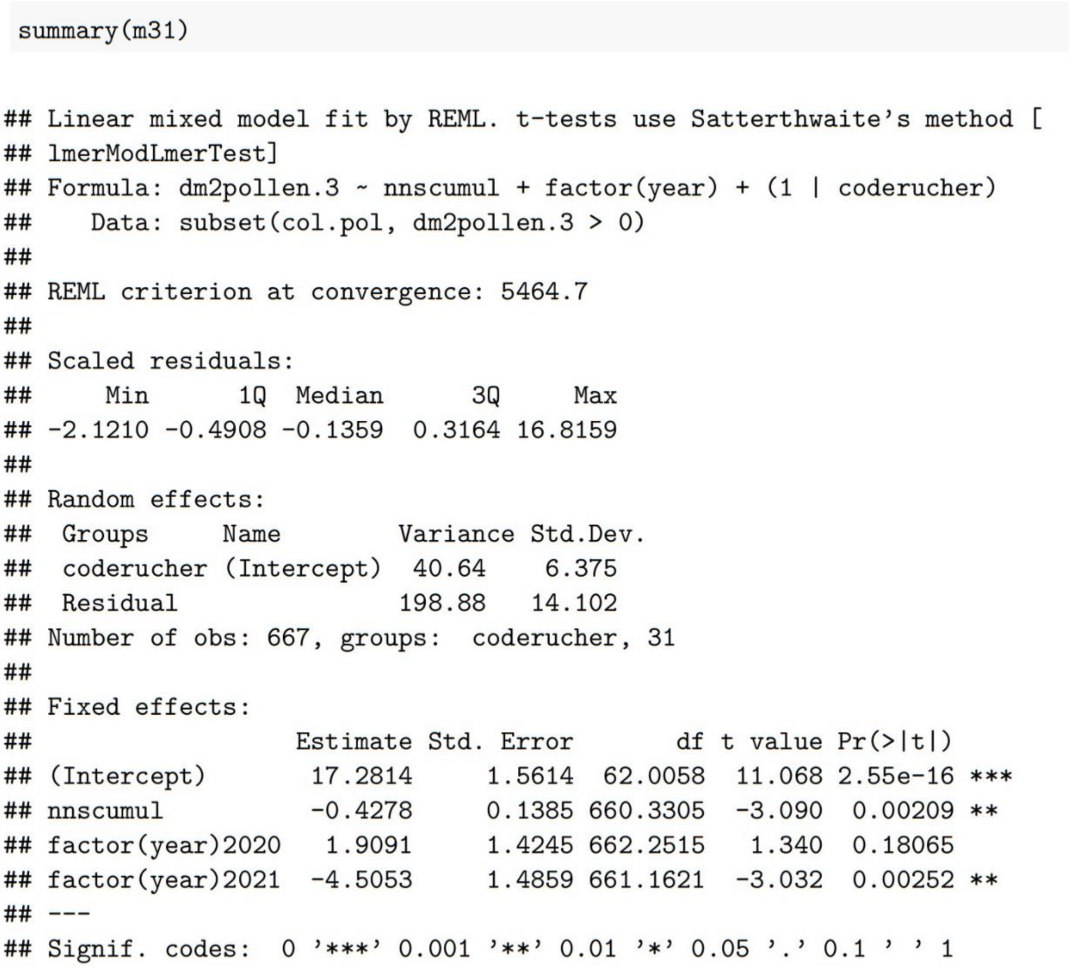
A.5.2 Liner mixed model fit by REML - Pollen stocks area (spring)



```
summary(m21)

## Linear mixed model fit by REML. t-tests use Satterthwaite’s method [
## lmerModLmerTest]
## Formula: dm2pollen.2 ~ nnscumul + factor(year) + (1 | coderucher)
##    Data: subset(col.pol, dm2pollen.2 > 0)
##
## REML criterion at convergence: 5179.1
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.9145 -0.6520 -0.1634  0.4892  4.6606
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  coderucher (Intercept) 25.95    5.094
##  Residual               97.70    9.884
## Number of obs: 691, groups:  coderucher, 31
##
## Fixed effects:
##                   Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)       15.62113    1.17798  53.14139  13.261  < 2e-16 ***
## nnscumul          -0.32146    0.08592 683.12676  -3.741 0.000199 ***
## factor(year)2020  -0.86861    1.01246 686.99722  -0.858 0.391237
## factor(year)2021   0.99618    1.02854 684.96953   0.969 0.333114
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
```

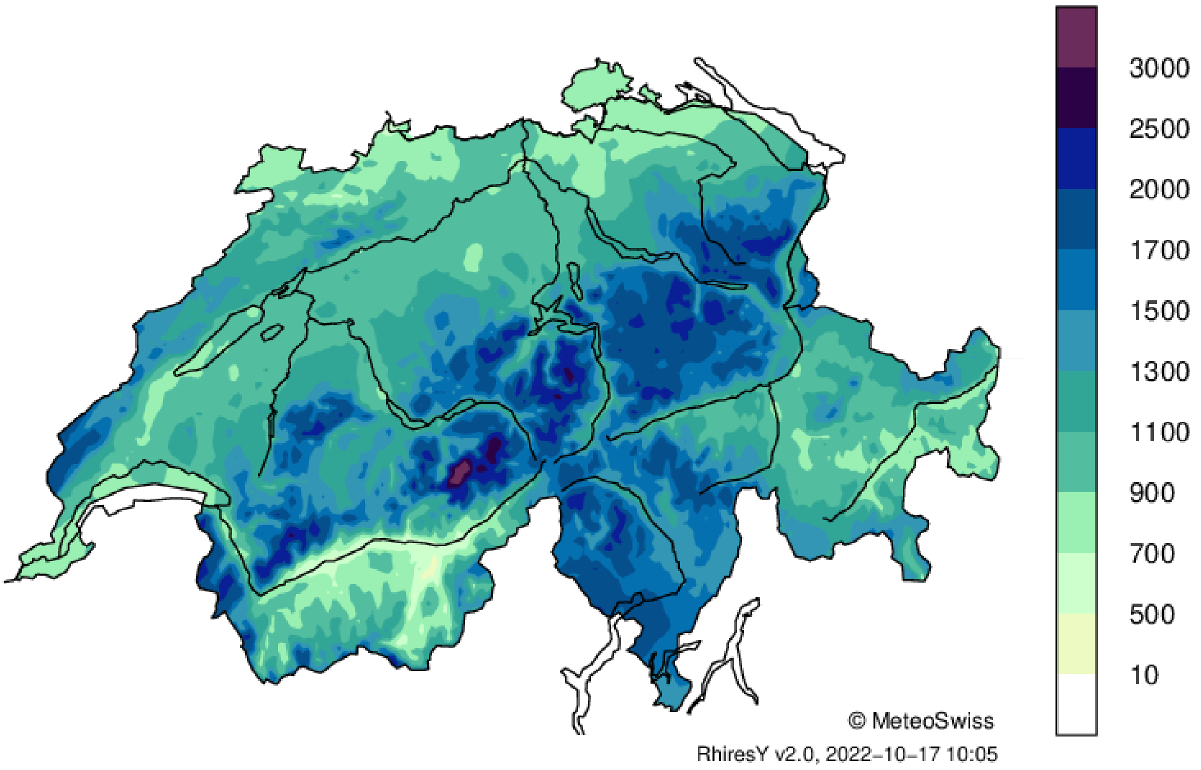
A.5.3 Liner mixed model fit by REML - Pollen stocks area (summer)



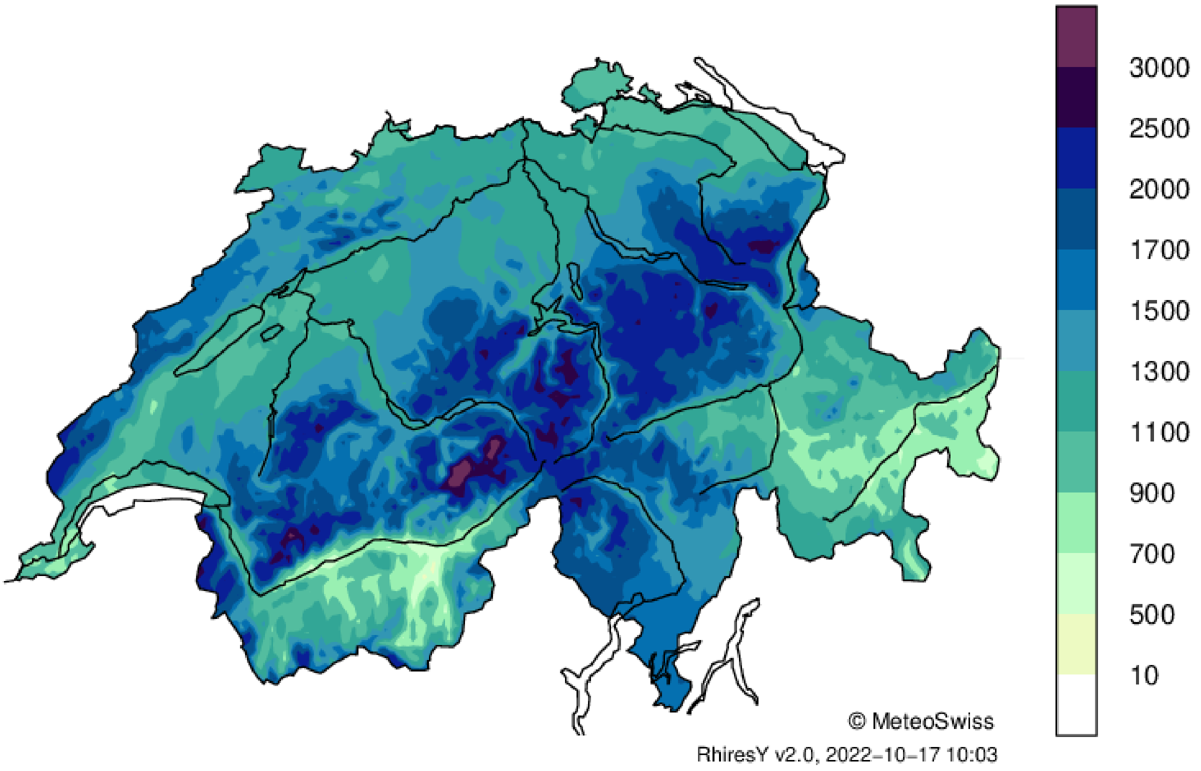
```
summary(m31)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite’s method [
## lmerModLmerTest]
## Formula: dm2pollen.3 ~ nnscumul + factor(year) + (1 | coderucher)
##    Data: subset(col.pol, dm2pollen.3 > 0)
##
## REML criterion at convergence: 5464.7
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.1210 -0.4908 -0.1359  0.3164 16.8159
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  coderucher (Intercept)  40.64    6.375
##  Residual               198.88   14.102
## Number of obs: 667, groups:  coderucher, 31
##
## Fixed effects:
##                  Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)       17.2814     1.5614  62.0058  11.068 2.55e-16 ***
## nnscumul          -0.4278     0.1385 660.3305  -3.090  0.00209 **
## factor(year)2020   1.9091     1.4245 662.2515   1.340  0.18065
## factor(year)2021  -4.5053     1.4859 661.1621  -3.032  0.00252 **
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
```

A.6.1 Meteorological report - Precipitation comparison (2020-2021)

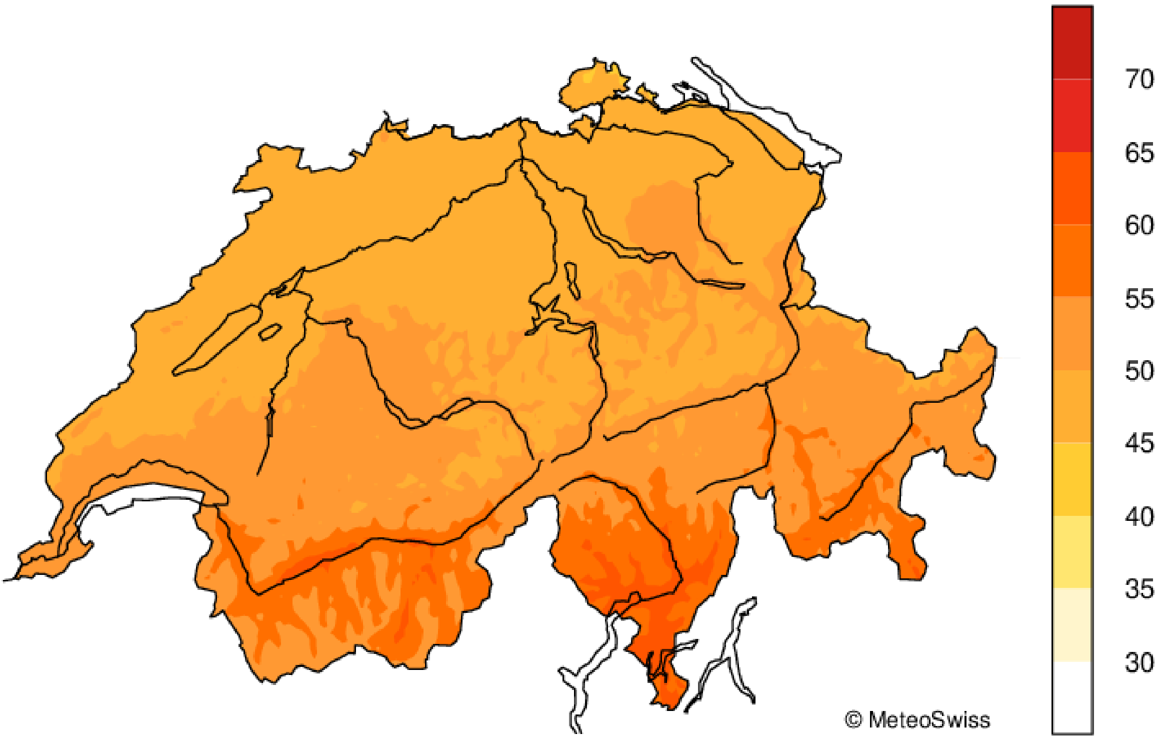

Yearly Precipitation (mm) 2020

© MeteoSwiss
RhiresY v2.0, 2022-10-17 10:05


Yearly Precipitation (mm) 2021

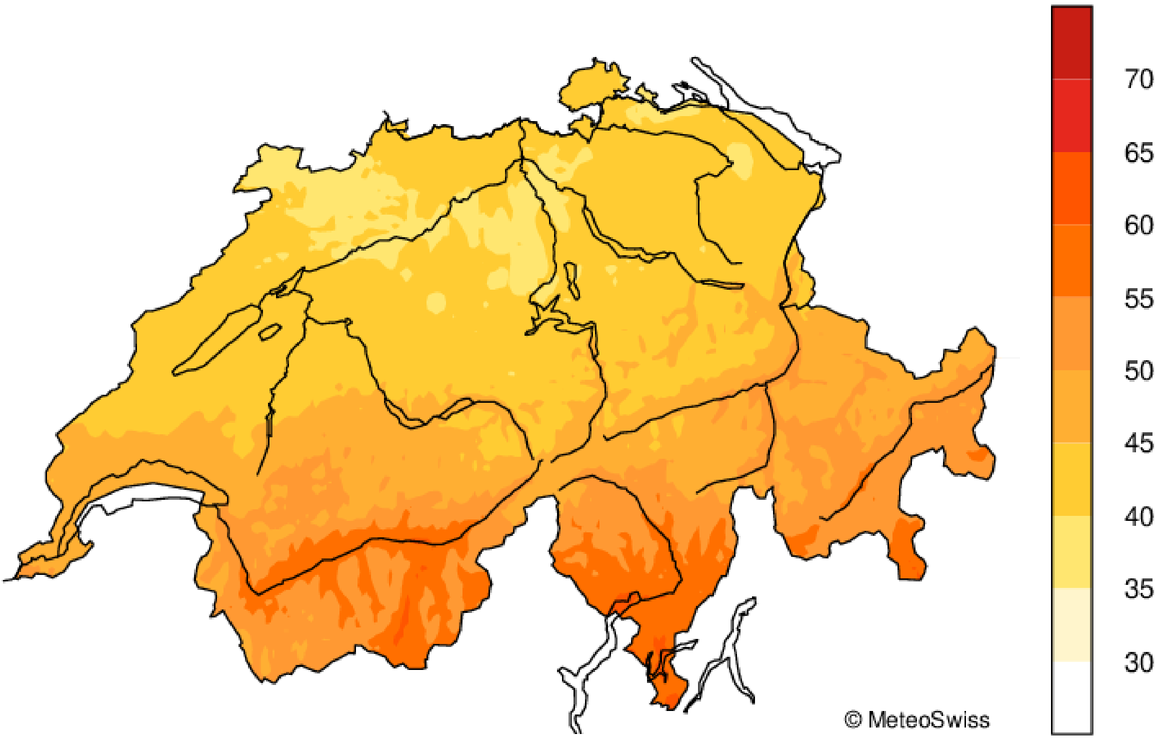
© MeteoSwiss
RhiresY v2.0, 2022-10-17 10:03

A.6.2 Meteorological report - Sunshine Duration comparison (2020-2021)


Yearly Relative Sunshine Duration (%) 2020

© MeteoSwiss

SrelY v1.5, 2022–10–18 07:08


Yearly Relative Sunshine Duration (%) 2021

© MeteoSwiss

SrelY v1.5, 2022–10–18 07:06