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## ► To cite this version:

Dimitry Wintermantel, Jean-François Odoux, Axel Decourtye, Mickaël Henry, Fabrice Allier, et al.. Neonicotinoid-induced mortality risk for bees foraging on oilseed rape nectar persists despite EU moratorium. Science of the Total Environment, 2020, 704, pp.135400. 10.1016/j.scitotenv.2019.135400 . hal-02382527

**HAL Id: hal-02382527**

**<https://hal.science/hal-02382527>**

Submitted on 7 Mar 2022

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1    **Neonicotinoid-induced mortality risk for bees foraging on oilseed rape nectar persists despite**  
2    **EU moratorium**

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## ABSTRACT

The implication of neonicotinoids in bee declines led in 2013 to an EU moratorium on three neonicotinoids in bee-attractive crops. However, neonicotinoids are frequently detected in wild flowers or untreated crops suggesting that neonicotinoids applied to cereals can spread into the environment and harm bees. Therefore, we quantified neonicotinoid residues in nectar from winter-sown oilseed rape in western France collected within the five years under the EU moratorium. We detected all three restricted neonicotinoids. Imidacloprid was detected in all years with no clear declining trend but a strong inter- and intra-annual variation and maximum concentrations exceeding reported concentrations in treated crops. No relation to non-organic winter-sown cereals was identified even though these were the only crops treated with imidacloprid, but residue levels depended on soil type and increased with rainfall. Simulating acute and chronic mortality suggests a considerable risk for nectar foraging bees. We conclude that persistent imidacloprid soil residues diffuse on a large-scale in the environment and substantially contaminate a major mass-flowering crop. Despite the limitations of case-studies and risk simulations, our findings provide additional support to the recent extension of the moratorium to a permanent ban in all outdoor crops.

**Keywords: Neonicotinoid, imidacloprid, foraging bees, oilseed rape nectar, risk assessment, environmental fate**

## 1. Introduction

Neonicotinoids comprise the dominant class of insecticides in the world with a market share of over 25% in 2015 (Casida, 2018). At their launch in the 1990s, they were considered more environmentally friendly than the prevailing insecticides, due to lower application rates and higher specificity to insects (i.e. lower toxicity to vertebras, including humans; Tomizawa and Casida, 2011). Neonicotinoids ensure efficient and lasting protection from insect pests, because the persistent and highly toxic active compounds translocate throughout the plant (Jeschke et al., 2011; Simon-Delso et al., 2015; Van der Sluijs et al., 2013). Relative specificity to insects results mostly from a higher affinity of the active compounds to the nicotinic acetylcholine receptors of insects than those of vertebrates (Tomizawa and Casida, 2005).

Paradoxically, the characteristics that initially contributed to the economic rise and environment-friendly perception of neonicotinoids are also the cause of environmental concerns. High persistence and water solubility do not only ensure systemic protection but also cause neonicotinoids to accumulate in the environment and contaminate ground and surface waters including rivers, lakes and puddles (Limay-Rios et al., 2016; Samson-Robert et al., 2014; Sánchez-Bayo et al., 2016; Schaafsma et al., 2015; Van der Sluijs et al., 2013). Neonicotinoids caused, however, most concern due to the chronic exposure of bees to sublethal doses in pollen and nectar, which led in December 2013 to a European Union-wide moratorium on the use of three neonicotinoids – imidacloprid, thiametoxam and clothianidin – in bee-attractive crops.

The moratorium did, however, not ban these neonicotinoids in crops such as winter-sown cereals or sugar beets from which residues can spread to nearby non-target plants. The contamination of wild flowers at field borders is widespread (Botías et al., 2015; David et al., 2016; Long and Krupke, 2016; Tsvetkov et al., 2017) with concentrations sometimes exceeding those of the treated crop (David et al., 2016). Wild flowers can in fact be the main source of neonicotinoid in bee-collected pollen (David et al., 2016; Tsvetkov et al., 2017). Hazardous neonicotinoid concentrations were also found in flowers and honeybee-collected nectar of untreated oilseed rape (Henry et al., 2015; Thompson et al., 2013; Woodcock et al., 2017). In fact, in the year after the moratorium came into effect, maximum neonicotinoid prevalence in UK honey samples coincided with oilseed rape flowering and residue concentrations increased with the area of oilseed rape surrounding the regarded honeybee hives (Woodcock et al., 2018) indicating that the crop took up soil residues that persisted for more than a year.

Contamination of non-target plants can originate from treated crops in the surroundings or from the cultivation of a treated crop on the same field in previous years (Henry et al., 2015; Wood and Goulson, 2017). Neonicotinoid seed-dressed crops take up only a small portion of the active ingredient (~5%); the remainder stays on the field unless it is transported by wind or water (Wood and Goulson, 2017). Neonicotinoids are persistent in the environment with half-lives in aerobic soil conditions (Van der Sluijs et al., 2013) ranging from a few months to years (Bonmatin et al., 2015). Consequently, neonicotinoids are often found in soil, sometimes even several years after applications ceased (Hladik et al., 2018; Jones et al., 2014). Neonicotinoids in agricultural soils do not only pose a direct threat to ground-nesting bees (Chan et al., 2019), but can also be taken up by plants and then threaten foraging bees.

To assess neonicotinoid spread in the environment more extensively, we examined nectar collected from a total of 291 winter-sown oilseed rape fields over the five years under the EU moratorium (2014-2018) using neonicotinoid residue analyses with particularly low limits of detection and quantification (Martel et al., 2013). The fields, located in a 435 km<sup>2</sup>-large agricultural study area in western France (Fig. 1), could not be sampled in multiple years due to crop successions, but were sampled 1-6 times during one oilseed rape flowering period. We related the prevalence (presence/absence) and concentration of imidacloprid, the most prevalent neonicotinoid in our study, to temporal and environmental (soil type, weather, land use) variables to identify the main drivers of accidental imidacloprid contamination in a mass-flowering crop. Finally, we simulated imidacloprid-induced acute and chronic mortality for nectar foraging honeybees, bumblebees and solitary bees using a risk assessment scheme adapted from the one used by the European Food Safety Authority (EFSA) in order to identify whether the EU restrictions on neonicotinoid use in bee-attractive crops have eliminated the risk of imidacloprid-induced mortality for bees feeding on oilseed rape nectar.

## **2. Materials and methods**

### **2.1. The study site**

The study was conducted in the 435 km<sup>2</sup>-large Long-Term Socio-Ecological Research (LTSER) Zone Atelier “Plaine & Val de Sèvre” site, central western France (46°23’N, 0°41’W), which is characterized by a high proportion of arable land and an oceanic climate with mean monthly temperatures ranging from 5-20 °C and 820 mm of precipitation well distributed over the year. In the LTSER site the broad soil type (Fig. 1) and the precise land use have been documented and mapped on vector-based GIS shapefiles since 1994

79 (Bretagnolle et al., 2018). Within the study period, between 2014 and 2018, the land area was covered on average to 33.0% with wheat  
80 6.1% barley, 1.4% other cereals, 9.7% maize, 9.3% sunflower, 7.2% oilseed rape, 8.0% grassland, 7.1% legumes (mostly alfalfa:  
81 3.0%) and 4.6% other crops. Organic farmland covered 6.5% of the area.

## 82 **2.2. *Oilseed rape nectar sampling***

83 Between 2014 and 2018, a total of 291 winter-sown oilseed rape fields were selected for nectar sampling. The field selection criteria  
84 differed between years and included flower timing (early and late flowering oilseed rape fields) and crop choice in previous years.  
85 Specifically, we strived for uniform distributions of the number of times winter-sown cereals were cultivated in the previous 10 years  
86 (i.e. from 0-8) or the number of years between the last wheat and the sampled oilseed rape cultivation. To estimate repeatability and  
87 temporal variation in neonicotinoid residue concentrations, fields were sampled 1-6 times per year (Fig. S1) on different days between  
88 23 March and 10 June (Table S1) and between 9 a.m. and 7 p.m. (Fig. S2), yielding a grand total of 536 nectar samples. The number  
89 of samples per field depended on the number of workers available for nectar collection, funding and weather conditions (sampling  
90 could not be done during rainy weather or drought). Using 5.0  $\mu\text{L}$  capillaries (Drummond Scientific, Broomall, PA, USA),  
91 approximately 25  $\mu\text{L}$  of nectar per sample was collected directly from randomly selected open flowers that were at least 10 m from  
92 field margins to avoid edge effects.

## 93 **2.3. *Neonicotinoid residue analysis***

94 Around 2  $\mu\text{L}$  of nectar were used to determine the sugar content by hand-held refractometers (BS Eclipse BS 45-81 / 45-82,  
95 Bellingham + Stanley Ltd., UK). The remaining nectar was analysed using liquid chromatography with electrospray tandem mass  
96 spectrometry by the EU reference laboratory for neonicotinoid multi-residual analyses (ANSES, Sophia-Antipolis, France; Martel et  
97 al., 2013) to identify (limit of detection (LOD) = 0.1  $\text{ng mL}^{-1}$ ) and quantify (limit of quantification (LOQ) = 0.3  $\text{ng mL}^{-1}$ ) the five  
98 neonicotinoids that were approved in the EU during the study period: acetamiprid, clothianidin, imidacloprid, thiacloprid,  
99 thiamethoxam. In this study, we focus on the three neonicotinoids that were banned in bee-attractive crops (clothianidin, imidacloprid  
100 & thiamethoxam) to determine whether the EU moratorium has eliminated their risk for bees foraging on oilseed rape nectar.

101

## 2.4. Weather data

Daily minimum and maximum air temperature and precipitation data were obtained from a weather station held by Météo France south of Niort and within the LTSER site, with data made available at the US National Oceanic and Atmospheric Administration. The total precipitation and growing degree days (oilseed rape: base temperature = 5 °C; wheat: base temperature = 0 °C (Ruiz Castillo and Gaitán Ospina, 2016)) over the growing seasons of the sampled oilseed rape and of wheat cultivated in the preceding year were calculated and related to imidacloprid prevalence. The oilseed rape growing season was estimated to range from 1 August of the previous year to the mean sampling day of the sampling year. The wheat growing season ranged from 1 October two years before nectar sampling to 1 July of the year before nectar sampling.

## 2.5. Statistical analyses

Among the neonicotinoids restricted from use in bee-attractive crops, only imidacloprid was detected frequently enough to allow for meaningful statistical analyses. Both the prevalence (absence/presence) and concentration of imidacloprid were analysed. For the latter, only the concentrations of positive samples were included (i.e. samples <LOD were excluded) to assess effects on concentration independently of effects on prevalence and to avoid zero-inflated datasets. However, to exclude artefacts that may arise when residues below the LOD are undetected and prevalence and concentration in positive samples show opposing trends, we refit the selected model on concentration with the whole dataset (i.e. including negative samples) and report these results in Table S2 and Fig. S3. Samples < LOQ but > LOD were set to 0.2 ng mL<sup>-1</sup> as determined by the equation (LOD+LOQ)/2, which is a more conservative assumption than the one used in the EFSA first-tier risk assessment in which LOQ is assigned to samples < LOQ but > LOD (EFSA, 2013). In the analyses on the whole dataset, samples <LOD were set to 0.05 ng mL<sup>-1</sup> (i.e. LOD/2). Analyses of repeatability of imidacloprid prevalence and concentration of different samples from the same field were restricted to the years 2016, 2017 and 2018 as typically only one sample per field was taken in 2014 and 2015. Repeatability was estimated by the intraclass coefficient (ICC) using the rpt function of the rptR package in R (Stoffel et al., 2017). ICCs were estimated on the logit link scale for prevalence and on the normal scale for logarithmically transformed (log<sub>10</sub>) concentration in positive samples.

The relationship between imidacloprid residues and temporal parameters (sampling year, sampling Julian day number, sampling time of day) and environmental parameters (soil type, precipitation, mean air temperature, non-organic winter-sown cereal cultivation in

126 the surrounding of the focal fields or on the same field in previous years) were analysed using generalized linear mixed-effects models  
 127 with field identity as a random factor, a logit-link function and a binomial error distribution. For the concentration data, a linear model  
 128 without random factors (LM, with Gaussian error distribution) was used, as the number of positive samples per field was too low to  
 129 allow for the inclusion of a random effect for field identity. Akaike Information Criterion (*AIC*) was used to select the most  
 130 parsimonious models. First, full models for prevalence and concentration were built that contained sampling year, sampling Julian day  
 131 number (incl. a quadratic effect), sampling time of day (incl. a quadratic effect) as fixed effects and the percentage of area covered by  
 132 non-organic winter cereals in 20 m buffers surrounding the sampled fields in the sampling year. The full models contained additional  
 133 fixed effects describing (i) precipitation, (ii) mean temperature, (iii) soil type, and (iv) non-organic winter cereal cultivation in  
 134 previous years on the sampled fields, which were pre-selected in a comparison of a range of univariate models (i.e. with only one  
 135 fixed effect) with a null model (i.e. without fixed effects). The compared variables were (i) the amount of precipitation in periods of 1-  
 136 10 days that ended 0-4 days before sampling day (hereafter '*lag*'), (ii) mean air temperature in periods of 1-5 days with a lag of 0-3  
 137 days, (iii) soil type distinguishing between red and calcareous soil and soil type distinguishing between red, shallow calcareous and  
 138 deep calcareous soil and (iv) the number of years passed since the last non-organic cereal cultivation and the number of non-organic  
 139 cereal cultivations in the previous 1-5 years. For each of the four categories, one variable was included in the full models as long as  
 140 the variables yielded a lower *AIC* than the regarded null model. In addition, two-way interactions between soil type and weather  
 141 variables were included in the full models. The two full models were then compared to all possible reduced models with the same  
 142 random effect (i.e. field identity for prevalence and none for concentration in positive samples) except that models with quadratic  
 143 terms or two-way interactions were only considered if they also contained the non-quadratic term or the main effects of these  
 144 variables. Explained variability of selected models was determined by coefficients of determination or their equivalent for models fit  
 145 on (restricted) maximum likelihood. These pseudo  $R^2$  values were determined using Nagelkerke's method.

146 All analyses were done in R version 3.5.0. GLMMs for model selection were fit using *glmmTMB* of the *glmmTMB* package.  
 147 However, for illustrations the selected model was re-fit using the *glmer* function of the *lme4* package because *glmmTMB* does  
 148 currently not allow to set random effects to zero. Pseudo coefficients of determination for GLMMs were obtained using the  
 149 *r.squaredGLMM* function of the *lmttest* package. Both marginal and conditional pseudo coefficients of determination are reported to  
 150 show the theoretical variance explained by fixed factors only ( $R^2_m$ ) and by the entire model ( $R^2_c$ ), respectively.



## 2.6. Risk assessment for nectar foragers

This risk assessment was restricted to nectar foragers feeding on imidacloprid residues as thiamethoxam and clothianidin were only infrequently detected. Hereby, samples <LOD were set to  $0.05 \text{ ng mL}^{-1}$  (i.e. LOD/2). To determine the sensitivity of the risk estimates to this assumption, we report in Supplementary Data 1 also risk estimates when samples <LOD were set to zero or to the value of the LOD.

As EFSA is responsible for assessing the risk of plant protection products in the EU, we adapted their first-tier assessment for bees using our measured field-level imidacloprid residue and sugar content values rather than theoretical values. Similarly to EFSA, we computed exposure toxicity ratios (ETRs) for acute and chronic toxicity for 1000 hypothetical individual honeybees, bumblebees or solitary bees per field (EFSA, 2014, 2013). ETRs constitute the quotient of an expected environmental dose (i.e. the imidacloprid residue intake) and a median lethal dose. However, to obtain easily interpretable risk estimates, we refrained from comparing ETRs to EFSA's trigger values that indicate potentially unsafe levels in a worst-case scenario and are based on the lowest observed background mortality and arbitrary assessment factors. Instead, we related ETRs to a probability of death, which we used to simulate acute and chronic mortality for individual honeybees, bumblebees and solitary bees foraging for 10 days on oilseed rape nectar to determine whether imidacloprid exposure through oilseed rape can shorten their lifespans. To obtain estimates for each field, we assumed that bees would forage throughout the regarded timeframe on the same oilseed rape field. In fact, honeybees forage up to 10 days in their lives (Schippers et al., 2006), while bumblebees and solitary bees can forage over even longer periods (Evans et al., 2017; Michener, 2007). Honeybees, bumblebees and some solitary bees forage intensively on oilseed rape during its bloom (Baron et al., 2017; Holzschuh et al., 2016; Magrach et al., 2018; Perrot et al., 2018; Rollin et al., 2013; Stanley et al., 2013) and exhibit flower constancy (Amaya-Márquez, 2009; Gegear and Laverty, 2005; Grüter et al., 2011).

In a first step, neonicotinoid concentrations were converted to mass fractions in ppb (i.e.  $\mu\text{g kg}^{-1}$ ), by dividing them by the density of the nectar sample,  $\rho_{\text{nectar}}$  (in  $\text{kg L}^{-1}$ ), which was estimated by the sugar content  $\omega_{\text{sugar}}$  (in  $\text{kg kg}^{-1}$ ), and assumed densities of water ( $1 \text{ kg L}^{-1}$ ) and sugar ( $1.6 \text{ kg L}^{-1}$ ) as follows:

$$\rho_{\text{nectar}} = (1 + 0.6 \omega_{\text{sugar}}) \rho_{\text{nectar}} = (1 + 0.6 \omega_{\text{sugar}}) \quad (\text{equation 1}).$$

For each of the three regarded bee types (*Apis mellifera*, bumblebees and solitary bees), a theoretical normal distribution of daily sugar consumption amounts was derived from reported ranges of daily sugar consumption (in mg; Fig. S4; EFSA, 2013; Rortais et al., 2005). The normal distributions were centred on the mean of the reported minimum and maximum sugar consumption amounts and standard deviations were estimated by a quantile function (qnorm) with alpha being set to 0.01, so that 99% of the estimated daily sugar consumption amounts were within the reported ranges (EFSA, 2014):

$$s.d. = \frac{\text{mean} - \text{min}}{\text{qnorm}(1 - \alpha)} \quad s.d. = \frac{\text{mean} - \text{min}}{\text{qnorm}(1 - \alpha)} \quad (\text{equation 2}).$$

For 1000 bees per field and bee type, we randomly selected daily sugar consumption amounts from these probabilistic distributions and imidacloprid concentrations with corresponding sugar content values from the available measures (if a field was more than once sampled) to calculate daily residue intake ( $\mu\text{g bee}^{-1}$ ) as follows (EFSA, 2013):

$$\text{daily residue intake} = \frac{\text{neonicotinoid mass fraction}}{\text{sugar content}} \times \frac{\text{daily sugar consumption}}{\frac{10^6 \text{ mg}}{\text{kg}}} \quad \text{daily residue intake} = \frac{\text{neonicotinoid mass fraction}}{\text{sugar content}} \times \frac{\text{daily sugar consumption}}{\frac{10^6 \text{ mg}}{\text{kg}}} \quad (\text{equation 3}).$$

Acute and chronic ETRs for each bee type and field were calculated for the periods in which the corresponding lethal doses were determined (i.e. one day for the acute LD<sub>50</sub> on bumblebees, two days for the acute LD<sub>50</sub> on honeybees and solitary bees and 10 days for chronic LDD<sub>50</sub> on any bee type; Table 1). For this, residue intake over the regarded period was divided by the corresponding median lethal dose (expressed as an absolute amount for the regarded period rather than a daily amount). Bee mortality was subsequently simulated based on an assumed relationship between probability of death and ETR. For ETR $\geq$ 0.1, probability of death was assumed to follow a logistic regression with ETR=0.1, corresponding to 10% mortality (Sanchez-Bayo and Goka, 2014) and ETR=0.5 corresponding to 50% mortality, while for ETR<0.1 no mortality was assumed (Fig. S5). Mortality was simulated in ten 1-day periods for acute mortality of bumblebees, in five 2-day periods for acute mortality of honeybees and solitary bees and in one 10-day period for chronic mortality. Acute and chronic mortality were combined by considering any bee dead that was simulated to die within any of the ten 1-day periods / five 2-day periods due to acute toxicity or within the 10-day period due to chronic mortality. Finally, we calculated the proportion of bees that would die a premature death due to imidacloprid-induced toxicity per field and determined then the proportion of fields that had mortality rates higher than 50%.

### 3. Results

#### 3.1. Prevalence and concentration of neonicotinoids in oilseed rape nectar

All three neonicotinoids that were banned in oilseed rape and other bee-attractive crops throughout the study period were detected at least once within the five years (Fig. 2a). Imidacloprid was detected in all years and in 43% samples overall and was therefore clearly more prevalent than other neonicotinoids including thiacloprid, which was allowed for spray applications on oilseed rape during the study period and detected in 6.7% of samples (Fig. S6). Imidacloprid prevalence varied strongly between years. In 2014 and 2016, imidacloprid was detected in over 60% of the samples (Fig. 2a). Due to repeated sampling on the same field, the proportion of imidacloprid-positive fields was even higher, exceeding 90% in 2016. In contrast, in 2015, imidacloprid was detected in only 5.4% of the samples/fields. Imidacloprid concentrations spanned also a wide range. Although 96.5% of all samples (i.e. 91.8% of positive samples) contained less than 1 ng mL<sup>-1</sup> imidacloprid, extremely high concentrations of up to 70 ng mL<sup>-1</sup> were detected in a few samples (Fig 2b). Imidacloprid prevalence was generally fairly repeatable while concentrations in positive samples of the same field varied more (Table 2). In fact, all of the 19 samples with imidacloprid concentrations above 1 ng mL<sup>-1</sup> stemmed from different fields although these fields were sampled on average three times and tested positive in 79% of the cases.

#### 3.2. Relation between neonicotinoid residues and weather, time, soil type or cereal cultivation

We expected that temperature and precipitation influence the degradation and uptake of neonicotinoids. However, there was no clear link between inter-annual differences in imidacloprid prevalence and weather conditions (growing degree days and precipitation) in the oilseed rape growing season or the preceding wheat growing season (Fig. S7). To determine which factors influenced imidacloprid prevalence and concentration (in positive samples only), we conducted model selection analyses using variables on sampling time (year, Julian day number and time of day), non-organic cereal cultivation (in the surrounding of the focal fields or on the focal fields but in previous years), weather (precipitation and air temperature), soil type and two-way interactions between weather variables and soil type. The selected models explained a substantial amount of variation for both prevalence ( $R^2_m=0.42$  (only fixed effects),  $R^2_c=0.61$  (including field identity as random factor)) and concentration in positive samples ( $R^2=0.27$ ). Imidacloprid prevalence tended to decrease with mean air temperature and both imidacloprid prevalence and concentration (in positive samples) were related to year, tended to decrease with Julian day and increase with precipitation in the days before sampling, although the regarded period of rainfall

222 was considerably longer for prevalence than for concentration (Fig. 3, Table S2). Both imidacloprid prevalence and concentration  
223 were also higher on red soil (i.e. brunisol on ferralitic clay) than on calcareous soil with no differences between shallow and deep  
224 calcareous soil. In addition, the increase in imidacloprid concentration with precipitation was particularly pronounced in oilseed rape  
225 grown on red soil, as indicated by the two-way interaction between soil type and precipitation (Fig. 3; Table S2). However, this was  
226 only true for concentration in positive samples. Including samples below the limit of detection also suggests that imidacloprid residues  
227 were higher on red than on calcareous soil, but only on calcareous soil imidacloprid residues increased with rainfall in the regarded  
228 period before sampling (Table S2, Fig. S3). Despite being the only crops treated with imidacloprid, non-organic winter cereals  
229 cultivated in the surroundings of the regarded oilseed rape fields or on the same fields but in previous years had no measurable impact  
230 on imidacloprid prevalence or concentration in those samples tested positive for the substance.

### 231 **3.3. Risk to foragers**

232 We simulated the risk of imidacloprid-induced mortality for individual honeybees, bumblebees and solitary bees foraging on oilseed  
233 rape nectar over a period of 10 days using a scheme that has been adapted from EFSA's first-tier risk assessment (EFSA, 2014, 2013)  
234 to obtain easily interpretable risk estimates per field based on our measured data. For honeybees, the risk peaked in 2014 and 2016  
235 (Fig. 4), with an estimated 50% of nectar foragers likely to die due to imidacloprid in 12% of fields in 2014 and 2016 (i.e. 9 and 7  
236 fields, respectively), and 5% of fields in the whole study period. For nectar-foraging bumblebees, we determined in 2014 and 2016 at  
237 least ten fields (13% and 20% of fields, respectively) and in 2018 one field with an estimated mortality of above 50% (Fig. 4,  
238 Supplementary Data 1). For solitary bees, the risk assessment indicated that in one field in 2016 half of the bees would die due to  
239 acute toxicity and in above 10% of fields in 2014 and 2016 (6 and 9 fields, respectively) due to chronic toxicity. Our estimates suggest  
240 that unlike honeybee mortality, wild bee mortality was mostly driven by chronic toxicity (Fig. 4). However, estimates of chronic  
241 mortality in bumblebees and solitary bees are based on dietary lethal doses that have been extrapolated from honeybees by EFSA  
242 using a safety factor of 10 (Table 1; EFSA, 2018). In addition, acute mortality of solitary bees was estimated using a lethal dose for  
243 contact rather than oral exposure (Uhl et al., 2018).

#### 4. Discussion

We found that neonicotinoid contamination of oilseed rape nectar is widespread despite EU-wide restrictions on the application of clothianidin, imidacloprid and thiamethoxam in bee-attractive crops. In particular imidacloprid was found in a varying and sometimes considerable fraction of fields in all years. The prevalence of neonicotinoid contamination varied strongly between years, but with no clear decline over time since the EU moratorium came into effect in December 2013. We detected imidacloprid not only frequently but sometimes also in very high concentrations. In two samples collected in 2016, over 45 ppb (52 ng mL<sup>-1</sup> and 70 ng mL<sup>-1</sup>) imidacloprid was found, which is more than five times the expected maximum concentration in nectar of imidacloprid-treated oilseed rape (Wood and Goulson, 2017).

During the study period, imidacloprid use was restricted in the study site to the seed-treatment of non-organic winter-sown cereals and even before the moratorium, imidacloprid was never used in oilseed rape and banned for seed-treatments in sunflower (1999) and maize (2004) in France (Maxim and van der Sluijs, 2007). However, we did not find any relation between imidacloprid residues in oilseed rape and the cultivation of non-organic winter-sown cereals in previous years on the sampled fields or in the sampling year on surrounding fields.

This suggests that imidacloprid spreads on a large scale in the environment, contaminating not only wild flowers at field borders of treated crops but also other crops that were planted outside the immediate vicinity or several years after the application of the insecticide. Neonicotinoids can travel from treated crops to insect-pollinated plants at the moment of sowing through contaminated dust (Girolami et al., 2013; Greatti et al., 2006; Krupke et al., 2012; Pistorius et al., 2010; Tapparo et al., 2012) or later on, through wind eroded soil (Limay-Rios et al., 2016; Schaafsma et al., 2015). Contaminated dust can contain extremely high concentrations of neonicotinoids (Bonmatin et al., 2015; Krupke et al., 2017; Wood and Goulson, 2017). Therefore, it is conceivable that dust drift caused the extremely high imidacloprid concentrations detected in two oilseed rape nectar samples of our study, although winter cereals in neighbouring fields were sown at least half a year before sampling. However, only a small proportion of neonicotinoid is released as dust (<2% in maize seed-coating; Tapparo et al., 2012) and dust drift from cereals is small compared to maize, which was not allowed to be treated with imidacloprid (Wood and Goulson, 2017). In addition, the adoption of improved seed drills in recent years has effectively limited dust drift (Wood and Goulson, 2017).

268 A more likely mechanism of large-scale imidacloprid spread is transport by water in leachate, run-off or contaminated irrigation water  
269 (Bradford et al., 2018; Huseeth and Groves, 2014; Kurwadkar et al., 2014), which neonicotinoids are prone to as they have to be water-  
270 soluble to be systemic (Bonmatin et al., 2015; Giorio et al., 2017). This is supported by our finding that imidacloprid prevalence and  
271 concentration increased with rainfall in the days before sampling and was higher on red soil (i.e. unsaturated brunisol on ferralitic  
272 clay) than on calcareous soil. Imidacloprid may be better retained on red soil because of the finer texture, higher content of the clay  
273 mineral kaolinite and higher water-holding capacity compared to the calcareous soil. Clay minerals and organic matter increase  
274 imidacloprid adsorption (Liu et al., 2002) and also soil texture affects the leaching potential of neonicotinoids, which is highest in  
275 sandy soils and lowest in loams (Wood and Goulson, 2017). Neonicotinoids are much more persistent under aerobic than anoxic  
276 conditions (Giorio et al., 2017). In fact, imidacloprid can persist several years after application as the half-life in soil ranges from 100  
277 to 1230 days (Giorio et al., 2017). Large-scale neonicotinoid spread and uptake from contaminated soil is supported by a Switzerland-  
278 wide survey that showed that neonicotinoids are prevalent in long-standing organic farmland and ecological focus areas with  
279 concentrations in soil and plant samples being correlated (Humann-Guillemot et al., 2019). At a much smaller spatial scale, lateral  
280 spread of imidacloprid residues around a horticultural crop, which affected ground-nesting insects, was associated with precipitation in  
281 a Japanese study (Sánchez-Bayo et al., 2007). However, although moisture content and soil temperature can influence degradation and  
282 leaching in soils (Bonmatin et al., 2015) and imidacloprid residues correlated in our study with rainfall in the days before sampling,  
283 inter-annual differences in imidacloprid prevalence could not be explained by air temperature or precipitation in the beginning of the  
284 year or in previous years. Although we cannot exclude differences in the cultivation of imidacloprid seed-treated non-organic winter-  
285 sown cereals between red and calcareous soils, this was likely a minor factor, as imidacloprid was exclusively used as seed-treatment  
286 in winter-sown cereals. Since the seeds were commercially sold with an imidacloprid coating, it is unlikely that large differences in the  
287 application rate of imidacloprid occurred.

288 We found that the detected imidacloprid concentrations pose a substantial risk to bees foraging on oilseed rape nectar. At 16 of 291  
289 fields, imidacloprid toxicity was estimated to kill 50% of honeybees foraging on oilseed rape nectar for 10 days. For solitary bees and  
290 bumblebees such a high mortality rate was determined at 15 and 23 fields, respectively. We acknowledge that our mortality estimates  
291 are rough expectations of risk levels rather than empirically supported predictions. We assumed that bees would forage for 10 days  
292 exclusively on single oilseed rape fields, for which we took nectar samples at 1-6 dates. This means we did not capture the whole

293 variability of imidacloprid residues, as neonicotinoid levels can vary strongly between fields and within a field on different or even the  
294 same date (see results and e.g. Osterman et al., 2019). In addition, not accounting for a potential preference (Kessler et al., 2015) for or  
295 avoidance against (Kang and Jung, 2017) imidacloprid may have affected the results (but see also Gels et al., 2002; Larson et al.,  
296 2013). Besides, many bees likely do not forage exclusively on oilseed rape. However, it is well-established that mass-flowering  
297 oilseed rape is attractive to honeybees (Requier et al., 2015; Rollin et al., 2013; Stanley et al., 2013), generalist bumblebees (Baron et  
298 al., 2017; Holzschuh et al., 2016; Magrach et al., 2018; Stanley et al., 2013) and solitary bees (Holzschuh et al., 2016; Magrach et al.,  
299 2018; Perrot et al., 2018) and blooms at a time in the season when honeybee and bumblebee colonies are growing rapidly with  
300 consequently high food demands (Requier et al., 2015; Westphal et al., 2009). In addition, individuals of many bee species exhibit  
301 high flower constancy (Amaya-Márquez, 2009; Gegear and Laverty, 2005; Grüter et al., 2011). Moreover, foraging on alternative  
302 floral resources does not necessarily reduce the risk of neonicotinoid exposure. Neonicotinoids are frequently found in wild flowers  
303 (Botías et al., 2015; David et al., 2016; Long and Krupke, 2016; Tsvetkov et al., 2017) and in our study oilseed rape fields were  
304 contaminated with neonicotinoids that they have not been treated with.

305 The assumed foraging timespan of 10 days is a worst-case scenario for honeybees (Rortais et al., 2005; Visscher and Dukas, 1997).  
306 However, wild bees can forage over longer periods (Evans et al., 2017; Michener, 2007), which may imply even higher probabilities  
307 of premature death. To translate estimated exposure to mortality rates, we assumed a logistic dose-response curve with 10% mortality  
308 at ETR=0.1 and 50% mortality at ETR=1, which is typical for pesticides in many species (Sanchez-Bayo and Goka, 2014) and this  
309 seems to fit well imidacloprid toxicity for honeybees (Cresswell, 2011). Thereby, we relied largely on the same lethal doses that the  
310 European Food Safety Authority (EFSA) uses for their risk assessments (EFSA, 2018). However, there is some variability in dose  
311 responses and median lethal doses (Cresswell, 2011; Decourtye and Devillers, 2010). In addition, toxicity can vary considerably  
312 between taxa and even between different subspecies of *Apis mellifera* (Suchail et al., 2001). This may further increase the mortality  
313 risk.

314 Our risk assessment suggests an even higher risk for individual wild bees than for honeybees. However, we used a contact rather than  
315 an oral acute lethal dose for solitary bees and chronic lethal dietary doses for bumblebees and solitary bees that were extrapolated  
316 from values derived from honeybees using a safety factor of 10 by EFSA (EFSA, 2018). This likely caused an over-estimation of  
317 mortality rates, although neonicotinoids tend to be more toxic to bees via oral than contact exposure (EFSA, 2018; Sanchez-Bayo and

318 Goka, 2014). Therefore, the mortality risk associated with nectar consumption is not necessarily higher for individual wild bees, but  
319 ground-nesting wild bees may also be exposed to neonicotinoid residues in agricultural soils (Chan et al., 2019). In addition,  
320 implications of losses of individual bees may be more severe for wild bee populations than for honeybees, as elevated forager losses  
321 translate for solitary bees directly into population declines. In contrast, social bees can compensate for the loss of individual foragers.  
322 This is particularly true for honeybees due to their large colony size as field studies showing more severe effects of neonicotinoids on  
323 bumblebee than on honeybee colonies suggest (Henry et al., 2015; Osterman et al., 2019; Rundlöf et al., 2015; Wintermantel et al.,  
324 2018; Woodcock et al., 2017).

325 Nonetheless, our risk estimates suggest an impact on honeybee colony functioning. Typically, honeybees forage 6.5-10 days in their  
326 lives (Khoury et al., 2011; Rortais et al., 2005; Visscher and Dukas, 1997) and a reduction of the average foraging lifespan by 2.8 days  
327 has been estimated to precipitate colony failure (Khoury et al., 2011). We estimated that in eight fields (four fields each in 2014 and  
328 2016) 50% mortality of nectar foragers due to acute toxicity was reached after 4 days. Even in fields with more moderate loss rates,  
329 long-term effects may occur. Elevated forager mortality may trigger for instance bees to forage at a younger age with negative  
330 consequences for brood care, foraging efficiency and worker longevity (Khoury et al., 2011; Perry et al., 2015), causing colonies to  
331 enter a positive feedback loop accelerating colony weakening (Perry et al., 2015). For instance, reduced foraging efficiency can result  
332 in increased forager recruitment and consequently reduced brood care (Gill et al., 2012). All these aspects can also be affected by  
333 exposure to sublethal levels of neonicotinoids (Godfray et al., 2015; Pisa et al., 2017). In fact, a 6-year survey of Greek apiaries  
334 indicated that sublethal neonicotinoid concentrations in nearby nectar sources led to a substantial decline in colony size and honey  
335 production (Chambers et al., 2019) and field experiments showed that neonicotinoids in nearby oilseed rape plants can lead to  
336 substantial reproductive impairments for bumblebees and solitary bees (Rundlöf et al., 2015; Wintermantel et al., 2018; Woodcock et  
337 al., 2017).

338 We found that both imidacloprid prevalence and concentration in positive samples increased with rainfall in the previous days, likely  
339 due to increased uptake from the soil. Bees, in particular honeybees, avoid foraging during rainy days (Javorek et al., 2002).  
340 Therefore, they may forage more intensively when rainfall ceases, which implies that intensive foraging and high prevalence and  
341 concentration would coincide, which further increases the risk. We regard here only the risk of imidacloprid-induced mortality and



neglect potential interactions with other factors affecting bees such diseases or lack of flowers (Chambers et al., 2019; Goulson et al., 2015).

Despite the limitations of our risk simulation, we conclude that the EU moratorium restricting imidacloprid, thiamethoxam and clothianidin use in bee-attractive crops has not eliminated the risk for bees foraging on oilseed rape nectar. This questions the concept of banning harmful pesticides only for the use in insect-pollinated crops. In fact, both systemic and non-systemic pesticides, including neonicotinoids, have been found in bee-collected pollen from untreated plants and were shown to pose a risk to bees (McArt et al., 2017; Nicholls et al., 2018; Tosi et al., 2018). For bumblebees, neonicotinoid exposure through pollen and nectar declined post-ban (2015) in rural areas, but not in peri-urban areas (Nicholls et al., 2018). These and our results, provide support to the total ban of all neonicotinoids in France and the EU-wide ban of the outdoor use of imidacloprid, thiamethoxam and clothianidin in all crops that have come into effect in September and December 2018, respectively. Neonicotinoids are, however, still used extensively outside the European Union as well as in permanent greenhouses within the European Union (except in France), from which they may leach or be discharged into nearby water systems. Therefore, there remains a need to examine the movement of neonicotinoids in the environment. The extent by which neonicotinoids spread through leaching, runoff and dust drift as well as the factors governing neonicotinoid uptake by plants should be studied in more detail. The large variability in neonicotinoid prevalence and concentration that we determined suggests that pesticides, especially systemic pesticides, which are water-soluble and mobile, should be extensively assessed under differing field conditions particularly in respect to their transportation pathways and fate before conclusions on their safety can be drawn.

## **5. Acknowledgements**

The study was partly funded by the former French region 'Poitou-Charentes' and INRA SPE (PhD grant to DW), the European 'Interreg' program 'Poll-Ole-Gi SUDOE, SOE1/P5/EO129', the project RECOTOX NéoNet (2018), the 'Projet Pollinisateurs' funded by the French Ministry of Environment, and the European Community program for French beekeeping (797/2004) coordinated by the French Ministry of Agriculture (RISQAPI, Écophyto-DEPHY-Abeille). We thank the staff of INRA-APIS le Magneraud, CNRS-CEBC and ITSAP, including several associated Bachelor and Master students, for participating in oilseed rape nectar sampling. Many thanks to the farmers of the 'Zone Atelier Plaine & Val de Sèvre' and Marine Gourrat and Fabien Vialloux for contacting and

366 cooperating with farmers. Special thanks to Yoanna Marescot for being deeply involved in the coordination of the field work. We are  
367 grateful for the residue analysis conducted by ANSES and the fruitful exchanges with Anne-Claire Martel. In addition, we thank Ben  
368 Woodcock, Noa Simon-Delso and Jens Pistorius for their valuable advice on the statistical analyses and the writing of this study.

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577 **7. Tables**

578 **Table 1.** Acute median lethal doses (LD<sub>50</sub>) and chronic dietary median lethal doses (LDD<sub>50</sub>) used in the risk assessment for nectar-  
579 foraging bees foraging on imidacloprid residues in oilseed rape nectar.

Endpoint	Honeybee	Bumblebee	Solitary bee	
				581
Acute LD <sub>50</sub> (in µg bee <sup>-1</sup> )				582
	0.0037 (48 h) <sup>a,b</sup>	0.04 (24 h) <sup>b</sup>	0.03 (48 h) <sup>a</sup>	583
				584
Chronic 10 d-LDD <sub>50</sub> (in µg bee <sup>-1</sup> d <sup>-1</sup> )	0.00282 <sup>a</sup>	0.000282 <sup>c</sup>	0.000282 <sup>c</sup>	585 <sup>a</sup> (EFSA, 2018)

586 <sup>b</sup> (Marletto et al., 2003)

587 <sup>c</sup> (Uhl et al., 2018)

588 <sup>d</sup> Contact rather than oral exposure

589 <sup>e</sup> The endpoint has been extrapolated from the endpoint for honeybees using a safety factor of ten.

590

591

592 **Table 2.** Repeatability in imidacloprid prevalence and log<sub>10</sub> concentrations in positive samples. Repeatability refers to the closeness of  
 593 the agreement among imidacloprid residues from samples of the same field and was measured as intraclass coefficient (ICC). Only  
 594 fields with at least two (positive) measurements were considered.

Year <sup>a</sup>	Prevalence		Log <sub>10</sub> concentration	
	<i>ICC</i> <sup>b</sup> (95% <i>CI</i> <sup>c</sup> )	<i>P</i> <sup>b, d</sup>	<i>ICC</i> (95% <i>CI</i> <sup>c</sup> )	<i>P</i> <sup>d</sup>
2016	0.13 (0-0.26)	<b>0.014</b>	0 (0-0.19)	1
2017	0.73 (0.34-0.98)	<b>&lt;0.001</b>	0 (0-0.60)	0.44
2018	0.09 (0-0.44)	0.24	0.24 (0-0.88)	0.31
all	0.32 (0.13-0.41)	<b>&lt;0.001</b>	0 (0-17)	1

595 <sup>a</sup> In 2014 and 2015, fields were (typically) sampled only once and could therefore not be separately assessed.

596 <sup>b</sup> Estimation was done on the logit scale.

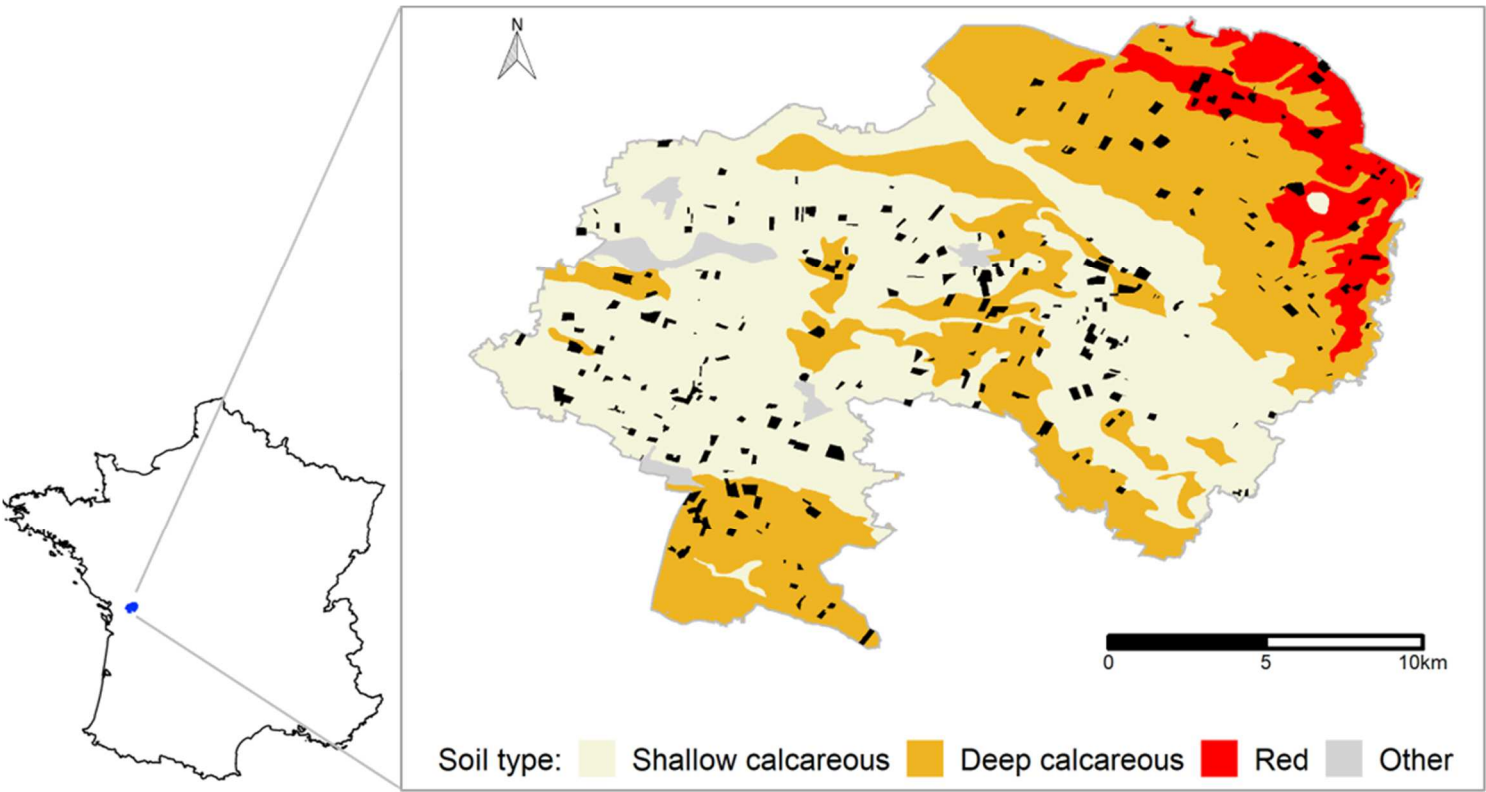
597 <sup>c</sup> 95% *CI* were obtained by bootstrapping with 1500 simulations.

598 <sup>d</sup> *P*-values were computed by permutation tests with 1500 simulations.

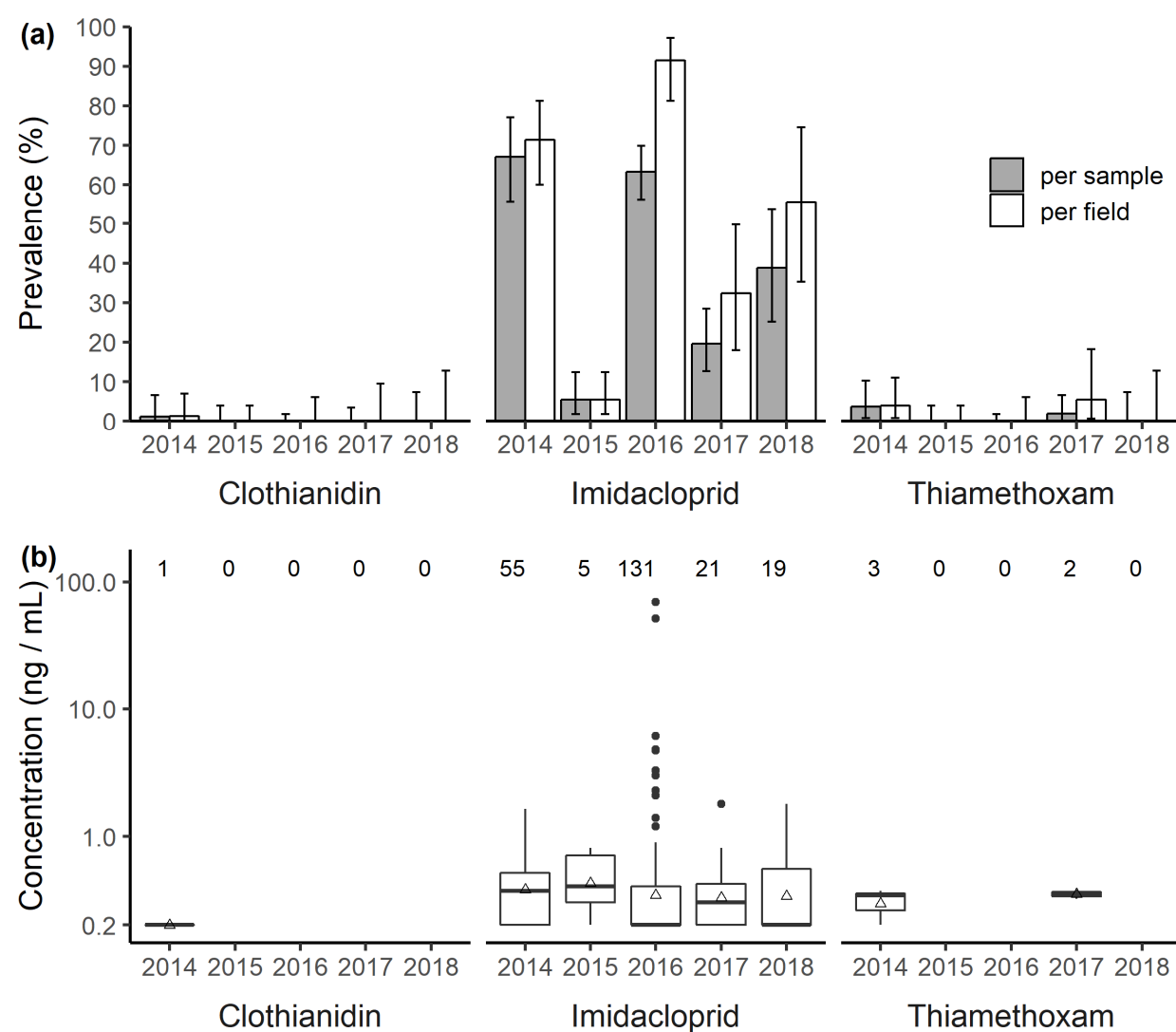
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8. Figures



**Figure 1.** Study site. Location and main soil types of the Long-Term Socio-Ecological Research (LTSER) site ‘*Plaine & Val de Sèvre*’ and the oilseed rape fields (black) that nectar was collected from within the study period (2014-2018).

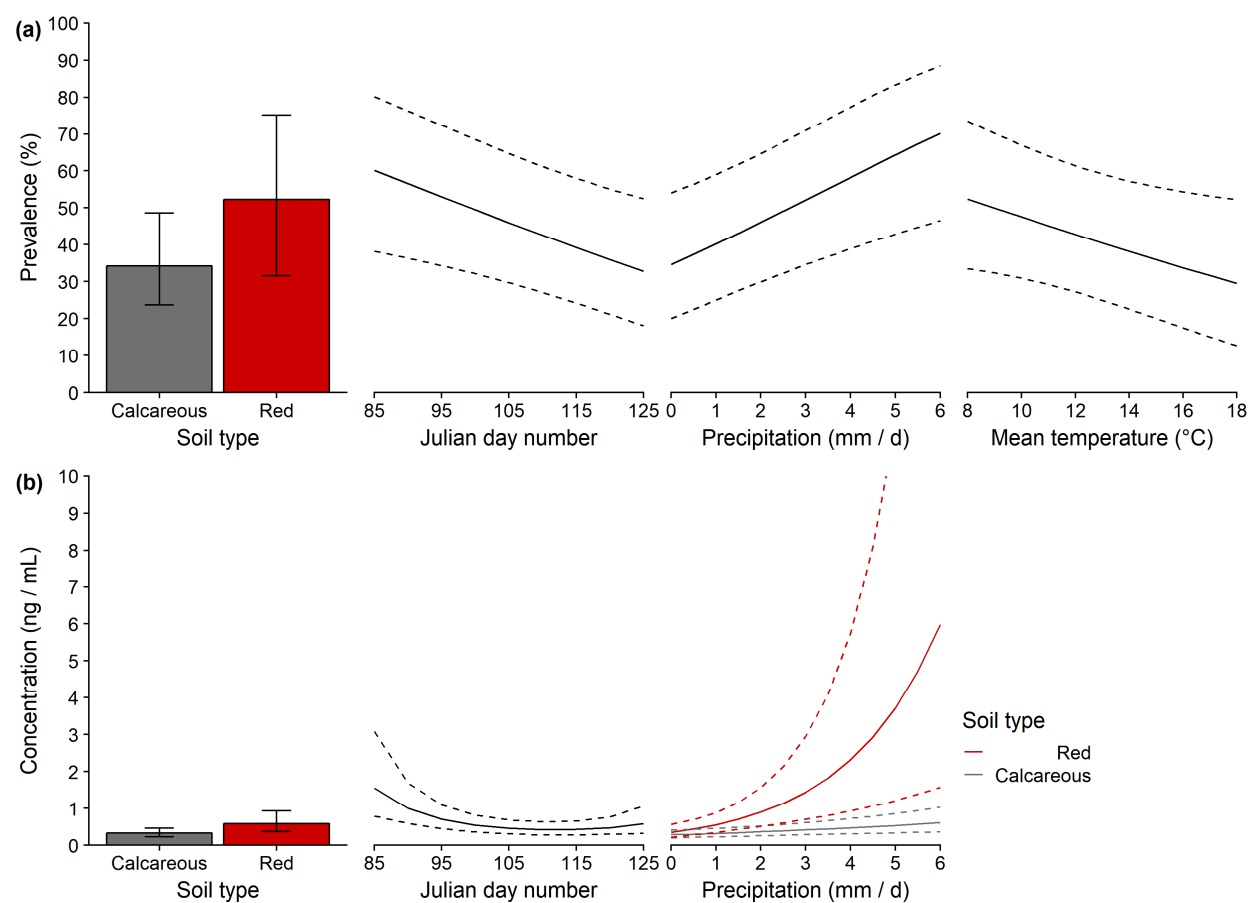


607

608 **Figure 2.** Prevalences and concentrations of clothianidin, imidacloprid and thiamethoxam in oilseed rape nectar by year. (a)  
 609 Prevalences are shown per sample (grey) and per field (white). Error bars denote 95% confidence intervals computed by binomial  
 610 tests. (b) Tukey boxplots show neonicotinoid concentrations in positive samples on a log<sub>10</sub>-scale with horizontal lines denoting median  
 611 values and triangles mean values. The bottom and the top of the boxes show the first and the third quartiles, respectively. Whiskers  
 612 illustrate minimum and maximum values within 1.5 interquartile ranges. The dots denote outliers. The number of positive samples is  
 613 shown above the boxplots. Samples < LOQ but > LOD were set to 0.2 ng mL<sup>-1</sup> as determined by the equation (LOD+LOQ)/2.

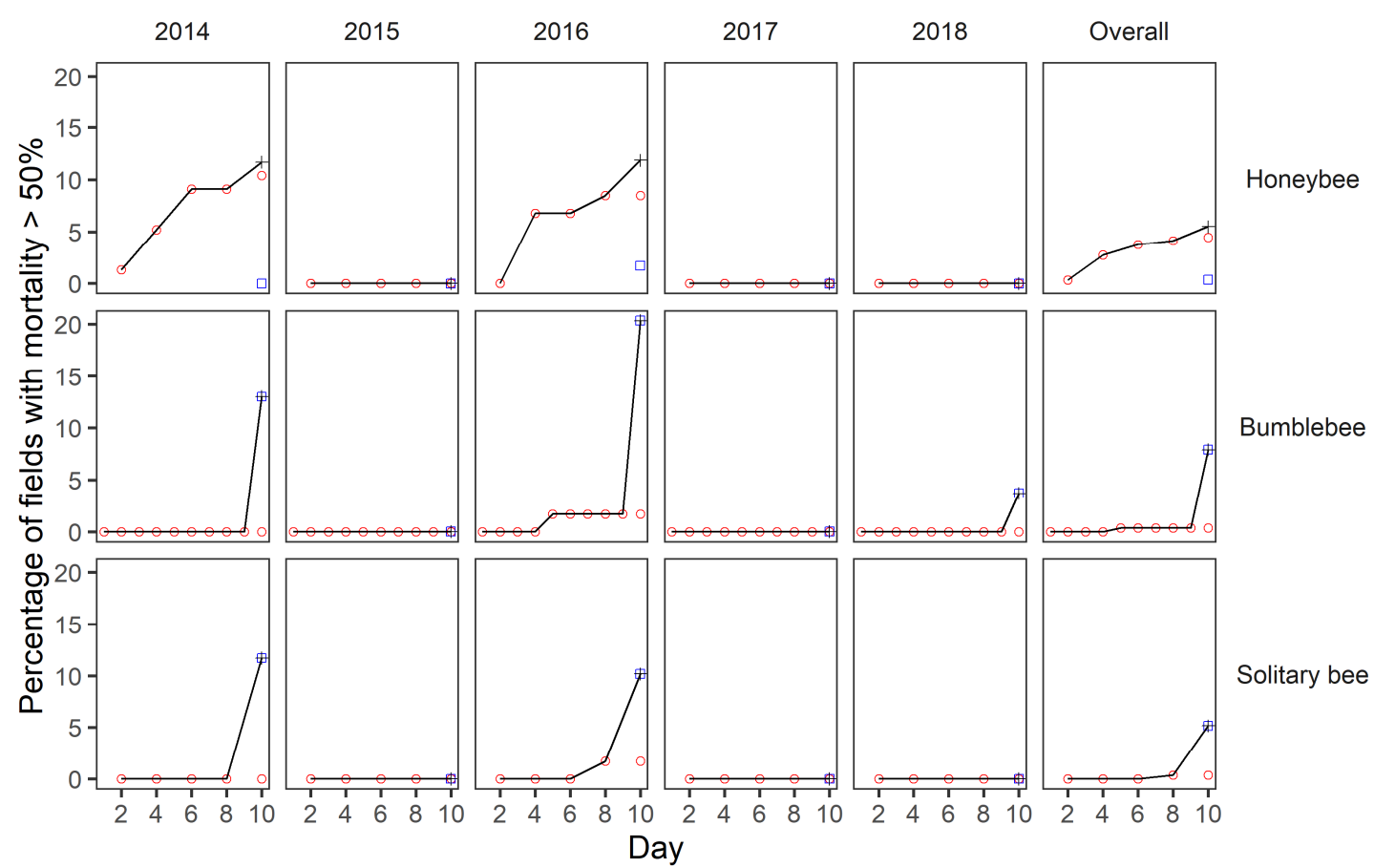
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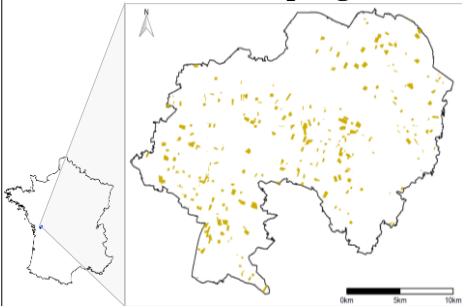
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616 **Figure 3.** Imidacloprid prevalence and concentration in positive samples in oilseed rape nectar in relation to selected predictors. Solid  
617 lines and bars denote estimates and dotted lines and error bars 95% intervals derived from (a) a generalized linear mixed-effects model  
618 (GLMM) with a binomial error distribution and a log-link for prevalence or (b) a linear model (LM) with a Gaussian error distribution  
619 for concentration in samples in which imidacloprid was detected. The GLMM for prevalence contained field identity as random factor  
620 and sampling Julian day number, precipitation in a 10-day period ending 4 days before sampling, mean air temperature in a 4-day  
621 period ending 3 days prior to nectar sampling and soil type as fixed factors. The LM for concentration contained sampling Julian day  
622 number both as linear and quadratic terms, and an interaction (including main effects) between precipitation in a 1-day period ending  
623 3 days before sampling and soil type as predictors.



**Figure 4.** Mortality risk for nectar foragers from imidacloprid exposure through oilseed rape. Percentage of fields at which over 50% of honeybees, bumblebees and solitary bees are estimated to die due to acute (red circle) and chronic (blue square) imidacloprid toxicity over 10 days. Combined mortality (due to acute or chronic toxicity) is indicated by black crosses and black lines (chronic mortality is only estimated after 10 days).

## Nectar sampling:



291 oilseed rape fields between 2014-2018



**Neonicotinoid &  
sugar content  
determination**



## Findings:

- **Imidacloprid poses mortality risk to foraging bees**
- **Rain increases imidacloprid contamination**

