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## **Neonicotinoid use on cereals and sugar beet is linked to continued low exposure risk in honeybees.**

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2     **Abstract**

3     Risks posed to bees from neonicotinoid seed treatments (clothianidin, thiamethoxam,  
4     imidacloprid) led in 2013 to the European Union instigating a moratorium for their use on mass-  
5     flowering crops, including oilseed rape in the UK. This restriction did allow for the continued  
6     use of these seed treatments, in particular clothianidin, on non-flowering crops like winter wheat.  
7     To determine the impacts of the moratorium, we assessed neonicotinoid concentrations pre-  
8     (2014) and post- (2015-17) moratorium in 347 honey samples collected across Great Britain.  
9     While the probability of detecting clothianidin declined immediately following the moratorium,  
10    detection rates remained constant over the following three years (mean= 0.10 ppb, maximum =  
11    2.8 ppb). In contrast, after three years thiamethoxam residues entirely disappeared while  
12    detection of imidacloprid was infrequent but persistent over the whole period. For those hives  
13    where neonicotinoids were detected, there was no evidence that the concentrations in the honey  
14    declined over the three years following the ban. Using metabarcoding approaches, we identified  
15    plants foraged upon by honeybees during the production of honey. After the moratorium came  
16    into effect, the highest neonicotinoid residues were associated with honey produced by foraging  
17    on both oilseed rape and several wild plants found in arable field margins. Concerns about soil  
18    persistence and uptake by non-target flowering plants ultimately led to a full European Union  
19    ban in 2018. Our results suggest that before this full ban came into effect, the use of clothianidin  
20    on non-flowering crops maintained a low-level probability of encountering this neonicotinoid  
21    within honey. However, these concentrations were low and would have been unlikely to pose  
22    significant risks to honeybees.

23

**Keywords:** *Apis mellifera*; Clothianidin; Imidacloprid; EU Moratorium; metabarcoding;

Thiamethoxam.

24

25

26 **1. Introduction**

27 Worldwide declines in both honeybees and wild bees have been associated with habitat  
28 loss, disease, invasive species, poor husbandry and climate change (Genersch, 2010; Potts *et al.*,  
29 2010; vanEngelsdorp and Meixner, 2010; Vanbergen and The Insect Pollinators Initiative, 2013;  
30 Powney *et al.*, 2019). However, the impact of agrochemicals remains one of the most frequently  
31 cited drivers of population decline (Johnson *et al.*, 2010; Mullin *et al.*, 2010; Potts *et al.*, 2010;  
32 Woodcock *et al.*, 2016). While far from the only agrochemical impacting on managed and wild  
33 bee populations, the widespread use of systemic neonicotinoid seed treatments on mass-  
34 flowering crops has been identified as representing a significant risk to population persistence  
35 (Cresswell, 2011; Blacquiere *et al.*, 2012; Goulson, 2013; Woodcock *et al.*, 2016). In the case of  
36 managed honeybees, low-level and long-term exposure to neonicotinoids in pollen and nectar  
37 has been shown to reduce colony viability through a variety of mechanisms, of which a reduction  
38 in worker homing ability and subsequent survival is the most frequently identified (Cresswell,  
39 2011; Henry *et al.*, 2012; Woodcock *et al.*, 2017).

40 To address reported negative impacts on bees, the European Union (EU) implemented in  
41 2013 a moratorium on the use of clothianidin, thiamethoxam and imidacloprid as seed treatments  
42 on mass flowering crops, including oilseed rape and sunflowers (EU moratorium 485/2013). As  
43 winter-sown crops intended for harvest in 2014 had been sown by this date, the effective  
44 cessation of use in mass flowering crops came into effect for the 2015 season. While studies

45 have questioned the economic value of neonicotinoids (e.g. Budge *et al.*, 2015), they still  
46 represent globally one of the most widely used insecticides (Simon-Delso *et al.*, 2015). Their  
47 initial ban on mass flowering crops was perceived within the farming community as a significant  
48 blow to their ability to control key pests, including pyrethroid resistant flea beetles on oilseed  
49 crops (Noleppa and Hahn, 2013). While the EU moratorium focused on mass flowering crops,  
50 winter-sown cereals and sugar beet were not considered a threat as they were not directly  
51 attractive to bees (Grimwood and Downing, 2017). As such, the moratorium did not include the  
52 these crops in the EU (EU moratorium 485/2013).

53 Clothianidin, and to a lesser extent imidacloprid, have been used in Great Britain (GB) to  
54 control aphids transmitting cereal viruses, with a combined 37% of the 1823,000 ha of winter  
55 wheat and 27% of the 422,000 ha of winter barley treated in 2015 (Garthwaite *et al.*, 2015).  
56 Similarly, 97% of the 90,000 ha of sugar beet was treated with clothianidin or thiamethoxam  
57 following the moratorium on mass flowering crops. Following the EU moratorium, clothianidin  
58 was the most widely used active ingredient (*a.i.*) in GB applied by area to 93.9% of all cereal and  
59 sugar beet crops in 2015 (Garthwaite *et al.*, 2015). While the direct treatment of mass flowering  
60 crops may be the immediate risk to bees, neonicotinoid treatment of other crops can still result in  
61 an exposure risk. Soils on field margins can be contaminated by residues applied to the crop and  
62 absorbed by wild flowering plants, while flowering crops grown as part of a rotation can also  
63 absorb soil residues that persist from the previous year (Botías *et al.*, 2015; Botias *et al.*, 2016;  
64 Woodcock *et al.*, 2018; Wintermantel *et al.*, 2020). Guttation fluids exuded from wheat leaves  
65 may also pose an additional mechanism of exposure to bees (Reetz *et al.*, 2011). These are all  
66 potentially pathways of secondary exposure for foraging bees (Goulson, 2013; Botías *et al.*,  
67 2015; Botias *et al.*, 2016). Indeed the potential risks associated with these exposure routes led to

68 the EU decision to fully ban the use of neonicotinoids under field conditions for both flowering  
69 and non-flowering crops in 2018 (EU Regulations 2018/783-785).

70 The EU moratorium can be considered a test case for how effective the restricted use of  
71 these insecticides has been in decreasing exposure of bees to neonicotinoids as result of their  
72 agricultural use. In particular it provides insights not only into the rate at which these insecticides  
73 dissipate within the agri-environment, but also whether their continued use on non-flowering  
74 cereal crops posed a threat to foraging bees. Using honey samples collected across GB from  
75 before (2014) and for three years after the moratorium (2015-17), we assess potential for  
76 exposure to honeybees from clothianidin, thiamethoxam and imidacloprid. We predicted that  
77 while the moratorium would lead to a general reduction in the probability of encountering  
78 neonicotinoid residues in honey, the continued widespread use of clothianidin on cereals would  
79 maintain a low- level exposure through its systemic non-target uptake in wild flowers and  
80 untreated mass-flowering crops on which bees feed. We also predicted that honey resulting from  
81 bees foraging on flowering crop (e.g. oilseed rape) or wild plants found in association with  
82 agricultural land would be the most likely to contain neonicotinoid residues.

83

## 84 **2. Materials and methods**

### 85 ***2.1. Honey samples***

86 The last mass flowering crops (oilseed rape in GB) treated with neonicotinoid seed treatments  
87 were harvested in 2014. We obtained 347 honey samples intended for human consumption from  
88 England, Wales and Scotland for 2014 (N=21), 2015 (N=109), 2016 (N=107) and 2017 (N=110)  
89 (Fig 1). Honey harvesting involves the removal of multiple frames from an individual hive  
90 followed by the stripping of the wax capping to allow subsequent spinning in an extractor to

remove the honey. For the purposes of this study, each honey sample originates from a single hive and represents a minimum 5 ml sub-sample removed from the homogenized mix within this collecting container. Each sample is therefore associated with a unique hive, sampling date and location. Multiple samples from the same sample year and location were not included in the analysis. Once provided honey samples were stored in -80 °C freezer. Samples from 2014 and 2015 were collected as part of a previous study (Woodcock *et al.*, 2018) and provided between 23/2/2016 - 20/5/2016. The second tranche of honey samples harvested from 2017-2018 were provided by beekeepers from 25/1/2018 -10/6/2018. In 2015 four English counties (Cambridgeshire, Suffolk, Hertfordshire and Bedfordshire) were given an emergency authorization derogation that allowed farmers to treat 5% of oilseed rape crops with clothianidin and thiamethoxam (Fig. 1) (Grimwood and Downing, 2017). Although the derogation was granted in 2015 the treated crops would not have flowered until 2016. We therefore excluded samples collected from these counties and a 5 km buffer surrounding them for 2016 (n=15) and 2017 (n=9). A 5 km buffer reflects honeybee foraging ranges (e.g. Steffan-Dewenter and Kuhn, 2003; Woodcock *et al.*, 2017).

106

## 107 **2.2. Residue analysis**

108 We tested for residues of clothianidin, thiamethoxam (UKAS accredited ISO17025:2005  
109 standards) and imidacloprid in each honey sample (see Woodcock *et al.*, 2018). To do this  
110 isotopic labelled standards were added to samples extracted using a methanol: water solution  
111 (50:50; v:v). Using SPE (Oasis HLB) each extract was cleaned and then submitted to liquid  
112 chromatography coupled to a triple quadrupole Quantum Ultra TSQ mass spectrometer using an  
113 ion max electrospray ionisation source (Thermo Fisher Scientific, Hemel Hempsstead; UK). A

114 Phenomenex Synergi Fusion column (2.5 µm particle size, 50 mm × 2 mm I.D., Phenomenex)  
115 was used to perform analyte separation. This was then compared to a mobile phase water:  
116 methanol gradient. Neonicotinoids residues in these samples were then quantified by the  
117 recovery rates corrected internal standard method. The limit of detection (LoD) was set to be  
118 three times the signal to noise ratio, while the limit of quantification (LoQ) was defined to be the  
119 LoD plus the expanded uncertainty. The LoD and LoQ for the three compounds was determined  
120 to be 0.38 and 0.53 ng g<sup>-1</sup> for the analysis of 2014 and 2015 honey samples, and 0.04 and 0.05 ng  
121 g<sup>-1</sup> for the 2016-17 samples (following the introduction of new analytical equipment). For  
122 consistency, we standardized all analysis to the 2014-15 values (LoD<sub>2014-15</sub> and LoQ<sub>2014-15</sub>), but  
123 report trends where the LoD<sub>2016-17</sub> and LoD<sub>2016-17</sub> thresholds were applied (Table A.1). Residues  
124 below the LoD were assigned a non-detect zero concentration. In the following analysis we focus  
125 on both the presence of residues as well as their concentration in honey. An alternative approach  
126 would have been to derive Risk Quotients that consider the ratio of exposure (e.g. ng a.i. bee<sup>-1</sup>) to  
127 toxicity data (EPA, 2014). However, even though honey is a direct product of collected nectar,  
128 the process of dehydration makes it hard to directly equate residues in honey to those found  
129 within the nectar from which it originates (Rortais *et al.*, 2005). As such the direct application of  
130 risk quotients based on residue concentration in honey are likely unreliable, especially were they  
131 to be compared directly to limits of concern threshold derived under the assumption of direct  
132 exposure though nectar consumption (EPA, 2014). For this reason we have not directly derived  
133 Risk Quotients in the current analysis. Note, Clothianidin is a break-down product of  
134 thiamethoxam and so some residues may not directly originate from direct use of this product,  
135 but instead from applications of thiamethoxam (Maienfisch, 2006).

136

137    ***2.3. Forage plant identification by DNA barcoding of pollen in honey***

138 Honey samples are not pure but contain contaminates that include pollen grains originating from  
139 the plants on which bees foraged while collecting nectar. By extracting these pollen grains from  
140 the honey we used metabarcoding approaches to identify what species these forage plant were.  
141 To do this we used pollen extracted from honey collected during the second round of sampling  
142 (2016-2017). A full methodology is given in Appendix A with a brief overview provided here. A  
143 vacuum filtration system (Nalgene) filtered honey samples across a mixed cellulose esters  
144 membrane (pore size=1.2 µm). Total DNA was extracted from filters using the DNeasy  
145 PowerPlant Pro Kit (Qiagen, Hilden, Germany) before homogenization and centrifuging.  
146 Resultant DNA was quantified using a Nanodrop One spectrophotometer (Thermoscientific,  
147 Waltham, MA, USA) and then amplified in a reaction containing Q5 High Fidelity Polymerase  
148 and 5X buffer (Kozich *et al.*, 2013). Primers were used to amplify and sequence (using Illumina  
149 MiSeq V3 chemistry) the solution using a universal eukaryotic internal for pollen (Sickel *et al.*,  
150 2015). All amplicons were normalised and sequenced using a Illumina MiSeq platform using  
151 MiSeq Reagent Kit v3 (Illumina Inc., San Diego, CA, USA). To identify taxonomically similar  
152 units amplicon sequence variants were phlytotted and rarefied to the species level using the  
153 phyloseq package within R 3.6.3 (McMurdie and Holmes, 2013). Not all bee keepers provided  
154 sufficient honey to allow for both neonicotinoid residue and DNA analysis leaving 135 samples  
155 with sufficient volume for processing (2016=58; 2017=77).

156

157    ***2.4. Agricultural land use as an index of neonicotinoid exposure***

158 The most likely route of exposure to neonicotinoids following the moratorium was through  
159 flowering crops and wild plants attractive to bees grown on or near agricultural land (Botías *et*

160 *al.*, 2015; Woodcock *et al.*, 2018). Although honeybees can forage much further, they typically  
161 feed within 2 km of hives (Steffan-Dewenter and Kuhn, 2003). Using the CEH Land Cover map  
162 in ArcGIS v10.4 we defined the total area of arable land in a 2 km radius around each hive  
163 (CEH, 2016).

164

165 **2.5. Statistical analysis**

166 For each of the neonicotinoid active ingredients we scored them as either being absent (0) or  
167 present (1) based on whether the residue within the honey sample was greater than the LoD (0.38  
168 ng g<sup>-1</sup>). We assessed how variation in this binary response was explained by four competing  
169 models: m1) a null intercept only model; m2) a categorical year effect; m3) the percentage cover  
170 of arable crops surrounding the hive; m4) year + arable crop cover. Due to the large number of  
171 zeros (i.e. non-detects) for the response variable (>85%) no interaction term was considered. We  
172 modeled this binary response variable using rare events logistic regression (LR) implemented in  
173 the Zelig package of R 3.5.1 (Choirat *et al.*, 2017). This applied a prior correction to adjust the  
174 intercept term based on the true population fraction ( $\tau$ ) where neonicotinoid residues were found.  
175 The competing LR models were compared using Akaike's information criterion (AIC) which  
176 provides a measure of model fit that penalizes for the number of estimated parameters. Using  
177 Morans' I we found no evidence of spatial autocorrelation (clothianidin,  $p=0.08$ ; thiamethoxam,  
178  $p=0.35$ ; imidacloprid,  $p=0.85$ ). General linear models (GLM) were then used to assess the  
179 response of residue concentrations in honey to the year in which it was collected, the cover of  
180 arable crops surrounding sites and the interaction between these two factors. Moran's I test failed  
181 to identify any spatial structure in the data (clothianidin,  $p=0.82$ ; thiamethoxam,  $p=0.60$ ;  
182 imidacloprid,  $p=0.46$ ). All GLMs used a Gaussian distribution and identity link with model

183 simplification by deletion of least significant effects (Halekoh and Højsgaard, 2014) in R version  
184 3.6.1 (Team, 2019). Standard diagnostics checks of underlying model assumptions were  
185 undertaken including checks for variance inflation (largest VIF < 3.0, Zuur *et al.*, 2010).

186 The final analysis was used to identify associations between the communities of  
187 flowering plants (determined using DNA barcoding of pollen within the honey) on which bees  
188 were foraging and neonicotinoid residues contained within those same honey samples. An  
189 unconstrained Detrended Correspondence Analysis (DCA) was performed on a matrix of arable  
190 associated plant species for each of the 135 honey samples analysed from 2016 and 2017. The  
191 analysis was restricted to UK mass flowering crops (oilseed rape and field bean (*Vicia faba*;  
192 Fabaceae)) and nectar producing flowering plants associated with arable and horticultural  
193 farming systems (as defined in Hill *et al.*, 2004) present in at least 10 honey samples. These  
194 represented the plant species most likely to present a risk of non-target exposure to neonicotinoid  
195 residues (Jones *et al.*, 2014; Botías *et al.*, 2015; Botias *et al.*, 2016; Woodcock *et al.*, 2018).  
196 Honey samples from counties that were granted a derogation in 2016 were excluded. Monte  
197 Carlo permutation tests (n=999) were used to identify associations between the DCA axis and  
198 residues of neonicotinoids found within each of the honey samples. This was assessed separately  
199 for clothianidin, thiamethoxam, imidacloprid, as well as the summed neonicotinoid residues  
200 across all three products. Year was included as a factor in all models and non-significant terms  
201 ( $p>0.05$ ) were removed. Constrained ordinations (e.g. CCA) were not appropriate as there was  
202 no *a priori* hypothesis that neonicotinoid residues acted to structure plant communities. Where  
203 significant associations between residues and floral community structure were identified these  
204 were shown as a smoothed surface on a DCA ordination plot. This analysis was undertaken using  
205 the Vegan package (Oksanen *et al.*, 2019) with the R 3.6.3 statistical platform.

206

207 **3. Results**

208 Overall, detection of clothianidin, thiamethoxam and imidacloprid residues in honey was  
209 infrequent; with detectable residues respectively in 14.8%, 4.6% and 3.4% of the honey samples  
210 when applying a LoD of 0.38 ng g<sup>-1</sup> (Table 1). In the year before the moratorium (2014) 52.3%  
211 of honey samples contained at least one of the neonicotinoids above this LoD. As the  
212 moratorium came into effect, this fell to 22.9% in 2015 and subsequently to 18.4% and 11.8% in  
213 2016 and 2017. Using the lower LoD (0.04 ng g<sup>-1</sup>) available for samples from 2016 and 2017,  
214 respectively 52.2% and 39.6% of samples were still found to contain at least one of the three  
215 neonicotinoids. However, this increased detection rate had little overall effect on the reported  
216 concentrations of the neonicotinoid compounds, which increased by no more than 0.05 ng g<sup>-1</sup>  
217 because of the inclusion of these lower LOD data (Table A.1).

218

219 **3.1. Clothianidin**

220 Clothianidin was the most widely used active ingredient, both on oilseed rape before the  
221 moratorium and as a seed treatment on non-flowering winter-sown cereal crops following the  
222 ban. It was also found to be the most frequently detected residue in honey both before and after  
223 the moratorium (Table 1, Figure 2, Figure A.1). Even so, only 20.0% (mean probability 0.20, -  
224 SE=0.10, +SE=0.20) of honey samples in 2014 (pre-moratorium) had levels of clothianidin  
225 above the limit of detection, with this declining by two-thirds (0.65, -SE=0.03, +SE=0.05) during  
226 the first year of the ban (LR: z=-1.94, p=0.05; Table 2). However, there was no evidence of a  
227 decline in the detection rate of clothianidin into the second (LR: z=-1.08, p=0.27) or third years  
228 of the moratorium (LR: z=-0.60, p=0.54). The probability of finding clothianidin residues was

229 positively correlated with extent of arable land cover surrounding the sample hives (Figure 3,  
230 Table A.2).

231 Focusing on those hives where clothianidin was detected in the honey, the mean  
232 concentrations of clothianidin were similar before ( $0.76 \text{ ng g}^{-1} \pm 0.09$ ) and after ( $0.75 \text{ ng g}^{-1}$   
233  $\pm 0.08$ ) the moratorium came into effect. We found no evidence that the concentrations of  
234 clothianidin in honey for those samples where the residue was detected declined with year  
235 (GLM:  $F_{1,43}=0.40$ ,  $p>0.05$ ). Unexpectedly, there was a negative correlation between the risk  
236 quotients associated with clothianidin in honey and the cover of arable crops (GLM:  $F_{1,40}=8.93$ ,  
237  $p=0.002$ ), although there was no interaction between year and this covariate (GLM:  $F_{1,40}=1.84$ ,  
238  $p=0.11$ ).

239

240 **3.2. Thiamethoxam**

241 Thiamethoxam was infrequently detected in honey samples and had a mean ( $\pm \text{S.E}$ ) pre-  
242 moratorium concentration in honey (taken across all samples including non-detects) of  $0.11$   
243  $\pm 0.08 \text{ ng g}^{-1}$ . This fell following the moratorium to the point that thiamethoxam could not be  
244 detected above the LoD ( $0.38 \text{ ng g}^{-1}$ ) in 2017 (Table 1; Figure 2). For those samples that  
245 contained thiamethoxam residues, the average concentrations in the post-moratorium period  
246 remained broadly equivalent to those of the pre-moratorium period ( $0.62 \text{ ng g}^{-1} \pm 0.09$ ). When we  
247 were able to apply lower LoDs ( $0.04 \text{ ng g}^{-1}$ ) for samples in 2017, thiamethoxam could still be  
248 detected in 5% of the samples, although taken across all samples, this was at a low mean  
249 concentration of  $0.004 \text{ ng g}^{-1}$  ( $\pm \text{SE } 0.002$ ) (Table A.1).

250 While detectable thiamethoxam residues had effectively disappeared by 2017, prior to  
251 this there was no statistically significant change in the probability of detecting residues from the

252 pre-moratorium period into either the first (LR:  $z=-1.10$ ,  $p=0.26$ ) or second years (LR:  $z=-1.22$ ,  
253  $p=0.21$ ) of the ban (Figure 2; Table 2; Table A.2). The probability of finding thiamethoxam  
254 residues was positively correlated with the extent of arable land around the hive (Figure 3; Table  
255 2, Table A.2). The concentration of thiamethoxam in honey for those sites where the residue was  
256 present did not change significantly between years (GLM:  $F_{1,11}=0.15$ ,  $p=0.92$ ), nor did they show  
257 any response to the cover of arable land surrounding hives (GLM:  $F_{1,13}=1.04$ ,  $p=0.22$ ) or an  
258 interaction between these two factors (GLM:  $F_{1,9}=0.56$ ,  $p=0.38$ ). However, the absence of  
259 thiamethoxam in 2017 suggests that by this point there was no exposure to this active ingredient  
260 for honeybees assuming a Lod of  $0.38 \text{ ng g}^{-1}$ .

261

### 262 **3.3. Imidacloprid**

263 Imidacloprid was the least frequently detected of the three neonicotinoids and was largely  
264 undetectable by the third year of the moratorium. Although concentrations as high as  $1.61 \text{ ng g}^{-1}$   
265 were recorded (Table 1), mean concentrations in each of the years following the moratorium  
266 were low ( $<0.05 \text{ ng g}^{-1}$ ). Focusing on those sites where imidacloprid was detected in the honey,  
267 the mean concentrations were similar before ( $0.51 \text{ ng g}^{-1} \pm 0.18$ ) and after ( $0.70 \text{ ng g}^{-1} \pm 0.14$ ) the  
268 moratorium. The probability of detecting imidacloprid did not vary significantly between years  
269 or with the cover of arable crops (Table 2, Table A.2). Similarly, the concentrations of  
270 imidacloprid in honey for those sites where it was detected were not seen to respond to year  
271 (GLM:  $F_{1,6}=0.48$ ,  $p=0.42$ ), cover of arable land (GLM:  $F_{1,9}=0.01$ ,  $p=0.99$ ) or the interaction  
272 between these two factors (GLM:  $F_{1,6}=0.57$ ,  $p=0.36$ ).

273

### 274 **3.4. Associations between forage plants and neonicotinoid residues in honey**

275 Metabarcoding of the 135 honey samples collected in 2016 and 2017 identified 459 plant  
276 taxa, of which 29 were widely associated with arable or horticultural land (Hill *et al.*, 2004). This  
277 included the mass flowering crops oilseed rape and field beans. After undertaking a DCA of  
278 these arable and horticulture associated plant species the summed concentration of all three  
279 neonicotinoids (clothianidin, thiamethoxam and imidacloprid) were found to be significantly  
280 associated with the first and second ordination axis ( $p=0.04$  for 999 permutations). There was,  
281 however, no association with these axes when the neonicotinoids were considered separately, or  
282 for the effect of sample year. The DCA biplot suggests that honey containing oilseed rape pollen  
283 was more likely to contain the highest concentrations of neonicotinoids (0.4. ng g<sup>-1</sup>; Fig. 4).  
284 However, several wild plants often found in close association with arable fields were also  
285 associated with some of the highest recorded neonicotinoid residues (> 0.3 ng g<sup>-1</sup>). This included  
286 species of *Papaver* (Papaveraceae) and *Cirsium arvense* (Asteraceae). Axis scores for the DCA  
287 are given in Table A.3.

288

#### 289 **4. Discussion**

290 For all three neonicotinoids there was evidence that residues persisted following the EU  
291 moratorium on their use in mass flowering crops, although the probability of encountering these  
292 residues within honey did decrease. In the case of clothianidin it is likely that its continued use  
293 on cereal crops after this moratorium came into effect maintained a continued, although  
294 significantly reduced, probability of encountering it within honey samples. Clothianidin was the  
295 most widely detected neonicotinoid in honey, both before and after the moratorium, with  
296 detection rates of thiamethoxam and imidacloprid being at least two-fold lower. Worldwide the  
297 relative prevalence of these three widely used neonicotinoids in honey is highly variable in

298 response to the local prevalence of agricultural use (Codling *et al.*, 2016; Mitchell *et al.*, 2017).  
299 In the context of GB, the pre- and post-moratorium dominance of clothianidin in honey similarly  
300 reflects its widespread use as a seed treatment in mass flowering and non-flowering crops during  
301 this period (Garthwaite *et al.*, 2015). Importantly, the detection of clothianidin and thiamethoxam  
302 residues above the limit of detection declined following the moratorium, although the nature of  
303 the decline differed between the active ingredients. In the case of clothianidin the initial decline  
304 following the moratorium plateaued. For thiamethoxam, there was little evidence of an  
305 immediate reduction in its detection, but residues were undetectable by the third year of the  
306 moratorium.

307 The widespread use of clothianidin as a seed treatment on cereals and sugar beet crops  
308 following the 2013 EU moratorium provided a mechanism that may have acted to maintain the  
309 persistence of this product in honey. Although used on crops not attractive to bees, there is likely  
310 an indirect risk linked to soil contamination (Botías *et al.*, 2015; Botias *et al.*, 2016; Woodcock *et*  
311 *al.*, 2018). Less than 20% *a.i.* of neonicotinoid seed treatments may be taken up by treated crops,  
312 leaving a relatively high proportion within surrounding soils or water following its application  
313 (Sur and Stork, 2003). Although under idealized conditions soil detoxification rates may be  
314 relatively rapid, there is considerable evidence to suggest that residues of all three compounds  
315 may remain present for several years under field conditions (Goulson, 2013; Bonmatin *et al.*,  
316 2015; Hilton *et al.*, 2015). This may result in a potentially large reservoir for uptake by non-  
317 target flowering plants and (un-treated) flowering crops planted later in the rotation. Subsequent  
318 expression of residues in nectar may therefore be an exposure risk for honeybees (Goulson,  
319 2013; Botías *et al.*, 2015; Botias *et al.*, 2016). Indeed Jones *et al.* (2014) found that in arable soils  
320 neonicotinoid residues may be detected even where their use had not occurred within the

321 previous three years. The correlations between detection of clothianidin and thiamethoxam  
322 residues and the area of arable land surrounding hives is consistent with this mode of exposure.  
323 However, while the probability of detecting these compounds increased with arable cover, the  
324 actual concentrations of clothianidin for those sites where residues were detected was negatively  
325 correlated with this same covariate. This may reflect unexplained patterns in historical usage  
326 surrounding those sites that meant that arable cover did not adequately describe soil  
327 accumulation of this product resulting from current and historical use (Jones *et al.*, 2014;  
328 Wintermantel *et al.*, 2020).

329 When considering carbohydrate sources as food for honeybees the majority of studies  
330 looking at the effect of neonicotinoids use artificial sucrose media as a means of exposing them  
331 under laboratory conditions (Mitchell *et al.*, 2017). As such, information on the direct  
332 consequences of residues in honey is limited. However, in the case of imidacloprid concentration  
333 as low as 0.25 ng g<sup>-1</sup> in honey were shown to be lethal to older bees, while honey containing 0.7  
334 ng g<sup>-1</sup> could have negative effects for overwintering honeybees (Rondeau *et al.*, 2014). Although  
335 not directly relating to clothianidin, imidacloprid is a closely related active ingredient with  
336 comparable LD<sub>50</sub> values (EFSA, 2013a, b). Average concentrations of clothianidin in honey of ≥  
337 0.75 ng g<sup>-1</sup>, both before and after the moratorium, may therefore pose a level of risk to  
338 honeybees. However, while the average concentration of clothianidin where detected did not  
339 change over time, in practice the proportion of samples containing clothianidin more than halved  
340 after the moratorium came into effect. Following the moratorium only 17% of the honey  
341 samples contained clothianidin with an overall mean concentration of 0.1 ng g<sup>-1</sup> when non-detect  
342 values were taken into account. It is reasonable therefore to assert that the risk to honeybees  
343 posed by its agrochemical use has on average declined.

344 Of those compounds considered, imidacloprid was the most infrequently encountered  
345 both immediately before and after the moratorium. This in part reflects trends in its use, which  
346 had been declining in GB even before the moratorium came into effect (Budge *et al.*, 2015;  
347 Garthwaite *et al.*, 2015). When compared to the post moratorium use of clothianidin in 2015,  
348 only 219 kg *a.i.* of imidacloprid was used compared to some 73,237 kg of clothianidin applied  
349 on GB cereal crops (Garthwaite *et al.*, 2015). This low but continued use of imidacloprid may  
350 explain why there was no evidence of a temporal change in detection rates in honey while  
351 simultaneously permitting its infrequent but continued appearance within the crop. Given the  
352 infrequency of this product's use over the considered time period its hard to make reliable  
353 predictions about its risk to bees in the context of other systems, however, at least in Africa and  
354 South America it is one of the dominant neonicotinoid seed treatment residues found in honey  
355 (Mitchell *et al.*, 2017).

356 The results from the metabarcoding of honey samples support these proposed  
357 mechanisms whereby residues in soil can continue to be available to honeybees following their  
358 systemic uptake in non-target plant species (Botías *et al.*, 2015; Botias *et al.*, 2016). Analyses  
359 suggested that the highest combined neonicotinoid residues found within honey were associated  
360 with samples that contained oilseed rape pollen. This suggests that the crop can uptake soil  
361 residues resulting when they are grown in a rotation following treated wheat crops (Woodcock *et*  
362 *al.*, 2018) or from longer lasting residues from historic use (Jones *et al.*, 2014). While oilseed  
363 rape continues to provide a mechanism for non-target exposure to honeybees, pollen of several  
364 wild flowers found in association with arable fields were also associated with honey containing  
365 higher neonicotinoid residues. Perhaps most notable here is the creeping thistle (*C. arvense*)  
366 which is one of the most frequently encountered weed species in arable land (Hill *et al.*, 2004).

367 Indeed, this species is so prevalent it is classified as an injurious arable weed in the UK (Weeds  
368 Act, 1959). While the risk posed to honeybees remains low given the concentrations we report  
369 within honey these results support the conclusions of previous studies that pollinator exposure to  
370 neonicotinoids is not restricted to its direct use on mass flowering crops (Goulson, 2013; Botías  
371 *et al.*, 2015; Botías *et al.*, 2016).

372

## 373 **5. Conclusions**

374 In 2018 the EU extended the moratorium on the use of neonicotinoids on mass flowering  
375 crops to include cereals and sugar beet (EU Regulations 2018/783-785). We show that the  
376 implementation of the moratorium resulted in a reduction in the probability of detecting all three  
377 of the tested neonicotinoids within honey, even if this did not result in a clear reduction in the  
378 concentrations of those products in honey when they were detected. The reduced frequency with  
379 which neonicotinoids are detected does suggest a reduction in risk posed to honeybees. The  
380 implications of these findings for other bee species are however less clear as the direct  
381 consequences of neonicotinoids for these species will depend on individual species responses  
382 dictated by unique toxicokinetic and toxicodynamic processes (Heard *et al.*, 2016). In addition,  
383 solitary bee species that do not have the same demographic regulation responses as seen in large  
384 honeybee colonies and wider evidence suggests considerable within species variation in their  
385 responses to neonicotinoid exposure, although the extent this is to do with behavior or  
386 physiology is not clear (Goulson, 2013; Henry *et al.*, 2015; Woodcock *et al.*, 2016; Woodcock *et*  
387 *al.*, 2017). While it has been suggested that there exist viable chemical or cultural control  
388 methods that could replace the use of neonicotinoids in 96% of cases (Jactel *et al.*, 2019), the  
389 majority of farmer interest organizations highlight a loss in the economic viability of many crops

390 as a result of the loss of these compounds (Noleppa and Hahn, 2013). Our results suggest that the  
391 EU moratorium on mass flowering crops has been effective in reducing exposure in bees to  
392 neonicotinoids, but support the need for further work to identify whether their use in non-  
393 flowering winter-sown crops like cereals may still be undertaken with an acceptable risk.

394

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400 Achieving Sustainable Agricultural Systems ([www.assist.ceh.ac.uk](http://www.assist.ceh.ac.uk)). CEH Land Cover® plus:  
401 Crops map is © NERC (CEH) 2016, © RSAC 2016 and © Crown Copyright 2007 (License  
402 number 100017572).

403

404 **Appendix A.** Supplementary methodology for pollen metabarcoding.

405 **Appendix B.** Supplementary figures and tables

406

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540

541 **Figure captions**

542 **Figure 1.** Distribution of honey samples collected across Great Britain from 2014-2017. A  
543 derogation for the use of clothianidin and thiamethoxam was granted for the 2016 harvest season  
544 for four counties. Samples from 2016 and 2017 within these counties and a 5 km buffer  
545 surrounding them were excluded.

546

547 **Figure 2.** Probability of detecting clothianidin and thiamethoxam residues in honey samples ( $>$   
548 LoD  $0.38 \text{ ng g}^{-1}$ ) from the pre-moratorium use of neonicotinoids on mass flowering crops (2014)  
549 though to post-moratorium period of 2015-2017. Rare events logistic regression parameter  
550 estimates ( $\pm \text{SE}$ ) are given. Although clothianidin was banned for use on mass flowering crops it  
551 continued to be used as a seed treatment on winter wheat from 2015-2017.

552

553 **Figure 3.** Probability of detecting clothianidin and thiamethoxam residues in honey samples ( $>$   
554 LoD  $0.38 \text{ ng g}^{-1}$ ) in response to the area of arable land within 2 km surrounding hives. Raw  
555 binomial residue values have been presented but are offset from the 0 and 1 true position for  
556 clarity. The model predicted response curve ( $\pm \text{SE}$ ) is also given.

557

558 **Figure 4.** Plot of detrended correspondence analysis of arable and horticultural forage plants  
559 identified from honey using DNA metabarcoding base on 2016 and 2017 samples. The  
560 significant association between the summed neonicotinoid residues found within the honey  
561 samples and floral community structure is plotted in red as a smoothed surface on the DCA  
562 ordination. These contour lines indicate those foraging plants most associated with honey  
563 containing different summed concentrations of neonicotinoid residues ( $\text{ng g}^{-1}$ ). Only arable and

564 horticultural associated plants present in at least 10 honey samples are shown on the ordination  
565 plot.

566

567

568

569 **Tables**

570 **Table 1.** Summary statistics for the residues of clothianidin (CTD), thiamethoxam (TMX) and  
 571 imidacloprid (IMI) identified from honey samples from 2014-17. Where: LoD= residue limit of  
 572 detection set at 0.38 w/w ng g<sup>-1</sup>; N= number of samples with residues above the limit of  
 573 detection.

574

		<b>2014</b> <b>(pre-</b> <b>moratorium)</b>	<b>2015</b>	<b>2016</b>	<b>2017</b>
<b>Number of honey samples</b>		21	109	92	101
<b>Percentage of Residues &gt; LoD</b>	<b>CTD</b>	38.1% (N=8)	16.6% (N=18)	10.9% (N=10)	11.9% (N=12)
	<b>TMX</b>	14.3% (N=3)	6.5% (N=7)	5.5% (N=5)	0.0% (N=0)
	<b>IMI</b>	9.6% (N=2)	5.6% (N=6)	2.2% (N=2)	1.0% (N=1)
<b>Mean concentration in honey (ng g<sup>-1</sup>)</b>	<b>CTD</b>	0.29 (SE 0.09)	0.12 (SE 0.03)	0.07 (SE 0.03)	0.10 (SE 0.04)
	<b>TMX</b>	0.11 (SE 0.08)	0.05 (SE 0.02)	0.03 (SE 0.01)	0.00 (SE 0.00)
	<b>IMI</b>	0.05 (SE 0.04)	0.04 (SE 0.02)	0.02 (SE 0.01)	0.01 (SE 0.01)
<b>Maximum recorded concentration</b>	<b>CTD</b>	1.02 ng g <sup>-1</sup>	1.69 ng g <sup>-1</sup>	1.94 ng g <sup>-1</sup>	2.78 ng g <sup>-1</sup>
	<b>TMX</b>	1.41 ng g <sup>-1</sup>	1.41 ng g <sup>-1</sup>	0.82 ng g <sup>-1</sup>	0 ng g <sup>-1</sup>
	<b>IMI</b>	0.64 ng g <sup>-1</sup>	1.61 ng g <sup>-1</sup>	0.98 ng g <sup>-1</sup>	0.78 ng g <sup>-1</sup>

575

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578

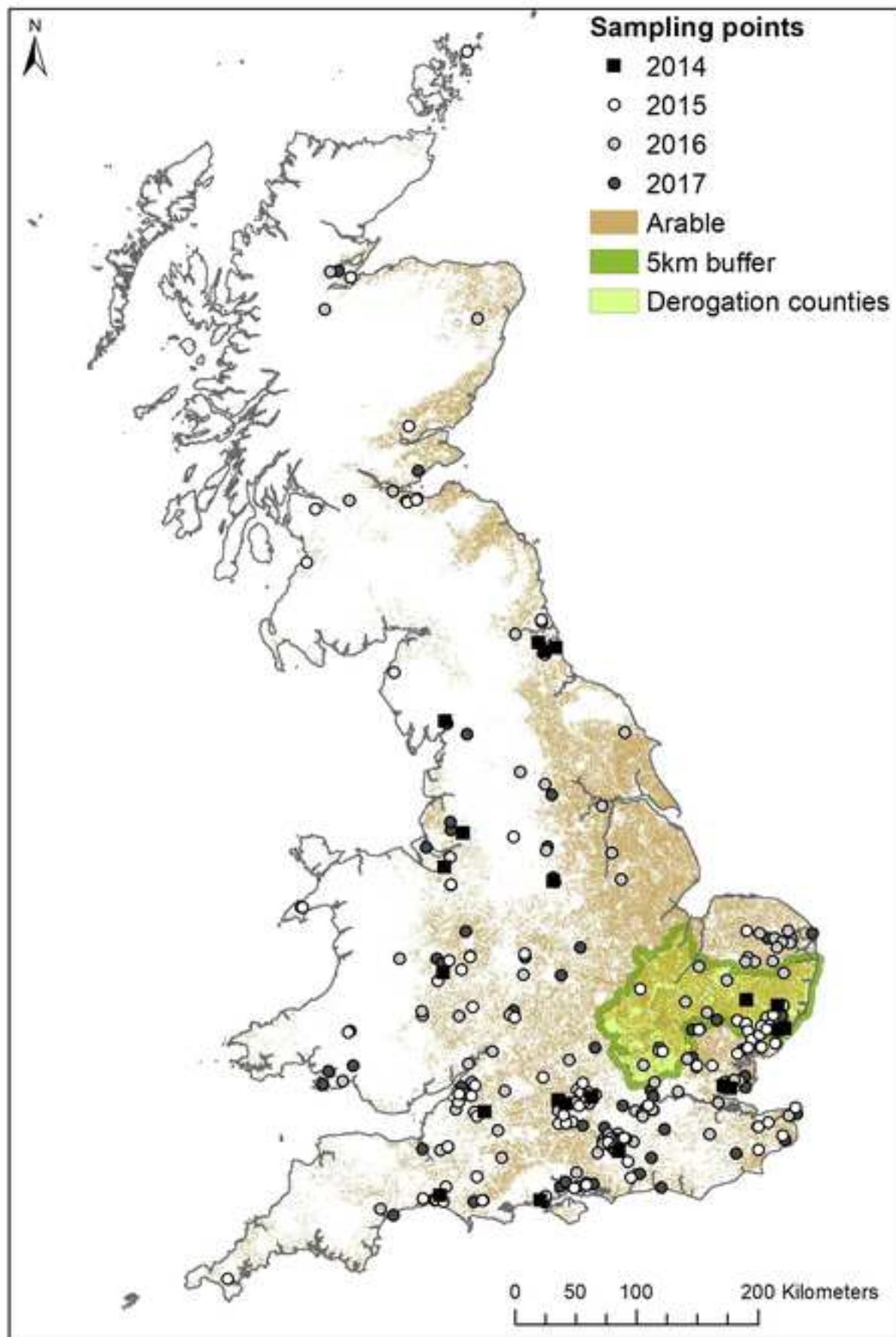
579 **Table 2.** Rare events logistic regression model comparisons assessing the probability of  
580 detecting clothianidin, thiamethoxam and imidacloprid within honey above the limit of detection  
581 ( $>0.38 \text{ ng g}^{-1}$ ). Each binomial descriptor was tested against four models and assessed against the  
582 fixed effects of year (yr.) and percentage cover of arable land (ar.). Relative explanatory power  
583 of the model to the data is assessed using AIC (smaller the better) where  $\Delta\text{AIC}$  is the difference  
584 between the best and worst fit.

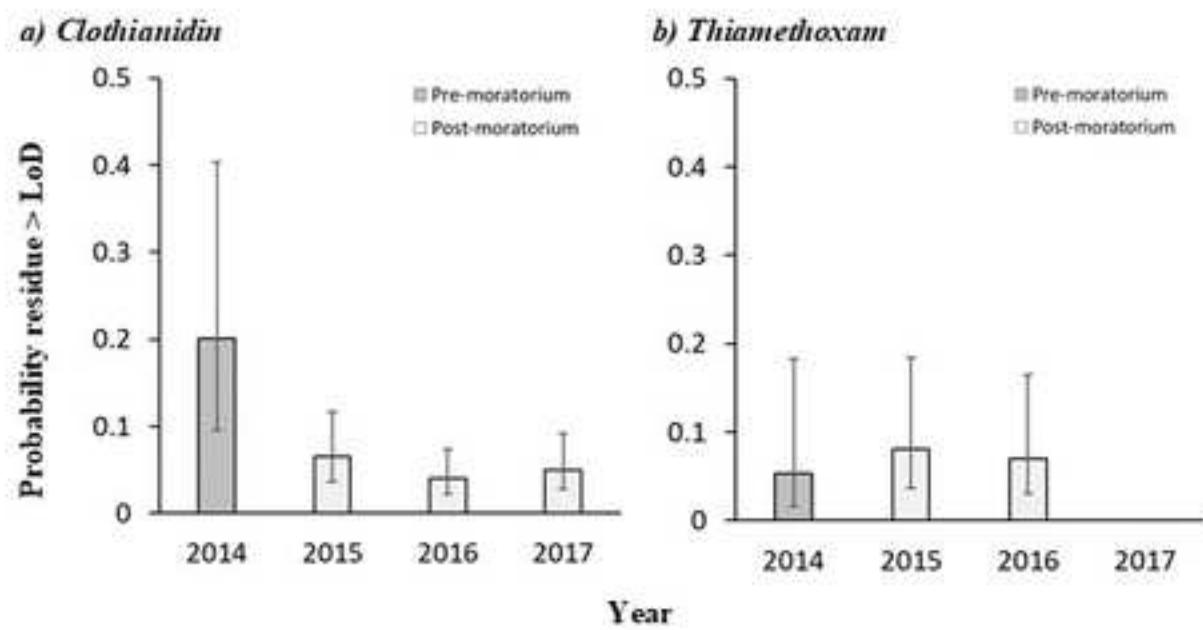
585

	<b>Clothianidin</b>	<b>Thiamethoxam</b>	<b>Imidacloprid</b>
$\mu = a_0$	AIC=273.5 ( $\Delta\text{AIC}=11.0$ )	AIC=123.4 ( $\Delta\text{AIC}=8.8$ )	AIC=97.8 ( $\Delta\text{AIC}=0.0$ )
$\mu = a_0 + \beta_1 * \text{yr}$	AIC=270.5 ( $\Delta\text{AIC}=8.0$ )	AIC=116.1 ( $\Delta\text{AIC}=1.5$ )	AIC=98.2 ( $\Delta\text{AIC}=0.4$ )
$\mu = a_0 + \beta_1 * \text{ar.}$	AIC=263.7 ( $\Delta\text{AIC}=1.2$ )	AIC=119.8 ( $\Delta\text{AIC}=5.3$ )	AIC=100.0 ( $\Delta\text{AIC}=2.2$ )
$\mu = a_0 + \beta_1 * \text{yr} + \beta_2 * \text{ar.}$	AIC=262.5 ( $\Delta\text{AIC}=0.0$ )	AIC=114.6 ( $\Delta\text{AIC}=0.0$ )	AIC=100.1 ( $\Delta\text{AIC}=2.3$ )

586

Figure 1

[Click here to access/download;Figure;Fig. 1.jpg](#)



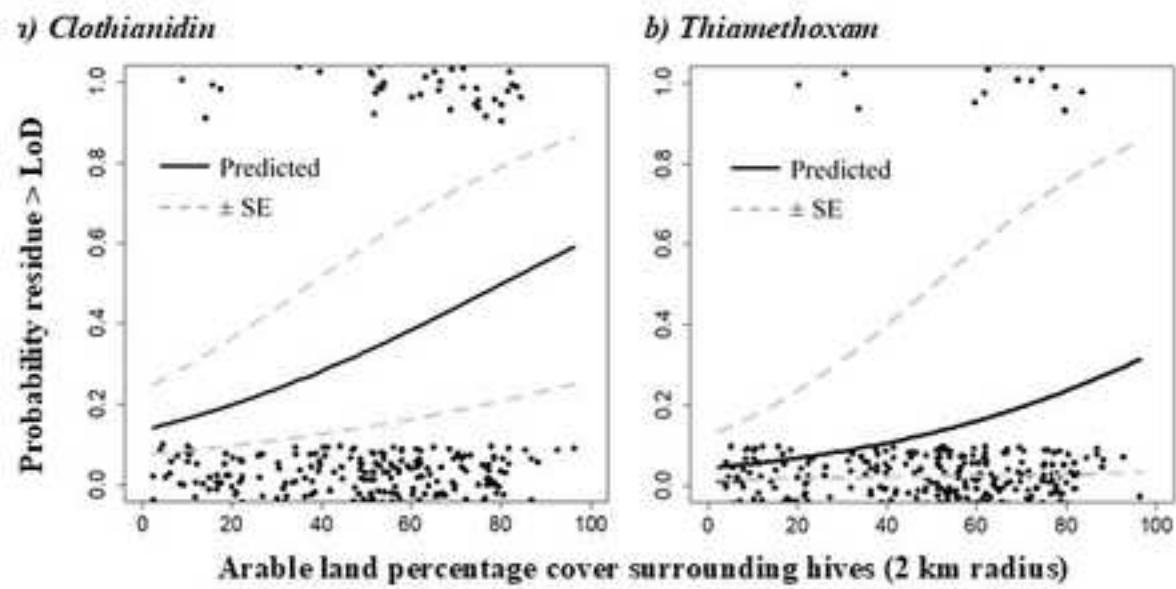
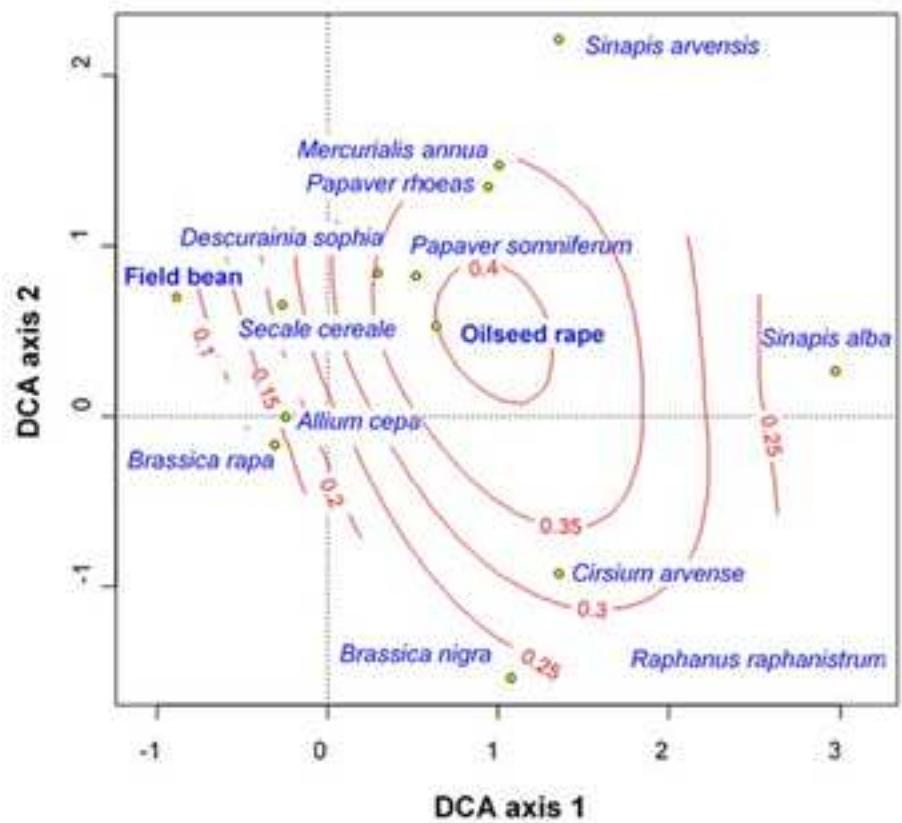


Figure 4

[Click here to access/download;Figure;Fig. 4.JPG](#)



## Appendix A. Supplementary methods for DNA barcoding

### Extraction of plant DNA from honey

Experimental samples were thoroughly mixed by hand using a clean laboratory spatula. Approximately 15 g of honey was weighed into a separate sterile 50 ml falcon tube and diluted to 50 mls using molecular grade water. Honey dilutions were heated at 55 ° C for 1hr, with occasional mixing in order to thoroughly dissolve honey and equally disperse any plant material. After removal of wax from the surface of the tubes, samples were individually filtered using a reusable bottle top vacuum filtration system (Nalgene), fitted with 47mm diameter mixed cellulose esters (MCE) membrane filters with a pore size of 1.2 µm (Millipore, Burlington, Massachusetts). Filter units were washed between samples using detergent and rinsed with both deionised H<sub>2</sub>O and 10% bleach prior to drying with a clean paper towel. Filters were retained and stored at -80° C prior to DNA extraction.

Total DNA was extracted from half a filter using the DNeasy PowerPlant Pro Kit (Qiagen, Hilden, Germany), with the following additional steps to manufacturers protocol. To account for the small size of pollen grains, approx. 0.25 g of ≤ 106 µm autoclaved, acid washed glass beads (Merck, Darmstadt, Germany) were added to the PowerBead tubes- already containing 2.38 mm metal beads. Individual filters were sliced into smaller fragments using sterile dissection scissors and placed into the PowerBead tubes. To ensure complete cellular lysis filters were treated with 450µl Bead Solution, 50 µl Phenolic Separation Solution (PSS) and 5 µl of proteinase K solution (20 mg/ml). Samples were incubated at 60° C for 1 hr, prior to addition of Solution SL and RNase A Solution (Step 2 of the manufacturer's protocol) and tissue homogenization using a Fastprep 24 tissue disrupter (MP Biomedicals, Solon, Ohio, USA) for 1 min at speed setting 5.5K. Samples

were centrifuged at 1,400 rpm for 3 min and lysate transferred to a clean 2ml microcentrifuge tube, 250 µl of Solution IR was added and the manufacturer's recommended protocol followed. Finally, due to the presence of PCR inhibitors associated with honey samples an additional wash of 500 µl, 97% ethanol was employed prior to a drying spin of 3 minutes (16,000 x g) and sample elution using Solution EB. Resultant DNA was quantified using a Nanodrop One spectrophotometer (Thermoscientific, Waltham, MA, USA) and extractions normalised to a concentration of ~10 ng µl<sup>-1</sup>.

### **Amplicon generation and sequencing**

Approximately 20 ng DNA template was amplified in a 50 µl reaction containing 0.5 µl Q5 High Fidelity Polymerase (New England Biolabs, Hitchin, UK) and associated 5X buffer, 1 µl 10 mM dNTP Mix, molecular grade water and 50mM of a unique barcode-primer combination to allow separation of sequences associated with the different samples (Kozich *et al.*, 2013). Primers were designed to specially amplify and sequence (using Illumina MiSeq V3 chemistry) a universal eukaryotic internal transcribed spacer 2 region (ITS2) from pollen by Sickel *et al.* (2015). PCR conditions were as follows: initial denaturation at 98 °C for 30 s, 37 cycles of denaturation at 98 °C for 10 s, annealing at 49 °C for 20 s and elongation at 72 °C for 25 s; followed by a final extension step at 72 °C for 2 min. Amplicons were normalised using SequalPrep Normalisation Plate Kit, 96-well (Invitrogen, Carlsbad, CA, USA), gel purified and quantified using Qubit dsDNA Assay kit (Invitrogen, Carlsbad, CA). The resultant amplicon library was sequenced at a concentration of 5.4 pM with a 0.6 pM addition of Illumina generated PhiX control library. Sequencing was performed on an Illumina MiSeq platform using MiSeq Reagent Kit v3 (Illumina Inc., San Diego, CA, USA).

## **Creation of ITS2 training database**

A total of 1 958 909 sequences were downloaded from NCBI on 25 March 2020 using the following query “internal transcribed spacer [All Fields] AND 10:10000 [SLEN]”. The downloaded sequences were de-replicated with VSEARCH (Rognes *et al.*, 2016) which resulted in 1,411,443 sequences. ITS2 regions were subsequently retrieved using ITSx (Bengtsson-Palme *et al.*, 2013) which also removed flanking conserved regions. Sequences shorter than 100 bps and those classified as non-eukaryotes were removed. From the resulting ITS2 (966,676 sequences), RDP compatible training database was created with RDP Tools (Wang *et al.*, 2007) (available at <https://sourceforge.net/projects/honeypi>).

## **Processing of the sequenced amplicon data**

The raw amplicon sequences were quality filtered and adapters removed using TrimGalore (<https://github.com/FelixKrueger/TrimGalore>). DADA2 pipeline was subsequently used to generate an ASV abundance table containing chimera-removed, high-quality error-corrected sequences (Callahan *et al.*, 2016). For each ASV, conserved regions flanking ITS2 were removed with ITSx (Bengtsson-Palme *et al.*, 2013) and resulting sequences were taxonomically classified using the naive Bayesian classifier (Wang *et al.*, 2007) against the aforementioned custom ITS2 database. Unless stated otherwise, default parameters were used for the steps listed (the entire pipeline is available at <https://github.com/hsgweon/honeypi>).

## **Molecular statistics**

After quality filtering, a total of 3891733 sequences remained. In order to identify taxonomically similar units amplicon sequence variants (ASV's) were phylotyped at the species level using the function aggregate\_taxa in R package phyloseq V 1.30.0 (McMurdie and Holmes, 2013). Taxa unassignable at the Kingdom/Phylum level and Non-Angiosperm taxa (Fungi, Metazoa, Chlorophyta) were considered erroneous or non relevant to this study and therefore removed from the analysis. Additionally to account for sequence bias samples with <6000 sequences were removed from analysis and data was rarefied to an even depth of 6066, using phyloseq function 'rarefy\_even\_depth'.

## References

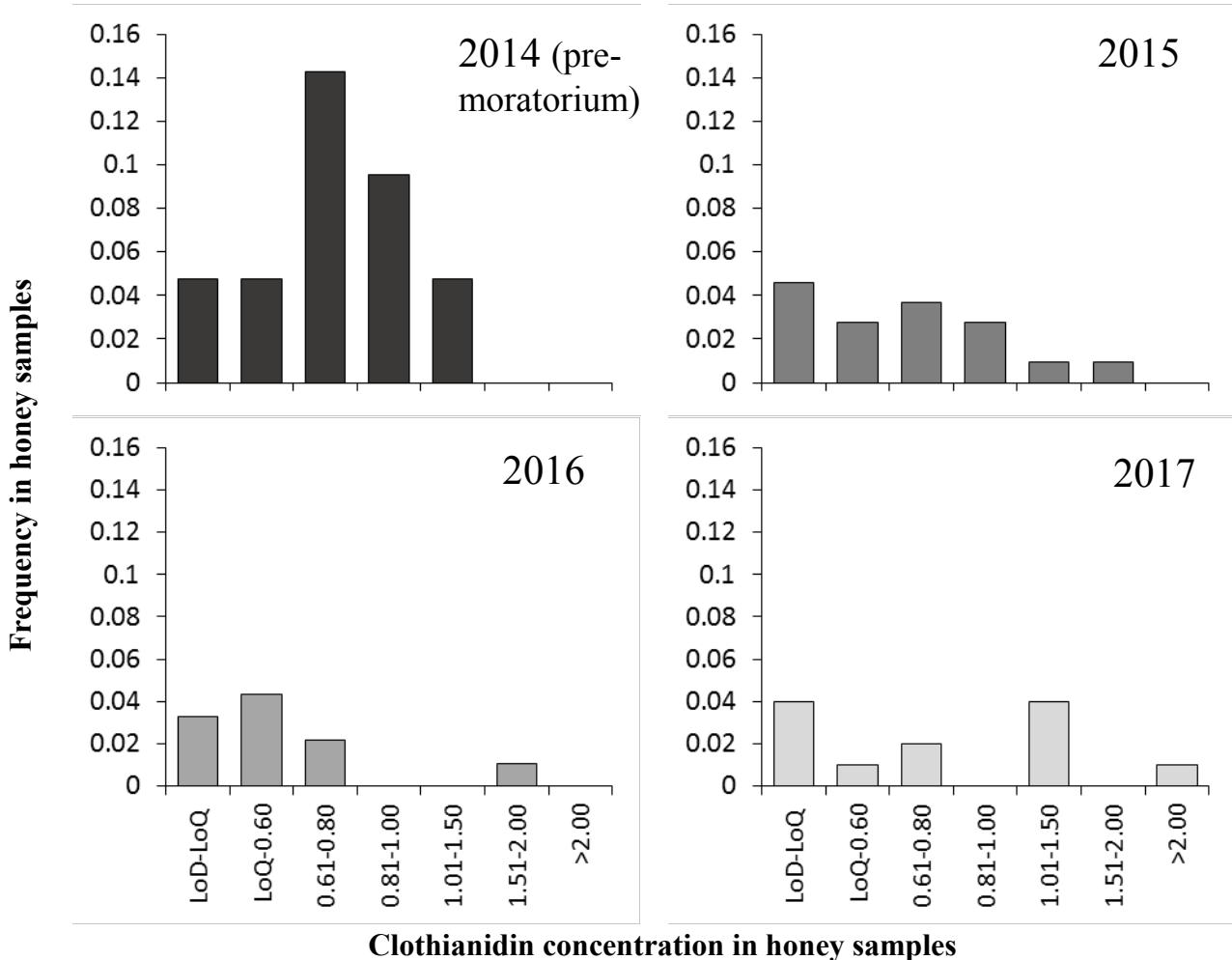
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## Appendix B. Supplementary figures and tables

**Fig A.1.** Frequency distribution for the occurrence of clothianidin in honey samples from 2014 - 2017. Limit of detection (LoD) was to  $0.38 \text{ ng g}^{-1}$  and the limit of quantification (LoQ) to  $0.53 \text{ ng g}^{-1}$ .



**Table A.1.** Summary statistics for the residues of clothianidin (CTD), thiamethoxam (TMX) and imidacloprid (IMI) from honey samples collected during 2016-17 where the LoD was set at either 0.38 ng g<sup>-1</sup> (used in the main paper) or 0.04 ng g<sup>-1</sup> (achieved only for 2016-17 samples).

In the main paper the LoD was standardized to the highest common value from 2014-2017 (0.38 ng g<sup>-1</sup>). N= number of samples with residues above the limit of detection.

		<b>2016</b>	<b>2016</b>	<b>2017</b>	<b>2017</b>
<b>LoD (ng g<sup>-1</sup>)</b>	<b>0.38</b>	<b>0.04</b>	<b>0.38</b>	<b>0.04</b>	
<b>Number of honey samples</b>	92	92	101	101	
<b>Percentage of Residues &gt; LoD</b>	<i>CTD</i> 10.9 % (N=10)	<i>TMX</i> 5.5 % (N=5)	<i>IMI</i> 12.0 % (N=11)	<i>CTD</i> 33.7 % (N=31)	<i>TMX</i> 0.0 % (N=0)
				<i>CTD</i> 11.9 % (N=12)	<i>TMX</i> 5.0 % (N=5)
				<i>IMI</i> 1.0 % (N=1)	<i>IMI</i> 9.9 % (N=10)
<b>Mean concentration in honey (ng g<sup>-1</sup>)</b>	<i>CTD</i> 0.07 (SE 0.03)	<i>TMX</i> 0.03 (SE 0.01)	<i>IMI</i> 0.02 (SE 0.01)	<i>CTD</i> 0.12 (SE 0.03)	<i>TMX</i> 0.04 (SE 0.01)
				<i>CTD</i> 0.10 (SE 0.04)	<i>TMX</i> 0.00 (SE 0.00)
				<i>IMI</i> 0.01 (SE 0.01)	<i>IMI</i> <0.01 (SE<0.01)
<b>Maximum recorded concentration</b>	<i>CTD</i> 1.94 ng g <sup>-1</sup>	<i>TMX</i> 0.82 ng g <sup>-1</sup>	<i>IMI</i> 0.98 ng g <sup>-1</sup>	<i>CTD</i> 2.78 ng g <sup>-1</sup>	<i>TMX</i> 0.00 ng g <sup>-1</sup>
				<i>IMI</i> 0.78 ng g <sup>-1</sup>	<i>IMI</i> 0.11 ng g <sup>-1</sup>
					<i>IMI</i> 0.78 ng g <sup>-1</sup>

**Table A.2.** Model outputs for rare events logistic regressions assessing the probability of detecting clothianidin, thiamethoxam and imidacloprid above the limit of detection ( $0.38 \text{ ng g}^{-1}$ ) within honey. Each binomial descriptor was tested against four models and assessed against the fixed effects of year (yr.) and percentage cover of arable land (ar.) with the fit of these assessed using AIC. Only parameter estimates for the best fit model are presented below.

***Clothianidin***

	<b>Estimate</b>	<b>Std. Error</b>	<b>z value</b>	<b>Pr(&gt; z )</b>
<b>Intercept</b>	-1.85	0.67	-2.75	0.005
<b>Year - 2015</b>	-1.02	0.52	-1.94	0.05
<b>Year - 2016</b>	-1.49	0.57	-2.61	0.01
<b>Year - 2017</b>	-1.27	0.55	-2.28	0.02
<b>Arable cover (2 km)</b>	0.02	0.01	2.89	0.003

***Thiamethoxam***

	<b>Estimate</b>	<b>Std. Error</b>	<b>z value</b>	<b>Pr(&gt; z )</b>
<b>Intercept</b>	-3.07	1.13	-2.71	0.006
<b>Year - 2015</b>	-0.82	0.74	-1.10	0.26
<b>Year - 2016</b>	-0.96	0.78	-1.22	0.21
<b>Year - 2017</b>	>0.001	0.001	2344.8	<2e-16
<b>Arable cover (2 km)</b>	0.02	0.01	1.60	0.10

***Imidacloprid***

	<b>Estimate</b>	<b>Std. Error</b>	<b>z value</b>	<b>Pr(&gt; z )</b>
<b>Intercept</b>	-3.37	0.30	-11.01	<2e-16

**Table A.3.** Axis scores (axis 1-4) for the Detrended Correspondence Analysis of the arable and horticultural plant species foraged upon by honeybees to produce the honey samples collected in 2016 and 2017.

	<b>Axis 1</b>	<b>Axis 2</b>	<b>Axis 3</b>	<b>Axis 4</b>
Eigenvalues	0.562	0.349	0.294	0.264
DCA values	0.597	0.301	0.170	0.100
Axis lengths	3.736	2.995	3.088	2.789

Metric	Description
year	year honey was collected in
2016_derrogation	Location where there was a 2016 c
Easting	National grid reference location (e
Northing	National grid reference locations (r
TMX_raw_ng_g	The raw thiamethoxam before app
CTD_raw_ng_g	The raw clothianidin before appro
IMI_raw_ng_g	The raw imidacloprid before appro
TMX_above_LOD@0.38_ng_g	Processed thiamethoxam residue c
CTD_above_LOD@0.38_ng_g	Processed clothianidin residue dat
IMI_above_LOD@0.38_ng_g	Processed imidacloprid residue dat
TMX_prob	Presence (1) or absence (0) of thia
CTD_prob	Presence (1) or absence (0) of clot
IMI_prob	Presence (1) or absence (0) of imid
Arable_2km	Percentage cover of all arable crop
Columns O to AB	Sequence counts for arable and ho

lerogation allowing the use of neonicotinoids on oilseed rape.  
esting) - note that to prefer anonymity of bee keepers the resolution here is reduced.  
northing)  
ropriate limits of detection and quantification were applied.  
ropriate limits of detection and quantification were applied.  
ropriate limits of detection and quantification were applied.  
data after applying limits of detection and quantification described in main manuscript.  
a after applying limits of detection and quantification described in main manuscript.  
ta after applying limits of detection and quantification described in main manuscript.  
methoxam residue > LoD (0.38 ng g<sup>-1</sup>)  
nianidin residue > LoD (0.38 ng g<sup>-1</sup>)  
lacloprid residue > LoD (0.38 ng g<sup>-1</sup>)  
is within 2 km radius of hive where honey was collected.  
rticulture associated plant species identified from honey samples using metabarcoding. Only species in 1

0 or more sites are included.

year	2016_derr	Easting	Northing	TMX_raw_r	CTD_raw_r	IMI_raw_n	TMX_abov	CTD_above
2014	0	345000	99000	0	0	0	0	0
2014	0	347000	282000	0	0	0	0	0
2014	0	348000	369000	0	0.611525	0	0	0.61
2014	0	349000	490000	0	0	0	0	0
2014	0	364000	398000	0	0.392273	0	0	0.38
2014	0	382000	168000	0	0	0	0	0
2014	0	426000	554000	0	0.967997	0	0	0.97
2014	0	428000	95000	0	0.943602	0	0	0.94
2014	0	430000	547000	0	0	0	0	0
2014	0	438000	358000	0.47	0	0	0.38	0
2014	0	440000	550000	0	0	0	0	0
2014	0	442000	178000	0	0.68838	0	0	0.69
2014	0	448000	174000	0	1.02063	0	0	1.02
2014	0	470000	180000	0	0	0	0	0
2014	0	492000	135000	0	0	0	0	0
2014	0	578000	189000	0	0	0	0	0
2014	0	584000	187000	0	0	0.63822	0	0
2014	0	597000	260000	1.413201	0.587471	0	1.41	0.59
2014	0	623000	255000	0.485412	0	0	0.38	0
2014	0	624000	236000	0	0	0	0	0
2014	0	628000	237000	0	0.70888	0.517751	0	0.71
2015	0	170000	30000	0	0	0	0	0
2015	0	231000	336000	0	0	0	0	0
2015	0	235000	620000	0	0	0	0	0
2015	0	242000	664000	0	0	0	0	0
2015	0	269000	233000	0	0	0	0	0
2015	0	271000	856000	0	0	0	0	0
2015	0	307000	530000	0	0	0	0	0
2015	0	317000	671000	0	0	0	0	0
2015	0	318000	670000	0	0	0	0	0
2015	0	319000	733000	0	0	0	0	0
2015	0	325000	672000	0	0	0	0	0
2015	0	331000	97000	0	0	0.4378	0	0
2015	0	340000	95000	0	0	0	0	0
2015	0	341000	95000	0	0	0	0	0
2015	0	343000	276000	0	0	0	0	0
2015	0	348000	94000	0	0	0	0	0
2015	0	348000	369000	0	0.591578	0	0	0.59
2015	0	349000	106000	0	0	0	0	0
2015	0	350000	488000	0	0	0	0	0
2015	0	351000	139000	0	0	0	0	0
2015	0	352000	292000	0	0	0	0	0
2015	0	353000	378000	0	0	0	0	0
2015	0	353000	355000	0	0	0	0	0
2015	0	359000	176000	0	0	0	0	0
2015	0	360000	182000	0	0	0	0	0

2015	0	362000	285000	0	0	0	0	0
2015	0	367000	1041000	0	0	0	0	0
2015	0	369000	296000	0	0.46933	0	0	0.38
2015	0	370000	181000	0	0	0	0	0
2015	0	371000	254000	0	0	0	0	0
2015	0	373000	168000	0	0	0	0	0
2015	0	373000	190000	0	0	0.410083	0	0
2015	0	375000	164000	0	0.44103	0	0	0.38
2015	0	375000	114000	0	0	0	0	0
2015	0	376000	167000	0	0	0	0	0
2015	0	380000	95000	0	0	0	0	0
2015	0	405000	394000	0	0	0	0	0
2015	0	406000	246000	0	0	0	0	0
2015	0	414000	298000	0	0	0	0	0
2015	0	426000	554000	0	0	0	0	0
2015	0	428000	573000	0	0	0	0	0
2015	0	429000	196000	0	0	0	0	0
2015	0	430000	547000	0	0	0	0	0
2015	0	431000	545000	0	0	0	0	0
2015	0	432000	99000	0.774158	0	0	0.77	0
2015	0	438000	358000	0	0	0	0	0
2015	0	440000	550000	0	0	0	0	0
2015	0	442000	158000	0.390786	0.721314	0	0.38	0.72
2015	0	442000	178000	0	0.443704	0	0	0.38
2015	0	446000	165000	0	0	0	0	0
2015	0	448000	174000	0	0	0	0	0
2015	0	448000	159000	0	0.558819	0.52514	0	0.56
2015	0	453000	159000	0	1.37	0	0	1.37
2015	0	455000	105000	0	0	0.461928	0	0
2015	0	456000	175000	0	0	0	0	0
2015	0	458000	186000	0	0.622939	0	0	0.62
2015	0	459000	173000	0	0	0	0	0
2015	0	460000	184000	0	0	0	0	0
2015	0	462000	192000	0	0	0	0	0
2015	0	464000	177000	0	0	0	0	0
2015	0	464000	177000	0	0	0	0	0
2015	0	465000	108000	0	0	0	0	0
2015	0	467000	184000	0	0	0	0	0
2015	0	483000	143000	0	0	0	0	0
2015	0	483000	150000	0	0	0	0	0
2015	0	485000	146000	0	0	0	0	0
2015	0	486000	139000	0	0	0	0	0
2015	0	488000	138000	0	0	0	0	0
2015	0	489000	136000	0	0	0	0	0
2015	0	490000	145000	0	0.580695	0	0	0.58
2015	0	492000	135000	0	0	0	0	0
2015	0	492000	145000	0.694241	0	0	0.69	0

2015	0	494000	146000	0	0	0	0	0
2015	0	496000	146000	0	0	0	0	0
2015	0	498000	146000	0	0	0	0	0
2015	0	499000	127000	0	0	0	0	0
2015	0	502000	114000	0	1.000122	0	0	1
2015	0	509000	269000	0	0	0	0	0
2015	0	516000	173000	0	0	0	0	0
2015	0	528000	218000	0.452912	0	0	0.38	0
2015	0	556000	206000	0	1.685685	0	0	1.69
2015	0	558000	235000	0	0	0	0	0
2015	0	568000	206000	0	0	0	0	0
2015	0	578000	192000	0	0	0	0	0
2015	0	584000	187000	0	0	0	0	0
2015	0	589000	243000	0.695117	0.91241	0	0.7	0.91
2015	0	590000	216000	0	0	0	0	0
2015	0	597000	241000	0	0	0	0	0
2015	0	597000	317000	0	0.6	0	0	0.6
2015	0	598000	235000	0	0	0	0	0
2015	0	598000	221000	0	0	0	0	0
2015	0	601000	228000	0	0	0	0	0
2015	0	606000	155000	0	0.646477	0	0	0.65
2015	0	607000	137000	0	0	0	0	0
2015	0	608000	231000	0	0	0	0	0
2015	0	608000	232000	0	0	0	0	0
2015	0	609000	238000	0.402689	0.428018	0	0.38	0.38
2015	0	609000	221000	0	0.796251	0	0	0.8
2015	0	613000	236000	0	0	0	0	0
2015	0	615000	159000	0	0	0	0	0
2015	0	619000	248000	0	0	0	0	0
2015	0	620000	224000	0	0	0	0	0
2015	0	623000	235000	0	0	0	0	0
2015	0	623000	255000	0	0	0	0	0
2015	0	624000	236000	0	0	0	0	0
2015	0	627000	149000	0	0.448162	1.614362	0	0.38
2015	0	627000	255000	1.409374	0	0.475204	1.41	0
2015	0	628000	237000	0	0.831807	0	0	0.83
2015	0	637000	171000	0	0	0	0	0
2016	0	250000	829000	0	0	0	0	0
2016	0	254000	860000	0	0	0	0	0
2016	0	264000	193000	0	0	0	0	0
2016	0	270000	671000	0	0	0	0	0
2016	0	295000	88000	0	0.24	0	0	0
2016	0	305000	679000	0	0.29	0	0	0
2016	0	312000	294000	0	0	0	0	0
2016	0	318000	670000	0	0	0.32	0	0
2016	0	319000	733000	0	0.36	0	0	0
2016	0	325000	672000	0	0	0	0	0

2016	0	330000	251000	0	0.17	0	0	0
2016	0	330000	247000	0	0	0	0	0
2016	0	341000	95000	0	0.24	0	0	0
2016	0	343000	276000	0.12	0.6	0.21	0	0.6
2016	0	345000	136000	0	0	0	0	0
2016	0	348000	369000	0.56	0	0	0.56	0
2016	0	349000	106000	0	0	0	0	0
2016	0	350000	488000	0	0	0	0	0
2016	0	358000	170000	0	0.08	0	0	0
2016	0	360000	247000	0	0.39	0	0	0.38
2016	0	364000	398000	0	0.12	0	0	0
2016	0	367000	1041000	0	0	0	0	0
2016	0	367000	182000	0	0	0	0	0
2016	0	368000	207000	0	0	0	0	0
2016	0	371000	192000	0	0.08	0	0	0
2016	0	376000	821000	0	0	0	0	0
2016	0	388000	218000	0	0	0	0	0
2016	0	392000	153000	0	0.09	0	0	0
2016	0	396000	130000	0	0.43	0	0	0.38
2016	0	398000	185000	0.19	0	0.23	0	0
2016	0	402000	248000	0	0.09	0	0	0
2016	0	407000	562000	0.16	0	0	0	0
2016	0	411000	448000	0	0	0	0	0
2016	0	413000	281000	0	0.08	0	0	0
2016	0	429000	571000	0	0	0.08	0	0
2016	0	431000	438000	0	0	0	0	0
2016	0	432000	383000	0	0.24	0	0	0
2016	0	439000	357000	0	0.28	0	0	0
2016	0	442000	169000	0	1.94	0	0	1.94
2016	0	442000	178000	0	0.26	0	0	0
2016	0	446000	165000	0	0.63	0	0	0.63
2016	0	448000	174000	0	0	0.26	0	0
2016	0	451000	210000	0	0	0	0	0
2016	0	456000	175000	0	0	0	0	0
2016	0	457000	118000	0	0	0	0	0
2016	0	457000	106000	0	0	0	0	0
2016	0	459000	187000	0	0.48	0	0	0.38
2016	0	467000	180000	0	0	0.06	0	0
2016	0	467000	175000	0	0	0	0	0
2016	0	470000	180000	0	0.17	0	0	0
2016	0	473000	182000	0	0	0	0	0
2016	0	474000	134000	0	0	0	0	0
2016	0	478000	420000	0.1	0.08	0	0	0
2016	0	479000	142000	0.44	0	0	0.38	0
2016	0	479000	145000	0	0	0	0	0
2016	0	485000	146000	0	0	0.19	0	0
2016	0	486000	146000	0	0	0.27	0	0

2016	0	486000	381000	0	0	0	0	0
2016	0	489000	136000	0	0	0	0	0
2016	0	491000	143000	0.49	0	0	0.38	0
2016	0	491000	144000	0	0	0	0	0
2016	0	491000	150000	0	0	0	0	0
2016	0	494000	359000	0.06	0.31	0	0	0
2016	0	496000	480000	0	0	0	0	0
2016	0	498000	146000	0	0.27	0	0	0
2016	0	499000	127000	0	0	0	0	0
2016	0	504000	143000	0	0	0	0	0
2016	0	506000	169000	0	0.53	0	0	0.53
2016	0	511000	167000	0	0	0.98	0	0
2016	0	511000	164000	0	0	0	0	0
2016	1	512000	207000	0	0	0.06	0	0
2016	0	520000	170000	0	0	0	0	0
2016	0	520000	169000	0	0.75	0	0	0.75
2016	1	521000	192000	0	0	0	0	0
2016	1	525000	218000	0	0.19	0	0	0
2016	1	525000	221000	0	0	0	0	0
2016	0	541000	185000	0	0	0.06	0	0
2016	1	547000	258000	0	0	0	0	0
2016	1	548000	212000	0	0	0.06	0	0
2016	1	558000	288000	0	0.42	0	0	0.38
2016	1	565000	250000	0	0	0	0	0
2016	0	567000	150000	0	0.56	0	0	0.56
2016	0	574000	175000	0	0.53	0	0	0.53
2016	0	578000	192000	0	0	0.91	0	0
2016	1	581000	276000	0	0.07	0	0	0
2016	0	587000	194000	0.82	0	0	0.82	0
2016	0	591000	194000	0.66	0	0	0.66	0
2016	0	596000	291000	0	0	0	0	0
2016	1	596000	239000	0.28	0.4	0	0	0.38
2016	0	598000	221000	0	0	0	0	0
2016	0	598000	295000	0	0	0	0	0
2016	0	604000	292000	0	0	0	0	0
2016	0	607000	137000	0	0.25	0	0	0
2016	0	608000	315000	0	0	0	0	0
2016	1	608000	231000	0	0	0	0	0
2016	1	613000	246000	0	0.23	0	0	0
2016	1	616000	240000	0.06	0	0.23	0	0
2016	0	619000	292000	0	0	0	0	0
2016	0	620000	311000	0	0	0	0	0
2016	0	622000	303000	0	0.06	0	0	0
2016	1	624000	237000	0	0	0	0	0
2016	0	626000	308000	0	0	0	0	0
2016	1	627000	283000	0	0.16	0	0	0
2016	0	631000	317000	0	0	0	0	0

2016	0	632000	165000	0	0	0	0	0
2016	0	633000	307000	0.09	0	0	0	0
2016	0	634000	310000	0	0.31	0	0	0
2017	0	231000	336000	0	0	0	0	0
2017	0	242000	664000	0	0	0	0	0
2017	0	248000	191000	0	0	0	0	0
2017	0	253000	201000	0	0	0	0	0
2017	0	261000	860000	0	0	0	0	0
2017	0	264000	193000	0	0	0	0	0
2017	0	270000	235000	0	0	0	0	0
2017	0	273000	206000	0	0	0	0	0
2017	0	295000	88000	0.08	0.77	0	0	0.77
2017	0	306000	83000	0	0	0.19	0	0
2017	0	312000	294000	0	0	0	0	0
2017	0	318000	670000	0	0	0.28	0	0
2017	0	319000	733000	0	0	0	0	0
2017	0	326000	696000	0	0	0	0	0
2017	0	330000	137000	0	0	0	0	0
2017	0	333000	386000	0	0	0	0	0
2017	0	340000	96000	0	0	0.14	0	0
2017	0	341000	95000	0	0.11	0	0	0
2017	0	341000	95000	0	0.62	0	0	0.62
2017	0	342000	294000	0	2.78	0.78	0	2.78
2017	0	343000	276000	0.05	0.44	0	0	0.38
2017	0	345000	99000	0	0.27	0	0	0
2017	0	345000	136000	0	0	0	0	0
2017	0	346000	290000	0	0	0	0	0
2017	0	348000	369000	0	0.25	0	0	0
2017	0	349000	490000	0	0	0.15	0	0
2017	0	351000	139000	0	0	0	0	0
2017	0	353000	407000	0	0	0	0	0
2017	0	353000	400000	0	0.08	0	0	0
2017	0	353000	355000	0	0.23	0	0	0
2017	0	362000	189000	0	0.06	0	0	0
2017	0	366000	316000	0	0	0	0	0
2017	0	367000	1041000	0	0	0	0	0
2017	0	367000	479000	0	0	0	0	0
2017	0	369000	296000	0	0.08	0	0	0
2017	0	373000	94000	0	0	0	0	0
2017	0	375000	164000	0	1.02	0	0	1.02
2017	0	376000	821000	0	0	0	0	0
2017	0	380000	95000	0.11	0.43	0	0	0.38
2017	0	382000	168000	0	0	0	0	0
2017	0	392000	153000	0	0	0	0	0
2017	0	405000	252000	0	0.06	0	0	0
2017	0	407000	562000	0	0	0	0	0
2017	0	411000	448000	0	0	0	0	0

2017	0	415000	295000	0	0	0	0	0
2017	0	429000	571000	0	0	0	0	0
2017	0	429000	196000	0	0	0	0	0
2017	0	431000	93000	0	0	0	0	0
2017	0	431000	438000	0	0	0	0	0
2017	0	432000	383000	0	0	0	0	0
2017	0	433000	386000	0	0	0.24	0	0
2017	0	436000	429000	0	0	0	0	0
2017	0	438000	361000	0	0	0	0	0
2017	0	444000	107000	0	0	0	0	0
2017	0	444000	281000	0	0	0	0	0
2017	0	448000	110000	0	0	0	0	0
2017	0	448000	174000	0	1.03	0	0	1.03
2017	0	455000	105000	0	0	0	0	0
2017	0	459000	173000	0	0	0	0	0
2017	0	460000	303000	0	0	0.1	0	0
2017	0	460000	184000	0	0.15	0	0	0
2017	0	462000	157000	0	0.38	0	0	0.38
2017	0	465000	109000	0.06	1.23	0	0	1.23
2017	0	466000	175000	0	0	0	0	0
2017	0	467000	175000	0	0	0	0	0
2017	0	471000	109000	0	0.17	0	0	0
2017	0	472000	221000	0	0	0	0	0
2017	0	480000	155000	0	0	0	0	0
2017	0	492000	145000	0	0.18	0	0	0
2017	0	495000	173000	0	0	0	0	0
2017	0	498000	146000	0	0.23	0	0	0
2017	0	509000	117000	0	0.23	0	0	0
2017	0	511000	167000	0	0	0.25	0	0
2017	0	515000	172000	0	0	0	0	0
2017	0	516000	173000	0	0	0	0	0
2017	0	518000	186000	0	0	0	0	0
2017	0	518000	186000	0	0	0	0	0
2017	0	519000	130000	0	0	0	0	0
2017	0	520000	180000	0	0	0	0	0
2017	0	527000	106000	0	0	0	0	0
2017	0	529000	153000	0	0	0	0	0
2017	0	552000	235000	0	0	0	0	0
2017	1	552000	213000	0	0.1	0	0	0
2017	0	556000	205000	0	1.03	0	0	1.03
2017	1	558000	288000	0	0.23	0	0	0
2017	0	558000	235000	0	0.06	0	0	0
2017	0	558000	236000	0	0	0	0	0
2017	1	572000	243000	0	0.35	0	0	0
2017	0	578000	189000	0	0.07	0	0	0
2017	0	584000	189000	0	0	0.22	0	0
2017	0	588000	134000	0	0.58	0	0	0.58

2017	0	594000	219000	0	0.25	0	0	0
2017	0	595000	197000	0	0	0	0	0
2017	0	596000	188000	0	0	0	0	0
2017	0	606000	156000	0	0	0	0	0
2017	0	607000	137000	0	0.38	0	0	0.38
2017	0	609000	221000	0	0	0	0	0
2017	0	614000	311000	0	0	0	0	0
2017	0	615000	159000	0	0	0.1	0	0
2017	1	623000	255000	0	0.12	0	0	0
2017	1	623000	255000	0.11	0.15	0	0	0
2017	1	626000	247000	0	0.06	0	0	0
2017	0	627000	149000	0	0.25	0	0	0
2017	1	627000	255000	0	0	0.18	0	0
2017	1	627000	283000	0	0.42	0	0	0.38
2017	1	628000	237000	0.07	1.25	0	0	1.25
2017	0	629000	145000	0	0	0	0	0
2017	0	633000	307000	0	0.26	0	0	0
2017	0	638000	166000	0	0	0	0	0
2017	0	651000	315000	0.05	0	0	0	0

IMI_above_TMX_prob	CTD_prob	IMI_prob	Arable_2kr	Brassica_n;	Vicia_faba	Brassica_ra	Mercurialis
0	0	0	0	73.14 NA	NA	NA	NA
0	0	0	0	88.12 NA	NA	NA	NA
0	0	1	0	71.52 NA	NA	NA	NA
0	0	0	0	51.73 NA	NA	NA	NA
0	0	1	0	52.18 NA	NA	NA	NA
0	0	0	0	60.49 NA	NA	NA	NA
0	0	1	0	51.72 NA	NA	NA	NA
0	0	1	0	50.93 NA	NA	NA	NA
0	0	0	0	59.08 NA	NA	NA	NA
0	1	0	0	50.68 NA	NA	NA	NA
0	0	0	0	57.19 NA	NA	NA	NA
0	0	1	0	80.13 NA	NA	NA	NA
0	0	1	0	66.87 NA	NA	NA	NA
0	0	0	0	56.08 NA	NA	NA	NA
0	0	0	0	22.19 NA	NA	NA	NA
0	0	0	0	25.78 NA	NA	NA	NA
0.64	0	0	1	16.71 NA	NA	NA	NA
0	1	1	0	83.47 NA	NA	NA	NA
0	1	0	0	79.51 NA	NA	NA	NA
0	0	0	0	66.67 NA	NA	NA	NA
0.38	0	1	1	52.7 NA	NA	NA	NA
0	0	0	0	39.32 NA	NA	NA	NA
0	0	0	0	48.48 NA	NA	NA	NA
0	0	0	0	26.73 NA	NA	NA	NA
0	0	0	0	36.28 NA	NA	NA	NA
0	0	0	0	31.53 NA	NA	NA	NA
0	0	0	0	37.44 NA	NA	NA	NA
0	0	0	0	59.26 NA	NA	NA	NA
0	0	0	0	61.51 NA	NA	NA	NA
0	0	0	0	55.83 NA	NA	NA	NA
0	0	0	0	77.2 NA	NA	NA	NA
0	0	0	0	8.45 NA	NA	NA	NA
0.38	0	0	1	41.87 NA	NA	NA	NA
0	0	0	0	50.9 NA	NA	NA	NA
0	0	0	0	52.35 NA	NA	NA	NA
0	0	0	0	74.84 NA	NA	NA	NA
0	0	0	0	46.42 NA	NA	NA	NA
0	0	1	0	71.52 NA	NA	NA	NA
0	0	0	0	53.57 NA	NA	NA	NA
0	0	0	0	55.45 NA	NA	NA	NA
0	0	0	0	47.21 NA	NA	NA	NA
0	0	0	0	71.21 NA	NA	NA	NA
0	0	0	0	51.31 NA	NA	NA	NA
0	0	0	0	61.93 NA	NA	NA	NA
0	0	0	0	8.39 NA	NA	NA	NA
0	0	0	0	21.6 NA	NA	NA	NA

0	0	0	0	69.66	NA	NA	NA
0	0	0	0	37.62	NA	NA	NA
0	0	1	0	82	NA	NA	NA
0	0	0	0	57.84	NA	NA	NA
0	0	0	0	56.78	NA	NA	NA
0	0	0	0	52.03	NA	NA	NA
0.38	0	0	1	64.16	NA	NA	NA
0	0	1	0	17.33	NA	NA	NA
0	0	0	0	78.43	NA	NA	NA
0	0	0	0	27.18	NA	NA	NA
0	0	0	0	81	NA	NA	NA
0	0	0	0	20.79	NA	NA	NA
0	0	0	0	46.11	NA	NA	NA
0	0	0	0	34.25	NA	NA	NA
0	0	0	0	51.72	NA	NA	NA
0	0	0	0	53.82	NA	NA	NA
0	0	0	0	70.37	NA	NA	NA
0	0	0	0	59.08	NA	NA	NA
0	0	0	0	49.87	NA	NA	NA
0	1	0	0	30.5	NA	NA	NA
0	0	0	0	50.68	NA	NA	NA
0	0	0	0	57.19	NA	NA	NA
0	1	1	0	74.24	NA	NA	NA
0	0	1	0	80.13	NA	NA	NA
0	0	0	0	42.76	NA	NA	NA
0	0	0	0	66.87	NA	NA	NA
0.53	0	1	1	76.59	NA	NA	NA
0	0	1	0	60.01	NA	NA	NA
0.38	0	0	1	46.42	NA	NA	NA
0	0	0	0	67.6	NA	NA	NA
0	0	1	0	84.5	NA	NA	NA
0	0	0	0	52.79	NA	NA	NA
0	0	0	0	77.82	NA	NA	NA
0	0	0	0	65.86	NA	NA	NA
0	0	0	0	48.6	NA	NA	NA
0	0	0	0	48.73	NA	NA	NA
0	0	0	0	46.16	NA	NA	NA
0	0	0	0	38.39	NA	NA	NA
0	0	0	0	23.17	NA	NA	NA
0	0	0	0	30.14	NA	NA	NA
0	0	0	0	15.05	NA	NA	NA
0	0	0	0	19.29	NA	NA	NA
0	0	0	0	11.16	NA	NA	NA
0	0	0	0	4.6	NA	NA	NA
0	0	1	0	15.56	NA	NA	NA
0	0	0	0	22.19	NA	NA	NA
0	1	0	0	33.67	NA	NA	NA

0	0	0	0	44.78	NA	NA	NA
0	0	0	0	40.79	NA	NA	NA
0	0	0	0	79.36	NA	NA	NA
0	0	0	0	52.95	NA	NA	NA
0	0	1	0	62.03	NA	NA	NA
0	0	0	0	80.45	NA	NA	NA
0	0	0	0	14.85	NA	NA	NA
0	1	0	0	61.66	NA	NA	NA
0	0	1	0	39.69	NA	NA	NA
0	0	0	0	78.53	NA	NA	NA
0	0	0	0	60.34	NA	NA	NA
0	0	0	0	62.02	NA	NA	NA
0	0	0	0	16.71	NA	NA	NA
0	1	1	0	66.17	NA	NA	NA
0	0	0	0	55.55	NA	NA	NA
0	0	0	0	78.61	NA	NA	NA
0	0	1	0	60.54	NA	NA	NA
0	0	0	0	74.46	NA	NA	NA
0	0	0	0	45.73	NA	NA	NA
0	0	0	0	19.55	NA	NA	NA
0	0	1	0	51.36	NA	NA	NA
0	0	0	0	58.48	NA	NA	NA
0	0	0	0	62.07	NA	NA	NA
0	0	0	0	61.07	NA	NA	NA
0	1	1	0	69.08	NA	NA	NA
0	0	1	0	68.71	NA	NA	NA
0	0	0	0	68.4	NA	NA	NA
0	0	0	0	22.45	NA	NA	NA
0	0	0	0	57.71	NA	NA	NA
0	0	0	0	69.47	NA	NA	NA
0	0	0	0	63.78	NA	NA	NA
0	0	0	0	80.65	NA	NA	NA
0	0	0	0	66.67	NA	NA	NA
1.61	0	1	1	78.62	NA	NA	NA
0.38	1	0	1	77.51	NA	NA	NA
0	0	1	0	52.7	NA	NA	NA
0	0	0	0	16.63	NA	NA	NA
0	0	0	0	16.9	0	0.371342	0.007099
0	0	0	0	61.3	NA	NA	NA
0	0	0	0	3.9	NA	NA	NA
0	0	0	0	46.82	NA	NA	NA
0	0	0	0	53.3	0	0.001145	0.035811
0	0	0	0	66.7	0	0	0.399486
0	0	0	0	59.9	0	0	0.120146
0	0	0	0	51.4	0	0	0.426542
0	0	0	0	77.2	NA	NA	NA
0	0	0	0	7.9	0	0.143902	0.233997

0	0	0	0	71.9	NA	NA	NA	NA
0	0	0	0	77.62	0.000765	0.007513	0.148998	0.00313
0	0	0	0	49.5	0	0	0	0
0	0	1	0	75.2	0	0.031289	0.021427	0.050109
0	0	0	0	71.7	0	0.04612	0.318065	0.003936
0	1	0	0	72.1	0.000267	0.00912	0.180095	0
0	0	0	0	54	0.004098	0	0.035159	0.00586
0	0	0	0	55.1	NA	NA	NA	NA
0	0	0	0	17.6	NA	NA	NA	NA
0	0	1	0	81.5	NA	NA	NA	NA
0	0	0	0	48.6	0	0.028958	0.385922	0.004519
0	0	0	0	41.3	0	0.01134	0.012312	0.018699
0	0	0	0	44.7	0	0.006268	0.066452	0.018682
0	0	0	0	47.5	NA	NA	NA	NA
0	0	0	0	52.4	0.006247	0	0.037694	0.129599
0	0	0	0	53.9	0	0.006015	0.119225	0.008275
0	0	0	0	22	NA	NA	NA	NA
0	0	0	0	56.3	NA	NA	NA	NA
0	0	1	0	71.43	0.007571	0.025166	0.033929	0.022222
0	0	0	0	76.9	NA	NA	NA	NA
0	0	0	0	69.7	NA	NA	NA	NA
0	0	0	0	51.7	NA	NA	NA	NA
0	0	0	0	18.3	NA	NA	NA	NA
0	0	0	0	10.6	NA	NA	NA	NA
0	0	0	0	31.4	0	0.002328	0.14507	0.002472
0	0	0	0	5.5	NA	NA	NA	NA
0	0	0	0	18.7	NA	NA	NA	NA
0	0	0	0	55.4	0	0	0.005006	0.001203
0	0	1	0	53.83	0.007367	0.006392	0.060022	0.002194
0	0	0	0	79.9	NA	NA	NA	NA
0	0	1	0	39.7	0.007333	0.016646	0.02076	0.047338
0	0	0	0	65	0	0.042538	0.012041	0.037904
0	0	0	0	44.9	NA	NA	NA	NA
0	0	0	0	64.3	NA	NA	NA	NA
0	0	0	0	47.4	NA	NA	NA	NA
0	0	0	0	22	NA	NA	NA	NA
0	0	1	0	74.6	0.008799	0.00632	0.090402	0.009914
0	0	0	0	41.7	NA	NA	NA	NA
0	0	0	0	22.2	NA	NA	NA	NA
0	0	0	0	53.1	NA	NA	NA	NA
0	0	0	0	57.31	NA	NA	NA	NA
0	0	0	0	65.71	NA	NA	NA	NA
0	0	0	0	96.4	NA	NA	NA	NA
0	1	0	0	62.43	0.007545	0.000794	0.093418	0.003872
0	0	0	0	65.59	NA	NA	NA	NA
0	0	0	0	11.7	0.006698	0.002625	0.026024	0.012763
0	0	0	0	10.2	0	0	0.002588	0

0	0	0	0	86.8	NA	NA	NA
0	0	0	0	4	NA	NA	NA
0	1	0	0	20.17	0	0.002163	0.042581
0	0	0	0	21.6	NA	NA	NA
0	0	0	0	39.5	0	0.121363	0.187062
0	0	0	0	92.6	0.002699	0	0.10928
0	0	0	0	86.8	NA	NA	NA
0	0	0	0	36.3	0	0.021267	0.01973
0	0	0	0	55.8	NA	NA	NA
0	0	0	0	40.4	0	0.001964	0.080202
0	0	1	0	34.91	0.000511	0.023359	0.192098
0.98	0	0	1	12	0	0.001934	0.244763
0	0	0	0	26	NA	NA	NA
0	0	0	0	61.8	0	0.140371	0.277569
0	0	0	0	10.2	NA	NA	NA
0	0	1	0	7.5	0	0.001998	0.09411
0	0	0	0	2.9	0	0	0.015379
0	0	0	0	42.8	0	0.019068	0.382079
0	0	0	0	55	0	0.002946	0.104347
0	0	0	0	2.3	0	0	0.589834
0	0	0	0	12.1	NA	NA	NA
0	0	0	0	37.7	NA	NA	NA
0	0	1	0	70.2	0.002423	0.003931	0.064357
0	0	0	0	85.1	NA	NA	NA
0	0	1	0	66.98	0.003367	0.003816	0.284231
0	0	1	0	63.3	0.005686	0.011753	0.059007
0.91	0	0	1	62.01	NA	NA	NA
0	0	0	0	33	0	0	0.015395
0	1	0	0	59.48	0.003582	0.003059	0.006118
0	1	0	0	85.38	0.023126	0.007201	0.05281
0	0	0	0	69.2	0	0.003618	0
0	0	1	0	80	0.010992	0.052497	0.046432
0	0	0	0	56	0.004743	0.010294	0.111944
0	0	0	0	75.9	NA	NA	NA
0	0	0	0	71.6	0	0.002486	0.04969
0	0	0	0	57.7	0.013282	0.009433	0.021771
0	0	0	0	78.6	NA	NA	NA
0	0	0	0	60.4	0	0.003536	0.041925
0	0	0	0	51.6	0	0.002952	0.009547
0	0	0	0	69.9	NA	NA	NA
0	0	0	0	76.2	NA	NA	NA
0	0	0	0	20.9	0	0.034241	0.336745
0	0	0	0	73.3	0	0.004984	0.085914
0	0	0	0	51	0	0.000954	0.086617
0	0	0	0	36.3	NA	NA	NA
0	0	0	0	77.1	NA	NA	NA
0	0	0	0	48.6	NA	NA	NA

0	0	0	0	79.05	0	0.017037	0.096967	0
0	0	0	0	31.8 NA	NA	NA	NA	
0	0	0	0	61.8	0	0.008985	0.173936	0.005706
0	0	0	0	47.2	0	0.001542	0.032956	0.001696
0	0	0	0	36.8	0	0	0.000685	0
0	0	0	0	35.8 NA	NA	NA	NA	
0	0	0	0	10.2	0	0.006253	0.349273	0.000844
0	0	0	0	48.9 NA	NA	NA	NA	
0	0	0	0	3.9 NA	NA	NA	NA	
0	0	0	0	40.8	0	0	0.026428	0
0	0	0	0	14.6 NA	NA	NA	NA	
0	0	1	0	53.4 0.004222	0.004446	0.030182	0.007096	
0	0	0	0	34.1	0	0	0.191707	0
0	0	0	0	59.9 NA	NA	NA	NA	
0	0	0	0	51.5	0	0	0.445688	0.001081
0	0	0	0	76.9 NA	NA	NA	NA	
0	0	0	0	57.3	0	0	0	0
0	0	0	0	26.7	0	0.005268	0.075707	0.002083
0	0	0	0	9.3 NA	NA	NA	NA	
0	0	0	0	54.9 0.009196	0	0.031825	0	
0	0	0	0	49.3	0	0	0.026077	0.017225
0	0	1	0	53 0.009758	0	0.010773	0.009055	
0.78	0	1	1	14.1	0	0.001938	0.001974	0
0	0	1	0	74.9	0	0	0.032496	0
0	0	0	0	75.1	0	0.019828	0	0.002203
0	0	0	0	71.7 NA	NA	NA	NA	
0	0	0	0	77.7 NA	NA	NA	NA	
0	0	0	0	72.2	0	0.000716	0.240726	0.04895
0	0	0	0	51.1	0	0	0.012744	0
0	0	0	0	45.3	0	0.000574	0.337801	0.000862
0	0	0	0	48.5 NA	NA	NA	NA	
0	0	0	0	65.8	0	0.007856	0.005904	0
0	0	0	0	63.6 0.006202	0	0.020156	0.012202	
0	0	0	0	57.3 0.004963	0.014363	0.036289	0	
0	0	0	0	88 NA	NA	NA	NA	
0	0	0	0	41.4	0	0	0.256243	0
0	0	0	0	7.8 NA	NA	NA	NA	
0	0	0	0	81.8 NA	NA	NA	NA	
0	0	0	0	71.3 NA	NA	NA	NA	
0	0	1	0	8.8	0	0.001814	0.14173	0.01354
0	0	0	0	53.8 NA	NA	NA	NA	
0	0	1	0	82.6 0.01096	0.007403	0.066875	0.008481	
0	0	0	0	62.1	0	0.024436	0.312224	0.010982
0	0	0	0	56.2 NA	NA	NA	NA	
0	0	0	0	74.8	0	0.1655	0.320231	0
0	0	0	0	51.9 NA	NA	NA	NA	
0	0	0	0	18.3	0	0	0.01459	0

0	0	0	0	39.7	NA	NA	NA	NA
0	0	0	0	31.3	0	0	0.533831	0
0	0	0	0	69.8	0	0	0	0
0	0	0	0	42	0	0	0.396621	0
0	0	0	0	5.5	0	0	0.145112	0
0	0	0	0	18.6	0	0	0.063826	0.001753
0	0	0	0	3.4	0	0	0.207909	0
0	0	0	0	44.1	0	0.002743	0.046218	0
0	0	0	0	37.6	0	0	0.146772	0.004233
0	0	0	0	6	0	0	0.11644	0
0	0	0	0	77.7	NA	NA	NA	NA
0	0	0	0	26.2	0	0	0.178026	0.002589
0	0	1	0	65.1	0	0.036199	0.050814	0.057547
0	0	0	0	44.5	0	0	0.352934	0.002575
0	0	0	0	51.8	0	0	0.399293	0.004874
0	0	0	0	5.1	0	0	0.12815	0
0	0	0	0	76	0.005002	0.00148	0.525101	0
0	0	1	0	66.5	0	0.01222	0.02847	0.005256
0	0	1	0	51.1	0.000922	0.015941	0.190058	0.004523
0	0	0	0	34.1	0	0.131088	0.095417	0.028272
0	0	0	0	22.3	0	0.022097	0.166989	0.005602
0	0	0	0	17.1	0	0	0.010938	0.013929
0	0	0	0	76.4	0	0.246162	0.009789	0.000901
0	0	0	0	30.2	NA	NA	NA	NA
0	0	0	0	32.3	0	0	0.01256	0
0	0	0	0	35.1	NA	NA	NA	NA
0	0	0	0	36.4	0	0	0.456784	0.003936
0	0	0	0	39.7	0	0	0	0.005599
0	0	0	0	12.1	0	0	0.133255	0.001975
0	0	0	0	20	NA	NA	NA	NA
0	0	0	0	13.5	0	0.000694	0.012876	0.004163
0	0	0	0	6.4	0	0	0.275092	0.007666
0	0	0	0	6.4	NA	NA	NA	NA
0	0	0	0	38.7	0	0	0	0.012372
0	0	0	0	7	NA	NA	NA	NA
0	0	0	0	2.4	0	0	0.218455	0
0	0	0	0	37.8	NA	NA	NA	NA
0	0	0	0	70.1	0	0.339117	0.154803	0
0	0	0	0	82.5	0	0.015927	0.030678	0.007502
0	0	1	0	75	0.001204	0	0.007287	0.016476
0	0	0	0	70.2	NA	NA	NA	NA
0	0	0	0	78.4	0	0.184976	0.333397	0.002201
0	0	0	0	81.8	0	0.005067	0.354894	0.002695
0	0	0	0	83.6	NA	NA	NA	NA
0	0	0	0	21.4	NA	NA	NA	NA
0	0	0	0	17	0.002896	0.000499	0.006641	0.010886
0	0	1	0	57.2	0.013031	0.005805	0.156559	0.006423

0	0	0	0	81.8	0.01018	0.013795	0.119417	0.006981
0	0	0	0	61.1	0	0.010941	0.426054	0
0	0	0	0	46.5	0	0.008083	0.005042	0.003951
0	0	0	0	51.2	0	0.010124	0.471031	0
0	0	1	0	57.6	0	0.00126	0.175922	0.004803
0	0	0	0	69.5	0.005779	0.00787	0.047056	0.01552
0	0	0	0	63.1	NA	NA	NA	NA
0	0	0	0	18.3	NA	NA	NA	NA
0	0	0	0	83.4	0	0.000913	0.548697	0
0	0	0	0	83.4	0	0.141495	0.446999	0.00422
0	0	0	0	39.2	NA	NA	NA	NA
0	0	0	0	78.4	0	0.001754	0.120441	0.002691
0	0	0	0	77.4	0	0.000482	0.547629	0.000722
0	0	1	0	77.3	0.013589	0	0.034597	0.004314
0	0	1	0	49.4	0.006072	0.014795	0.203831	0.030402
0	0	0	0	54.5	0	0.014672	0.227827	0.004286
0	0	0	0	31.9	NA	NA	NA	NA
0	0	0	0	11.2	0	0.005496	0.105829	0.004736
0	0	0	0	59	NA	NA	NA	NA







NA								
0.018225	0.006052	0	0.004521	0	0	0	0	0.002017
0	0	0	0	0	0	0	0	0
0.045362	0	0.037373	0	0	0	0	0	0
0.010171	0	0	0	0	0	0	0	0
0	0	0	0	0.009861	0	0	0	0
0.028418	0	0	0.009383	0	0	0	0.002068	0
NA								
NA								
NA								
0.007628	0.001627	0	0	0	0	0	0	0
0.015645	0.006804	0.010507	0	0	0	0.009118	0	0
0.043351	0.01141	0.013218	0.001728	0	0	0	0	0
NA								
0.032241	0	0	0	0.020276	0	0	0.013606	0
0.004479	0.017105	0	0.002005	0	0	0.014205	0	0
NA								
NA								
0.056923	0.012829	0.009464	0	0	0	0	0.012268	0
NA								
NA								
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NA								
NA								
0.007387	0.0048	0.002961	0	0	0.003535	0	0	0
NA								
NA								
0.000987	0.00337	0	0	0.303278	0.000891	0	0	0
0.023727	0.013868	0	0.001896	0	0	0	0	0
NA								
0.025278	0.007735	0	0.008199	0.01055	0	0.018069	0	0
0.008199	0	0	0	0	0.001545	0	0	0
NA								
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NA								
0.007621	0.007714	0	0	0	0	0	0	0
NA								
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NA								
0	0.029981	0	0	0.003276	0	0	0	0
NA								
0.035574	0	0.004435	0.009504	0	0	0	0	0
0	0	0	0	0.076739	0.002365	0	0	0



	0	0	0	0	0	0.001911	0	0	0
NA									
0.004985	0.026759		0	0	0.008264	0	0	0	0
0.008094	0.003199		0	0.002236		0	0	0	0
	0	0.065798		0	0	0	0.012052	0	0
NA									
0.008437	0.003201		0	0.000893		0	0	0	0
NA									
NA									
	0	0	0	0	0.002646		0	0	0
NA									
0.003952	0.011633		0	0.001437		0	0	0.091489	0
	0	0.038607		0	0.075335	0.020218	0.010397	0	0
NA									
	0	0	0	0	0	0	0	0	0
NA									
	0	0	0	0	0	0	0.036696	0	0
	0	0	0	0	0	0	0	0	0
NA									
	0	0	0.011691		0	0	0	0	0
	0	0	0.118301		0	0.027871	0	0	0
0.009133	0.022274	0.021311		0	0	0	0	0	0
	0	0	0	0	0	0.009742	0	0	0
0.000891		0	0	0	0	0	0	0	0
0.000904		0	0.001356		0	0	0	0	0
NA									
NA									
0.012176		0	0	0.004816		0	0	0	0
	0	0	0	0	0	0	0	0	0
0.003415	0.003		0	0	0	0	0	0	0
NA									
	0	0	0	0	0	0.002661	0	0	0
0.028044	0.004112		0	0.008022		0	0	0	0
	0	0.008534		0	0	0	0	0.005961	0
NA									
	0	0.024511		0	0	0.004029	0	0.051416	0
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NA									
0.061332	0.002826		0	0.004471		0	0	0	0.002236
NA									
0.009667		0	0	0.001437		0	0	0	0.00327
0.008291	0.004443	0.003442		0	0	0	0.007353	0	0
NA									
	0	0	0	0	0.000633		0	0	0
NA									
0	0	0	0	0	0	0	0.016917	0	0

NA								
0.000718	0	0	0	0	0	0	0	0
0.001224	0.043634	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0.069039	0.009162	0	0.018446	0	0	0	0	0.003722
0.003162	0.055938	0	0.000563	0.010893	0.000595	0.007043	0	0.001315
0.007944	0	0.001501	0	0	0.003663	0.00609	0	0
0	0.002331	0	0	0.003086	0	0	0.001714	0
0	0	0.006085	0	0.002169	0	0	0	0
0	0	0	0	0	0	0	0	0
NA								
0	0.007409	0	0	0.029923	0.010034	0	0	0
0.033704	0.005862	0.019486	0	0.013862	0	0	0	0
0.001785	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0.002341	0.00086
0	0.008209	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0.035785	0.004511	0.006088	0.006351	0	0	0	0	0
0.012955	0.003074	0	0	0	0	0	0.001888	0
0.035008	0	0	0.004307	0	0	0	0	0.008614
0.001971	0	0	0	0	0	0	0	0
0.004512	0	0.006427	0	0	0.004118	0	0	0
0	0	0	0	0	0	0	0	0
NA								
0.000256	0	0	0	0	0.000803	0.001716	0	0
NA								
0	0.001165	0.002425	0	0	0	0	0	0
0.000407	0	0.001627	0	0.076232	0	0.066879	0	0
0.020507	0	0	0.003646	0	0	0	0	0
NA								
0.011796	0.003084	0	0	0	0	0	0	0
0.002298	0	0	0.001159	0	0	0	0	0
NA								
0.013005	0.01765	0	0	0	0	0.083224	0.005658	0
NA								
0	0	0	0	0	0	0	0	0.002413
NA								
0	0	0	0	0	0	0	0.000765	0
0.003695	0.001791	0	0	0.294995	0	0	0	0
0.015082	0	0.003073	0.001901	0	0	0.061246	0	0
NA								
0.004713	0	0.000287	0	0	0	0	0	0
0.002695	0.001617	0	0	0	0	0	0	0
NA								
NA								
0.010037	0.016479	0	0.001248	0	0.003346	0	0	0
0.035326	0	0.021863	0	0	0	0	0	0



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