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Country-specific effects of neonicotinoid pesticides on honeybees and wild bees

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Abstract:

Neonicotinoid seed dressings have caused concern world-wide. We use large field experiments to assess effects of neonicotinoid-treated crops on three bee species across three countries (Hungary, Germany and the UK). Winter-sown oilseed rape was grown commercially with either seed coatings containing neonicotinoids (clothianidin or thiamethoxam) or no seed treatment (control). For honeybee we found both negative (Hungary and UK) and positive (Germany) effects during crop flowering. In Hungary, negative effects on honeybees (associated with clothianidin) persisted over winter and resulted in smaller colonies in the following spring (24% declines). In wild bees (*Bombus terrestris* and *Osmia bicornis*), reproduction was negatively correlated with neonicotinoid residues. These findings point to neonicotinoids causing a reduced capacity of bee species to establish new populations in the year following exposure.

One Sentence Summary:

Honeybee and wild bee exposure to neonicotinoid pesticides reduces their ability to establish populations.

Main Text:

Global declines in honeybees and wild bees have been linked to pathogens, climate change, habitat fragmentation and pesticide use (*1*-*3*). The potential threat from neonicotinoid seed coatings applied to flowering crops has been the subject of considerable debate (*4*-*9*). Neonicotinoids have been shown to increase mortality in honeybees by impairing their homing ability (*4*) and to reduce the reproductive success of bumblebees (*5, 8, 10*) and solitary bees (*8, 11*), while other studies have identified no effects (*8, 12, 13*). There is limited information from replicated studies on longer-term survival of honeybee colonies following exposure (see (*12*)). Landscape-scale experiments under real world agricultural conditions are needed to integrate spatial, temporal and species-specific variation to understand the impacts of neonicotinoids on bees (*8, 12, 14*-*16*). Such studies should explore the impacts of different neonicotinoid formulations, land use and regional climate. In a large-scale experiment spanning three European countries, we tested the hypotheses that: (i) exposure to seed treatments containing neonicotinoids affected the reproductive potential of managed and wild bee species and (ii) if such effects differ between countries.

At each of 33 sites (Germany=9, Hungary=12, UK=12) an average of 63.1 ha (SE±2.8 ha) of winter-sown oilseed rape (OSR) was established in 2014 (Fig.1 & S1, Table S1). We clustered sites into triplets (>3.2 km between sites) and randomly allocated sites to one of three treatments: 1) Clothianidin applied at 11.86-18.05 g ha^{-1} *a.i.* with a fungicide (Thriam and prochloraz) and non-systemic pyrethroid (beta-cyfluthrin) (trade name Modesto); 2) Thiamethoxam applied at 10.07-11.14 g ha^{-1} *a.i.* and combined with the fungicides fludioxonil and metalaxyl-M (trade name Cruiser); 3) Control oilseed rape receiving a commercial fungicide (Thriam and Dimethomorph in Germany & Hungary, Thriam and Prochloraz in the UK), but no neonicotinoid seed treatment. All treatments received typical commercial inputs of pesticide (e.g. Lambda-cyhalothrin) and fertilizer, with these standardized across a triplet. Standardized colonies of honeybees (*Apis mellifera*) and wild bees (bumblebee *Bombus terrestris* and solitary bee *Osmia bicornis*) were introduced to each site. For honeybees we quantified the impacts of the treatments on colony viability during the crop flowering period and in the year following exposure (hive survival and overwintering worker, brood and storage cell numbers). Overwintering fitness defines the multi-year persistence of honeybees. For *B. terrestris* we measured impacts on within-year reproductive output (colony weight gain, and worker, queen and drone production) and for *O. bicornis* the number of reproductive cells produced (Table S2). Neonicotinoids can be persistent and widespread in agro-ecosystems (*17, 18*), so we quantified residues both in the nests of bee species and those expressed in the crop.

We found that neonicotinoid seed treatment affected the inter-annual viability of honeybee colonies following the winter period in a country-specific manner. In Hungary worker numbers were 24% lower where clothianidin was compared to the control (treatment×country: χ^2_6=1.47, p=0.01, explained variance=59.4%; Fig.2), with no significant effect of thiamethoxam. Clothianidin was more likely to be expressed in the crop where it was applied as a seed treatment, which identified a mechanism of exposure to the bees (χ^2_2=6.46, p=0.04), but this was not so for thiamethoxam (Table S3). In the UK high hive mortality precluded a formal statistical analyses of overwintering worker numbers. However, median worker numbers were zero for all four clothianidin-treated sites, but above zero for two of the control and one of the thiamethoxam sites (Table S2; Fig.2). Worker numbers following the winter in Germany showed no treatment effect (Table S4). Overwintering honeybee brood, stored hive products (pollen and nectar) and the likelihood of hives surviving the winter were not affected by seed treatments (Table S3).

Neither *B. terrestris* queen nor *O. bicornis* egg cell production were directly affected by the seed treatments or its interaction with country (Table S5). However, they were negatively correlated with peak (χ^2_1=2.09, p=0.03, explained variance=13.5%; Fig.3a) and median (χ^2_1=4.34, p=0.04, explained variance=0.8%; Fig.3b) neonicotinoid nest residues (combined clothianidin, thiamethoxam and imidacloprid). Imidacloprid was not applied as part of the study and its presence is most likely a result of environmental contamination from previous widespread agronomic use (*17, 18*). Residues of neonicotinoids detected in stored hive products did not differ in response to seed treatments for any bee species (Table S6). This may be due to the amalgamation of stored hive products at the site level for residue analysis, which may have obscured within site heterogeneity in residues. The negative correlation for *B. terrestris* queen production remained significant when we excluded sites with imidacloprid residues (χ^2_1=2.14, p=0.02), although this was not the case for *O. bicornis* (χ^2_1=0.05, p=0.81). Country-specific responses to neonicotinoid seed treatment were found for *B. terrestris* drone production, with positive and negative effects from exposure to thiamethoxam in Germany and the UK respectively (treatment×country: χ^2_6=13.1, p=0.04, explained variance=13.6%; Fig.2).

We also found seed treatment effects during the crop flowering period that lasted between 3 to 6 weeks (Table S4 & 5). Significant interactions between seed treatment and country were identified for peak worker (χ^2_6=16.6, p<0.01, explained variance=45.3%), egg cell (χ^2_6=4.13, p=0.01, explained variance=49.9%) and combined pollen and nectar storage cell (χ^2_6=40.5, p<0.001, explained variance=53.6%) numbers. These responses describe within-year colony performance. Neonicotinoid exposure resulted in both negative (Hungary and UK) and positive (Germany) effects on colony size (see Fig.2; pairwise treatment comparison given in Table S4 & 5). *Bombus terrestris* worker and peak colony weight showed no seed treatment response.

Our quantification of neonicotinoid effects on the inter-annual viability of honeybees and wild bee populations represents a fundamental advance in our understanding of the impacts of these pesticides. For solitary bees and bumblebees (queen production) neonicotinoid impacts were associated with the residues found in nests rather than the experimental seed treatments. For *B. terrestris* the few treatment effects and the presence of imidacloprid in stored pollen and nectar (Table S7-S9) suggests that negative impacts of neonicotinoids may be driven by persistence of residues in the wider landscape, rather than current management alone (*18, 19*). The EU moratorium meant that no neonicotinoids were applied to oilseed in the surrounding landscapes during the experiment, so such residues may originate from previous agricultural use leading to expression in non-target plants (*17-19*), guttation fluids or contaminated water (*19, 20*). While the reproductive potential of *O. bicornis* was also negatively affected by neonicotinoid residues in nests, the explained variation of these effects was small. However, a failure to detect small population changes may be due to limited experimental replication restricting statistical power. Our results suggest that even if their use were to be restricted, as in the recent EU moratorium, continued exposure to neonicotinoid residues resulting from their previous widespread use has the potential to impact negatively wild bee persistence in agricultural landscapes (*14, 18, 19*).

Taken together, our results suggest that exposure to neonicotinoid seed treatments can have negative effects on the inter-annual reproductive potential of both wild and managed bees, but that these effects are not consistent across countries. The country-specific responses of honeybees and bumblebees strongly suggests that the effects of neonicotinoids are a product of interacting factors (*20*-*23*). This study has identified between country differences in the use of oilseed rape crop as a forage resources by bees (affecting exposure to crop residues) and incidence of disease within hives. Both factors were higher for Hungarian and UK honeybees (Table S10 & S11). Overall neonicotinoid residues were detected infrequently and rarely exceeded 1.5 ng g^{-1} w/w. As such, direct mortality effects caused by exposure to high concentrations of neonicotinoids are likely to be rare (Table S12). However, our results suggest that exposure to low levels of neonicotinoids may cause reductions in hive fitness that are influenced by a number of interacting environmental factors. Such interacting environmental factors can amplify the impact of honeybee worker losses (e.g. through sub-lethal toxicity effects) and reduce longer-term colony viability (*4, 16*). Importantly, our common experimental approach applied across three countries revealed varying impacts and may explain the inconsistent results of previous studies conducted in single countries or at few sites (*4, 5, 8, 12, 13, 15*).

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Supplementary content

References (*24-30*)

Materials and methods

Figs.S1-2

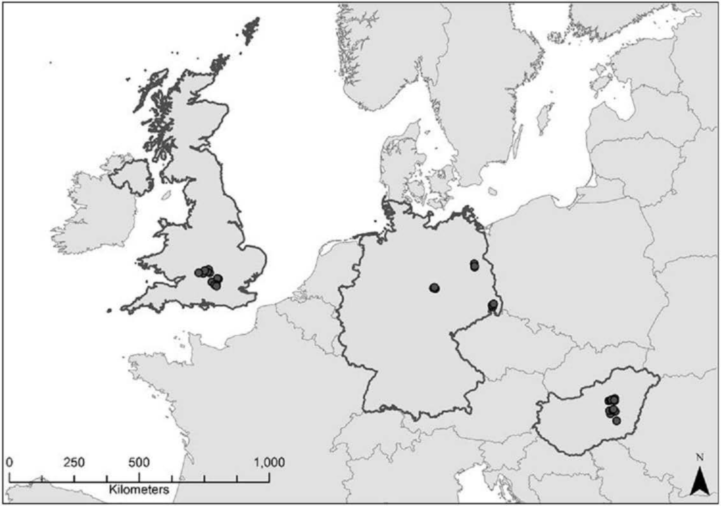
Tables S1-12

Fig.1. Location of the 33 experimental sites in the UK, Hungary and Germany. See Fig S2 for a diagrammatic representation of the experimental setup.

Fig.2. Summary effect sizes for the response of honeybees and wild bees to the neonicotinoid seed treatments. An effect size represents the difference between the mean population response for a given seed treatment and the control within a country, with this difference divided by the pooled standard deviation. Where: * indicates a significant differences between the control and seed treatment (either TMX=thiamethoxam, CTD=clothianidin) determined from the predicted marginal means of the model '*y ~ seed treatment*country +*

block/country'. † indicates where UK colony survival was too low for a formal analysis. Note effect sizes differ between countries.

Fig.3. Wild bee reproductive success in response to neonicotinoid nest residues. Separate graphs are shown for the response of *B. terrestris* queen production and *O. bicornis* reproductive cell production to neonicotinoid residues found in nests. The significance of these relationship is based on a likelihood ratio test comparison of H0: '*y* ~ *country*' and H1: '*y* ~ *Neonicotinoid+country*'. Neonicotinoid residues are based on summed concentrations of clothianidin, thiamethoxam and imidacloprid. *Expl.Var*=Explained variance.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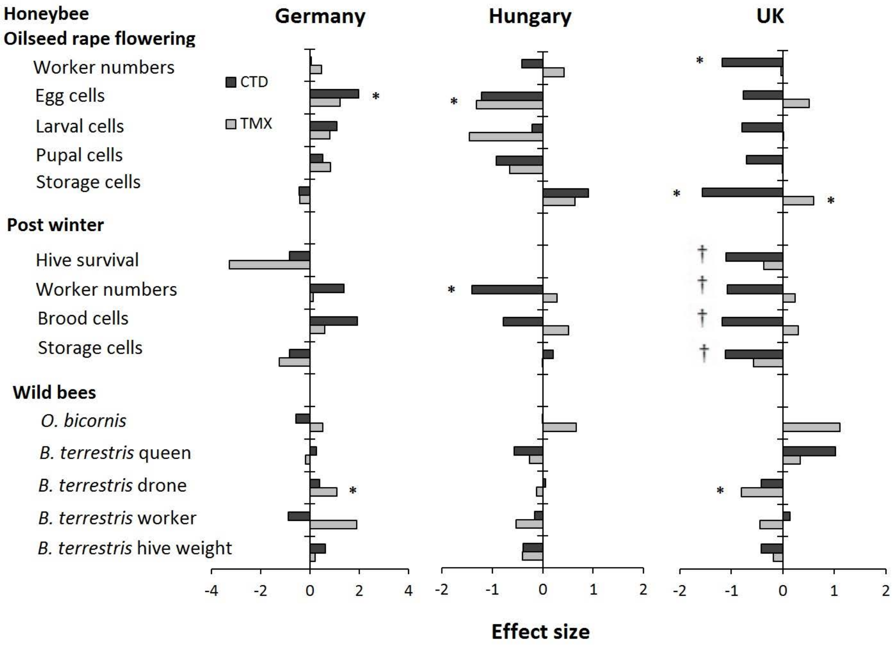
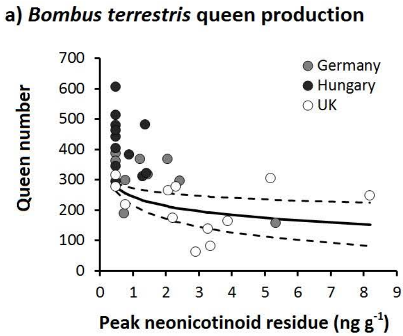
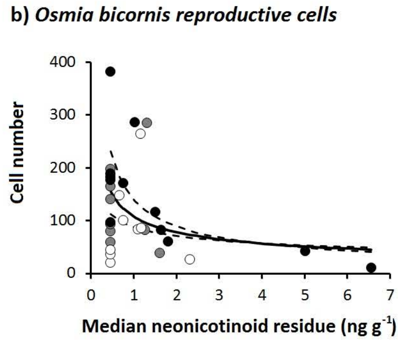

Honeybee
Oilseed rape flowering
Worker numbers
Egg cells
Larval cells
Pupal cells
Storage cells
Post winter
Hive survival
Worker numbers
Brood cells
Storage cells
Wild bees
O. bicornis
B. terrestris queen
B. terrestris drone
B. terrestris worker
B. terrestris hive weight
Germany
Hungary
UK
CTD
TMX
*
†
-4
-2
0
2
4
-2
-1
0
1
2
-2
-1
0
1
2
Effect size


a) Bombus terrestris queen production
Queen number
Peak neonicotinoid residue (ng g-1)
Germany
Hungary
UK
0
100
200
300
400
500
600
700
0 1 2 3 4 5 6 7 8 9


b) Osmia bicornis reproductive cells
Cell number
Median neonicotinoid residue (ng g-1)
0
100
200
300
400
0 1 2 3 4 5 6 7