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Short communication

Size matters: Significant negative relationship between mature plant mass and residual neonicotinoid levels in seed-treated oilseed rape and maize crops

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ABSTRACT

Neonicotinoid insecticides have been under scrutiny in recent years due to their potential to harm bees. The European Union recently imposed a two year moratorium (2014–2015) on their application as a seed-treatment for certain bee-attractive crops. In this study we investigated the effect of mature plant size on residual neonicotinoid concentration in two widely grown, bee-attractive crops: oilseed rape (*Brassica napus*) and maize (*Zea mays*). Plants were collected from four commercial farms in Sussex, United Kingdom, three growing oilseed rape and one maize. All were grown from seeds treated with the neonicotinoid thiamethoxam. For both crops there was a significant negative relationship between mature plant mass and residual neonicotinoid (thiamethoxam and its metabolite clothianidin) concentrations ($p < 0.001$). Concentrations in plant tissues roughly halved with a four-fold increase in plant weight. These results indicate that agronomic practices that result in larger mature plants might have the potential to reduce the exposure of bees to neonicotinoid contamination of pollen and nectar.

1. Introduction

Neonicotinoids, a relatively new class of insecticides in use since 1991 (Elbert et al., 2008) have been under scrutiny in recent years due to research indicating negative impacts on non-target species, both directly (bees: e.g. Whitehorn et al., 2012; aquatic invertebrates: Beketov and Liess, 2008) and indirectly (insect-feeding birds: Hallmann et al., 2014). Presently, there is a moratorium on neonicotinoid use as seed treatments, or as granules, on certain "bee attractive crops" such as maize, sunflower and oilseed rape in the European Union (EU) due to "high acute risks" to bees (Europa, 2013). In the EU, neonicotinoid insecticides were primarily applied as a broad-spectrum seed-treatment to protect crops from insect pests during their early growth phase (Elbert et al., 2008). However, residues in the nectar and pollen of mature flowering plants may be ingested by foraging bees.

Neonicotinoids concentrations present in pollen and nectar is the principal determinant of toxicity to individual bees or their colonies (Carreck and Ratnieks, 2014). Although the acute lethal dose for honey bees has been established under laboratory conditions (LD_{50}, thiamethoxam: 5 ng/bee, clothianidin: 3.8 ng/bee; EFSA 2013), determining the effects of chronic exposure in the field is more challenging. Thus it is unclear what levels would be acceptable in the nectar and pollen of flowering crops (However, see Sanchez-Bayo and Goka, 2014). The recent change in the active ingredient commonly used in seed-treatments (from imidacloprid to clothianidin and thiamethoxam; Goulson, 2013) further compounds the uncertainties around this issue. As a guideline however, the chronic dose of the better studied compound, imidacloprid is 20 μg/kg (LC_{50}: Mommaerts et al., 2010) and the acute lethal dose is 3.7 ng/bee (LD_{50},Schmuck et al., 2001). Nevertheless, it would seem likely that lowering residual neonicotinoid concentrations in the pollen and nectar of seed-treated crops would help to mitigate harm to bees (see meta-analysis in Cresswell 2011).

We hypothesised that this could be achieved via agronomic practices that increase mature plant size, thereby diluting neonicotinoid residues in the pollen and nectar. To test this hypothesis we measured residual concentrations of the neonicotinoid thiamethoxam and its metabolite clothianidin over a range of mature plant sizes in two widely-cultivated (FAO, 2014), bee-attractive crops: oilseed rape (*Brassica napus*) and maize

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(*Zea mays*). The flowers of these species are visited by honey bees and other bees and insects to collect pollen (oilseed rape and maize) and/or nectar (oilseed rape only).

2. Methods

We studied plants grown commercially on farms in Sussex and planted in the spring (maize) and late summer (oilseed rape) of 2013, before the EU moratorium took effect.

We analysed neonicotinoid residues in plant tissue samples of mature plants of different sizes, as gathering sufficient amounts of nectar and pollen per size class for chemical analysis would have been extremely challenging in the case of oilseed rape. Previous studies have found similar concentrations in plant tissue (leaves/panicles), nectar and pollen (Schmuck et al., 2001; Bonmatin et al., 2005). This is because neonicotinoids are water-soluble and thus readily translocated throughout the plant (Elbert et al., 2008). As such, this methodology was appropriate for determining the effect of plant size on residues in a way that is relevant to both nectar and pollen.

Flowering oilseed rape plants grown from seeds that had been treated with thiamethoxam (Cruiser OSR, Syngenta Ltd., Basel) were gathered from three farms in East Sussex, near Brighton, UK, between 8 and 11 June, 2014. To minimise within-field environmental variability, we collected all plants from a 15 m^2 section of each field. Plants were cut at ground level and weighed using a portable scale (sensitivity 1 g). We then removed the upper-leaves and panicles of ten plants in each of five weight ranges (i.e. 0–100 g, 100–200 g, 200–300 g, 300–400 g, 400–500 g). Samples were subsequently stored at −20 °C. During August 2014 fifteen oilseed rape sub-samples were prepared from these, five from each weight category per farm. Each sub-sample consisted of an equal amount of material from ten plants homogenised in water (1 g fresh weight of flower panicles plus 1 g of leaves × 10 plants, plus 20 g of water).

Nine whole seed-treated (Cruiser 5FS, Syngenta) flowering maize plants were collected from three fields on 29 August, 2013 at Sefter Farm, near Bognor Regis, West Sussex. One plant from each of three size categories (small, medium and large) was selected per field and cut at ground level. Samples were later weighed and stored at −20 °C. Whole plant samples were analysed.

The 15 oilseed rape samples and nine whole maize plants were analysed by SAL (Scientific Analysis Laboratory Ltd., Cambridge) an accredited (UK Accreditation Service) contract analytical laboratory. SAL routinely analyses plant and food materials for the farming and food industries. Their extraction method is based on the Quechers extraction technique (e.g. Kamel, 2010), which uses water and acidified acetonitrile as an extraction solvent. Magnesium sulphate and ammonium acetate buffer were added to induce solvent partitioning. Quantitation was assessed against a series of known calibration standards dissolved in a methanol-water solution. Deuterated clothianidin (Clothianidin-d3) was used as an internal standard pre-extraction, to correct for losses during extraction and to compensate for matrix effects (suppression or enhancement) during analysis. Values for oilseed rape neonicotinoid concentrations received from SAL were doubled to allow for the water we added to the samples. Neonicotinoid concentrations are presented as the sum of thiamethoxam and clothianidin in μg per kg (parts per billion). Statistical analyses were conducted using 'R' software (version 3.1.1; R-Project, 2014). Linear Model (LM) analysis were simplified using backwards elimination of non-significant variables and model comparison using ANOVA. Oilseed rape LM analysis used the mean weights of the ten plants in each sample. All values are presented as mean ± 1 standard deviation.

3. Results

In the oilseed rape samples, neonicotinoid concentrations differed significantly among the three farms sampled (df = 2, F = 33.06, $p < 0.001$). Therefore, the data were not pooled across farms and 'farm' was held as a co-variable in LM analysis. Neonicotinoid concentrations decreased significantly with increasing oilseed rape plant weight (df = 1, F = 28.073, $p < 0.001$), Fig. 1. In the maize samples neonicotinoid concentrations did not differ significantly between the three fields (df = 2, F = 0.074, p = 0.930). Therefore, the data were pooled. Neonicotinoid concentrations were $\log_{10}$ transformed prior to analysis (West et al., 2001) as this gave a substantially better statistical fit (R-squared: linear: 0.592, exponential: 0.826). Neonicotinoid concentrations were found to decrease significantly with increasing maize plant weight (df = 8, F = 33.42, $p < 0.001$), Fig. 2.

4. Discussion

Our results clearly show that residual neonicotinoid concentrations in the tissues of mature flowering plants are negatively correlated with plant mass in both oilseed rape and maize. For both crops, concentrations roughly halved with a four-fold increase in plant weight (Figs. 1 and 2). If the residual levels in pollen and nectar are also proportional to plant size, as the results of this study suggest, our data may also provide an insight into the wide range of

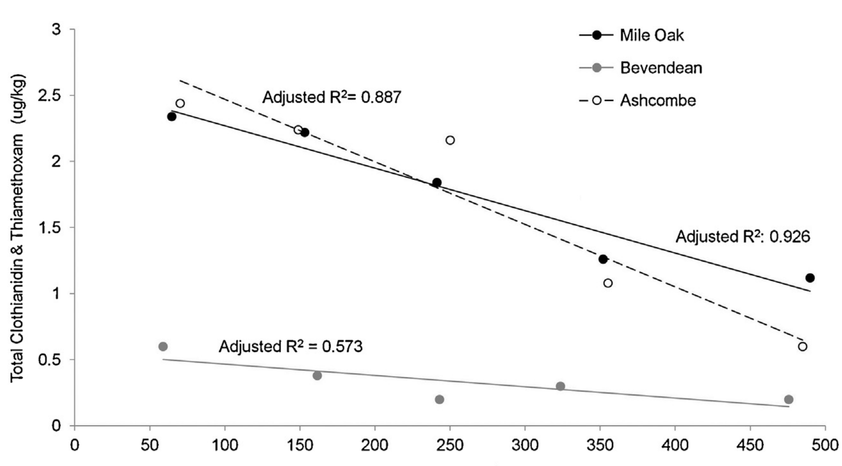

Fig. 1. Variation in neonicotinoid concentrations in plant tissues with oilseed rape plant weight for three farms: Mile Oak, Bevendean and Ashcombe.