Title: Neonicotinoid insecticide residues in New Zealand maize paddock soil

Authors: Chris Pook and Iana Gritcan

Affiliation: Institute for Applied Ecology New Zealand, School of Sciences, Auckland University of Technology, Wellesley, Auckland 1142, New Zealand

Corresponding Author: Chris Pook

Corresponding Author address: Dr Chris Pook

School of Sciences

Auckland University of Technology

Wellesley Campus C42

Auckland 1142

New Zealand

Corresponding Author E-mail: cpook@aut.ac.nz, drchrispook@gmail.com

Abstract: Neonicotinoid are the most commonly used class of insecticides. Between 2005 and 2010 neonicotinoid use in the USA and UK more than doubled. Anecdotal evidence suggests similar trends exist in New Zealand, where neonicotinoid seed coatings are now often applied prophylactically in contravention of the principles of Integrated Pest Management. This widespread use of neonicotinoid insecticides is controversial due to a lack of understanding about their persistence in the environment and the long-term consequences of their use. We present a novel, simple, low-cost method for the extraction and quantification of five neonicotinoids from soil with a detection limit <1 ng g^{-1}. We have applied this method to soil collected from maize paddocks in New Zealand and found clothianidin and imidacloprid in 48 out of 50 samples. Neonicotinoid concentrations ranged from 0.5 to 9.4 ng g wet $weight^{-1}$ imidacloprid and 2.1 to 26.7 ng g wet $weight^{-1}$ clothianidin. These concentrations are likely to be hazardous to non-target organisms exposed to them. This is the first study to report the prevalence of neonicotinoid residues in New Zealand's environment.

Keywords: sustainable agriculture; integrated pest management; beneficial insects; ecotoxicology; pesticide; ecotoxicology, pesticides, emerging pollutants, soil ecotoxicology, persistent compounds

Introduction

Neonicotinoids are the most commonly used type of insecticide (Douglas and Tooker 2015). Where neonicotinoid use is documented—for example in the United States of America and the United Kingdom—both the mass of active ingredient applied and the diversity of applications continue to increase (DEFRA 2014, Douglas and Tooker 2015). Recent research shows these compounds are more persistent in soil than previously understood (Goulson 2013, de Perre *et al.* 2015). Very low concentrations of neonicotinoid residues in plants, soil, and groundwater are associated with reductions in the diversity and abundance of non-target insects and insectivorous birds (Goulson 2013, Van Dijk *et al.* 2013, Hallmann *et al.* 2014). Direct, mechanistic links between environmentally relevant concentrations of neonicotinoids and population-level effects upon non-target organisms are now being established (Laycock *et al.* 2012, Whitehorn *et al.* 2012, Pisa *et al.* 2015). In 2013 the European Commission placed restrictions on the use of three neonicotinoids following assessments carried out by the European Food Safety Authority. Due to the controversy around this ubiquitous class of insecticides, it is important to continue to investigate the consequences of their large-scale use.

The ultimate environmental fate of neonicotinoid residues has not been established and the threat they pose to non-target species is not well understood (Goulson 2013). Neonicotinoids are most commonly applied as a coating onto planted seeds, where they then disperse into the soil. Their persistence in soil is highly variable with reported half-lives of up to seven years (Goulson 2013, Jones *et al.* 2014). The small size of neonicotinoid molecules and their polarity makes them systemic, facilitating their uptake into plants' roots and dispersal throughout their tissue where they act against biting, chewing and boring insect pests. These properties limit their bioaccumulation in food chains; however, these properties also allow them to dissolve in groundwater and mobilise, resulting in their presence in soils, water, and organisms distinct from their site of application (eg. Main *et al.* 2015). Sur & Stork (2003) reported that 80-98% of imidacloprid seed treatment was not taken up by the target plant. This material will leach through the soil in surface and groundwater flows instead, contaminating plants, soil, waterways and wetlands distinct from their site of application (Bonmatin *et al.* 2015, de Perre *et al.* 2015, Main *et al.* 2015). So the fate of neonicotinoids in the environment can be categorised as either persisting *in situ*, broken down, or exported. Neonicotinoids breakdown quickly when exposed to sunlight and they can be metabolised by plants and animals (Sur and Stork 2003, Suchail *et al.* 2004). Export is a relative process depending on the scale in question, but results from either biological processes (uptake by mobile organisms or biological transport systems) or physical ones, via dissolution and by the mobilisation of

sediment or biological material to which residues are adsorbed. Residues dispersed in this manner to field margins can be taken up by wild plants at concentrations similar to those present in the crop (Botías *et al.* 2015). How far neonicotinoid residues can disperse and for how long they can persist is not known.

Many different animals inhabit agricultural ecosystems and provide ecosystem services that contribute to crop productivity such as pollination, pest predation, soil engineering, and nutrient cycling. For example, the presence of moderate numbers of soil Collembola have been shown to increase plant productivity (Harris and Boerner 1990). The mechanisms underlying these effects are complex and may involve interactions between invertebrates, vertebrates, fungi bacteria and plants. As a result of exposure to neonicotinoid residues insects can suffer impaired reproductive performance, impaired foraging or defensive behaviour, loss of prey or hosts, and death (Kunkel *et al.* 2001, Pisa *et al.* 2015). This will impact ecosystem services provided by beneficial insects as well as those provided by any commensal, mutualistic or symbiotic partners with implications for the productivity of the agricultural system.

Assessment of the risks associated with use of a pesticide is contingent upon understanding the prevalence, persistence, and availability of that compound in the environment. Residues of the neonicotinoid imidacloprid in arable soil at the end of a growing season have been reported to be in the range of one to 100 ng g^{-1} (Bonmatin *et al.* 2005, Krupke *et al.* 2012, Goulson 2013, Jones *et al.* 2014, Botías *et al.* 2015, Schaafsma *et al.* 2015). The New Zealand Environmental Protection Agency [NZEPA] has established an Environmental Exposure Limit for imidacloprid in soil of 1 µg per kg dry weight. However, no monitoring programs appear to have been implemented and we are unaware of any research published on the distribution, persistence, and fate of neonicotinoid insecticides applied in New Zealand.

The current standard for the quantitative analysis of organic biocide residues involves solvent extraction from environmental or biological samples followed by separation by liquid- or gas-chromatography and detection by tandem mass spectrometry (eg. Payá *et al.* 2007). Many such methods are time-consuming and costly to apply at scale. We have developed a novel, simple, low-cost extraction technique for five neonicotinoid residues in arable soils. Imidacloprid, clothianidin, thiacloprid, and thiamethoxam are the most common neonicotinoid seed treatments used in the United Kingdom [UK] and United States of America [USA] (DEFRA 2014, Douglas and Tooker 2015). Acetamiprid is the least used and is not currently licensed for use in New Zealand [NZ]. We report here the results of a pilot study to test where we have applied the method to measured concentrations of neonicotinoid insecticides in soil samples collected from New Zealand maize paddocks prior to planting, when the lowest concentrations of neonicotinoid residues can be expected.

Methodology and materials

Reagents and analytical standards

Optima LC-MS grade acetonitrile and analytical reagent grade ethyl acetate, boric acid, sodium chloride and magnesium sulphate heptahydrate were obtained from Thermo Fisher (Thermo Fisher Scientific, New Zealand). Mass-spectrometry grade formic acid and analytical standards of thiamethoxam, clothianidin, imidacloprid, and imidacloprid-d4 were obtained from Sigma Aldrich (Sigma Aldrich, New Zealand). Ultrapure water was obtained from a Purite Select Fusion system (Total Lab Systems, New Zealand).

Extraction of neonicotinoid residues from soil

Approximately 1.5 g of wet soil was placed in a 15 mL polypropylene centrifuge tube and spiked with 10 µL of 20 mg L^{-1} imidacloprid-d4 in 50% acetonitrile. Then, 5 mL of ultrapure water was added and the sample was vortexed thoroughly to mix and disperse the soil before 2 mL of ethyl acetate was added and the mixture was vortexed again. Finally, 2 g of salt mixture (eight parts $MgSO_4 \cdot 7H_2O$, two parts NaCl and three parts H_3BO_3) was added and the tube vortexed thoroughly to allow it to dissolve. Extracts were incubated at room temperature for 15 minutes with regular vortexing before being centrifuged at 4,000 RCF for 5 minutes at room temperature. A 1.4 mL volume of the upper, organic layer was removed and placed in a 2 mL microcentrifuge tube with 0.4 mL of 1% formic acid in ultrapure water. This mixture was vortexed briefly before the ethyl acetate layer was evaporated *in situ* using a centrifugal concentrator (Centrivap Console, Labconco, USA). The remaining aqueous solution was centrifuged at 10,000 RCF for 5 minutes at 4 °C (Z216MK microcentrifuge, Hermle Labortechnik, Germany) before a 100 µL volume was transferred to a low volume glass insert inside an amber 1.8 mL autosampler vial and capped for injection to LC-MS/MS.

Liquid chromatography with tandem mass spectrometry – LC-MS/MS

Neonicotinoids were quantified using an Agilent 1260 Series liquid chromatograph comprising a G1311C quaternary pump, G1329B thermostatted autosampler and a G1330B thermostatted column compartment (Agilent Technologies, Santa Clara, USA). Mobile phase A was 0.1% formic acid in ultrapure water, mobile phase B was 0.1% formic acid in acetonitrile, the injection volume was 5 µL and the column, a ZORBAX Rapid Resolution HT SB-C18 measuring 2.1x30 mm, with 1.8 µm diameter packing material, was maintained at 30 °C. The chromatographic gradient started at 5% B, ramped to 33% B at 3 minutes, 80% B at 4 minutes, held at 80% B for 0.2 minutes and then returned to 5% B at 5 minutes. The total run time was 10.5 minutes.

Neonicotinoids were quantified with an Agilent 6420 triple quadrupole mass spectrometer fitted with an Agilent Multimode Ionisation source operating in positive electrospray mode and using Multiple Reaction Monitoring [MRM]. MRM transitions were established using Agilent MassHunter Optimiser software and are presented in Supplementary Data Table 1.

Supplementary Data Table 1: Multiple Reaction Monitoring (MRM) transitions for LC-MS/MS of neonicotinoid pesticides. The dwell time for each MRM was 100ms and the cell accelerator voltage was 7.

neonicotinoid	MRM transition	fragmentor voltage	collison energy
thiamethoxam	292.0 → 211.1	100	14
clothianidin	250.0 → 169.1	100	14
imidacloprid	256.1 → 209.1	123	18
imidacloprid-d4	260.1 → 213.1	91	10
thiacloprid	253.0 → 126.0	122	22
acetamiprid	223.1 → 126.0	91	10

Instrument Detection Limits and extraction validation

Five 1.5 g samples of arable soil that showed no trace of neonicotinoid contamination were spiked with a 10 µL volume of a 5 mg L^{-1} solution of the six targets in acetonitrile and shaken for 60 seconds. These were then extracted and analysed as described above. Instrument Detection Limits [IDL] were calculated by the method given in Wells et al. (Wells *et al.* 2011) in accordance with US Guidelines Establishing Test Procedures for the Analysis of Pollutants (United States Government Code of Federal Regulations, title 40, sec 1.136, appendix B).

Field Sampling, Locations and Processing

A total of 45 soil samples were collected from nine maize paddocks around the Waikato, East Cape, and Bay of Plenty regions of New Zealand's North Island. The approximate location of each paddock and the seed treatment, where it could be established, is shown in Table 1. Paddocks had been planted with maize in late Spring 2014 (Sept to Nov), harvested in Autumn 2015 (April to June) and left fallow for the winter. Paddocks were sampled on the 28th and 29th of September, 2015. Five replicate soil samples were taken from each paddock using a clean, stainless steel trowel to a depth of 100 mm, placed into a zip-lock bag and shaken to homogenise the contents. Samples were collected every 10 metres along a transect from the

corner of the paddock towards the centre, starting from 10 metres in to the paddock. Samples were immediately refrigerated at 4°C until analysis.

Soil water and organic matter content

Approximately one gram of homogenised, wet soil was weighed into a foil boat and lyophilised for 24 hours to obtain the dry weight. The foil boat was then placed in a muffle furnace and heated to 590°C for two hours to obtain the ash weight of the soil. The organic content of the soil was calculated by subtraction of the ash weight from the dry weight.

Table 1: Approximate locations (decimal degrees) of maize paddocks in New Zealand's North Island sampled for soil neonicotinoid residue analysis. Neonicotinoid seed treatments are stated, where known.

site	town	coordinates	seed treatment
A	Matamata	-37.80, 175.77	Bayer Poncho (clothianidin)
B	Awakeri	-38.00, 176.90	Bayer Poncho (clothianidin)
C	Poroporo	-38.00, 176.96	Bayer Poncho (clothianidin)
D	Te Teko	-38.07, 176.82	unknown
E	Poroporo	-38.00, 176.93	unknown
F	Whakatane	-37.95, 176.95	unknown
G	Te Puke	-37.76, 176.30	Bayer Poncho (clothianidin)
H	Te Puke	-37.76, 176.30	Bayer Poncho (clothianidin)
I	Te Karaka	-38.47, 177.88	Bayer Poncho (clothianidin)

Results

Method validation

Instrument Detection Limits ranged from 0.201 ng g^{-1} for imidacloprid to 0.516 ng g^{-1} for thiamethoxam. Recoveries (mean ±SD) for the six targets spiked into uncontaminated soil were consistent and ranged from 85.3% ±2.4 for thiamethoxam to 110.2% ±5.4 for acetamiprid (Table 2).

Table 2: Recovery and Instrument Detection Limits [IDL] for six neonicotinoid insecticide residues in soil using the method reported here.

neonicotinoid	mean % recovery (standard deviation)	IDL (ng g wet weight^{-1})
acetamiprid	110.2 (5.4)	0.096
clothianidin	103.0 (13.5)	0.413
imidacloprid	109.9 (19.4)	0.250
imidacloprid-D4	106.0 (1.7)	0.246
thiacloprid	93.9 (7.6)	0.153
thiamethoxam	85.3 (2.4)	0.208

Neonicotinoid residues in maize paddock soil samples

Of the five neonicotinoids targeted for quantification with this method, we detected only two—clothianidin and imidacloprid—in the maize paddock soil samples. However, we detected these two neonicotinoids in almost every sample analysed at concentrations up to 109.3 ng g^{-1} clothianidin at site I and 13.7 ng g^{-1} imidacloprid at site F. Imidacloprid concentration was below the IDL in just two samples: one at site G and one at site I. Imidacloprid concentrations (mean ±SD) varied from 0.5 ±0.5 ng g^{-1} at site I to 9.4 ±3.1 ng g^{-1} imidacloprid at site E. Clothianidin concentrations ranged from 2.1 ±2.4 ng g^{-1} at site E to 26.7 ±46.5 ng g^{-1} at site I. The highest concentration for total neonicotinoids was also at site I with 27.3 ±46.26 ng g^{-1}. The mean concentration across all sites was 8.16 ±16.78 ng g^{-1} clothianidin, 5.06 ±3.73 ng g^{-1} imidacloprid and 13.22 ±8.12 ng g^{-1} for total neonicotinoids. These results are displayed in Figure 1.

Soil water content and organic matter content

Soil water content (mean ±SD) was 32.2 ±8.0 % and organic content was 12.3 ±4.7 %. Linear models revealed no significant relationships between neonicotinoid concentrations and soil water or organic content (statistics not shown). There was a significant linear relationship between soil water and organic matter content, shown in Figure 2.

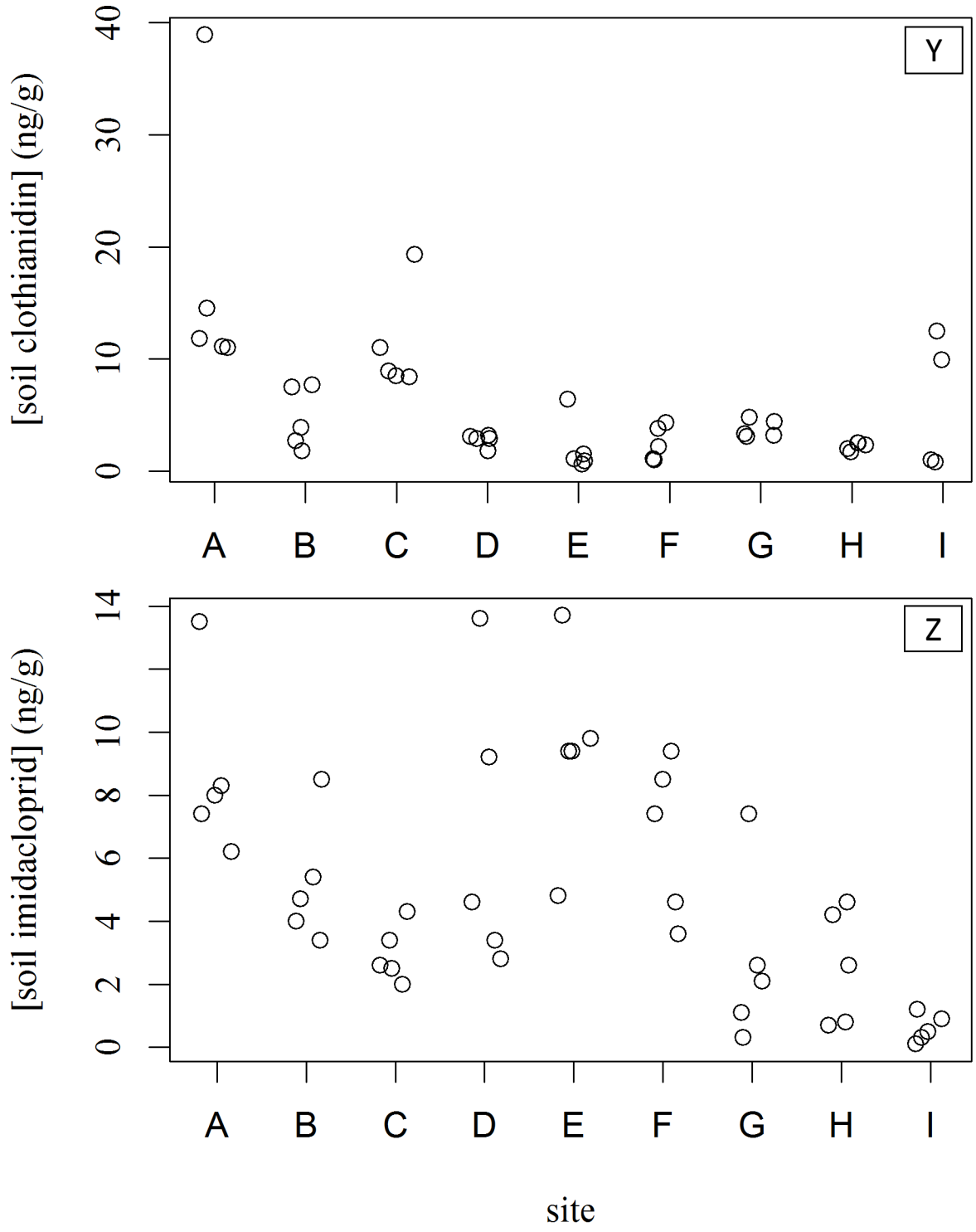

Figure 1: Concentrations of clothianidin (Y) and imidacloprid (Z), in ng g^{-1} wet weight of soil from nine maize paddocks in New Zealand's North Island. To better visualise the distribution of the data one outlying data point at 109.3 ng g^{-1} for site I has been excluded from plot Y.

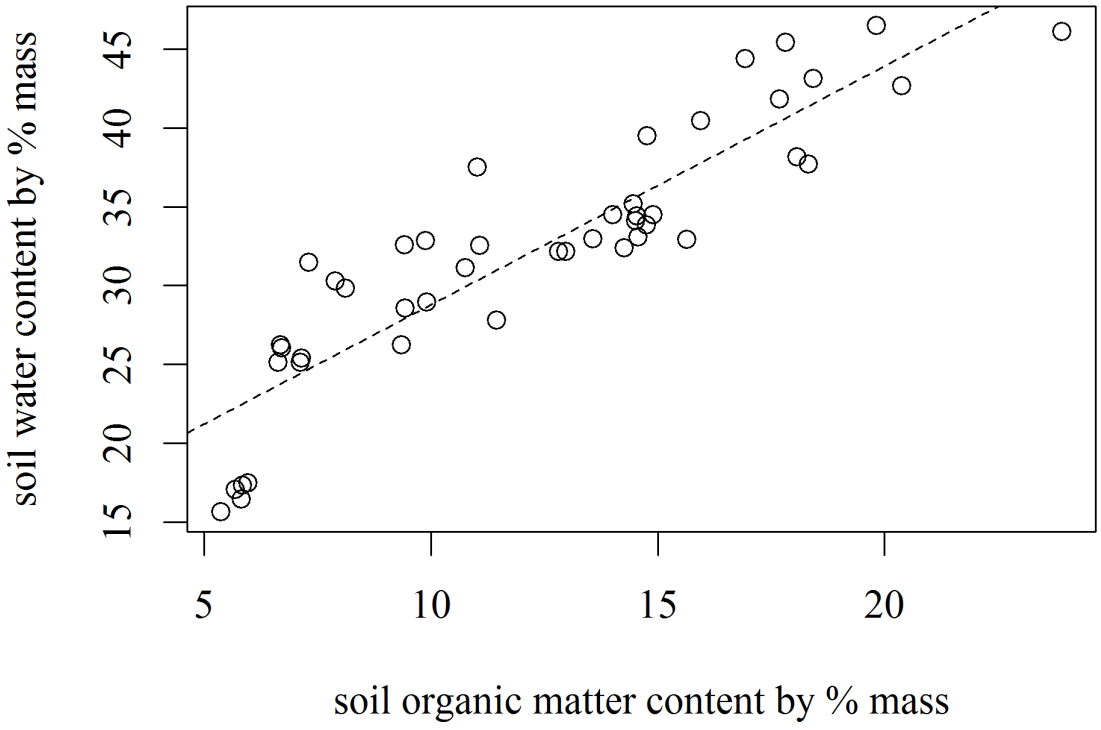

Figure 2: Plot of soil water content and organic matter content for all of the samples analysed. The dotted line was fitted using a linear model (water content = 1.5142 × organic content + 13.6481, $r^2 = 0.8047$, $F_{1,43} = 182.4$, $p < 0.001$).

Discussion

The widespread use of neonicotinoid insecticides is controversial due to a lack of understanding about their persistence in the environment and the long-term consequences of their use. It is therefore important to monitor their prevalence and effects. We present a novel, simple, low-cost method for the extraction of five neonicotinoids from soil with a detection limit <1 ng g^{-1}. We have applied this method to soil collected from maize paddocks in New Zealand and found clothianidin and imidacloprid in 48 out of 50 samples. Neonicotinoid concentrations ranged from 0.5 to 9.4 ng g wet $weight^{-1}$ imidacloprid and 2.1 to 26.7 ng g wet $weight^{-1}$ clothianidin. This is the first study to report the prevalence of neonicotinoid residues in New Zealand's environment.

The concentration of neonicotinoids found here compare well with reported neonicotinoid residues in arable soil (Bonmatin *et al.* 2003, Krupke *et al.* 2012, Jones *et al.* 2014, Botías *et al.* 2015, Schaafsma *et al.* 2015). The New Zealand Environmental Protection Agency [NZEPA] has set an Environmental Exposure Limit [EEL] for imidacloprid in soil of 1ng g dry

weight^{-1}. We have found imidacloprid concentrations that exceed that value by as much as 14 times at eight out of nine sites sampled. The NZEPA has set no EEL for clothianidin in soil, however, clothianidin concentrations exceeded the EEL for imidacloprid at all nine sites and clothianidin appears to be equally as toxic to insects as imidacloprid (Pisa *et al.* 2015, Cavallaro *et al.* 2017). Therefore it appears that potentially hazardous concentrations of neonicotinoid residues persist at all of the sites sampled.

As our samples were collected immediately prior to planting of new seed, they represent the lowest concentrations of neonicotinoid residues to be found throughout the year. It is not surprising that clothianidin concentrations exceeded imidacloprid as all of the paddocks we could establish seed treatment histories for had received the former. Clothianidin is the most commonly applied neonicotinoid seed treatment in the USA and UK (DEFRA 2014, Douglas and Tooker 2015). Residues are likely to accumulate from successive years of planting, which could be why we have found multiple neonicotinoids in almost all of our soil samples. This suggests that most of the imidacloprid residues we have measured are sourced from seed treatment applications nearly two years earlier. Other possible explanations for multiple residues are that some of the residues detected may have leached from seed coating applications in adjacent paddocks or that they originate from other types of application, such as foliar sprays. Although we did not detect thiamethoxam, this neonicotinoid decomposes or is metabolised to form clothianidin. Acetamiprid was also not detected, but is not currently licensed for use in any New Zealand products. Clothianidin is reported to have a higher capacity for leaching through soils and so this may indicate that residues we have measured here have leached from elsewhere, although given the known application histories this seems unlikely (Bonmatin *et al.* 2015). The retention and persistence of neonicotinoid residues is influenced by soil characteristics, with higher organic matter contents being associated with greater retention (Bonmatin *et al.* 2015). However, we found no relationship between the organic matter content of soils and the concentrations of imidacloprid or clothianidin residues. This could be a result of insufficient replication at each site or a consequence of the differential application of neonicotinoids across sites. Although we were able to obtain neonicotinoid application histories for several sites, we could not obtain them for sites D, E and F and therefore their treatment history remains unknown. However, the concentrations of residues found here suggest that it is likely that neonicotinoids were applied.

On the assumption of a normal planting rate for New Zealand of 90,000 seeds ha^{-1} (Stone *et al.* 2000), maize coated with Bayer's Gaucho seed treatment according to the manufacturer's guidelines carries 452 µg imidacloprid per seed. That represents an application rate of 41 grams of active ingredient per hectare. This accords with the findings of Jones *et al* (2014), who reported application rates on wheat, sugarbeet and canola of 10-100 g Ha^{-1}. If the

insecticide is evenly dispersed in the top 20 cm of soil it will result in a mean concentration of 20.5 µg L^{-1}. Concentrations we have measured are approximately 50% of that estimate, indicating that neonicotinoids are highly persistent in New Zealand maize paddock soil.

Because the seed coated with neonicotinoids represents a point source, their distribution in undisturbed soil might be patchy. While we took care to homogenise soil samples, it is possible that our subsampling incorporated plant matter derived from the original seed or soil particles that were proximate to the seed. This could explain the high concentrations of imidacloprid and clothianidin found in some samples, one of which exceeded our estimate for the initial mean concentration. Further analysis is needed to assess whether these indicate variation in the application rate or the soil conditions influencing neonicotinoid persistence in those samples.

Some studies have detailed the hazards posed by residues from neonicotinoid seed treatments to non-target species (Krupke *et al.* 2012, Goulson 2013, Bonmatin *et al.* 2015, Botías *et al.* 2015, Pisa *et al.* 2015). Botias et al (2015) demonstrated that neonicotinoid residues from seed coatings applied to canola can be measured in the soil beyond the margins of the field, at concentrations similar to those reported here. Beyond the margins, they are taken up by wild plants and transferred to the pollen and nectar at concentrations higher than those found in the flowers of the crop itself (Botías *et al.* 2016). This represents a significant threat to honeybees foraging in the area as wildflower pollen constituted the majority of the pollen they returned to the hive (Botías *et al.* 2015). The concentrations of soil neonicotinoid residues measured here are similar to those measured by Botias et al (2015). If the same mechanisms are at work in the margins of the paddocks sampled here then imidacloprid and clothianidin residues available to bees and other pollinators may be high enough throughout the year to compromise a number of sublethal endpoints including navigation, communication, and reproduction (Henry *et al.* 2012, Laycock *et al.* 2012, Whitehorn *et al.* 2012, Botías *et al.* 2016).

It is not clear how long-term neonicotinoid use is affecting the productivity of arable soil ecosystems in New Zealand or elsewhere. Populations of New Zealand maize pest species, such as the Australian soldier fly, *Inopus rubriceps*, and cosmopolitan armyworm, *Mythimna separate*, have been alleged to spike as a result of the removal of natural predators and parasites through the application of insecticides (Chapman 1984). Soil engineers, such as earthworms and microarthropods, such as Collembola, are major service providers in arable ecosystems, enhancing soil productivity by mobilising nutrients through their diet of organic detritus and increasing microbial activity and soil porosity. Earthworms are unlikely to experience acute toxicity from neonicotinoid residues either at the concentrations that we have

estimated are present immediately after seed sowing or that have been reported in the literature (Pisa *et al.* 2015). However, little data exists regarding the hazard to earthworms of chronic exposure to these toxicants and little information on chronic toxicity of neonicotinoids to microarthropods (Dilling *et al.* 2009, Pisa *et al.* 2015). Several species of insect associated with New Zealand maize crops are known to parasitise or predate upon major maize pests. The parasitic wasp, *Apanteles rubricus,* and metallic green rove beetles, *Thyreocephalus spp.*, parasitise or predate upon many of the major New Zealand pest species (Early 1984). These, and other beneficial species, will be exposed to neonicotinoids either through their hosts and prey or through contact with contaminated soil and plant material (eg. Kunkel *et al.* 2001). For example, it has been demonstrated that thiamethoxam can be harmlessly accumulated in the tissue of slugs at concentrations that are lethal to arthropod predators (Douglas *et al.* 2015).

It is established that productivity gains from prophylactic application of pesticides will eventually be outweighed by losses associated with the effects upon ecosystem service provision and the development of resistance (Heckel 2012). The concentrations of neonicotinoid residues we have measured are symptomatic of this. Animals that habitually ingest or burrow through soil, sediment, or tissue cannot avoid exposure to pervasive toxicants, such as neonicotinoids (Pook *et al.* 2009). Chronic exposure to sublethal concentrations of a toxicant are an evolutionary pressure that selects for resistive mechanisms (Orr 1998). Resistance to imidacloprid has already been documented in the USA in Colorado potato beetle, *Leptinotarsa decemlineata*, across Southeast Asia in the brown planthopper *Nilaparvata lugens*, and in Australian green peach aphids, *Myzus persicae* (Alyokhin *et al.* 2007, de Little *et al.* 2016, Garrood *et al.* 2016). The latter species is found throughout New Zealand and is an economically important pest on many crops. However, there is no empirical data on the resistance of this or any other New Zealand pest, predator or parasite to neonicotinoids.

Finally, the novel extraction method deployed here is effective and enables sensitive analysis of environmentally relevant concentrations of neonicotinoid residues in arable soil. The process is simpler than many other soil extraction methods (eg. Botías *et al.* 2015) with only one extraction step, requires no clean-up using the costly dSPE materials that some commonly used methods require, and uses a single concentration step. The final sample matrix is aqueous and can be injected directly to reverse-phase liquid chromatography. We have injected volumes of 25 µL without observing matrix effects (data not shown) with implications for improving the sensitivity further.

Conclusions

This is the first study to report quantities of neonicotinoid residues in New Zealand's environment. We have found that these residues persist in maize paddock soil throughout the year at concentrations that are likely to be hazardous to non-target invertebrates. They either persist from year to year and/or are mobile enough to disperse from paddock to paddock to create multi-residue hazards. Significant knowledge gaps exist in our understanding of the effects of long-term prophylactic application of these compounds. Soil residues of neonicotinoid insecticides should be considered emerging contaminants and the following knowledge gaps should be addressed as a matter of priority:

- Are soil neonicotinoid residues a direct threat to non-target species, such as pollinators and other beneficial insects?
- What are the indirect impact of neonicotinoid residues upon the productivity and ecosystem service provision of the soil community?
- Are current neonicotinoid use patterns likely to accelerate the evolution of resistance to neonicotinoids in pest species?

Acknowledgements

This work would not have been possible without the assistance and guidance of Don MacLeod and Barry Foster of the Apiculture NZ Technical Focus Group, to whom the authors are extremely grateful.

References Cited

Alyokhin, Dively, Patterson, Castaldo, Rogers, Mahoney and Wollam (2007). *Resistance and cross-resistance to imidacloprid and thiamethoxam in the Colorado potato beetle* Leptinotarsa decemlineata. Pest Management Science 63(1): 32-41.

Bonmatin, Giorio, Girolami, Goulson, Kreutzweiser, Krupke, Liess, Long, Marzaro, Mitchell, Noome, Simon-Delso and Tapparo (2015). *Environmental fate and exposure; neonicotinoids and fipronil*. Environmental Science and Pollution Research 22(1): 35-67.

Bonmatin, Moineau, Charvet, Colin, Fleche and Bengsch (2005). *Behaviour of Imidacloprid in Fields. Toxicity for Honey Bees*. Environmental Chemistry. E. Lichtfouse, J. Schwarzbauer and D. Robert, Springer Berlin Heidelberg**:** 483-494.

Bonmatin, Moineau, Charvet, Fleche, Colin and Bengsch (2003). *A LC/APCI-MS/MS Method for Analysis of Imidacloprid in Soils, in Plants, and in Pollens*. Analytical Chemistry 75(9): 2027-2033.

Botías, David, Hill and Goulson (2016). *Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects*. Science of The Total Environment 566–567: 269-278.

Botías, David, Horwood, Abdul-Sada, Nicholls, Hill and Goulson (2015). *Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for Bees*. Environmental Science & Technology 49(21): 12731-12740.

Cavallaro, Morrissey, Headley, Peru and Liber (2017). *Comparative chronic toxicity of imidacloprid, clothianidin, and thiamethoxam to* Chironomus dilutus *and estimation of toxic equivalency factors*. Environmental Toxicology and Chemistry 36(2): 372-382.

Chapman (1984). *Pasture pests*. New Zealand pest and beneficial insects. R. R. Scott. Christchurch, New Zealand, Lincoln University College of Agriculture**:** 119-142.

de Little, Edwards, van Rooyen, Weeks and Umina (2016). *Discovery of metabolic resistance to neonicotinoids in green peach aphids (*Myzus persicae*) in Australia*. Pest Management Science: n/a-n/a.

de Perre, Murphy and Lydy (2015). *Fate and effects of clothianidin in fields using conservation practices*. Environmental Toxicology and Chemistry 34(2): 258-265.

DEFRA (2014). *Pesticide Usage Survey Statistics - https://secure.fera.defra.gov.uk/pusstats*.

Dilling, Lambdin, Grant and Rhea (2009). *Community Response of Insects Associated with Eastern Hemlock to Imidacloprid and Horticultural Oil Treatments*. Environmental Entomology 38(1): 53-66.

Douglas, Rohr and Tooker (2015). *EDITOR'S CHOICE: Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield*. Journal of Applied Ecology 52(1): 250-260.

Douglas and Tooker (2015). *Large-Scale Deployment of Seed Treatments Has Driven Rapid Increase in Use of Neonicotinoid Insecticides and Preemptive Pest Management in U.S. Field Crops*. Environmental Science & Technology 49(8): 5088-5097.

Early (1984). *Parasites & Predators*. New Zealand pests & beneficial insects. R. R. Scott. Christchurch, New Zealand, Lincoln University College of Agriculture**:** 271-308.

Garrood, Zimmer, Gorman, Nauen, Bass and Davies (2016). *Field-evolved resistance to imidacloprid and ethiprole in populations of brown planthopper* Nilaparvata lugens *collected from across South and East Asia*. Pest Management Science 72(1): 140-149.

Goulson (2013). *REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides*. Journal of Applied Ecology 50(4): 977-987.

Hallmann, Foppen, van Turnhout, de Kroon and Jongejans (2014). *Declines in insectivorous birds are associated with high neonicotinoid concentrations*. Nature advance online publication.

Harris and Boerner (1990). *Effects of belowground grazing by collembola on growth, mycorrhizal infection, and P uptake of* Geranium robertianum. Plant and Soil 129(2): 203-210.

Heckel (2012). *Insecticide Resistance After Silent Spring*. Science 337(6102): 1612.

Henry, Béguin, Requier, Rollin, Odoux, Aupinel, Aptel, Tchamitchian and Decourtye (2012). *A Common Pesticide Decreases Foraging Success and Survival in Honey Bees*. Science 336(6079): 348.

Jones, Harrington and Turnbull (2014). *Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years*. Pest Management Science 70(12): 1780-1784.

Krupke, Hunt, Eitzer, Andino and Given (2012). *Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields*. PLOS ONE 7(1): e29268.

Kunkel, Held and Potter (2001). *Lethal and Sublethal Effects of Bendiocarb, Halofenozide, and Imidacloprid on* Harpalus pennsylvanicus *(Coleoptera: Carabidae) Following Different Modes of Exposure in Turfgrass*. Journal of Economic Entomology 94(1): 60.

Laycock, Lenthall, Barratt and Cresswell (2012). *Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*)*. Ecotoxicology 21(7): 1937-1945.

Main, Michel, Headley, Peru and Morrissey (2015). *Ecological and Landscape Drivers of Neonicotinoid Insecticide Detections and Concentrations in Canada's Prairie Wetlands*. Environmental Science & Technology 49(14): 8367-8376.

Orr (1998). *The Population Genetics of Adaptation: The Distribution of Factors Fixed during Adaptive Evolution*. Evolution 52(4): 935-949.

Payá, Anastassiades, Mack, Sigalova, Tasdelen, Oliva and Barba (2007). *Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection*. Analytical and Bioanalytical Chemistry 389(6): 1697-1714.

Pisa, Amaral-Rogers, Belzunces, Bonmatin, Downs, Goulson, Kreutzweiser, Krupke, Liess, McField, Morrissey, Noome, Settele, Simon-Delso, Stark, Van der Sluijs, Van Dyck and Wiemers (2015). *Effects of neonicotinoids and fipronil on non-target invertebrates*. Environmental Science and Pollution Research 22(1): 68-102.

Pook, Lewis and Galloway (2009). *The metabolic and fitness costs associated with metal resistance in* Nereis diversicolor. Marine Pollution Bulletin 58(7): 1063-1071.

Schaafsma, Limay-Rios, Baute, Smith and Xue (2015). *Neonicotinoid Insecticide Residues in Surface Water and Soil Associated with Commercial Maize (Corn) Fields in Southwestern Ontario*. PLOS ONE 10(2): e0118139.

Stone, Pearson, Sorensen and Rogers (2000). *Effect of row spacing and plant population on maize yield and quality*. Agronomy New Zealand 30: 67-75.

Suchail, Debrauwer and Belzunces (2004). *Metabolism of imidacloprid in* Apis mellifera. Pest Management Science 60(3): 291-296.

Sur and Stork (2003). *Uptake, translocation and metabolism of imidacloprid in plants*. Bulletin of Insectology 56(1): 35-40.

Van Dijk, Van Staalduinen and Van der Sluijs (2013). *Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid*. PLoS ONE 8(5): e62374.

Wells, Prest and Russ (2011). *Signal, Noise, and Detection Limits in Mass Spectrometry*. Agilent Technologies Incorporated. 5990-7651EN.

Whitehorn, O'Connor, Wackers and Goulson (2012). *Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production*. Science 336(6079): 351-352.