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Correspondence/Rebuttal

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Response to Comment on "Neonicotinoid Residues in Wildflowers, A Potential Route of Chronic Exposure for Bees"

It should first be noted that Thompson and Campbell are employees of Syngenta, a major manufacturer of neonicotinoids, and thus they have a clear incentive for disputing our finding that neonicotinoids are contaminating soils and wildflowers. They raise five questions regarding our methods and findings, which we address in turn:

1. SAMPLES OF POLLEN FROM WILDFLOWERS CONTAINED HIGH RESIDUES OF THIAMETHOXAM WHILE RESIDUES IN NECTAR ARE NOT DETECTABLE

Many previous studies report nectar residues being consistently lower than pollen residues in the same plant species.[1−3] This is also evident in our samples from the oilseed rape (OSR) crop, where 100% of pollen samples contained thiamethoxam, whereas only 53.9% of the nectar samples had detectable levels of this compound. However, as clearly explained in our manuscript, our wildflower nectar and pollen samples were hand collected from different subsets of plant species—because some species produce plentiful pollen but little nectar, and others vice versa. Since the samples are from different species and were sometimes pooled, it is not surprising that they differ in levels of contamination. However, it should be mentioned that frequency of detections for thiamethoxam in pollen and nectar collected from wildflowers growing in OSR margins were 58.1 and 20.8% respectively, showing a similar relative proportion to what was found for OSR pollen and nectar (see above).

2. RESIDUES IN POLLEN AND NECTAR IN WILD FLOWERS ARE NOT CONSISTENT WITH THE RESIDUES REPORTED IN FIELD MARGIN SOIL

The variable and sometimes high concentrations of neonicotinoids in wildflowers that we describe are consistent with a growing body of literature on the subject. Stewart et al. (2014)[4] reported high heterogeneity in the concentrations of neonicotinoids detected in wildflowers growing near seed-treated crops, where again a few wild plant samples showed greater neonicotinoid concentrations than the ones found in the seed-treated crop (i.e., maximum levels detected in wildflowers collected adjacent to recently planted fields of seed-treated crops were 256 ng/g for thiamethoxam, 53 ng/for clothianidin, 48 ng/g for imidacloprid, compared to 1.1 ng/g of thiamethoxam, 23 ng/g for clothianidin and 2.9 ng/g of imidacloprid detected as maximum levels in seed-treated plants). Additionally, Greatti et al. (2006),[5] Krupke et al. (2012)[6] and Rundlöf et al. (2015)[7] confirmed that wild plants growing near seed-treated fields are frequently contaminated with neonicotinoids through deposition on the flowers, uptake by the root system, or both, showing levels as high as 123.7 ng/g (imidacloprid), 9.4 ng/g and 6.5 ng/g (clothianidin) respectively. The route of contamination has yet to be established—it may be via neonicotinoid dust created during drilling, leaching of neonicotinoids in soil water, or contaminated soil surface dust blowing into field margins. Our soil samples from the field margins were each comprised of a pool of 15 subsamples collected all along the margin (margin length range: 165−980 m). Because these are pooled samples we would not detect local hotspots of neonicotinoid contamination which might underlie the observed heterogeneity in concentrations in wildflowers. Additionally, it is highly likely that some plant species have a higher propensity to take up neonicotinoids than others (as has been found when comparing crop species)[8] which would again create heterogeneity in neonicotinoid concentrations in our samples. For all of these reasons, we would not expect concentrations in wildflowers to match those in our field margin soil samples.

3. HIGH LEVELS OF THIAMETHOXAM WERE REPORTED WHILE PLANT METABOLITES WERE ABSENT

Our findings are entirely consistent with previous studies, which have found that high levels of the parent compound (in our case thiamethoxam) are not always associated with detectable levels of its main metabolite (clothianidin), both in plants[4] and in bees.[9] Neonicotinoids are stable and persistent compounds in both soil and plant tissues, so we would not expect rapid breakdown to their metabolites.

4. THIAMETHOXAM RESIDUES ARE LIKELY TO RESULT FROM CROSS-CONTAMINATION DURING FLOWER POLLEN COLLECTION

This is a criticism that could be levied at any study of pesticide residues, but it is unclear why Thompson and Campbell have specific reason to doubt our methodology and results. We are very confident that cross-contamination from crop flowers to wildflowers did not happen because we followed all the necessary precautions to prevent this from happening (i.e., crop flowers and wildflowers were never collected on the same days, all samples were individually wrapped in aluminum foil and stored separated in sealed plastic bags, all OSR samples were kept on different shelves than wildflower samples in the −80 °C freezer, wildflowers were sieved before OSR flowers were, all the material used for both collection and sieving was thoroughly cleaned between samples following standard laboratory procedures).

In addition, cross-contamination from the crop cannot explain the much higher levels of thiamethoxam found in some wildflowers. If cross-contamination from the crop had occurred, we would expect clothianidin and thiacloprid (which were present in 90.5% and 85.6% of the crop pollen samples, respectively) to be found frequently in the wildflowers, which they were not. Nor is cross-contamination consistent with our

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detection of imidacloprid in many wildflower samples but never in the crop.

5. USE OF THE DATA IN SUBSEQUENT ANALYSIS OF POTENTIAL EXPOSURE

Thompson and Campbell claim that the use of mean pollen values effectively misleads and exaggerates the residue levels reported in wild flower pollen even if the data are taken at face value. However, median values are all reported in our paper, as well as means (Table 1 and Table 2).[10] We mention our average detection levels throughout the manuscript just as the majority of similar papers published in the subject.[1,4,11−13] It is also worth noting that bees collect and process pollen from numerous flowers, effectively pooling samples in the colony, so that the best predictor of the exposure of their offspring is the mean concentration in pollen (bees will not be exposed to a median value).

Thompson and Campbell also misinterpret our results regarding the proportion of neonicotinoid residues contained in wildflower pollen brought back to the hive. As they mention, more than 90% of the trapped pollen during OSR flowering had a wildflower origin, with 9.9% coming from OSR flowers, but that is just data about pollen type diversity collected by the bees. The 97% figure comes from a different calculation, which is the proportion of neonicotinoid residues present in wildflower pollen in relation to total amount of residues detected in the trapped pollen. As it is clearly explained in our paper, we individually analyzed the residue levels for all pollen types collected by bees, and we found that from the total amount of residues detected in trapped pollen collected during 4 days (287 ng), only 3% were contributed by the OSR pollen (9.2 ng), with 97% being in wildflower pollen (277.8 ng). Our results robustly show that the vast majority of neonicotinoid residues brought back to the hives were contained in the pollen that bees collected from wildflowers. Therefore, this unintended contamination of nontarget plants, predominantly by a compound manufactured by Thompson and Campbell's employer, represents a major source of exposure to neonicotinoid residues for bees. The effects of this exposure have not been evaluated in any field trial to date, all of which have focused solely on exposure from the treated crop.

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Notes

The authors declare no competing financial interest.

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