




## Scientific note: Imidacloprid found in wild plants downstream permanent greenhouses in Sweden

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In 2018, a few months after the European Food Safety Authority had identified high risk to bees for several field uses of three neonicotinoids (EFSA 2018a, b, c), the European Commission decided to permanently ban them from outdoor plant protection (European Commission 2018a, b, c). This was an expansion of an earlier ban on their use in crops attractive to bees (European Commission 2013). The new ban covered all outdoor cultivations, only giving some room for emergency authorization (European Commission 2020). However, all three substances were still allowed in permanent greenhouses, under the condition that treated plants remain in a greenhouse during their entire life cycles (European Commission 2018a, b, c). The three neonicotinoids were imidacloprid, clothianidin and thiamethoxam.

A recent screening of pesticides in streams adjacent to commercial greenhouses in southernmost Sweden identified imidacloprid as one of the most

common pesticides in the water (Kreuger et al. 2019). Spray application and soil treatment with imidacloprid have never been allowed in outdoor cultivations in Sweden, but until the EU-wide ban, farmers could sow seeds coated with imidacloprid (Pedersen et al. 2009). However, the concentrations of imidacloprid found in surface water downstream greenhouses were in several cases up to two orders of magnitude higher than those found within the Swedish monitoring program in high-intensity agricultural areas (Boye et al. 2019). There was also a link between detections in surface water and registered applications in the greenhouses, indicating the discharge of contaminated water, for example during backwashing of irrigation systems (Kreuger et al. 2019). Because neonicotinoids are systemic and plants can absorb them via their roots (Sur and Stork 2003), we suspected that the presence of imidacloprid in water could result in uptake by plants growing nearby the streams, constituting a potential route of exposure to bees and other insects. On the 4<sup>th</sup> and 5<sup>th</sup> of June 2019, we revisited six of the sites from the stream water screening study (Kreuger et al. 2019) and collected anthers (as a

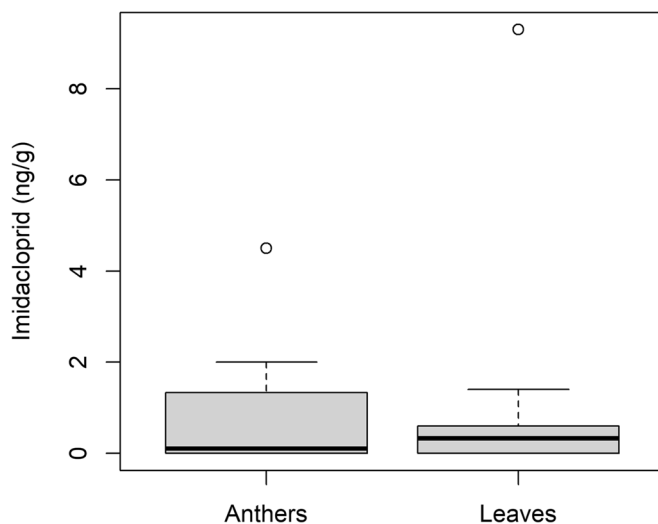
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substitute for pollen) from wild plants growing adjacent to the streams to measure their imidacloprid content. We collected entire anthers, with a lot of pollen, to obtain sufficient amounts to allow quantification of imidacloprid ( $n = 8$ ). As not all plants were flowering at the time of collection we also collected leaves to investigate if imidacloprid was present systemically in the plants ( $n = 15$ ). The samples were kept in a cooler box, with ice blocks, during the day of collection, and were then transferred to a  $-20$  freezer.

In brief, the analytical procedure was as follows: Stable isotope-labelled internal standards, including imidacloprid- $d_4$ , were added to all samples. Leaf and anther samples were homogenized with drying agent ( $Na_2SO_4$ ) using Precellys tubes with 2.8-mm ceramic beads and a Precellys Evolution Homogenizer (Bertin instruments, Montigny-le-Brettonneux, France). Extraction was performed in the same tubes and instrumental setup using two portions of acetonitrile. Leaf sample extracts were further cleaned by dispersive solid-phase extraction with octadecylsilane (C18) and primary and secondary amine (PSA) adsorbents (roQ kit KSO 8921, Phenomenex, USA), evaporated to dryness, and finally reconstituted in acetonitrile and water. Study samples, blank samples, recovery samples and calibration samples were analyzed with liquid chromatography and tandem mass spectrometry (6460

triple quadrupole from Agilent Technologies, Palo Alto, USA).

We detected imidacloprid at five out of six sites, with detections in 50% of the samples for both anthers and leaves (Online Resource 1-2, figure 1). The detection of imidacloprid in anthers indicates that bees foraging for pollen can get exposed to imidacloprid despite the extensive ban on outdoor plant protection. As the high concentrations of imidacloprid in the sampled streams have previously been linked to application in greenhouses (Kreuger et al. 2019), it is possible that this was also the source of the imidacloprid that we detected in plants along the same streams. However, because Sweden has now further restricted the use of imidacloprid, by only allowing it in greenhouses where no leakage or unintentional discharge can occur (Kemikalieinspektionen 2019), we cannot verify this. While acknowledging that the sample size in our study was low, we note that the concentrations of imidacloprid in anthers (up to 4.5 ng/g, Online Resource 1) were within the range where chronic exposure to imidacloprid reduces reproduction in solitary bees and bumblebees ( $\sim 1.5$  ng/g, Woodcock pers. comm.; Woodcock et al. 2017). As there may be other flowering plants from which the bees can forage, these concentrations are likely higher than the resulting chronic exposure that the bees experience. However, it is also possible



**Figure 1.** Concentrations (ng/g) of imidacloprid in anthers ( $n=8$ ) and leaves ( $n=15$ )

that the identified concentrations do not cover the entire range of imidacloprid concentrations that can be found in wild flowering plants.

While we cannot verify the source of imidacloprid in the wild plants, our results suggest that exposure via pollen from wild plants along streams that have been contaminated through discharge from greenhouses is possible. In order to reduce the risk of exposing bees and other insects to systemic pesticides, such as imidacloprid, there is a need to assess this pathway further.

## SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1007/s13592-021-00876-4>.

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## AUTHOR CONTRIBUTION

LH conceived the idea and planned the study together with OJ and MR. LH performed field work. JK and OJ performed lab work. LH wrote the manuscript with contribution from HS, JK, OJ and MR.

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## DATA AVAILABILITY

Data are presented in Online Resource 1-2.

## CODE AVAILABILITY

No analyses have been performed. All data are presented in Online Resource 1.

## DECLARATIONS

**Ethics approval** Not applicable (no humans or animals involved)

**Consent to participate** Not applicable (no humans or animals involved)

**Consent for publication** Not applicable (no humans or animals involved)

**Conflict of interest** The author declare no competing interests.

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