

## Abstract

The monarch butterfly (*Danaus plexippus*) is a species of special concern. Southern Ontario is one of the most important breeding areas in Canada for the eastern monarch butterfly and an area of intensive corn and soybean agriculture. Exposure to neonicotinoid insecticides (NNIs) in intensive agricultural landscapes may play a role in reducing monarch numbers through contamination of the common milkweed (*Asclepias syriaca*), the plant species most favoured for egg laying and larval food source. Recent studies show that exposures of monarch larvae to concentrations of the NNI as low as 1 ng/g of clothianidin reduces larval size and condition (Pencenka et al., 2015). In this study, we sampled milkweed leaves and soils at four field sites in two separate watersheds in southwestern Ontario to assess contamination of milkweed plants adjacent to corn and soy fields with NNIs. Samples were prepared for analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS). The levels of clothianidin (CTN) and thiamethoxam (TMX) in soil samples adjacent to agricultural fields at all sites were very low or below detection limits. Both NNIs were detected in the leaves of milkweed but the concentrations were highly variable. The highest concentration of CTN detected in milkweed leaf was 23.9 ng/g dry weight, above the reported threshold for lethal effects in larvae of the monarch butterfly. No NNIs were detected in milkweed leaves collected from a non-agricultural reference site. These data indicate that exposures of larvae of the eastern monarch butterfly to NNIs in Southwestern Ontario may play a role in the reduced numbers of this species of special concern.

## Objectives

To determine if previous laboratory determined detrimental levels of NNIs were present in common milkweed leaves next to intensive agricultural fields in southwestern Ontario, having the potential for sublethal or lethal effects on larval survival in this part of the breeding range.

## Field Collection



Figure 1: Location of field sites in southern Ontario on the north shore of Lake Erie (A) and example photo of a field site location (B).

Field locations with detectable NNI concentrations in adjacent watersheds (Struger et al. (2017), Metcalfe et al (2019)) were pre-screened after snow melt. Two field locations were selected near McKillop Drain (Wallacetown, ON), and two near Lebo Drain (Leamington, ON). A reference site was located north of Peterborough, ON in an open meadow remote from agricultural areas.

## Sample Preparation and Analysis

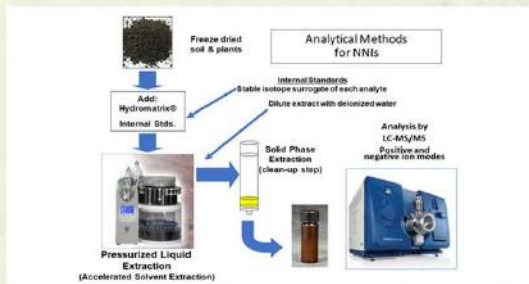


Table 1: Target compounds for LC-MS/MS analysis of extracts prepared from samples of milkweed leaves and soils, and their analytical surrogates.

Compound	Class
Imidacloprid	NNI insecticide
Imidacloprid-hydroxy	Transformation product of imidacloprid
Imidacloprid-olefin	Transformation product of imidacloprid
Acetamiprid	NNI insecticide
Clothianidin	NNI insecticide; transformation product of thiamethoxam
Thiamethoxam	NNI insecticide
Imidacloprid-d4	Internal standard
Acetamiprid-d3	Internal standard
Clothianidin-d3	Internal standard
Thiamethoxam-d3	Internal standard

## Conclusions

Thiamethoxam and clothianidin were only detected at trace concentrations in some soil samples. Concentrations in milkweed leaves were highly variable among and between sites, however the majority of leaf samples contained clothianidin and total NNIs at concentrations above the 1 ng/g threshold reported to produce effects on the condition and size of monarch larvae. Concentrations in some plant samples approached or exceeded the LC<sub>50</sub> for monarch larvae exposed to clothianidin in laboratory studies (Pecenka and Lundgren, 2015). These preliminary results indicate the potential for exposure of early life stages of the eastern monarch butterfly to harmful concentrations of NNIs within its breeding territory in southern Ontario. This preliminary survey of NNIs in wild plants that are preferred for forage by larvae of the eastern Monarch butterfly shows that there is potential for NNIs to impact population recruitment for this species at risk in Ontario.

## References

- Metcalfe CD, Helm PA, Paterson G, Kaltenecker G, Murray C, Nowierski M, Sultana T. 2019. Pesticides related to land use in the watersheds in the Great Lakes basin. *Sci. Total Environ.* 648:681-692.
- Pecenka, JR, JG Lundgren. 2015. Non-target effects of clothianidin on monarch butterflies. *Sci. Nat.* 102:19-27.
- Struger J, Grabuski J, Cagampan S, Sverko E, McGoldrick D, Marvin CH. 2017. Factors influencing the occurrence and distribution of neonicotinoid insecticides in surface waters of southern Ontario, Canada. *Chemosphere* 169:516-523.

## Results

### SOILS:

Table 2: Mean concentration (ng/g dry weight) of thiamethoxam and clothianidin in soils at various collection dates in 2017 and 2018. ND = Not detected (below limits of detection), P = Present (below limits of quantitation; NA = Not analyzed because sample sites could not be accessed).

Collection Date	Thiamethoxam (TMX)				Clothianidin (CTN)				
	Site 1	Site 2	Site 3	Site 4	Collection Date	Site 1	Site 2	Site 3	Site 4
17-Jun	1.5	1.4	P	ND	17-Jun	ND	ND	ND	ND
17-Jul	ND	ND	ND	NA	17-Jul	1.1	2.4	1.0	NA
17-Sep	ND	ND	ND	ND	17-Sep	1.1	1.1	1.6	1.5
18-Jun	ND	ND	ND	ND	18-Jun	1.0	1.0	P	ND
18-Aug	6.6	P	0.9	1.2	18-Aug	10.2	2.0	0.9	5.5

### MILKWEED LEAF:

The NNIs, imidacloprid, acetamiprid and transformation products of imidacloprid were not detected in any of the leaf samples. Average concentrations in leaf samples do not indicate the high degree of variability of concentrations of TMX and CTN among leaf samples (Figure 2).

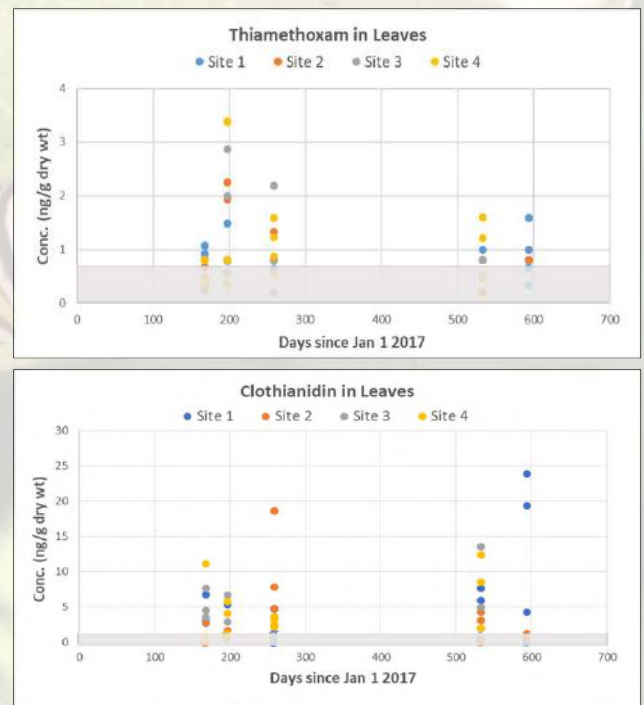


Figure 2: Concentrations of thiamethoxam and clothianidin in milkweed leaf samples showing high variability among individual samples at all sites and collection times. Grey bar indicates instrument limit of quantification.