

# Protecting Pollinators from Neonicotinoid Insecticide Applications in Turfgrass

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## Abstract

Imidacloprid is one of the most commonly used insecticides in the turfgrass industry. Part of the neonicotinoid class, it has a broad spectrum of activity, but is also highly mobile and toxic to pollinators like *Apis mellifera*. In turfgrass, it is often applied as a broadcast spray, sometimes in close proximity to flower beds, raising the specter of unintentional contamination of critical pollinator resources. We conducted a survey of the pollinators visiting these beds and sought to evaluate the likelihood and severity of floral contamination in response to the distance of imidacloprid application from flower beds. The treatments included applications over top of the bed, up to the edge of the bed, up to within 2' of the bed, and an untreated control. We sampled flowers from *Monarda didyma* at four different dates through July and August, and flowers of *Aster dumosus* at one date in September. Halictids dominated the pollinator community detected at the site, with several species in the genera *Augochlora*, *Lassioglossum*, and *Agopostemon* being the most prevalent. Concentrations of imidacloprid in *M. didyma* flowers did not vary significantly between treatments at any point during the season, but there was a significant spike in imidacloprid concentrations across all treatments approximately 6 weeks after the applications. In *A. dumosus* flowers, concentrations of imidacloprid were significantly higher in plots where treatments were made up to the edge of the bed compared to the untreated control, but there were no other significant differences between treatments. Regardless of treatment, imidacloprid concentrations in flowers often exceeded levels considered safe for honey bees. These findings raise questions about the movement of imidacloprid in soil, or as drift, and highlight the need for more research to understand the utility and safety of pollinator gardens in urban areas.

## Introduction

Neonicotinoids are one of the most widely used classes of pesticides in the world. They are commonly utilized in agriculture, urban, and turfgrass pest management programs. The first neonicotinoid – imidacloprid – was introduced into the market in 1991, and as of 2008 was “the most successful, highly efficacious and best-selling insecticide worldwide” (Jeschke, 2008). These insecticides can be applied in many ways, including broadcast sprays, granules, and seed treatments. While more briefly effective as residual sprays, neonicotinoids are especially known for their ability to be translocated into plant tissue. Stored in the plants themselves, these insecticides can then be ingested by pests feeding on the treated plant.

While this class of insecticides has proven extremely useful and successful in pest management, it has also been shown to be highly toxic to pollinators. In honey bees (*Apis mellifera*), imidacloprid has a contact LD50 of 60 ng/bee, and an ingested LD50 of 13 ng/bee (Sanchez-Bayo, 2014). At sub-lethal doses, it may impair the cognitive abilities of honey bees, influencing navigation, food

collection, and predator avoidance (Tan, 2014). Little work has been conducted on the LD50 of imidacloprid for native solitary bees, but one study has found that imidacloprid can have detrimental effects to larval development of *Osmia lignaria* when pollen provisions were contaminated with 30 ppb or higher concentrations of imidacloprid (Abbott, 2008).

Neonicotinoids have been found in pollen and nectar of treated plants. With virtually all corn seed treated with neonicotinoids (Krupke 2012), corn alone can provide a large source of contaminated pollen. More concerning, however, is the discovery of neonicotinoids in non-target flowering plants near treated areas. Clothianidin has been found in the leaf tissue, pollen, and nectar of flowering plants adjacent to cornfields. Not only have neonicotinoids been found in plants adjacent to treated fields, but also those near organic fields – and with similar concentrations to those near conventional farms (Mogren, 2016).

There have been few studies looking at the movement of imidacloprid and other neonicotinoids through soil, and none looking at the horizontal movement of imidacloprid in a turfgrass setting. What studies have been conducted have found that

imidacloprid is prone to leaching, and can move vertically through the soil (Gupta, 2001).

Little work has been conducted on the presence of neonicotinoids in plants adjacent to turfgrass, despite the recommendation and use of pollinator areas in golf courses and home lawns and gardens (Larson, 2017). The presence of thatch and dense roots found in turf likely limit the horizontal movement of neonicotinoids in these systems, but this has never been explicitly examined. We decided to test if imidacloprid can move through treated turf to adjacent flower beds, and quantify how far from flower beds applications would need to be in order to prevent contamination. We paired this with a pollinator survey to identify the pollinators most likely to be affected by contaminated flowers in urban landscapes located in northern Indiana.

## Materials and Methods

### Flower Plots:

Plots were arranged in a randomized complete block design, with 3 replicates (Fig. 1). Treatments included a broadcast spray of imidacloprid (Merit 2F) in a 1.5 × 1.5 m band, 1) directly over the flower bed, 2) on the turf up to the edge of the bed, 3) on the turf up to within two feet of the bed, and 4) an untreated control. Two species of flowers were planted within each plot: *Aster dumosus* and *Monarda didyma*. Both *A. dumosus* and *M. didyma* were established perennials that were planted in 2014. Plots were irrigated to minimize plant stress. Weeds were controlled by hand-pulling and mulching, supplemented with spot treatments of herbicides (Glyphosate Round-Up® and Triclopyr Confront®). Soil was a sandy clay/loam (48:28:24, sand:silt:clay).

### Imidacloprid Application:

Imidacloprid (Merit 2F) was applied on June 16, using a hand-held boom-sprayer calibrated to deliver 18.7 L/Ha. A rate of 1844.5 ml/Ha was used, as this was the high end of what the label allowed. 2 “drift guards” were created out of PVC tubes and shower curtains, and were used to protect adjacent plots from drift while spraying.

### Sample Collection:

*M. didyma* samples were collected on July 1, July 15, July 29, and August 12. *A. dumosus* samples were collected on September 23. Clean nitrile gloves were worn, and each treatment had an assigned pair of scissors that would be rinsed in acetonitrile before

each trip. Flowers were stored in plastic Ziploc bags, and frozen (-20°C) until processed.

### Sample Preparation:

Reproductive parts of the flowers (parts where nectar and pollen could be found) were collected for analysis. The samples were handled with a fresh pair of gloves for each sample, and all utensils were rinsed in acetonitrile before use on each sample. Reproductive tissues were removed, weighed and approximately 2.0 g of each sample was placed individually into 7mL Precellys lysing kit tubes with 4.0 ml of acetonitrile. Samples were then homogenized at 6000 rpm, with 3 rounds running 20 seconds and 90 second intervals between. Homogenized samples were then placed back into the freezer until imidacloprid concentrations could be quantified.

### Sample Analysis:

Samples were sent to the plant metabolomics laboratory at Bindley bioscience center to be analyzed via liquid chromatography. They were analyzed using a modified QuEChERS protocol with Bindley’s TSQ Endura Triple Quadrupole mass spectrometer. This technique has been used successfully in other experiments to quantify neonicotinoids (including imidacloprid) in plant tissues (Alford, 2017).

### Data Analysis:

Data was analyzed using Statistica 13. Repeated measures ANOVA was used to examine variation in imidacloprid concentrations over time in the reproductive tissues collected from *M. didyma*. One-way ANOVA was used to examine variation in imidacloprid concentrations in *A. dumosus* reproductive tissues on the single collection date. Post-hoc means separation was performed using Tukey’s HSD.

### Pollinator Survey:

Pollinators were surveyed via pollinator bowls. The set up consisted of a 3.25 oz plastic bowl spray painted fluorescent yellow, and attached to 4 ft fence posts. 4 of these were placed intermittently between the flower plots. The bowls were filled at the beginning of each week with soapy water, and checked 2-3 times a week. On Fridays, they would be checked, and the bowls emptied and brought indoors to prevent being blown away over weekends.

Specimens were then stored in 70% EtOH and identified to family, omitting minute insects.

## Results

### Imidacloprid Concentration Analysis:

*M. didyma* samples showed high levels of imidacloprid throughout the flowering period (July 1 – August 12). The first 3 sample dates had concentrations above 500 ng/g tissue, and the final date still measured just under 500 ng/g tissue. On the third sampling date, concentrations ranged from 1313-3202 ng/g tissue (Figure 2). While there was a significant difference between the third date and all other sampling dates, there was no significant difference between any of the insecticide treatments during any of the sampling dates (Table 1). All imidacloprid concentrations in the *M. didyma* samples were well above the honey bee LD50 for ingestion and contact.

*A. dumosis* samples were only collected on 23 September. Concentrations ranged from 27-101 ng/g tissue (Figure 3). Samples collected from the plots where insecticide was applied up to the edge of the bed contained significantly higher concentrations of imidacloprid than the untreated control. Otherwise, treatment had no significant effect on concentrations of imidacloprid in the flower tissues examined (Table 2). On average, the concentration of imidacloprid in the *A. dumosis* in bed and edge treatments were above the ingested and contact LD50 of honey bees. The concentrations found in the control and buffer treatments were generally below the contact LD50, but still above the ingested LD50 of honey bees.

### Pollinator Survey:

The pollinator survey recovered 9 important pollinator families, along with multiple families of beetles and parasitoid wasps (Table 3). Halictidae was by far the most common family with 31 specimens collected throughout the summer. *Lassioglossum*, *Augochlora*, and *Agopostemon* were the most common genera of Halictids caught in the pollinator bowls. Vespidae was the second most common family collected with a total of 5 individuals being observed in the pollinator bowls over the course of the growing season.

Relatively few members of Apidae were caught in the pollinator bowls and no bumblebees were collected in these traps during the growing season.

## Discussion

The results reported herein paint an alarming picture for pollinators visiting flowering plants near turfgrass treated with imidacloprid. Within the parameters of our study, we were unable to find a safe distance within which imidacloprid could be applied without contaminating adjacent flowering plants. On top of that, imidacloprid concentrations in flowers collected from untreated *M. didyma* controls were similar to concentrations detected in all other treatments, including those receiving a direct foliar application. In this case, imidacloprid concentrations were well above the contact LD50 for *A. mellifera*, throughout the entire blooming period for this species.

The late season blooms of asters serve as an important source of nectar and pollen during the fall (Caron, 2013). Still, even 15 weeks after application, imidacloprid was detected in the reproductive tissue of this species at levels higher than the LD50 for ingestion by *A. mellifera*. Concentrations in some treatments (edge and in bed treatments) were above the contact LD50 for *A. mellifera*.

These results suggest that imidacloprid may be prone to horizontal movement through soil in turf settings, and can persist in either the soil or in the plants for an extended period of time. Each of our flower beds were separated by a 3 ft buffer zone, which suggests that imidacloprid can travel horizontally through soil for at least 3 ft. Such high concentrations of imidacloprid in the flower tissue raises serious questions about the safety of pollinators visiting flower beds near treated turf. Our results suggest that keeping flowering plants near treated turf may pose significant risks to any visiting pollinators, acting as accidental trap crops for pollinating insects.

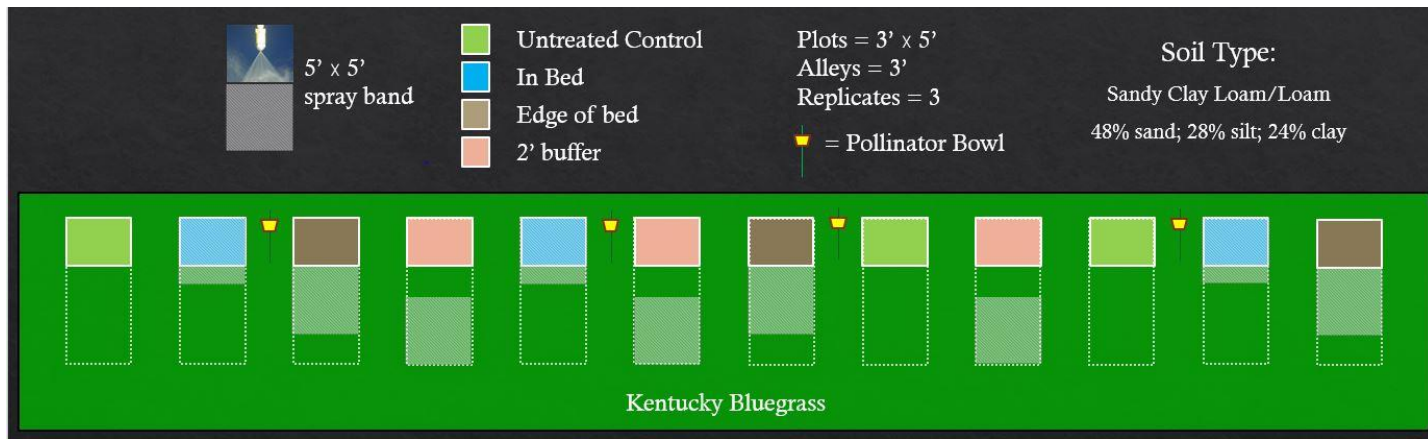
Overall, our results raise concerns about the establishment of pollinator conservation areas near neonicotinoid-treated turf. In particular, more research is needed to examine the horizontal mobility of imidacloprid in turfgrass systems. Future studies should incorporate larger distances between plots and employ larger spray distances (buffer zones) to more clearly delineate the distances required for safe use of neonicotinoids near flowering plants. Because soil type could influence the movement and uptake of neonicotinoids, measuring imidacloprid concentrations in the soil at various distances from the application will be useful for characterizing the potential for horizontal movement of these insecticides in different turfgrass settings. We would

also want to modify the plants utilized. Including a flowering annual plant species that blooms throughout the entire season will provide a continuous view of how imidacloprid concentrations in the plants fluctuate throughout the year. This information could help inform landscape design decisions for incorporating flowering plants in turfgrass dominated landscapes.

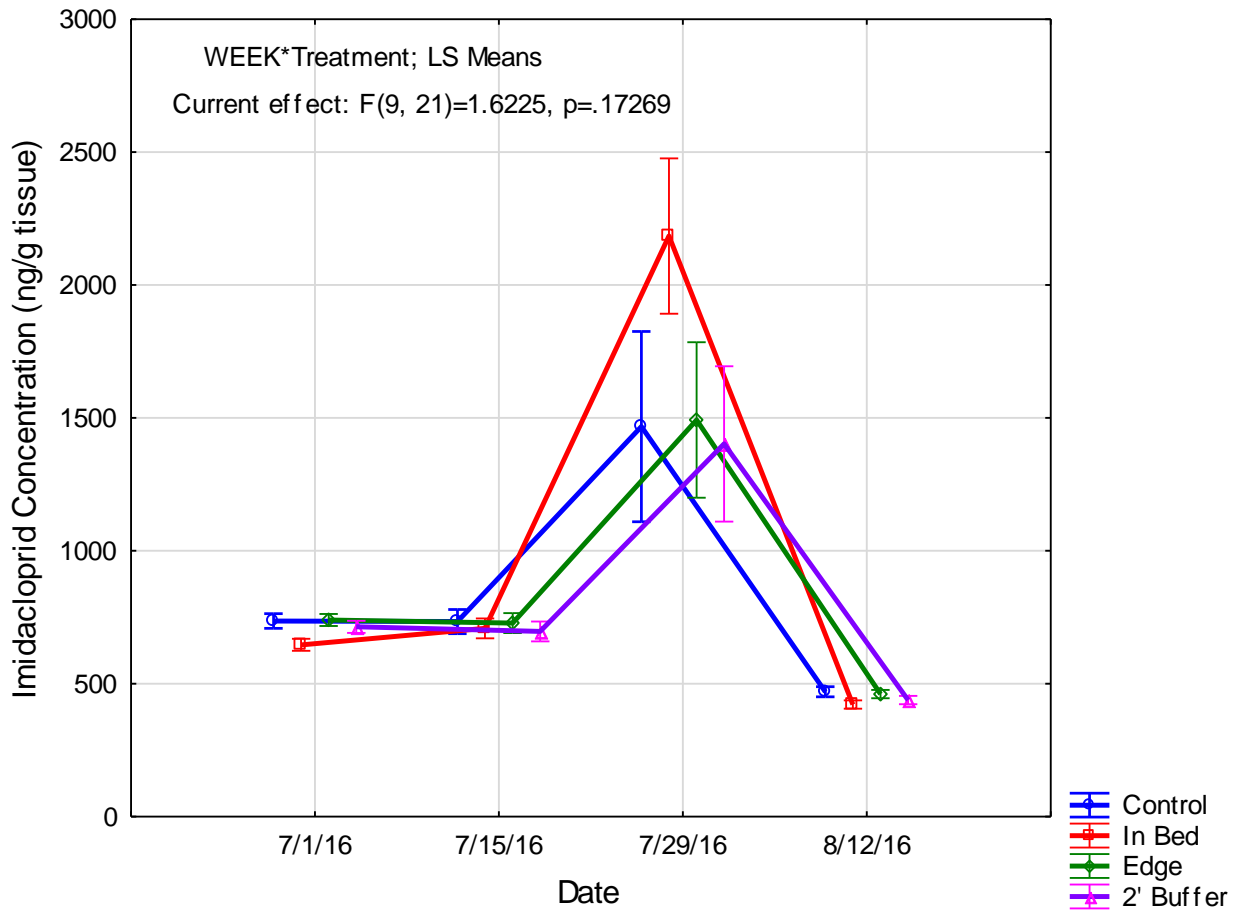
A longer-term examination of pollinator communities visiting these sites and use of larger bee

bowls could also be informative. By using larger bee bowls, we should be able to have a more robust survey that can account for larger arthropod pollinators, such as bumble bees. Repeating the survey can also be useful for determining if pollinator diversity and abundance is affected over the long-term by neonicotinoid treatments, and characterize how pollinator populations in these systems may fluctuate over time.

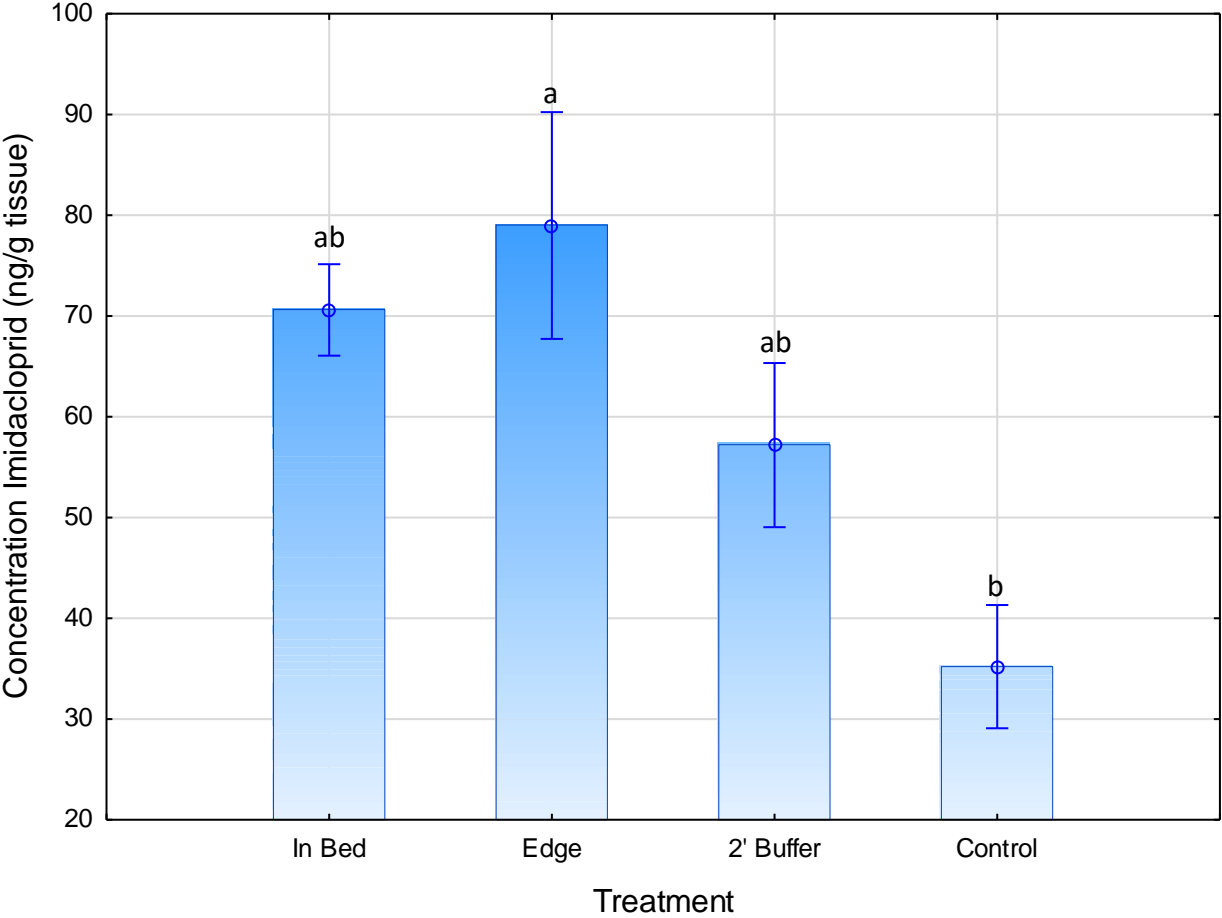
**Figure 1.** Plot map depicting the arrangement of flower beds, treatments, and pollinator bowl locations in an experiment designed to characterize pollinator communities and quantify contamination of flowers stemming from imidacloprid applications made to surrounding turfgrass at various distances form the flower beds.



**Figure 2.** Imidacloprid concentrations in the blooms of *Monarda dydima* over time following application of the insecticide Merit 2F to turfgrass at various distances (Treatment) from the flower bed. Note that all concentrations are above the contact LD50 of *A. mellifera* (60 ng/g).



**Figure 3.** Imidacloprid concentrations in the blooms of *A. dumosis* 15 weeks after application of the insecticide Merit 2F to turfgrass at various distances (Tretament) from the flower bed.



**Table 1.** ANOVA table for repeated-measures analysis examining variation in imidacloprid concentrations in the blooms of *Monarda dydima* plants over time as a result of imidacloprid applications made to turfgrass at various distances (Treatment) from the flower bed.

Effect	df	MS	F	p
Date	3	2898187	42.69568	0.000000
Date × Treatment	9	110133	1.62246	0.172690
Error	21	67880		

**Table 2.** ANOVA table for analysis of imidacloprid concentration in flower tissues of *A. dumosis* as a result of imidacloprid applications made to turfgrass at various distances (Treatment) from the flower bed.

Effect	df	MS	F	p
Intercept	1	39034.92	220.2292	0.000002
Treatment	3	1090.98	6.1551	0.022470
Error	7	177.25		

**Table 3.** Number of specimens from various families of invertebrate pollinators collected in pollinator bowls placed in ornamental plantings located near turfgrass treated with the insecticide imidacloprid (Merit 2F).

Family	June 6-10	June 13-17	June 20-24	June 27-July 1	July 4-8	July 11-15	July 25-29	August 1-5	Totals
Halictidae	3	3	1	3	2	4	7	8	31
Syrphidae				1					1
Apidae						1	1		2
Braconidae	1					1			2
Vespidae						2		3	5
Megachilidae									0
Ichneumonidae				1					1
Pompilidae					2				2
Andrenidae							3		3
Other	8	10	3	6	3	7	12	33	82



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