Purdue University Undergraduate Capstone Research

Quantifying the concentration of thiamethoxam in soybeans over time and assessing the potential for effects on target pests below ground

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Abstract

Neonicotinoids, such as thiamethoxam, are used as a seed treatment in many types of crops. They are incredibly versatile and extremely water soluble, and these traits are what has led to their popularity. Because they travel with the xylem, the insecticide travels from the root toward the upper part of the plant. Over time, the concentration of insecticide declines to ineffective levels before target above ground pests are in the field. The concentration of thiamethoxam and its metabolite clothianidin decrease within the first three weeks of soybean growth in the root. Levels of the insecticide decline to minute levels before below ground pests, such as white grub, are in the field feeding on the root.

Introduction

Neonicotinoids account for one-third of the worldwide insecticide market (Simon-Delso et al. 2015). The physiochemical properties of neonicotinoids have many advantages over other insecticides and low operator and consumer risks (Simon-Delso et al. 2015). Neonicotinoids are extremely water soluble (Krupke et al. 2017). The solubility of neonicotinoids ranges from 184 mg/L to 590,000 mg/L. At 20 degrees Celsius at pH 7, the water solubility of thiamethoxam is 4,100 mg/L and clothianidin is 340 mg/L (Bonmatin et al. 2015).which is why they are used as seed treatments. Once the plant begins to germinate, it takes up water, as well as the insecticide. Neonicotinoids work by mimicking the action of neurotransmitters in the central nervous system, continuously stimulating neurons leading to death (Pisa et al. 2015).

Neonicotinoids have many key attributes, including high persistence, systemicity, and assumed lower impacts on fish and other invertebrates. More research needs to be done on their impacts in the area. Because neonicotinoids such as thiamethoxam and clothianidin travel in the xylem, they travel with the water in the plant. A study by Krupke et al. (2017), found that the neonicotinoids used as a seed treatment decline to levels not significantly different from untreated plants.

For this project, the concentration of thiamethoxam and clothianidin in two different parts of young soybean plants over a two-week period were studied. The potential for effects on target pests that feed on the root were then assessed. I hypothesized that there would be a difference in concentrations of insecticide within different parts of the plant over time. Further, I hypothesize that there will be differences in concentrations between treatments over time.

Materials and Methods

This experiment was conducted at Throckmorton Purdue Agricultural Center in Tippecanoe County, Indiana. It began May 22, 2015 and ended August 12, 2015.

Plot Area

Each treatment was 8 rows wide by 80 meters long. There were 4 replications done with the treatments to obtain the different orders of treatment applications. I will only be focusing on Rep 2 for this project. Figure 1 is a layout of the plot area.

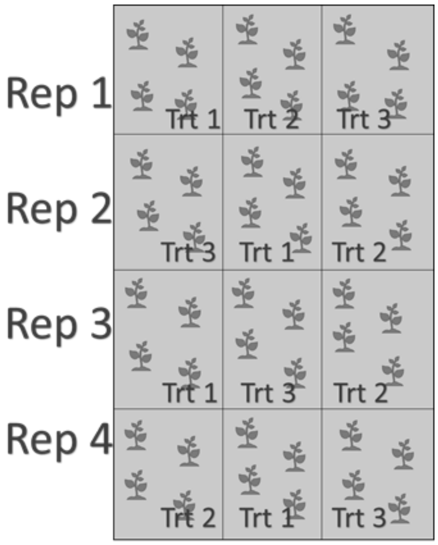

Figure 1. Schematic representation of plot layout.

Applications

Three different soybean seed treatments were used. All the seeds were planted the 22nd of May 2015. The first treatment was naked seed (untreated control). The second treatment applied was the fungicides mefenoxam (brand name Apron) and fludioxonil (brand name Maxim). Each seed contained an average of 14,500 ng/g active ingredient/seed mefenoxam and 5,700 ng/g ai/seed fludioxonil. The third treatment was a combination of thiamethoxam (brand name Cruiser Maxx) and the fungicides from the second treatment. Cruiser Maxx contains on average 76,200 ng/g ai/seed thiamethoxam, 5,700 ng/g ai/seed mefenoxam, and 3,900 ng/g ai/seed fludioxonil. The application amounts were applied according to the pesticide labels.

Collection

Five collection dates within the first three weeks of planting were chosen to monitor the amount of pesticide in the soybean plant over time. The dates of collection were the 1st, 4th, 9th, 12th, and 15th of June 2015. These dates were chosen because previous reports state that the window of pest control by seed treatments is offered within three weeks after planting (Krupke et al. 2017). Four plants were sampled per treatment on each date for a total of 60 plant samples. The plants were then separated into root and newest trifoliate, and each part was weighed separately.

Sample Processing

Each sample was placed in a 7 mL Precellys ((Montigny-le-Bretonneux France)) tubes that contained 1.5 g of ceramic beads to assist in homogenization. 4 mL of acetonitrile (ACN) was then added to each tube. The tubes were then placed in a Precellys 24 tissue homogenizer machine. The machine was set to 6,000 rpm, 3- 20 second cycles. With 90 second pauses between each cycle to prevent the tissue sample from degradation. Once the machine finished running, the tubes were then carefully poured into an identically labeled 15 mL Falcon (Pittsburg, Massachusetts, USA) tube. Four mL of acetonitrile was then added to the now empty Precellys tube and the tube was carefully shaken and poured into the corresponding falcon tube. Two mL of ACN was then added to each falcon tube to make a total of 10 mL in each tube. The tubes were placed in a -20 degree Celsius freezer until the samples could be processed at Bindley Bioscience Center in Purdue Discovery Park.

A salt mixture of 0.25 g sesquihydrate, 0.5 g sodium chloride, and 2.0 g magnesium sulfate was added to each tube. A standard spike of 10 µL d3-clothianidin and d3-thiamethoxsam was added to each tube. The tubes were then vortexed thoroughly, and centrifuged at 4 degrees Celsius at 2,500 rpm for 10 minutes. 1 mL of the supernatant was then transferred to an identically labeled 2 mL Agilent dispersive Solid Phase Extraction (dSPE) tube and vortexed for 10 minutes. The tubes were then centrifuged for 5 minutes at 15,000 rpm. The supernatant was then transferred to an identically labeled 1.5 mL Eppendorf tube. The Eppendorf tubes were then run in a SpeedVac dryer until the liquid evaporated (about 2 hours). The dried pellet in each tube was then resuspended in 100 µL ACN. All the liquid from each tube was transferred to 96 well plates for analysis by the TripleQ machine. This process is adapted from Payá et al. (2007).

Statistical Analysis

A total of 120 samples were processed. A one-way multivariate analysis of variance (one-way MANOVA) was conducted to determine whether there was a significant difference within treatments over time. The dependent variable for this test was the sample concentration in each plant part, and the independent variables were the treatment type (treatment 1, treatment 2, or treatment 3) and days post plant (14, 17, 22, 25, and 28). Following a significant one-way MANOVA result of less than 0.05, two post-hoc tests (Wilks' Lambda (Λ) and Tukey HSD) were performed. An analysis of variance (ANOVA) was done to determine whether there was significant difference between treatments over time.

For the third treatment, the percentage of thiamethoxam and clothianidin that was taken up by the root was calculated. The trifoliate percentage as not tested, since it mirrors a recent study by Krupke et al. (2017).

$$Percentage\ taken\ up\ by\ root\ = \frac{Initial\ amount\ of\ insecticide\ applied\ (ng/g) - insecticide\ recovered\ (ng/g)}{Initial\ amount\ of\ insecticide\ applied\ (ng/g)}$$

Results

	Value	Sig.	Partial Eta Squared
Treatment 1	.087	.004	.457
Treatment 2	.037	.002	.479
Treatment 3	.055	.000	.517

Figure 2. Wilks' Lambda post-hoc analysis. All three treatments are statistically significant.

	Dependent Variable	Days Post Plant	Sig.
Treatment 1	Clothianidin Trifoliate	14 → 17	.005
	Thiamethoxam Root	14 → 17	.005
		17 → 22	.007
Treatment 2	Clothianidin Root	17 → 22	.019
Treatment 3	Clothianidin Trifoliate	14 → 17	.002
	Thiamethoxam Trifoliate	14 → 17	<0.001

Figure 3. Tukey HSD post-hoc analysis. Only statistically significant output is shown.

	Plant Part	F	
Treatment 1	Thiamethoxam Root	8.577	*.
	Thiamethoxam Trifoliate	1.752	.
	Clothianidin Root	2.204	
	Clothianidin Trifoliate	7.655	*.
Treatment 2	Thiamethoxam Root	3.286	.
	Thiamethoxam Trifoliate	2.798	.
	Clothianidin Root	6.531	.
	Clothianidin Trifoliate	1.001	.
Treatment 3	Thiamethoxam Root	9.794	*<0.
	Thiamethoxam Trifoliate	39.841	*<0.
	Clothianidin Root	4.352	.
	Clothianidin Trifoliate	9.441	*.

Figure 4. ANOVA analysis output. Significance is less than p-value 0.001. Significant values are marked with (*).

Days Post Plant	Thiamethoxam Concentration (ng/g ai/seed)	Thiamethoxam percentage remaining in root tissue	Clothianidin Concentration (ng/g ai/seed)	Clothianidin percentage remaining in root tissue
14	3854.727	4.704%	628.689	0.825%
17	2067.015	2.713%	401.388	0.527%
22	469.710	0.616%	154.049	0.202%
25	192.012	0.252%	74.280	0.097%
28	379.507	0.498%	133.761	0.176%

Figure 5. Percentage of thiamethoxam and clothianidin taken up by the root tissue vs how much was initially applied.

Discussion

According to the MANOVA and Wilks' Lambda analyses, all treatments reflect significant declines over time (Figure 2). Since the concentrations within all three treatments was significant, a Tukey HSD post-hoc test was run to determine the significantly different sample dates (Figure 3). It may be expected that the variance within treatments would only be found in treatment 3, but this is also found in treatments 1 and 2. Treatment 1 and treatment 2 were not treated with thiamethoxam or clothianidin, yet the insecticide was found in the plant tissue, this is discussed in more detail below.

The ANOVA output (Figure 4) describes the variance in the concentration between treatments in each part of the plant tested. Variance was calculated for all samples, and the significant values were found for all treatments in the root tissue, except for the concentration of clothianidin in treatment 1 (Figure 4). This means that there were statistically significant differences in the concentrations between certain treatments, and that the concentration of insecticide in the root tissue changed over time. Since I am focusing on the effects for pests below ground, I will omit data for the trifoliate concentrations in this test.

Insecticide was found in all treatments, even though only treatment 3 had been treated with insecticide. This may be because neonicotinoids are highly persistent and have been reported to last for up to three years in Indiana field soils (Krupke et al. 2012). When the field was planted the year before this experiment was done (with corn seed), the seeds had been treated with clothianidin. Soils samples should have been done to determine if levels in soil correspond with concentrations reported from Treatment 1 and 2, or if an error occurred in during sample processing.

Looking at Figure 5, it is shown that at the first sampling date, day post plant 14, the concentration of thiamethoxam declines to less than 5% of the original amount of insecticide applied.

Overall, the concentrations of thiamethoxam and clothianidin declined to levels that were indistinguishable from plants grown from untreated seed within 22 days after planting.

The typical soybean season in Indiana is from about May 4 to mid-November harvest. The seed treatments are only effective for about three weeks once the soybean has been planted. A common guild of soybean pests, the white grubs (*Cyclocephala, Phyllophaga, Popillia,* etc., spp*.)*, will feed on soybean from 3 weeks before planting to mid-June, and then again in mid-July to mid-September. A neonicotinoid seed treatment may manage pests for the first attack, but will have declined to levels unlikely to provide plant protection by the second attack. From the analysis shown here, the window of pest protection provided by neonicotinoid seed treatments extends from planting date to a maximum of 22 days post planting. According to the 2017 Edition of the Corn & Soybean Field Guide, seedcorn maggot (*Delia platura*) and wireworms (*Agriotes, Limonius,* etc., spp.), will feed from the time soybeans are planted to late June. When used as a seed treatment, levels of thiamethoxam and clothianidin may provide some protection against seedcorn maggot and wireworms and the first attack by white grubs. If white grubs infest the field later in the season, these insecticides will not provide protection.

Neonicotinoids can be useful, but only for a small window of time. It can be a beneficial tool when targeting pests early in the season, but the plants will be left vulnerable to late season pests.

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