

**Department of Pesticide Regulation
Environmental Monitoring Branch
1001 I Street, P.O. Box 4015
Sacramento, California 95812**

**Study 325: Analysis of Neonicotinoid and Fungicide Concentrations
in Soil Cores from Treated Lettuce Fields**

**Nels Ruud
Research Scientist III
Groundwater Protection Program**

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SUMMARY

This report summarizes the findings of Groundwater Study 325 (Study 325): analysis of neonicotinoid and fungicide concentrations in soil cores from a treated lettuce field at the United States Department of Agriculture-Agricultural Research Station (USDA-ARS) in Salinas, California during the summer of 2019 (Ruud, 2019). Soil cores were collected before and after a field experiment in which four different treatment combinations of imidacloprid, clothianidin, and azoxystrobin were applied during planting of lettuce seeds to a 1.4-acre test site. The four treatments were: 1) a control treatment (no pesticide application), 2) lettuce seeds treated with imidacloprid, 3) lettuce seeds treated with clothianidin, and 4) lettuce seeds treated with azoxystrobin and imidacloprid applied as a soil drench concurrently with planting of the seeds. The soil core collection was part of a larger experiment performed for the California Department of Pesticide Regulation's (DPR) Surface Water Protection Program by the University of California Cooperative Extension (UCCE) in Monterey County and the United States Geological Survey (USGS) to measure concentrations of the three pesticides in irrigation runoff during the seed establishment phase of the lettuce crop. For Study 325, the Groundwater Protection Program (GWPP) was primarily interested in evaluating imidacloprid concentrations in soil for the plots that received treatments of that neonicotinoid insecticide (i.e., Treatment 2 and Treatment 4). Consequently, GWPP's participation in the larger experiment was limited by funding provided to the USGS to measure post-experiment concentrations of imidacloprid in soil cores collected. Due to probable use of imidacloprid at the test site in other field experiments that pre-date the present one, measured background (i.e., prior to lettuce seed planting) and post-experiment (i.e., about one month after planting) concentrations of imidacloprid in the sampled soil cores were too similar in value and could not be differentiated. Consequently, the fate and transport of imidacloprid in the soil root zone of the planted lettuce crop due to the seed treatment and soil drench application methods could not be further evaluated in this study. Because of this issue, the GWPP opted out of participation in a repeat of the study by UCCE Monterey County and USGS staff that was conducted later in the summer of 2020.

BACKGROUND

Neonicotinoid insecticides are used to control various insect pests in lettuce fields by soil drench application at the time of planting or by seed treatment. Fungicides can also be applied as a seed treatment to protect against seedling rot and disease in lettuce. During the summer of 2019, DPR funded the UCCE in Monterey County and the USGS to conduct a field experiment assessing neonicotinoid and fungicide concentrations in irrigation runoff from treated lettuce fields during the seed establishment phase of the crop. For this study, two neonicotinoid insecticides (imidacloprid and clothianidin) and one fungicide (azoxystrobin) were applied during planting of the lettuce seeds as a seed treatment or soil drench. Since imidacloprid, clothianidin, and azoxystrobin are all considered highly mobile and persistent in soil, they are listed in regulation on the Groundwater Protection List (Title 3 of the California Code of Regulations, section 6800[b]) as pesticides with the potential to leach to groundwater in California. Therefore, in addition to measuring concentrations of the three pesticides in irrigation runoff from the field, GWPP staff took part in the experiment by collecting soil cores from the treatment plots to evaluate residual concentrations of imidacloprid in the shallow soil root zone at the end of the experiment. Measured concentrations of imidacloprid in the irrigation runoff and soil cores were intended for use to evaluate its fate and transport in the lettuce field through water balance and unsaturated zone modeling.

This report summarizes the results of Study 325. It includes a brief description of the field experimental design; the irrigation schedule for the planted lettuce seeds; the collection of pre- and post-experiment soil cores; and the measured imidacloprid concentrations from the laboratory analysis of the collected soil cores.

METHODS AND RESULTS

Experimental Design

The field experiment took place at the USDA-ARS in Salinas, California. A schematic of the experimental design for the treatment plots in the lettuce field is displayed in Figure 1. The experimental design involved four pesticide treatments replicated four times (Reps A, B, C, and D) resulting in 16 treatment plots (Figure 1). The entire lettuce field was about 1.4 acres in size and was divided into the 16 treatment plots with individual plot dimensions of 270 feet x 13.33 feet. Each plot had four 40-inch lettuce beds where each bed consisted of two rows planted in lettuce with 12-inch spacing between planted rows. On September 11, 2019, Romaine lettuce (cv. True heart) seeds were planted across the 16 treatment plots. Four pesticide application treatments were made at the time of lettuce seed planting following a randomized complete block design with four replicates:

1. Control (no pesticide application)
2. Lettuce seed treatment with imidacloprid
3. Lettuce seed treatment with clothianidin
4. Lettuce seed treatment with azoxystrobin and imidacloprid soil drench at planting

For Treatment No. 2, lettuce seeds planted per acre were treated with 2.43 ounces (oz) of the imidacloprid insecticide product Admire Pro^{®1} (0.077 pounds (lb) imidacloprid per acre). For Treatment No. 4, imidacloprid was applied as a soil drench at a rate of 10.25 oz of Admire Pro[®] per acre (0.365 lb imidacloprid per acre).

Irrigation Applications

Starting on September 13, 2019, the newly planted lettuce field was irrigated with a Rainbird 20JH[®] overhead impact sprinkler system. The dates and amounts of irrigation are presented in Table 1. The period over which the lettuce crop was irrigated was intended to coincide approximately with the time required since planting for the lettuce seeds to become established in the soil.

Table 1. Irrigation schedule for lettuce crop planted on September 11, 2019.

Date	Sprinkler Applied Water (inches)
9/13/2019	0.9
9/14/2019	1.1
9/16/2019	1.3
9/18/2019	1.9
9/20/2019	1.6
9/24/2019	2.0
9/27/2019	2.2
10/2/2019	0.3
10/4/2019	0.2
10/11/2019	1.0
10/15/2019	2.6
10/18/2019	2.0
Total	17.1

Soil Sampling Results

On August 27, 2019, and prior to planting the lettuce seeds, USGS staff collected background soil samples from 0 to 30 centimeters (cm) (0 to 12 inches) in depth from the treatment plot areas (Figure 1). For each of the four planned treatments, the soil samples from the four plot replicates (A, B, C, D) scheduled to receive the treatment were combined to result in a composited soil sample for that treatment (Table 2). The composited soil samples were analyzed by the USGS laboratory for background concentrations of imidacloprid, clothianidin, and azoxystrobin and the results were published by Woodward et al. (2021) and are

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reproduced in Table 2. The measured concentrations of imidacloprid in the composite samples ranged from 0.36 to 0.75 parts per billion (ppb), with an average of 0.57 ppb. A single composite sample collected from the Treatment No. 2 plots yielded a background clothianidin concentration of 0.36 ppb (Table 2). All other samples analyzed for clothianidin and azoxystrobin resulted in non-detections of those active ingredients (see Table 2).

Table 2. Pre-experiment background concentrations of imidacloprid, clothianidin, and azoxystrobin from composite samples collected by the USGS.

Treatment Number	Composited Replicates	Sampling Date	Clothianidin Soil Mass Conc. (ppb)	Imidacloprid Soil Mass Conc. (ppb)	Azoxystrobin Soil Mass Conc. (ppb)
1	A, B, C, D	8/27/2019	ND	0.36	ND
2	A, B, C, D	8/27/2019	0.36	0.62	ND
3	A, B, C, D	8/27/2019	ND	0.55	ND
4	A, B, C, D	8/27/2019	ND	0.75	ND

The final irrigation of the experiment occurred on October 18, 2019 (Table 1). On October 28, GWPP staff collected composite soil samples for each replicate (A, B, C, D) of Treatment No. 2 and each replicate (A, B, C, D) of Treatment No. 4. Each core was 42 inches long and was further divided into seven 6-inch segments (0-6, 6-12, 12-18, 18-24, 24-30, 30-36, 36-42 inches). The soil sampling, chain-of-custody documentation, and transport of the samples to the USGS laboratory for analysis were conducted following the standard operating procedure documented in Garretson (1999). The measured concentrations of imidacloprid at each depth for each treatment replicate are presented in Tables 3 and 4. Like the collection of soil samples to measure background concentrations, the USGS also collected post-experiment composite soil samples on October 18 for each of the four treatments (Table 5). Woodward et al. (2021) also published the laboratory results for the post-experiment samples collected by both the USGS and the GWPP.

Measurable imidacloprid concentrations in the samples collected by GWPP were generally found only in the first 0- to 6-inch and 6- to 12-inch depth segments (Table 3). Except for one detection of 0.56 ppb found in the 12- to 18-inch depth segment for Treatment No. 2 in Rep D (Table 3), imidacloprid was not detected by the analytical method below 12 inches for all the other treatment replicate samples. For Treatment No. 2 (seed treatment), the average imidacloprid concentration in the upper 0 to 12 inches was 0.42 ppb for the GWPP-collected soil samples (Table 4) and 0.35 ppb for the USGS-collected composite soil samples (Table 5). For Treatment No. 4 (soil drench), the average imidacloprid concentration in the upper 0 to 12 inches was 0.50 ppb for the GWPP soil samples (Table 4) and 0.35 ppb again for the USGS composite soil samples (Table 5).

Table 3. Post-experiment concentrations of imidacloprid in soil from composite samples collected by GWPP.

Treatment Number	Imidacloprid Application	Replicate Letter	Imidacloprid Concentration in Soil (ppb)						
			Soil Segment Depth						
			0-6 in	6-12 in	12-18 in	18-24 in	24-30 in	30-36 in	36-42 in
2	seed treatment	A	0.49	0.32	ND	ND	ND	ND	ND
2	seed treatment	B	0.45	0.36	ND	ND	ND	ND	ND
2	seed treatment	C	0.42	0.33	ND	ND	ND	ND	ND
2	seed treatment	D	0.52	0.47	ND	ND	ND	ND	ND
4	soil drench	A	0.31	0.36	ND	ND	ND	ND	ND
4	soil drench	B	0.35	0.34	ND	ND	ND	ND	ND
4	soil drench	C	0.54	0.51	ND	ND	ND	ND	ND
4	soil drench	D	0.65	1.38	0.56	ND	ND	ND	ND
2	seed treatment	A, B, C, D	Average=0.47	Average=0.37					
4	soil drench	A, B, C, D	Average=0.46	Average=0.65					

Table 4. Average post-experiment concentrations of imidacloprid from 0 to 12 inches in soil from composite samples collected by GWPP.

Treatment Number	Imidacloprid Application	Replicate Letter	Average Imidacloprid Concentration in Soil 0-12 inches (ppb)
2	seed treatment	A	0.40
2	seed treatment	B	0.41
2	seed treatment	C	0.38
2	seed treatment	D	0.50
4	soil drench	A	0.33
4	soil drench	B	0.35
4	soil drench	C	0.53
4	soil drench	D	0.81
2	seed treatment	A, B, C, D	Average=0.42
4	soil drench	A, B, C, D	Average=0.50

Table 5. Post-experiment concentrations of imidacloprid, clothianidin, and azoxystrobin in soil from composite samples collected by the USGS.

Treatment Number	Composited Replicates	Sampling Date	Clothianidin Soil Mass Concentration (ppb)	Imidacloprid Soil Mass Concentration (ppb)	Azoxystrobin Soil Mass Concentration (ppb)
1	A, B, C, D	10/28/2019	0.33	0.28	ND
2	A, B, C, D	10/28/2019	0.33	0.35	ND
3	A, B, C, D	10/28/2019	0.41	0.37	ND
4	A, B, C, D	10/28/2019	0.31	0.38	ND

Since the measured post-experiment imidacloprid concentrations for the seed treatment (Treatment No. 2) and soil drench (Treatment No. 4) of 0.42 and 0.50 ppb, respectively, in Table 4 are not remarkably different than the average pre-experiment background concentration of 0.57 ppb (Table 2), it is not possible to evaluate the impacts of the two application methods (seed treatment and soil drench) on the fate and transport of imidacloprid in the soil root zone using the experimental data. Unfortunately, the similarity between the pre- and post-experiment concentrations of imidacloprid at the test site prevents us from evaluating the impacts of the imidacloprid seed treatment and soil drench application methods on its behavior in the soil under Study 325.

CONCLUSIONS

This report summarizes the findings of Study 325: analysis of imidacloprid concentrations in soil cores from a treated lettuce field at the USDA-ARS in Salinas, California, during the summer of 2019. Soil cores were collected before and after a field experiment in which four different treatment combinations of imidacloprid, clothianidin, and azoxystrobin were applied during planting of lettuce seeds to a 1.4-acre test site. The four treatments were: 1) a control treatment (no pesticide application), 2) lettuce seeds treated with imidacloprid, 3) lettuce seeds treated with clothianidin, and 4) lettuce seeds treated with azoxystrobin and imidacloprid applied as a soil drench concurrently with planting of the seeds. The soil core collection was part of a larger experiment performed for DPR by the UCCE in Monterey County and the USGS to measure concentrations of the three pesticides in irrigation runoff during the seed establishment phase of the lettuce crop. Due to probable use of imidacloprid at the test site in other field experiments that pre-date the present one, measured background and post-experiment concentrations of imidacloprid in the sampled soil cores were similar in value and could not be differentiated. Consequently, the fate and transport of imidacloprid in the soil root zone of the planted lettuce crop due to the seed treatment and soil drench application methods could not be further evaluated in this study. Because of this issue, the GWPP opted out of participation in a repeat of the study that was conducted by UCCE Monterey County and USGS staff later in the summer of 2020.

FIGURE

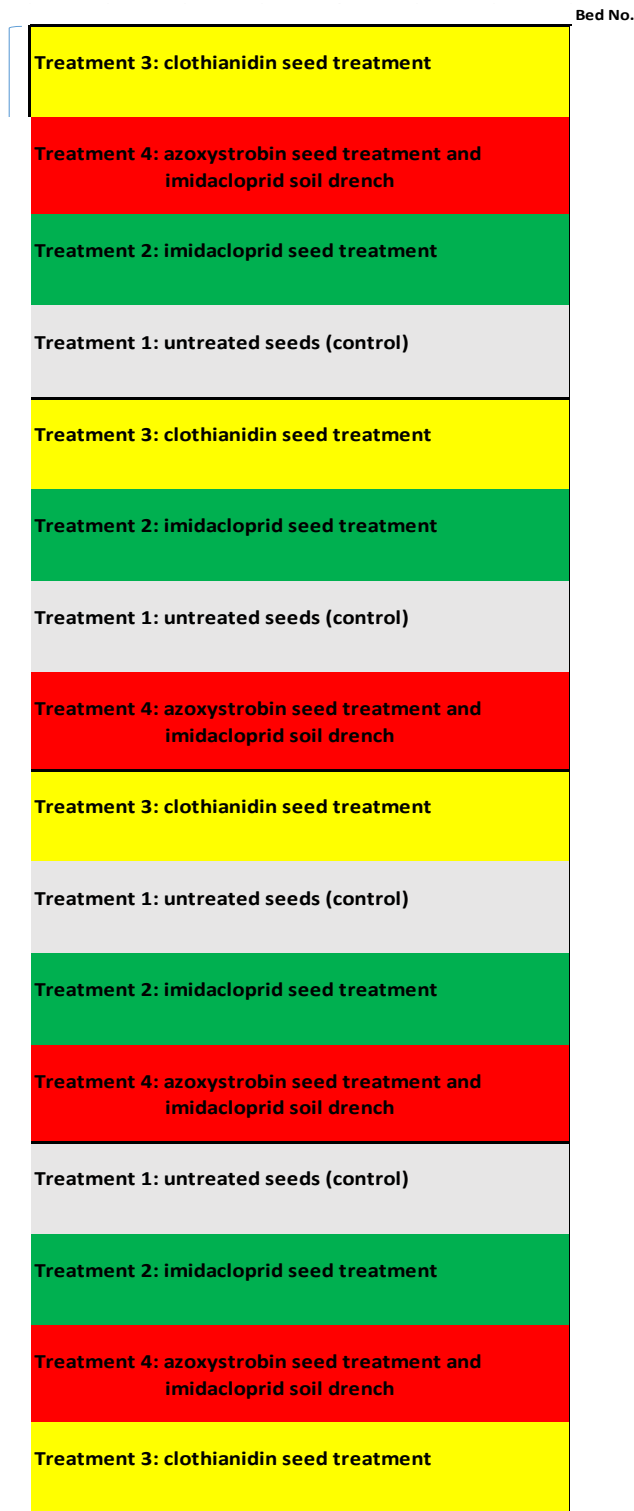


Figure 1. Experimental design layout for four replicates (A, B, C, D) of four pesticide treatments (1, 2, 3, 4) in the lettuce field at the USDA-ARS in Salinas, California. (Note: dimensions of the field in Figure 1 are not to scale.)

REFERENCES

- Garretson, C. 1999. SOP FSSO002.00. Soil sampling, including auger and surface soil procedures. California Department of Pesticide Regulation, Sacramento, California.
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