The Connecticut Agricultural Experiment Station

Quantifying Exposure of Bees to Neonicotinoids in Nectar and Pollen of Nursery Plants

Final Report on a Project Funded by the Connecticut Department of Energy and Environmental Protection

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Photo on cover page: Clipping anthers of rhododendron in order to analyze pollen for pesticides

Overall Summary:

This project had several aspects:

1. Measure and report on the concentration of imidacloprid in leaves, flowers, flower parts, nectar and pollen of rhododendrons treated with a soil application of imidacloprid according to current practices
2. Determine and report which nursery plants are most heavily used by honey bees for pollen collection and exposure to neonicotinoids through pollen in a nursery environment
3. Identify and report on current nursery practices that could lead to honey bee or bumble bee exposure to neonicotinoids

Summary of each project:

Imidacloprid in soil-treated rhododendron plants:

Imidacloprid was measured in potting soil, leaves, flowers, pollen and nectar of rhododendrons grown in 3 and 5 gallon pots with soil mix incorporated with imidacloprid at labeled rates at the cooperating nursery. Although the residues of imidacloprid in the potting mix were in the 100 - 600 ppb range, the residues in plant tissues and nectar were a small fraction of the concentrations found in potting mix, with the residue levels ranked leaves > pollen > flower > nectar. The residues in nectar ranged from 0.8 ppb to 10.0 ppb, with a mean of 3.2 ppb. The residues in pollen ranged from 14.2 ppb to 34.9 ppb, with a mean of 22.4 ppb. The imidacloprid olefin and hydroxyl metabolites can also be toxic to bees. The imidacloprid olefin metabolite was found in the potting mix but not in plant tissues or nectar. The hydroxyl metabolite at concentrations in tissues equivalent in ppb to those of the imidacloprid parent compound.

For context, the No Observed Adverse Effect Level on honey bee colonies for imidacloprid in nectar according to the EPA draft risk assessment is 25 ppb (EPA-HQ-OPP-2008-0844-0140). The level for pollen is not as well established, but may be as high as 100 ppb. Thus, the levels found here would not be of concern for the health of honey bee colonies. Other bees, such as bumble bees and solitary bees, may be affected at lower concentrations because they do not have large colony populations to buffer them from the sublethal neurological, behavioral, and immunological effects found in laboratory studies at low concentrations of imidacloprid and other neonicotinoids.

For more detail, please see Appendix 1: Concentrations of Imidacloprid in Rhododendrons Treated with a Soil Application According to Current Practice

Pesticides residues in pollen collected by honey bee colonies at commercial nurseries:

Three honey bee colonies were placed at each of three commercial plant nurseries in locations where they would not be directly sprayed. Pollen traps (devices to collect pollen from the legs of honey bee workers as they return from foraging) were installed in all the hives, and pollen was collected from two of the three hives at each location each week. The pollen was then analyzed for a range of pesticides using methods that would detect neonicotinoids down to a level of 1-2 parts per billion (ppb).

Of the neonicotinoids, imidacloprid was commonly found in pollen at two of the three nurseries, and rarely found at the third (See Table 1), while thiamethoxam and clothianidin were more often found at the remaining nursery. (Clothianidin is a metabolite of thiamethoxam, similar in toxicity to honey bees. Although it is labeled as an insecticide, it is not registered for use in nurseries, so it is presumably present as a metabolite.) Dinotefuran, another neonicotinoid similar in acute toxicity to honey bees to imidacloprid, was applied by one nursery, but was not detected in our pollen samples. Acetamiprid, which is a neonicotinoid but is much less toxic to bees than the above compounds, was applied by all three nurseries, but was only rarely detected, in one pollen sample from one nursery.

Table 1: Neonicotinoids detected in pollen trapped from honey bee hives at three commercial nurseries in Connecticut.

	Imidacloprid		Thiamethoxam		Clothianidin		Acetamiprid	
Nursery	% detections	Maximum (ppb)	% detections	Maximum (ppb)	% detections	Maximum (ppb)	% detections	Maximum (ppb)
C	3%	2.5	24%	305	24%	78		
M	54%	9.9						
P	32%	5.1	6%	4.4	3%	4.4	3%	1.6
Honey bee Oral LD_{50}	0.013 ug/bee		0.005 ug/bee		0.0035 ug/bee		14 ug/bee	

In general, neonicotinoids were found at relatively low concentrations, with all pollen samples at nurseries M and P below 20 ppb of neonicotinoids (adding concentrations of thiamethoxam, clothianidin and imidacloprid together), and 33 of 35 samples at nursery C below 20 ppb. However, there were a few samples at nursery C in August with exceptionally high levels of neonicotinoids, with the sample from Hive C on Aug. 17 reaching a total of 336 ppb of neonicotinoids (See Table 2).

Table 2: Neonicotinoids Levels in Trapped Pollen from Nursery C – August 2015, when pesticide concentrations were highest.

Date	Hive	Thimethoxam (ppb)	Clothianidin (ppb)
8/3/2015	A	2.5	2
	C	43	78
8/10/2015	A	1	1.2
	B	1.4	2
8/17/2015	B	81	20
	C	305	31
8/24/2015	A	41	4.5
	C	7.8	16

We investigated this sample further by separating the bulk sample of pollen into color categories, analyzing these color categories for pesticides, and also analyzing pollen loads from these color categories using palynology, the identification of pollen using morphological characteristics. We found that the high concentrations of thiamethoxam and clothianidin were associated with 3 of the

11 color categories in the sample, and were most closely associated with the amount of *Spiraea* pollen in the sample. This was confirmed by analyzing another sample from Hive C on Aug. 24 in the same way – again high concentrations of these neonicotinoids were associated with *Spiraea* pollen. (See Tables 3 and 4 below.)

Table 3. Relationship of neonicotinoid concentration and estimated honey bee toxicity to percentage of *Spiraea* pollen in trapped pollen samples sorted by color from Nursery C, Hive C, Aug. 17, 2015 (pollen sample with the highest neonicotinoid concentration and honey bee toxicity).

Color category	**% *Spiraea* pollen**	**Concentration of thiamethoxam (ppb)**	**Concentration of clothianidin (ppb)**	**Estimated % of honey bee LD_{50} (includes other pesticides)**
Bulk sample	**29%**	**305**	**31**	**70.5%**
Sorted colors:				
Mahogany rose	99%	680	143	180%
Warm sand	70	703	202	202
Almond buff	21	472	79	118
Mustard gold	2.4	15	0	3.6
Sunflower	1.2	14	0	2.8
Straw	0.7	8	0	1.6
Grape Leaf	0.6	6.8	0	1.4
Freesia	0.3	0	0	0
Butterscotch	0.1	8.3	0	1.7
Yolk yellow	0	6.1	0	1.2
Cathay spice	0	0	0	0

Table 4. Relationship of neonicotinoid concentration and estimated honey bee toxicity to percentage of *Spiraea* pollen in trapped pollen samples sorted by color from Nursery C, Hive C, Aug. 24, 2015. (Pollen sample from the following week, same location and hive.)

Color category	% *Spiraea* pollen	Concentration of thiamethoxam (ppb)	Concentration of clothianidin (ppb)	Estimated % of honey bee LD_{50} (includes other pesticides)
Bulk sample	**4%**	**7.8**	**15**	**6.1%**
Sorted colors:				
Mahogany rose	99%	89	221	81%
Almond buff	0.4	6	8.6	3.7
Cumin	0.4	0	6.3	1.8
Yolk yellow	0.2	0	0	0
Sunflower	0	6.4	0	1.3
Grape leaf	0	0	0	0
Freesia	0	0	0	0

We do not have detailed pesticide application information from Nursery C, but we did get information on insecticide treatments to *Spiraea* from the nursery manager. A treatment with acephate was made June 2 (of interest because substantial amounts of this systemic organophosphate and its metabolite methamidophos were also found in trapped pollen samples), and treatments with thiamethoxam were made to *Spiraea* on July 29 and on Aug. 12.

For detailed tables with data on insecticide residues in trapped pollen from all three nurseries, estimated % of honey bee LD_{50}, and pesticide application records, see Appendix 2: Analysis of Pesticides in Trapped Pollen Samples and Pesticide Application Records from Nurseries Where Pollen Was Collected (Supplementary Tables for Report to CT DEEP)

Nursery Practices with Respect to Neonicotinoids:

We have two sources of information about nursery practices with respect to neonicotinoids: 1) a survey of retail and wholesale nurseries carried out in the winter of 2015-2016, with 30 of the 78 nurseries responding, and 2) pesticide application records from our cooperating nurseries, in whatever level of detail they chose to provide.

Nursery Survey: Nineteen of the responding nurseries (70%) indicated that they had used at least one neonicotinoid insecticide over the 2015 growing season. Of those nurseries that used neonicotinoids, 16 (84%) used these products in foliar sprays, 11 (58%) used them in a “sprench” application (a coarse spray to wet foliage and potting media), and 3 (16%) used them incorporated into potting media prior to potting.

The nurseries used several methods to limit the exposure of pollinators to neonicotinoids on horticultural crops. Two thirds of growers using neonicotinoids reported that they never used these products when there were open blossoms on the crops being sprayed; one respondent simply noted that they apply the product at times of day when bees were not as active. Nearly as many (61%) reported using these products in greenhouses, where pollinators would not be present, including one respondent who applied these products in greenhouses 60 – 90 days before shipping. Many (39%) of growers only applied these insecticides when the plants were not mature enough to bloom. The least common measures were use of TriStar because of its greater safety to bees (17%) and incorporation of these insecticides into potting media (11%).

For a more detailed summary of this survey, see Appendix 3: Summary of Results – Connecticut Nursery Survey on Neonicotinoid Use, and for the detailed results, see Appendix 4: Connecticut Nursery Survey – Data

Pesticide Application Records from Participating Nurseries: We cannot generalize from practices and results of the three nurseries we studied in detail, but we observed relatively low neonicotinoid concentrations in pollen at Nursery M, where imidacloprid is primarily incorporated in potting soil, and at Nursery P, where neonicotinoids are used in a "sprench" application, mostly at least a few months from the season of bloom. At Nursery C, all neonicotinoids are used as foliar applications.

Detailed pesticide application records from the participating nurseries are in Appendix 2: Analysis of Pesticides in Trapped Pollen Samples and Pesticide Application Records from Nurseries Where Pollen Was Collected (Supplementary Tables for Report to CT DEEP)

Appendices:

1. Concentrations of Imidacloprid in Rhododendrons Treated with a Soil Application According to Current Practice
2. Analysis of Pesticides in Trapped Pollen Samples and Pesticide Application Records from Nurseries Where Pollen Was Collected
3. Summary of Results – Connecticut Nursery Survey on Neonicotinoid Use
4. Connecticut Nursery Survey – Data

Appendix 1:

Concentrations of Imidacloprid in Rhododendrons Treated with a Soil Application According to Current Practice

Methods

Analysis of imidacloprid contamination of rhododendron floral resources was determined from samples taken from a cooperating nursery that had a standard procedure to apply one gallon of imidacloprid (2 lb. per gallon in a flowable formulation) to 100 cubic yards of potting mix, in a preplant potting mix incorporation treatment. This nursery also used imidacloprid as a foliar spray for plants in which they suspected that the residues remaining in the plant might not be sufficient to maintain the required degree of control for the pests being targeted (principally rhododendron leafminer, but also rhododendron lace bug, rhododendron clearwing borer, and felt scales). This potting mix incorporation dosage is equivalent to 44 mg of active ingredient per gallon of potting medium (the range of dosages following label directions is 42 – 58 mg a.i. per one-gallon pot).

Two hundred *R. minus* 'PJM' rhododendrons in 3-gallon containers were provided by the nursery for us to collect nectar and pollen samples. Plants were held in a screen house to prevent pollinators from visiting the flowers and removing the nectar and pollen that we needed to collect. Various methods were used to collect nectar and pollen samples from these plants. Pipetting nectar directly from flowers held in a screen house was relatively inefficient, because few flowers contained nectar, and the nectar was very concentrated. Capillary tubes were not useful for nectar samples because sugars crystallized within the tubes, making the contained nectar inaccessible. Our most efficient method involved watering the plants thoroughly on the day before collecting samples, and covering individual plants in large plastic trash bags in a shed. The next morning, one droplet of nectar was found at the base of each corolla. A 200-µL pipet was used to collect these droplets – typically one plant had about 100 trusses with 10 flowers per truss. Out of these ~1,000 flowers we were able to obtain about 1 ml of nectar. Samples were held in 1.5 ml microcentrifuge tubes and kept in a freezer until analyzed for °Brix and insecticide residues. Pollen was collected with an air sampling device from which a vacuum picked up pollen, which was later scraped from a filter. A more efficient approach was to clip anthers from flowers, being sure not to collect the proximal half of the filament, which had sticky pubescence. Collected anthers were then held in a centrifuge tube and vibrated with a vortex mixer, causing the pollen to be released. The pollen was denser than the filament and empty anthers, and so could be retained in the bottom of the centrifuge tube.

Additional nectar and pollen samples were collected from *R. catawbiense* 'Boursault' plants grown in 5-gal pots kept within the cooperating nursery. Anthers were clipped directly in the field, but plants were also brought to a shed at the nursery so that the procedure mentioned above (watering, bagging, and leaving overnight) could be used to collect nectar.

Five plants each of 'PJM' and 'Boursault' were used as focal samples for comparing the residues found in their potting mix, leaves, petals, nectar, and pollen. Pollen samples of 1-g each required about 3 plants to collect sufficient quantities, and so the residue values for pollen are composite samples. Potting medium was removed with a trowel, leaves were sampled from around the plant (4 g fresh weight), and flower tissues (petals, 4 g per plant, fresh weight) were collected from single plants, so that the resulting data could be analyzed to determine the correlation among these residues. The intent was to determine whether readily collected plant tissues could be used as a predictor for the residues found in nectar or pollen.

Results and Discussion

The concentrations of imidacloprid residues found in floral resources and other collected samples are presented in Table 1. The size of the pot had a barely statistically significant influence on the residues found in flower samples ($F = 5.70$; df = 1,9; $P = 0.044$), which were the only residue data requiring log transformation prior to establish homogeneity of variance prior to conducting analysis of variance. Consequently, data presented in Table 1 have not been transformed.

Table 1. Residues of imidacloprid (ppb) found in 'PJM' and 'Boursault' rhododendron tissues, nectar, and potting media (ppb)

Pot size	Sample	Soil	Flower	Leaves	Nectar	Pollen
3	1	348	8.2	30.7	1.1	25.9
	2	172	15.2	45.3	6.5	34.9
	3	331	7.7	19.5	2.3	15.1
	4	597	16.0	110.4	3.6	
	5	601	8.2	33.9	1.4	
	Avg.	410	11.1	48.0	3.0	25.3
5	1	154	5.4	30.4	10.0	14.2
	2	117	8.9	22.6	0.8	18.4
	3	186	7.2	30.9	1.3	25.9
	4	271	3.3	34.7	4.4	
	5	376	6.7	52.1	1.0	
	Avg.	266	6.6	30.5	3.6	19.5
Avg.		315	8.7	41.0	3.2	22.4

Several points are notable about these data. First, although the imidacloprid potting mix residues were in the 100 - 600 ppb range, the residues in plant tissues and nectar were ranked leaves > pollen > flower > nectar, all of which were a small fraction of the concentrations found in potting mix. Relative to honey bee colony health, neither the residues found in nectar nor pollen would be of concern. The nectar concentration is below the threshold of ~20 ppb, above which sublethal effects to colonies may be observed. The NOEL (No Observable Effect Level) for pollen is not as well established as for nectar. For honey bees, a concentration of 100 ppb may be a suitable estimate: bees consume much less pollen than nectar, and the pollen is digested by worker bees before being fed to larvae. The concentrations we found in pollen may be of concern to the health of bumble bees and solitary bees, as they feed pollen mixed with honey directly to developing larvae. However, a threshold level for concern has not been established for these bees.

The concentrations of the imidacloprid olefin and hydroxyl metabolites are not given in Table 1. These metabolites can be toxic to insects, especially the olefin, which is about 15 times as toxic as the parent compound. Although the imidacloprid olefin was found at about 10% of the concentration of imidacloprid in the potting mix, it was not found in the plant tissues or nectar. The hydroxyl metabolite was found at concentrations in tissues equivalent in ppb to those of the imidacloprid parent compound.

A remarkable discovery is that the residues found in pollen samples were consistently 75% of the levels found in leaves from the same group of plants, with one outlier out of the six points. We could not exactly match residues from the same plants, because the pollen samples of necessity had to be collected as aggregate samples from about 3 plants for each sample. However, if the relationship (Pearson's r = 0.84, signifying that 70% of the experimental variation [R^2 = 0.71] is described by this correlation) is confirmed in further studies, then the need to expend so much effort to collect pollen samples for residue analyses could be bypassed by collecting leaf samples instead (Fig. 1).

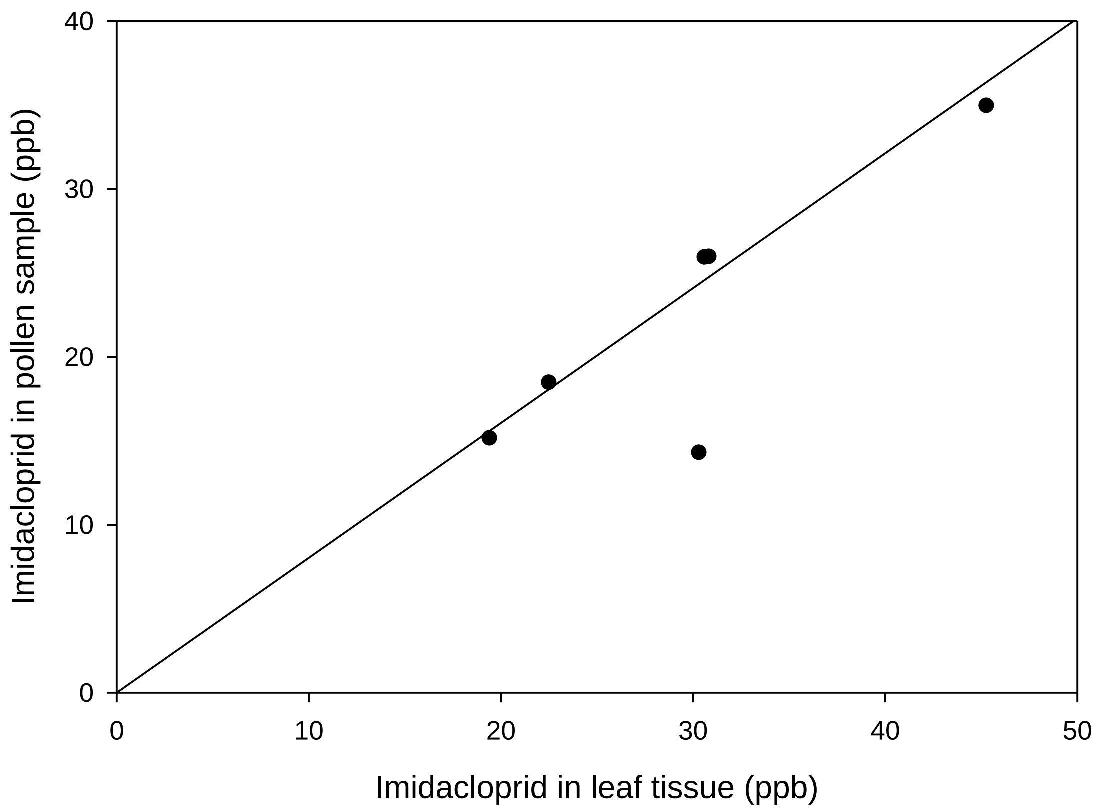

Fig. 1. The relationship between the residues found in pollen from aggregated samples and the leaf tissue from individual representative rhododendron plants from the same group. One point was excluded as an outlier from calculations used for the linear regression line presented in this figure.

Appendix 2: Analysis of Pesticides in Trapped Pollen Samples and Pesticide Application Records from Nurseries Where Pollen Was Collected

Supplementary Tables for report to CT DEEP

Insecticide and Acaricide Residues in Trapped Honey Bee Pollen from CT Nurseries

Notes:

- Some insecticides, particularly pyrethroids, are not detected at low levels by the analytical methods used. Pyrethroids are among the insecticides posing the greatest risk to honey bees (Sanchez-Bayo & Goka 2014).
- Clothianidin is a degradation product of thiamethoxam as well as being used as an insecticide on its own (Nauen et al. 2003).
- Methamidiphos is no longer used in the US, but is a metabolite of acephate (St. Amand & Girard, 2004).
- 5-Hydroxy imidacloprid is a metabolite of imidacloprid.
- Source for oral LD_{50} for honey bees is Sanchez-Bayo & Goka 2014.

Nursery C Insecticide/Acaricide Residues in Trapped Honey Bee Pollen (parts per billion).

Hive	Collection Date	Thiamethoxam	Clothianidin	Imidacloprid	Acephate	Methamidiphos	Chlorpyrifos	Diazinon	Flubendiamide
CA	5/13/2015								
CB	5/13/2015								
CA	5/22/2015			2.5			3.8		
CB	5/22/2015								
CA	5/29/2015								
CB	5/29/2015								
CC	5/29/2015						5	0.3	
CA	6/5/2015				394	19			
CC	6/5/2015					4.7	4.1		
CA	6/12/2015					1.1			
CB	6/12/2015					2			
CB	6/18/2015				9.4	12			
CC	6/18/2015				37	51			
CA	6/26/2015				99	55			
CB	6/26/2015				104	58			

CA	6/30/2015				136	72			
CB	6/30/2015				308	390			
CB	7/7/2015				44	49			
CC	7/7/2015				12	17			
CA	7/13/2015					2.8			
CC	7/13/2015								
CA	7/20/2015					3.8			
CB	7/20/2015								
CB	7/27/2015								
CA	8/3/2015	2.5	2						
CC	8/3/2015	43	78						7.4
CA	8/10/2015	1	1.2						
CB	8/10/2015	1.4	2						
CB	8/17/2015	81	20		31	15			
CC	8/17/2015	305	31		94	40			
CA	8/24/2015	41	4.5						
CC	8/24/2015	7.8	16						1.1
CA	8/31/2015							0.6	
CB	8/31/2015	2.7	1.2						
CA	9/8/2015							0.9	
CC	9/8/2015								1.1
CB	9/14/2015								
CB	9/21/2015								
	Maximum	305	78	2.5	394	390	4.1	0.9	7.4
	% detected	23.7%	23.7%	2.6%	28.9%	42.1%	7.9%	7.9%	7.9%
Oral LD_{50} for honey bees (ug ai /bee)		0.005	0.0035	0.013	0.23	0.2	0.24	0.21	> 200

Nursery M Insecticide/Acaricide Residues in Trapped Honey Bee Pollen (parts per billion).

Hive	Collection Date	Imidacloprid	Acephate	Methamidiphos	Carbaryl	Phosmet	Chlorpyrifos	Dimethoate	Spiromesifen	Spirotetramat	Coumaphos
MA	5/20/2015	2.8	107	65			6.2				
MB	5/20/2015	4.2		22			7.4				3.1
MB	5/27/2015			27			3.7				
MC	5/27/2015	3.8	8.7	16			22				
MA	6/3/2015	1.2	8.3	13		11.4	3.3				
MC	6/3/2015			2.2			4.5				
MA	6/10/2015	7.2	29	20			1				
MB	6/10/2015	5		6.4							
MB	6/17/2015	2.9									
MC	6/17/2015	2					16				
MA	6/17/2015	4.3	27	17			9.9				
MA	6/24/2015	2.9					7				
MC	6/24/2015	1.6					4.9				
MA	6/30/2015	4.9					1.5				
MC	6/30/2015	4.1		8.8			3.3				
MC	7/8/2015			5.3							
MA	7/15/2015		98	36							
MB	7/22/2015	7.5	111	118	1.6			2.7			
MC	7/22/2015		45	24							
MA	8/4/2015		36	35				1.3			
MB	8/4/2015	9.9	242	155	164			3.6	456		
MA	8/13/2015	2.4	27	34					53		
MC	8/13/2015			4.3						3.4	
MB	8/19/2015			4.2							
MC	8/19/2015			1.8							
MA	8/25/2015	1.8									
MB	8/25/2015										
MA	9/2/2015		23	4							
MB	9/2/2015	2.4	9.4								
MB	9/17/2015										

MC	9/17/2015										
MA	9/23/2015										
MB	9/23/2015										
MC	9/23/2015	3.8									
	Maximum	9.9	242	155	164	11.4	9.9	3.6	456	3.4	3.1
	% detected	54.3%	37.1%	60.0%	5.7%	2.9%	37.1%	8.6%	5.7%	2.9%	2.9%
Oral LD_{50} for honey bees (ug ai/bee)		0.013	0.23	0.2	0.15	0.37	0.24	0.17	790	195	4.6

Nursery P Insecticide/Acaricide Residues in Trapped Honey Bee Pollen (parts per billion).

Hive	Collection Date	Imidacloprid	5-Hydroxy-imidacloprid	Thiamethoxam	Clothianidin	Acetamiprid	Acephate	Methamidiphos	Diazinon	Chlorpyrifos	Methiocarb
PA	5/13/2015	5.1		4.4	4.4						
PA	5/21/2015										
PB	5/21/2015										
PC	5/28/2015	1.8							2.1	1	
PA	6/4/2015										
PC	6/4/2015									1.5	
PA	6/11/2015										
PB	6/19/2015	3.2									18
PC	6/19/2015	3.4	2								
PA	6/25/2015	3.5									
PB	6/25/2015	1.5									
PB	7/2/2015	1.7									
PC	7/2/2015										
PC	7/9/2015										
PA	7/23/2015						26	5.3			
PB	7/23/2015	4.9					194	49			
PB	7/30/2015							5.7			
PC	7/30/2015							19			
PB	8/7/2015	2.2						9.5			
PC	8/7/2015	1.5							0.2		
PA	8/13/2015							2.9			
PB	8/13/2015							5.4			
PC	8/13/2015			3.3				19			
PA	8/20/2015							5.7			
PB	8/20/2015							1.9			

PB	8/28/2015					1.6		2.3			
PC	8/28/2015								0.2		
PA	9/3/2015										
PC	9/3/2015								0.8		
PA	9/10/2015										
PB	9/10/2015							1.9	1.2		
	Maximum	5.1	2	4.4	4.4	1.6	194	49	2.1		18
	% detected	32.3%	3.2%	6.5%	3.2%	3.2%	6.5%	38.7%	16.1%	6.5%	3.2%
Oral LD_{50} for honey bees (ug ai/bee)		0.013	0.01	0.005	0.0035	14	0.23	0.2	0.2		0.47

References:

St-Amand, A.D. and Girard, L., 2004. Determination of acephate and its degradation product methamidophos in soil and water by solid-phase extraction (SPE) and GC-MS. *International Journal of Environmental Analytical Chemistry*, *84*(10), pp.739-748.

Nauen, R., Ebbinghaus-Kintscher, U., Salgado, V.L. and Kaussmann, M., 2003. Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pesticide Biochemistry and Physiology*, *76*(2), pp.55-69.

Sanchez-Bayo F, Goka K (2014) Pesticide Residues and Bees – A Risk Assessment. PLoS ONE 9(4): e94482. doi:10.1371/journal.pone.0094482 (supplementary table 2)

Estimate of Honey Bee Acute Toxicity. Estimated by calculating a Hazard Quotient for each pesticide (HQ = conc. In ppb/oral LD_{50} in ug/bee), adding together the Hazard Quotients (assuming effects of different pesticides are additive), and then calculating an estimated % LD_{50} by multiplying by the ug of pollen consumed per day by a nurse honey bee (the adult worker bee that directly consumes the most pollen). Because insecticides have much higher acute toxicity than fungicides, the sum of the HQs is mostly determined by insecticides.

Nursery C

Site & Hive	Date	Sum of pollen HQs	Estimated % LD50
CA	5/13/2015	0.68	0.00
CB	5/13/2015	0.57	0.00
CA	5/22/2015	215.43	0.22
CB	5/22/2015	4.52	0.00
CA	5/29/2015	2.99	0.00
CB	5/29/2015	1.92	0.00
CC	5/29/2015	24.73	0.02
CA	6/5/2015	1809.25	1.81
CC	6/5/2015	40.80	0.04
CA	6/12/2015	5.55	0.01
CB	6/12/2015	10.02	0.01
CB	6/18/2015	100.90	0.10
CC	6/18/2015	416.61	0.42
CA	6/26/2015	706.58	0.71
CB	6/26/2015	744.86	0.74
CA	6/30/2015	952.02	0.95
CB	6/30/2015	3292.01	3.29
CB	7/7/2015	446.10	0.45
CC	7/7/2015	142.28	0.14
CA	7/13/2015	14.12	0.01
CC	7/13/2015	0.64	0.00

CA	7/20/2015	20.12	0.02
CB	7/20/2015	2.19	0.00
CB	7/27/2015	0.36	0.00
CA	8/3/2015	1071.90	1.07
CC	8/3/2015	30887.69	30.89
CA	8/10/2015	542.90	0.54
CB	8/10/2015	851.72	0.85
CB	8/17/2015	22124.86	22.12
CC	8/17/2015	70471.91	70.47
CA	8/24/2015	9486.20	9.49
CC	8/24/2015	6132.07	6.13
CA	8/31/2015	7.72	0.01
CB	8/31/2015	888.62	0.89
CA	9/8/2015	4.71	0.00
CC	9/8/2015	0.78	0.00
CB	9/14/2015	11.85	0.01
CB	9/21/2015	1.99	0.00
Mean			3.99%
Median			0.07%

Nursery M

Site & Hive	Date	Sum of pollen HQs	Estimated % LD50
MA	5/20/2015	1031.92	1.03
MB	5/20/2015	464.86	0.46
MB	5/27/2015	151.45	0.15
MC	5/27/2015	502.09	0.50
MA	6/3/2015	238.44	0.24
MC	6/3/2015	29.78	0.03
MA	6/10/2015	784.11	0.78
MB	6/10/2015	420.41	0.42
MB	6/17/2015	223.31	0.22
MC	6/17/2015	222.36	0.22
MA	6/17/2015	574.41	0.57
MA	6/24/2015	253.17	0.25
MC	6/24/2015	144.88	0.14
MA	6/30/2015	400.03	0.40
MC	6/30/2015	375.96	0.38
MC	7/8/2015	32.84	0.03
MA	7/15/2015	606.28	0.61
MB	7/22/2015	1676.56	1.68
MC	7/22/2015	318.58	0.32
MA	8/4/2015	339.90	0.34
MB	8/4/2015	3705.13	3.71
MA	8/13/2015	472.24	0.47
MC	8/13/2015	22.84	0.02
MB	8/19/2015	21.07	0.02
MC	8/19/2015	15.38	0.02
MA	8/25/2015	138.78	0.14
MB	8/25/2015	1.22	0.00
MA	9/2/2015	120.48	0.12
MB	9/2/2015	230.62	0.23
MC	9/2/2015	2.10	0.00

MB	9/17/2015	0.19	0.00
MC	9/17/2015	0.24	0.00
MA	9/23/2015	2.66	0.00
MB	9/23/2015	349.32	0.35
MC	9/23/2015	9.96	0.01
Mean			0.40%
Median			0.23%

Nursery P

Site & Hive	Date	Sum of pollen HQs	Estimated % LD50
PA	5/13/2015	2530.33	2.53
PA	5/21/2015	0.59	0.00
PB	5/21/2015	0.92	0.00
PC	5/28/2015	154.99	0.15
PA	6/4/2015	0.36	0.00
PC	6/4/2015	11.86	0.01
PA	6/11/2015	0.10	0.00
PB	6/19/2015	284.75	0.28
PC	6/19/2015	415.79	0.42
PA	6/25/2015	269.23	0.27
PB	6/25/2015	115.60	0.12
PB	7/2/2015	142.25	0.14
PC	7/2/2015	5.78	0.01
PC	7/9/2015	0.15	0.00
PA	7/23/2015	156.87	0.16
PB	7/23/2015	1541.55	1.54
PB	7/30/2015	92.08	0.09
PC	7/30/2015	95.07	0.10
PB	8/7/2015	229.75	0.23
PC	8/7/2015	116.77	0.12
PA	8/13/2015	32.46	0.03
PB	8/13/2015	52.27	0.05
PC	8/13/2015	809.81	0.81
PA	8/20/2015	35.59	0.04
PB	8/20/2015	17.50	0.02
PB	8/28/2015	12.78	0.01
PC	8/28/2015	1.05	0.00
PA	9/3/2015	4.15	0.00
PC	9/3/2015	4.59	0.00
PA	9/10/2015	0.20	0.00

PB	9/10/2015	19.57	0.02
Mean			0.23%
Median			0.04%

Insecticide and Acaricide Application Records from CT Nurseries

Pesticide records were given to us in radically different forms and levels of detail from the three nurseries. We have tried to extract the basic information on what pesticides were applied at each nursery across the season in 2015.

Nursery C – insecticide and acaricide use

Nursery C sent us a summary record of which pesticides (including fungicides and growth regulators as well as insecticides and acaricides) were applied to each of 6 areas of the nursery each month. We have summarized this by active ingredient, month, and the number of areas to which the active ingredient was applied. (They didn't give us amounts used.)

Insecticide Active Ingredient	No. areas in April	No. areas in May	No. areas in June	No. areas in July	No. areas in August	No. areas in September	No. areas in October	Sum
abamectin	1		3	2	3	2	1	12
acephate		3	4	1	3			11
acetamiprid			2			3	1	6
bifenazate				1	2		1	4
chlorfenapyr				1	2	2		5
chlorpyrifos	2	2						4
oil			1		1	1		3
permethrin				4	3	3		10
thiamethoxam		2	3	2	4			11

Additional note: Because of the high levels of insecticides associated with *Spiraea* in the samples from August studied in detail, Rich Cowles asked Nursery C what insecticide treatments were used on this crop.

Specific treatments on *Spiraea*:

Acephate	1 lb per 100 gallons	6/2/15	Foliar application to salable *Spiraea* crop
Flagship (thiamethoxam)	4 oz. per 100 gallons	7/29/15	Foliar to part of grow-on crop
Flagship (thiamethoxam)	4 oz. per 100 gallons	8/12/15	Foliar to rest of grow-on crop

Nursery M insecticides (i), acaricides (m) and fungicides (f)– summarized by active ingredient, trade name, unit of measure, quantity used each month, and total for the year.

	Active	Trade name	Unit	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	total
i/m	abamectin	Avid 0.15 EC	gal		0.10	0.40	1.75	0.60	0.65		0.36	0.06	**3.92**
i	acephate	Orthene (Acephate Pro)	lb			66.00	29.30	39.00	25.70	0.16	0.50		**160.66**
i	acetamiprid	Tristar 30 SG	lb	1.50	3.00	1.50	1.00		1.50				**8.50**
i	azadirachtin	Azatin XL	qt				28.00						**28.00**
i/m	bifenthrin	Talstar G (Incorporated)	lb	5100	8750	8500	4250	2000	5000	1500			**35100**
i	chlorpyrifos	Dursban 50WSP	lb			68.00	70.00						**138.00**
i	dimethoate	Dimate 4EC	gal						32.50				**32.50**
i	dinotefuran	Safari 20 SG	lb										**0.00**
i	imidacloprid	Mallet 2F	qt		34.00	5.00	73.00	35.20	28.80				**176.00**
i/m	oil	Hort Oil (Dam oil Drex)	gal			9.50	30.50	45.00	5.00				**90.00**
i	permethrin	Astro 3.2EC	gal		0.20	1.00	2.25	1.50	2.05	0.23			**7.23**
i	pymetrozine	Endeavor WSP	oz						170				**170**
i	spinetoram/ sulfoxaflor	Xxpire WG	lb		0.20				7.68				**7.88**
i	Spirotetramat	Kontos	ml	263		690		1074					**2027**
i	thiamethoxam	Flagship 25WG	lb			1.50	4.70		1.30				**7.50**
i/f	carbaryl/ iprodione	Sevin SL Chipco Aventis	gal					1.00					**1.00**
m	Cyflumetofen	Sultan	pt			1.20		5.80	0.13				**7.13**
m	fenpyroximate	Akari 5SC	qt				3.70						**3.70**
m	hexythiazox	Hexygon DF	oz				65.00	12.50	2.50				**80.00**
m	Spiromesifen	Judo	oz						8.00				**8.00**

Nursery P - (area closest to honey bee hives) – insecticides and acaricides

Active ingredient	**Trade Name**	**Rate**	**unit**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**total**
Abamectin	Minx	8 oz/100gal	oz						208	384.08	212		804.08
Acephate	Acephate	1 lb/100 gal	lb	3.5				36		10		3	52.5
Acetamiprid	Tristar 8.5 SL	4 oz/100 gal or 8.5 oz/100 gal	oz					85	56				141
Bifenthrin	Talstar G	2 lb/ yard	lb	1680	3680	1680	2560	4160	880			320	14960
Bifenthrin	Up-Star Gold	20 oz/100 gal	oz				120	170				20	310
Capsaicin & plant extracts	Captiva	8 oz/100gal	oz							4			4
Chlorfenapyr	Pylon	3 oz/100 gal	oz					81					81
Cyfluthrin	Decathlon	1.9 oz/100 gal	oz			58.9	121.125	22.8		39.9		16.15	258.875
Etoxazole	Tetrasan	1 lb/100 gal	lb							38.01			38.01
Hexythiazox	Hexygon	2 oz/100 gal	oz					16	88	30			134
imidacloprid	Imidacloprid 2F	6 oz/100 gal	oz	30	30			72			1188	696	2016
insecticidal soap	M-pede	1 gal/100 gal	gal							16			116
Neem oil	Triact 70	1 gal/100 gal	gal				0.05						0.05
oil	Omni oil	1 gal/100 gal	gal									7	7
oil	Ultra fine oil	1.5 gal/100 gal	gal	55.5	46.5								102
Pymetrozine	Endeavor	2.5 oz/100 gal	oz			50	114.375	55					219.375
Spinosad	Conserve	6 oz/100 gal	oz			120.18		123				51	294.18
Spiromesifen	Judo	2 oz./100 gal	oz							1			1

Nursery P – Whole operation– neonicotinoids only

Active ingredient	Trade Name	Rate	Date	Crop	Amount sprayed (gallons)	Total amount of formulation used (oz)	Total ai used (lbs)
Thiamethoxam	Flagship	6 oz/100 gallon	2/6	Digitalis, Gallardia, Potentilla	7.5	0.45	4 oz. ai per lb
			3/20	Hydrangea	600	36	
			3/25	Hydrangea, Buxus	500	30	
			4/28	Hydrangea	1200	72	
			4/30	Hydrangea	500	30	
			8/11	Rhododendron	2100	126	
			8/12	Rhododendron	2300	138	
			11/3	Heuchera	125	7.5	
Total Flagship						439.95	6.87
Dinotefuran	Safari	20 oz/100 gallon	3/10	Bellis, Digitalis, Alcea	150	30	0.20 lbs ai per lb (Safari 20SG)
			3/11	Digitalis	50	10	
			3/26	Gaillardia, Agastache	300	60	
			4/6	Buddleia	25	5	
			4/7	Rose, Hydrangea	20	4	
			4/28	Rose	75	15	
			4/28	Asclepias	20	4	
			7/14	Ligularia	200	40	
			8/1	Kale	300	60	
			10/7	Rudbeckia	700	140	
			10/8	Rudbeckia	300	60	
			10/23	Veronica	500	100	
			11/3	Veronica, Bamboo	450	90	
Total Safari						618	7.73

Imidacloprid	Imidacloprid 2F	6 oz/100 gallon	3/18	Alcea, Gaillardia	25	1.5	2 lbs ai/gallon
			3/21	Hydrangea	2700	162	
			3/24	Hydrangea, Buxus	1500	90	
			4/2	Hydrangea	2900	174	
			4/3	Hydrangea, Berberis	4500	270	
			5/5	Crocosmia	25	1.5	
			7/7	Buxus, Ilex crenata	1250	75	
			7/9	Rhododendron	40	2.4	
			9/18	All liners in area	1300	78	
			9/23	Rhododendron	3400	204	
			9/24	Rhododendron, Euonymus	3000	180	
			9/25	Rhododendron, Euonymus	3000	180	
			9/26	Rhododendron	2600	156	
			9/28	Rhododendron, Euonymus	5300	318	
			9/29	Rhododendron, Euonymus	7200	432	
			10/2	All containers in area	50	3	
			10/2	New cuttings in area	20	1.2	
			10/6	Azalea	4200	252	
			10/7	Azalea	1000	60	
			10/8	Azalea	3500	210	
			10/9	Azalea	4500	270	
			10/10	Azalea	2800	168	
			10/12	Rhododendron	4000	240	
			10/13	Rhododendron, Azalea, Leucothoe	6100	366	
			10/16	Rhododendron	4700	282	
			10/17	Rhododendon	2600	156	
			10/19	Rhododendon	2500	150	
			10/20	Leucothoe	4500	270	
			10/21	Buxus	6000	360	
			10/22	Buxus	3800	228	

			10/24	Buxus	1500	90	
			10/26	Buxus	2500	150	
			10/29	Buxus, Weigela	3100	186	
			10/30	Weigela	500	30	
			11/3	Weigela, Rhododendron	6900	414	
			11/4	Physocarpus, Itea, Cephalanthus, Hydrangea	3600	216	
			11/5	Hydrangea	1800	108	
			11/6	Hydrangea	2000	120	
			11/9	Euonymus	3100	186	
			11/10	Rhododendron	2800	168	
			11/11	Euonymus, Azalea	2050	123	
			11/12	Rhododendron	1000	60	
			11/13	Hydrangea	1000	60	
			11/16	Hydrangea	1000	60	
			11/20	Ilex crenata, Hydrangea	1100	66	
Total Imidacloprid 2F						7377.6	115.28
Acetamiprid	Tristar 8.5 SL	8.5 oz/100 gallons	7/10	Cytisus,EAC, Prunus C.,	1000	85	0.76 lbs ai/gallon
		8.5 oz/100 gallons	7/14	Cytisus,EAC, Prunus C., Prunus M. Prunus G.,Fothergilla Aesculus,Acer, Wisteria, Chaenomeles,Betula,,Enkianthus Amelanchier	900	76.5	
		4 oz/100 gallons	8/18	Prunus	300	12	
		4 oz/100 gallons	8/20	Cytisus, EAC, Prunus C., Prunus M. Prunus G., Cotinus Chaenomeles, Corylus,Hamamelis	1100	44	
		4 oz/100 gallons	8/21	Cytisus, EAC, Prunus C., Prunus M. Prunus G., Cotinus Chaenomales, Corylus,Hamamelis	600	24	

				Acer, Fothergilla, wisteria Amelanchier			
		4 oz/100 gallons	8/22	Clethra,Vitis, Acer,Fothergilla, Chaeonomeles, Enkianthus	600	24	
						265.5 oz	1.58

Appendix 3

Summary of Results – Connecticut Nursery Survey on Neonicotinoid Use

A nursery survey was sent via e-mail during the winter of 2015 -2016 to all the retail and wholesale nurseries listed in the Connecticut Nursery and Landscape Association Yearbook, to assess their reliance on neonicotinoid insecticides. Of the approximately 78 nurseries contacted, 30 responded to the questionnaire (38% response rate).

Nineteen of the responding nurseries (70%) indicated that they had used at least one neonicotinoid insecticide over the 2015 growing season. Of those nurseries that used neonicotinoids, 16 (84%) used these products in foliar sprays, 11 (58%) used them in a "sprench" application (a coarse spray to wet foliage and potting media), and 3 (16%) used them incorporated into potting media prior to potting. No nurseries reported using these products in an ebb-and-flood chemigation.

Neonicotinoid insecticides were considered essential by 26% of users for management of scale insects, 26% for wood borers (such as bronze birch borer), 21% for targeting mealy bugs, 10% specifically for managing rhododendron leaf miners, 20% for other leaf miners, and 10% each for whiteflies and aphids. Pests for which growers rated neonicotinoids as either being essential or sometimes needed were ranked: aphids (57% of growers), scale insects (41%), white flies, wood borers, and mealy bugs (36%), other leaf miners (35%), red-headed flea beetle (26%), and white grubs (11%).

Nurseries responding to this survey and applying neonicotinoids grew a variety of crops, with 61% growing annuals, 79% growing non-coniferous shade trees, 89% growing non-coniferous shrubs, 83% growing conifers, and 94% growing non-woody perennials. Of these crops, the largest proportion of the crop type being treated with neonicotinoids were non-woody perennials and non-coniferous shrubs (56% each), followed by the remaining crop types (37 – 39% of the crop being treated).

Growers are aware of the controversy surrounding the use of neonicotinoids. 16% of growers currently using neonicotinoids had plans to discontinue their use, based upon customer demands. 10% of growers were using labels to indicate that plants had been treated with neonicotinoids, and 74% of growers using this class of insecticides had not been approached by customers asking for them to change their practices in using neonicotinoids.

Several methods were used to limit the exposure of pollinators to neonicotinoids being used on horticultural crops. Two thirds of growers that used neonicotinoids reported that they never used these products when there were open blossoms on the crops being sprayed; one respondent simply noted that they apply the product at times of day when bees were not as active. Nearly as many (61%) reported using these products in greenhouses, where pollinators would not be present, including one respondent who applied these products in greenhouses 60 – 90 days before shipping. Many (39%) of growers only applied these insecticides when the plants were not mature enough to bloom. The least common measures were use of TriStar because of its greater safety to bees (17%) and incorporation of these insecticides into potting media (11%).

Of the 11 growers (30% of total respondents) who reported not using neonicotinoids on their horticultural crops, their most commonly selected reasons for avoiding these insecticides were (1) they had effective alternatives (6 respondents, or 75% of this group), (2) they were opposed to using systemic insecticides (3 respondents, or 38%) and (3) customers had demanded they not use these products (2 respondents, or 25%). One of the growers not using neonicotinoids in their nursery did have a plant care division, which did apply imidacloprid to boxwoods for management of boxwood leaf miners.

Appendix 4

Connecticut Nursery Survey – Data

1. Did your nursery apply any neonicotinoids insecticides (imidacloprid [Marathon, Mallet, Imida 2F, etc...], dinotefuran [Safari], thiamethoxam [Flagship], or acetamiprid [TriStar]) in 2015?

Answer Choices –	Responses –
– Yes	70.37% 19
– No (Please skip to Question 7)	29.63% 8
Total	27

2. What method(s) of application were used to apply neonicotinoid insecticides? (Please check all that apply).

Answer Choices –	Responses –
– Foliar spray	84.21% 16
– Drench or "sprench" (high volume spray intended to wet the upper potting medium.	57.89% 11
– Preplant potting mix incorporation (granule or sprayed, then mixed into medium).	15.79% 3
– Ebb and flood system in a greenhouse	0.00% 0
Total Respondents: 19	

3. How important for your nursery are neonicotinoids for managing the following pests?

- Answered: 20
- Skipped: 7

	This pest is not a problem at our nursery	We never apply neonicotinoids to target this insect	We rarely apply neonicotinoids to manage this pest	Neonicotinoids are sometimes used to target this pest	Neonicotinoids are essential for us to manage this pest	Total
aphids	5.26% 1	15.79% 3	21.05% 4	47.37% 9	10.53% 2	19
whiteflies	31.58% 6	21.05% 4	10.53% 2	26.32% 5	10.53% 2	19
mealy bugs	26.32% 5	21.05% 4	15.79% 3	15.79% 3	21.05% 4	19
scale insects	15.79% 3	15.79% 3	26.32% 5	15.79% 3	26.32% 5	19
rhododendron leaf miner	57.89% 11	15.79% 3	10.53% 2	5.26% 1	10.53% 2	19
other leaf miners	35.00% 7	10.00% 2	20.00% 4	15.00% 3	20.00% 4	20
red headed flea beetle / strawberry root weevil	42.11% 8	31.58% 6	0.00% 0	21.05% 4	5.26% 1	19
white grubs	38.89% 7	38.89% 7	11.11% 2	5.56% 1	5.56% 1	18
wood borers (e.g., bronze birch borer)	47.37% 9	10.53% 2	5.26% 1	10.53% 2	26.32% 5	19

-

4. Please state to which type of plant material you applied neonicotinoid insecticides in 2015:

	We do not grow this category	We did not apply neonicotinoids	We applied neonicotinoids	Total
Annuals	38.89% 7	22.22% 4	38.89% 7	18
Non-woody perennials	5.56% 1	38.89% 7	55.56% 10	18
Shrubs (non-conifers)	11.11% 2	33.33% 6	55.56% 10	18
Trees (non-conifers)	21.05% 4	42.11% 8	36.84% 7	19
Conifers	16.67% 3	44.44% 8	38.89% 7	18

5. Have your customers been demanding that you stop using neonicotinoids, or that you label plants as having been treated with neonicotinoids?

- Answered: 19

- Skipped: 8

Answer Choices –	Responses –
– My customers have made this demand, and we are discontinuing use of neonicotinoids.	15.79% 3
– My customers have made this demand, and we are using labels to indicate treated plants.	10.53% 2
– My customers have not demanded that we change our practices.	73.68% 14
Total	19

6. What precautions do you take when applying neonicotinoids to protect pollinators?

Answer Choices –	Responses –
– We apply the insecticide as a preplant potting mix.	11.11% 2
– We use foliar applications only when there are no open blossoms on the crop being sprayed.	66.67% 12
– We use acetamiprid (TriStar) for foliar sprays because it is safer to bees.	16.67% 3
– We only apply these products to plants that are not mature enough to bloom, or are not attractive to bees.	38.89% 7
– We use them in greenhouses where bees are not exposed.	61.11% 11
Total Respondents: 18	

Comments for this question:

Only use them 60-90 days prior to shipment in winter in greenhouses
2/10/2016 9:49 AM View respondent's answers
We spray at times of day when bees are less active
2/9/2016 9:45 AM View respondent's answers
Imidacloprid appled in rain (drench) and not during bloom-time and Safari is a bark application
1/7/2016 8:01 AM View respondent's answers
We have not been routinely using neonics but we did use some to use up material we had on hand.
1/4/2016 3:43 PM View respondent's answers
we use as soil drench when possible
1/4/2016 10:44 AM View respondent's answers
We make a conscious effort to not apply when there are blooms or bees present.
12/31/2015 8:18 AM View respondent's answers
we are cautious and mindful to protect plant species known to attract pollinators. in our IPM approach systemic neonics are never our first choice
12/30/2015 2:02 PM View respondent's answers
do not use
12/29/2015 11:28 AM View respondent's answers

7. Note: Please skip this question if you answered "Yes" to Question 1.

The reason(s) why my nursery did not use neonicotinoid insecticides in 2015 was (were):

Answer Choices –	Responses –
– Our customers demanded that we not treat plants with these insecticides.	25.00% 2
– We are opposed to using systemic insecticides.	37.50% 3
– We have effective alternatives, and just don't need to use neonicotinoids.	75.00% 6

Comments for this question:

Showing **2** responses

1. In general- we do not neonicotinoids in our nursery operation. We do have a plant care division of our operation that does use imidacloprid to treat residential plant material. We have seen a rise in boxwood leaf miner in this area and use imidacloprid to control this pest and other boring insects.

2. We strongly oppose the use of neonicotinoid for grub control!!!!!!