Determination of Imidacloprid Residue Concentrations in Seedless Watermelon Flowers

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Galen P. Dively, Mike Embrey, Terry Patton, and Amy Miller
Department of Entomology, University of Maryland, College Park, MD 20742
(301) 405-7524, (202) 812-9828 cell, galen@umd.edu

Rationale: While the extent and causes of colony collapse disorder (CCD) are unknown, many believe that honey bees have reached a tipping point wherein the colony can no longer protect itself from a barrage of stress factors. The CCD Working Group developed an action plan of research that addresses four categories of factors that impact bee and colony health: 1) new or re-emerging pathogens; 2) bee pests; 3) environmental and nutritional stresses; and 4) pesticides. This study was the first step in our investigations to examine the potential sublethal effects of pesticides.

We focused on imidacloprid because it is widely used on cucurbits and other crops pollinated by bees. On cantaloupe, cucumber, and watermelon crops, imidacloprid is soil applied either at planting or as split treatments at planting and again at 2-5 weeks later through drip irrigation. The split treatment regime is common in Florida, California, Arizona, and Texas, where most cucurbit production is centered and where insect pests such as aphids and whiteflies require longer residual activity of systemic control. The labels for imidacloprid products used on cucurbits state that the insecticide should not be applied directly to the foliage during bloom. However, many cucurbits produce flowers within 2-3 weeks after transplanting and thus are likely to be exposed to split treatments applied during or close to bloom.

As a systemic insecticide, imidacloprid accumulates primarily in the vegetative parts of plants and much less in fruiting structures. However, it is possible that low levels of imidacloprid in pollen and nectar may sublethally expose honey bees, which could lead to chronic effects. Residue analysis studies have detected imidacloprid at levels of 2-5 ppb in pollen and >1.5 ppb in nectar of seed-treated corn, sunflowers and rape. These levels result from low rates of seed dressings applied many weeks prior to bloom. Thus, it is reasonable to assume that higher residues of imidacloprid may be present in pollen and nectar of cucurbits with higher field rates applied closer to bloom. Most published studies have shown that imidacloprid may cause disorientation and associative learning problems in honey bees at levels above 20 ppb. However, some recent studies suggest that bee behavior is affected at levels between 3-16 ppb. None of these studies examined chronic effects of dietary exposure to imidacloprid over multiple brood cycles.

In a field study planned for 2008, we will employ a functional colony experiment to examine potential chronic effects of sublethal exposure to imidacloprid on the colony performance and foraging behavior of honey bees. This work will compare colonies fed an imidacloprid-treated bee food over multiple broods with control colonies fed untreated food. The objective of our study in 2007 was to determine a realistic range of imidacloprid residue concentrations that pollination colonies might be subject to during a typical growing season.

Study Protocol: To estimate potential exposure levels of imidacloprid in a cucurbit crop, we treated ten replicate plots of seedless watermelons with the low labeled rate (Admire Pro, 7 oz per acre) of imidacloprid. Plots were located at three University of Maryland Research and Education facilities (Wye, Upper Marlboro, Beltsville). Each plot consisted of two subplots which received: 1) 7 oz. applied as a transplant drench, and 2) 3.5 oz. applied as a transplant drench, followed by 3.5 oz. three weeks later. We determined that these treatment regimes are the most widely used on cucurbit crops based on telephone surveys with University and private sector pest managers. At peak bloom, we collected flowers and extracted samples of flower tissue (sepals, receptacle) excluding the male and female parts, stamens (male parts of flower), carpels (female parts of flower), and water rinsate obtained by washing the reproductive parts of the flowers. Only staminate flowers were collected from four plots (Upper Marlboro, Beltsville), while both staminate and pistillate flowers were collected from six plots (Wye). In addition, a second set of samples were collected three weeks after peak bloom from six plots (Wye farm). Samples were sent to the USDA/Agricultural Marketing Service/National Science Laboratory in Gastonia, NC, where they were analyzed by LC/MS/MS for imidacloprid and its two major metabolites (5-OH-imidacloprid and imidacloprid olefin). For quality assurance, recovery rates were determined by analyzing matrix spiked samples and process control samples of untreated flower tissue. Corrections were made for recovery levels of around 65% which were consistent among sample runs.

Results: Table 1 summarizes the data obtained from samples collected during peak bloom. Three of the ten control samples from untreated watermelon plots had detectable levels of imidacloprid ranging from 1.8 to 2.3 ppb. This was probably due to pollen or nectar contamination from bees that had previously visited nearby treated cucurbit crops. No detectable levels of the two metabolites were found in any of the samples. Rinsate samples contained noticeable amounts of pollen, although removal of pollen from stamens was not complete. All rinsate samples had no detectable amounts of imidacloprid, except two that were collected from plots receiving split treatments. Concentrations of imidacloprid were the highest (14.4 to 122.6 ppb) in the sepal and receptacle parts of the flowers, and these levels were 6.2-fold higher in samples of both staminate and pistillate flowers. Dissected stamens (with some pollen attached) had significantly lower residue levels of imidacloprid (<5.2 ppb), whereas levels in samples of both stamens and carpels were 9.5 times higher. Noteworthy is that fact that overall levels were highest in samples obtained from both staminate and pistillate flowers compared to those from only staminate flowers. All types of samples collected three weeks after peak bloom (Table 2) had significantly lower levels of imidacloprid residues (<5 ppb).

Results of this study clearly show that imidacloprid residues are present in watermelon flowers; however, we could not separate levels of imdacloprid that may be present in pollen or nectar from systemic levels in the plant tissues. Data from the rinsate samples suggest that the potential exposure to residues via pollen should be very low. Since female flowers produce significantly more nectar to attract bees, the higher levels found in mixed samples of staminate and pistillate flowers suggest that nectar may be a more potential route of direct exposure to imidacloprid. However, the average exposure should be <20 ppb if imidacloprid is applied only at planting.

Table 1. Summary of imidacloprid concentrations detected in flowers during peak bloom in treated replicate plots of seedless watermelon. Samples of staminate flowers only are compared with samples of staminate/pistillate flowers.

Flower type	Treatment regime	Type of sample[1]	Mean ppb of imidacloprid	SEM	Minimum	Maximum
Staminate /pistillate	Untreated	F[2]	0.7	0.33	0.0	2.2
Staminate (four replicate plots)	Single transplant drench at planting (7 oz/acre)	F	8.9	5.21	2.5	19.2
		R	0.0	0.00	0.0	0.0
		S	2.6	0.50	1.7	3.9
	Split treatments (3.5 oz/acre at planting; 3.5 oz/acre 3 weeks later)	F	6.8	3.66	2.8	14.1
		R	0.0	0.00	0.0	0.0
		S	2.3	1.34	0.0	5.2
Staminate /pistillate (six replicate plots)	Single transplant drench at planting (7 oz/acre)	F	34.5	5.65	14.4	51.1
		R	0.0	0.00	0.0	0.0
		S/C	16.3	2.52	9.9	23.4
	Split treatments (3.5 oz/acre at planting; 3.5 oz/acre 3 weeks later)	F	63.2	19.17	18.5	122.6
		R	0.3	0.21	0.0	1.0
		S/C	30.3	9.43	7.1	57.6

[1] F = flower tissue (sepals, receptacle) excluding the male and female parts; S = Stamens (male parts of flower); C = Carpels (female parts of flower); and R = water rinsate obtained by washing the reproductive parts of the flowers.
[2] Control samples were collected from untreated watermelon and consisted of whole staminate and pistillate flowers cut at the base of the receptacle.

Table 2. Summary of imidacloprid concentrations detected in flowers collected three weeks after peak bloom in treated replicate plots of seedless watermelon.

Flower type	Treatment regime	Type of sample[1]	Mean ppb of imidacloprid	SEM	Minimum	Maximum
Staminate /pistillate (six replicate plots)	Single transplant drench at planting (7 oz/acre)	F	4.1	0.52	3.1	4.8
		R	0.0	0.00	0.0	0.0
		S/C	2.3	1.20	0.0	4.0
	Split treatments (3.5 oz/acre at planting; 3.5 oz/acre 3 weeks later)	F	3.3	0.37	2.8	4.0
		R	0.0	0.00	0.0	0.0
		S/C	0.7	0.67	0.0	2.0

[1] F = flower tissue (sepals, receptacle) excluding the male and female parts; S = Stamens (male parts of flower); P = Carpels (female parts of flower); and R = water rinsate obtained by washing the reproductive parts of the flowers.