# Environment and Natural Resources Trust Fund (ENRTF)
# 2010 Work Program

**For the Period Ending June 30, 2013**
**PROJECT TITLE**: **3e or 221G Mitigating Pollinator Decline in Minnesota**
**PROJECT MANAGER:** Vera Krischik
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**FUNDING SOURCE:** Environment and Natural Resources Trust Fund
**LEGAL CITATION: M.L. 2010, Chp. 362, Sec. 2, Subd. 3e,** $297,000 is from the trust fund to the Board of Regents of the University of Minnesota to assess the role of insecticides in pollinator health in order to help mitigate pollinator decline. This appropriation is available until June 30, 2013, by which time the project must be completed and final products delivered.

**APPROPRIATION AMOUNT: $$297,000**
**Date of Report:** August 5, 2013
**Date of Next Progress Report:** final
**Date of Work Program Approval:** July 30, 2010
**Project Completion Date:** June 30, 2013

**Overall Project Outcome and Results**

The commonly used systemic neonicotinyl class of insecticides (imidacloprid, thiamethoxam, clothianidin, and dinotefuran) is implicated in bee decline since insecticide residues accumulate in pollen and nectar. These residues can kill foraging bees and decrease pollination, seeds, and fruits of native plants and crops.

Neonicotinyls are applied in numerous methods (seeds, soil drenches, and tree trunk injections). Of the 442 million acres of U.S. cropland, 143 acres are treated with over 2 million pounds of neonicotinyl insecticides. In Minnesota in 2009 46,766 pounds of imidacloprid and 19,347 pounds of clothianidin were applied.

These research objectives were to understand the effects of imidacloprid residues on bee health. This research found that a standard, label rate of imidacloprid applied to soil of potted plants produced imidacloprid residues of 1973 ppb in mint and 1568 ppb in milkweed flowers. A residue in flowers of 185 ppb imidacloprid kills a bee.

Research on greenhouse colonies of bumblebees showed that 20-100 ppb imidacloprid or clothianidin provided in sugar syrup for 11 weeks increased queen mortality and decreased consumption, sugar syrup storage, colony weight, and male production. Consequently, 20 ppb had detrimental effects on bumblebees and will reduce pollination of native plants. Research on field colonies of honey bees showed that only 33% of the imidacloprid was stored in colony cells. At 200 ppb there was less brood, fewer returning foragers, and higher amounts of distorted wing virus, which can cause colony death.

This research demonstrated that applications of imidacloprid and clothianidin insecticides to soil result in high residues in nectar and pollen that will kill bees. Studies on bees showed how colonies died from these insecticides.

An 11 part website for outreach education in Minnesota on pollinator conservation was developed (www.entomology.umn.edu/cues/pollinators/index.html). A final report of 2010 LCCMR 2e or 221G was provided.

**Project Results Use and Dissemination**

The purpose of the research was to supply data to protect pollinators to ensure future seeds and fruits for wildlife and people. These research data are very important to groups trying to understand the impact of systemic, neonicotinyl insecticides on bee colonies and individual foragers. These data are used by bee keepers, advocacy groups, state agencies, and the US EPA for discussion on whether neonicotinyl insecticides are affecting bee health and whether their use needs to be restricted. In June 2013 The European Union's Food Safety Authority (EFSA) has restricted the use of neonicotinyl insecticides for 2 years on all flowering plants that bees utilize. The reports and discussion are on the LCCMR sponsored "Pollinator Conservation" website (www.entomology.umn.edu/cues/pollinators/index.html). This is a remarkable proactive decision to ensure the safety of pollinators.

An 11 part website on bee pollinator conservation was developed for outreach education in Minnesota (www.entomology.umn.edu/cues/pollinators/index.html). The website contains research results, manuscripts, workshop, bulletin on insecticides and bees, bulletin on pollinator conservation, and poster on bee plants. We will produce 4 manuscripts from these data and 3 are already in final form and available on the website.

These research data have been requested by groups that need to understand more about the risk of neonicotinyl insecticides to bees: US EPA, Center for Food Safety, PANNA (Pesticide Action Network), Xerces Society for Invertebrate Conservation, Washington State Department of Agriculture, Pesticide Research Institute, MN Honey Producers, Boulder County Bee Keepers, and Colorado State Beekeepers. The lab was interviewed by TV and radio many times: MN Public Radio (3), Harvest Public Media, Iowa Public Radio, WCCO, Kare 11 News, KSTP, Pioneer Press, Star Tribune, and the Minnesota State Fair. Krischik has provided her research results to the US EPA twice: an online slide show webinar to EPA scientists and a visit to UM by the US EPA Administrator for the Office of Chemical Safety and Pollution Prevention (OCSPP). Krischik's expertise from this research has made her a reviewer for 2 white papers from the Xerces Society of Invertebrate Conservation and another from the Friends of the Earth as well as peer reviewer on related scientific manuscripts.

A final report of 2010 LCCMR 2e or 221g containing data was provided to the LCCMR.

# Environment and Natural Resources Trust Fund (ENRTF)
# 2010 Work Program

**Date of Report:** August 5, 2013
**Date of Next Progress Report:** Final, project completed
**Date of Work Program Approval:** July 30, 2010
**Project Completion Date:** June 30, 2013

**I. PROJECT TITLE**: **Mitigating Pollinator Decline in Minnesota**

**Project Manager**: Vera Krischik
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**Location: University of Minnesota, Department of Entomology, St. Paul Campus**

| | | |
|---|---|---|
| **Total ENRTF Project Budget:** | **ENRTF Appropriation** | **$297,000** |
| | **Minus Amount Spent:** | **$297,000** |
| | **Equal Balance:** | **$0** |

## Legal Citation: M.L. 2010, Chp. 362, Sec. 2, Subd. 3e

## Appropriation Language:
$297,000 is from the trust fund to the Board of Regents of the University of Minnesota to assess the role of insecticides in pollinator health in order to help mitigate pollinator decline. This appropriation is available until June 30, 2013, by which time the project must be completed and final products delivered.

## II. Abstract: Overall Project Outcome and Results
## FINAL PROJECT SUMMARY AND RESULTS:
The commonly used systemic neonicotinyl class of insecticides (imidacloprid, thiamethoxam, clothianidin, and dinotefuran) is implicated in bee decline since insecticide residues accumulate in pollen and nectar. These residues can kill foraging bees and decrease pollination, seeds, and fruits of native plants and crops.

Neonicotinyls are applied in numerous methods (seeds, soil drenches, tree trunk injections). Of the 442 million acres of U.S. cropland, 143 acres are treated with over 2 million pounds of neonicotinyl insecticides. In Minnesota in 2009 46,766 pounds of imidacloprid and 19,347 pounds of clothianidin were applied.

These research objectives were to understand the effects of imidacloprid residues on bee health. This research found that a standard, label rate of imidacloprid applied to soil of potted plants produced imidacloprid residues of 1973 ppb in mint and 1568 ppb in milkweed flowers. A residue in flowers of 185 ppb imidacloprid kills a bee.

Research on greenhouse colonies of bumblebees showed that 20-100 ppb imidacloprid or clothianidin provided in sugar syrup for 11 weeks increased queen mortality and decreased consumption, sugar syrup storage, colony weight, and male production. Consequently, 20 ppb had detrimental effects on bumblebees and will reduce pollination of native plants. Research on field colonies of honey bees showed that only 33% of the imidacloprid was stored in colony cells. At 200 ppb there was less brood, fewer returning foragers, and higher amounts of distorted wing virus, which can cause colony death.

This research demonstrated that applications of imidacloprid and clothianidin insecticides to soil result in high residues in nectar and pollen that will kill bees. Studies on bees showed how colonies died from these insecticides.

An 11 part website for outreach education in Minnesota on pollinator conservation was developed (www.entomology.umn.edu/cues/pollinators/index.html).

**III. PROGRESS SUMMARY**

**Overall project summary of report final.**

Since 1990 the neonicotinyl insecticides, imidacloprid, thiamethoxam, clothianidin, and dinotefuran, were implicated in the decline of bees as they accumulate in pollen and nectar, are systemic, and are expressed for years from a single application. Neonicotinyls are applied in various ways (seed treatments, soil drenches, foliar sprays, irrigation systems, tree injections) on agricultural and landscape plants. Most genetically modified crops (corn, canola, and soybeans) use seed treatments of imidacloprid (Gaucho), clothianidin (Poncho), or thiamethoxam (Crusier). The annual market for neonicotinyl insecticides is in the billions of dollars due to their low mammalian toxicity, systemic nature, and extended efficacy. Most research focused on the effects of less than10 ppb of imidacloprid that was found in nectar and pollen of neonicotinyl-seed treated crops, such as canola, cotton, corn, soybean, and sunflower, on bee foraging, memory, and colony health.

This research on bumblebees and honey bees support accruing scientific data on the sublethal effects of imidacloprid on bee foraging. Bumblebee colony health was reduced at 20 ppb and honey bee colony health at 100 ppb. Applications of imidacloprid for landscape plants resulted in imidacloprid residues greater than 100 ppb and high enough to kill a foraging bee.

The ubiquitous use of neonicotinyl insecticides on crops and landscape plants throughout the season will lead to chronic sublethal and lethal effects on worker foraging and colony health. Social bee colonies, such as bumblebees and honey bees, rely on division of labor and need foragers to return nectar to the hive for the queen and brood. Native, annual bee colonies or bumblebee queens in spring and fall are even more vulnerable to neonicotinyl insecticides since the solitary queens can be impaired when foraging. Since most studies show reduction in foraging behavior below 10 ppb and residues in crop and landscape flowers are higher than 10 ppb, bees are likely to be experiencing chronic, sublethal doses with consequences on queen and colony health. The 20 year research focus on residue levels below 10 ppb of neonicotinyl insecticides found in nectar and pollen of seed-treated crops (corn, canola, and sunflower) has made us preoccupied with the lowest rates used in agriculture. Much higher rates and chronic exposure at low doses will lead to colony failure thru reduced navigation and foraging.

We performed this study to determine the imidacloprid residue in flowers grown in pots treated with a soil application of imidacloprid, and the effects of higher imidacloprid levels on honey bee and bumblebee colony health. We found that standard application rates of imidacloprid to soil produced high enough residues in flowers to kill bees at feeding. Our research demonstrated that a standard 1X imidacloprid rate to the soil produced in flowers residue of 1973 ppb in mint and 1568 ppb in milkweed flowers. The residue needed to kill a bee when feeding is an estimated oral LC50 for honey bees of 185 ppb (CA EPA 2009) or 192 ppb (Bayer, Fischer and Chalmers 2007). Consequently, a 1X label rate provides enough imidacloprid to the soil for sufficient imidacloprid residue to be found in flowers that would kill a bee.

For bumblebees, we demonstrates that 20 ppb imidacloprid or clothianidin fed to queenright colonies of *B. impatiens* for 11 weeks increased queen mortality and reduced colony consumption, sugar syrup storage in wax pots, colony weight, and male production. Consequently, low residue of 20 ppb will have detrimental effects on bumblebee colony health.

Behavioral studies in the laboratory called Proboscis Reflex Response (PERS) demonstrated that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory in bumblebees.

For honey bees, we demonstrated that sealed and total brood, pollen stores and returning foragers were reduced in 200 ppb imidacloprid compared to 0, 50, and 100 ppb treatments, but not queen production or numbers of bees in the colony. *Nosema* fungal pathogen numbers and distorted wing virus (DWV) were highest in late summer in 200 ppb treatments. At 100 ppb there was less pollen stores, fewer returning foragers, and higher amounts of DWV and black queen cell virus (BQCV). However, imidacloprid in stored nectar was 33 % less than that provided in sugar syrup: 50 ppb (24 ppb, 31%), 100 ppb (44 ppb, 33%), and 200 ppb (96 ppb, 34%) indicating mixing of provided sugar syrup with nectar from foraging in the field. Consequently, honey bees were only affected at the highest imidacloprid treatments of 100 ppb and 200 ppb as the bees were also foraging on flower nectar as well as sugar syrup.

**Tables and figures are at the end of this section**
**Summary of imidacloprid residue in flowers of plants grown in pots**
**Result 1-1: Residue analysis of imidacloprid in pollen and nectar**
We completed studies on the amount of imidacloprid residue in flowers from soil applications. *Agastache foeniculum* mint and *Asclepias curassavica* milkweed contained significant imidacloprid residue in flowers from an imidacloprid soil application. A standard 1X imidacloprid rate to the soil produced flowers residue of 1973 ppb in mint and 1568 ppb in milkweed (Table 1). The residue needed to kill a bee when feeding is an estimated oral LC50 for honey bees of 185 ppb (CA EPA 2009) or 192 ppb (Bayer, Fischer and Chalmers 2007). Consequently, a 1X label rate provides enough imidacloprid to the soil for sufficient imidacloprid residue to be found in flowers that would kill a bee. The woody native plant *Esperanza* yellowbells (YB) and Rose (R) had a residue of 106 ppb (YB) and 95 ppb (R) imidacloprid in the flowers, which should be a sublethal dose that affects behavior, but a 2X label rate produced 276 ppb (YB) and 332 ppb (R), a residue amount that would kill a bee. Previous research demonstrated 6000 ppb in *Fagopyrum esculentum* buckwheat and *Asclepias curassavica* milkweed flowers. In 2009 after a 1X label rate of imidacloprid to the soil, a homeowner's formulation of imidacloprid produced 812 ppb in rose flowers and a professional formulation 1175 ppb in rose flowers. All residues above 185 ppb are sufficient to kill a bee after a few seconds of feeding.

Mint flowers treated with an imidacloprid label rate had dead bees in the flowers, but a statistically significant number of dead bees were found on 2X label treatments. We found many bees dead on the ground but could not identify them to treatment. At 2X dose bees are killed while feeding on the flowers. Consequently, standard EPA registered doses of imidacloprid in homeowner and professional products translocate sufficient imidacloprid from the soil to the flowers to kill a bee (Table 1-3).

**Summary of bumblebee research**

**Result 1-2: Imidacloprid effects on bumblebee colony growth and survival**

We completed in 2011-2012 an 11-week greenhouse study on caged queenright colonies of *Bombus impatiens* Cresson (n=9 colonies/trt) that were fed 0, 10, 20, 50 and 100 ppb imidacloprid or clothianidin in sugar syrup (Table 1). Neonicotinyl treatments used in this study ranged from the highest amount found in seed-treatments (corn, canola and sunflower) (10 ppb) to levels found in landscape plants (20-100 ppb). Our study demonstrates that 20 ppb imidacloprid or clothianidin fed to queenright colonies of *B. impatiens* for 11 weeks increased queen mortality (Figure 1) and reduced colony consumption (Figure 2), colony weight (Figure 3), wax syrup pots that were added (Figure 4), and male production (Figure 5). Neither neonicotinyl decreased worker and queen production. Starting at 6 weeks, queen mortality was significantly higher in 50-100 ppb and by 11 weeks in 20-100 ppb imidacloprid- and clothianidin-treated colonies. As queens started to die at week 6, workers in 20-100 ppb treatments produced fewer males, but did continue to invest in new queen production.

We completed studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory.

**Summary of honey bee research**

**Result 1-3: Imidacloprid effects on honey bee colony growth and survival**

In 2011, in a 16 week field study, honeybee colonies (n=10 colonies/trt) were fed 0, 50, 100, 200 ppb imidacloprid in sugar syrup and colonies were assessed five times: June 8, July 6, August 3, August 31, and September 21 for 16 parameters of colony health: frames of bees, open brood, sealed brood, total brood, pollen stores, missing cell count, brood pattern, returning pollen foragers, percent returning foragers, sugar syrup consumption in 48 hrs and 1 week, dead bee counts, *Varroa* numbers, *Nosema* numbers, virus (distorted wing virus(DWV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV)) and queen mortality (Table 1, Table 2).

Frames of bees (Figure 1) was not affected by imidacloprid treatment. Sealed (Figure 2) and total brood (Figure 3), pollen stores (Figure 4), returning foragers (Figure 6), and colony consumption (Figure 7) were reduced in 200 ppb imidacloprid compared to 0, 50, 100 ppb treatments. Nosema numbers (Figure 5) and DWV (Figure 8) were highest in late summer in 200 ppb treatments. However, imidacloprid in stored nectar was 34 % less than that provided in sugar syrup at 50 (24, 31%), 100 (44, 33%), and 200 (96, 34%) ppb indicating mixing of provided nectar with nectar from foraging in the field (Table 1). The levels of imidacloprid found in nectar are below LD50 of 185-192 ppb found in studies (CA EPA 2009, Bayer, Fischer and Chalmers 2007) and should not kill bees on ingestion.

There was no correlation of treatment with dead bee counts and queen replacement, indicating a nontoxic effect of imidacloprid. Reduction in returning foragers, pollen stores, and colony consumption (Figure 7) indicate a sublethal effect of chronic exposure of imidacloprid on bee foraging. These data support accruing scientific data on the sublethal effects of imidacloprid on bee foraging. In addition, DWV virus was higher in 50, 100, and 200 ppb treatments compared to controls and BQV was higher in 100 ppb treatments (Figure 8).

**Summary of best bee plants**

**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**

We demonstrated that native plants have more beneficial insects visiting them than bedding plants (Figure 1, Table 1) and goldenrods *Solidago* and Joe-pyeweed *Eupatorium* were some of the best bee plants. We installed a demonstration plot on the Saint Paul campus in Fall 2012 by adding 300 *Agastache* mint bee plants to a10 yr old native plant restoration. We developed a section on the CUES on conserving bees thru proper plant selection, habitat restoration, and compatible pesticide use. We developed a bulletin and poster on best bee plants, identifying bees, and insecticides and bees.

**Summary of outreach and workshop**

**Result 3: Outreach talks and workshop, a Pollinator Conservation website was created**

A new pollinator conservation site was posted on the CUES website that contains 11 sections on education and research: value of bees, types of bees, bees and pesticides, colony collapse disorder, conservation and habitat, plants for pollinators, bumble bee conservation, US EPA and pollinators, European Union and pollinators, online 5 part workshop for Master Gardeners, and Krischik's research on bees. We developed a bulletin on ready to use consumer insecticides and their toxicity to bees, poster on best bee plants, and a pollinator conservation bulletin with agricultural and consumer insecticides and effects on bees See CUES website on Pollinator Conservation http://www.entomology.umn.edu/cues/pollinators/index.html

1. LCCMR Ready to use consumer insecticides and their toxicity to bees
2. LCCMR Best Bee Plant Poster
3. LCCMR Pollinator Conservation Bulletin
4. LCCMR Online Pollinator Conservation Workshop.
5. LCCMR Research and outreach products
6. LCCMR Website with research papers, EPA and EFSA EU documents and discussions on bee conservation

The PI gave discussed her research results with Steve Ellis and Jeff Anderson, two Minnesota beekeepers and presented talks at the annual meeting of the Minnesota Honey Producers. The funds on the grant supported a Master Graduate Student who worked on the bumble bees and graduated. Numerous talks were provide on the research: Wild Ones Native Plant Society, Minneapolis; Fruit and Vegetables Conference, St. Cloud; MN Rose Society, Minneapolis; Anoka County Master Gardeners Meeting, Maplewood; MN State Fair Bee keepers, St. Paul; MN Honey Producers summer meeting Duluth, Detroit Lakes; Minnesota Green Expo, Minneapolis; MNLA (MN Nursery and Landscape Association), Minneapolis, St. Paul.

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**Tables and figures**

**Result 1-1: Residue analysis of imidacloprid in pollen and nectar**

**Table 1**

2011 Imidacloprid residue plants

| Dose in mg/soil | Dead bees on Agasatche | *Agastache* spp. nectar ppb | *Asclepias* spp. nectar ppb | *Esperanza* spp. nectar ppb | *Rosa* spp. pollen ppb |
|---|---|---|---|---|---|
| 0 | 0.6b | 6b | 3c | 0c | 26b |
| 25 | 0.6b | 52b | 80c | 8c | 36b |
| 50 | 0.5b | 133b | 175bc | 21c | 30b |
| 300 1X 3 gal pot | 1.1ab | 1973b | 1568bc | 106c | 95b |
| 600 2X 3 gal pot | 2.4a | 5265ab | 2950b | 276b | 332b |
| 1200 | 2.4a | 9335a | 8337a | 9162a | 720a |
| | F=3.2, 0.01 | F=3.7, 0.017 | F=25.8, 0.0001 | F=166, 0.0001 | F=5.7, 0.0025 |

**Table 2**

**2009, 2010, 2011 Imidacloprid residue rose**

| Dose in mg/soil Marathon 1%G | Rose 2009 field | Rose 2010 GH | Rose 2011 field |
|---|---|---|---|
| 0 | 9d | 0c | 26b |
| 25 | na | 5c | 36b |
| 50 | na | 7c | 30b |
| Homeowner 1X 270 mg | 812c | na | na |
| Homeowner 2X 270 mg | 1648a | na | na |
| 300 1X 3 gal pot | 1175b | 32bc | 95b |
| 600 2X 3 gal pot | na | 161ab | 332b |
| 1200 | na | 268a | 720a |
| | F=256, 0.0001 | F=4.9, 0.0045 | F=5.7, 0.0025 |

**Table 3**

**2005, 2007, 2011 Imidacloprid residue buckwheat, milkweed**

| Dose in mg/soil Marathon 1%G | Buckwheat 2005 Nectar ppb | Milkweed 2007 Nectar ppb | 2011 Milkweed Nectar ppb |
|---|---|---|---|
| 0 | | | 3c |
| 25 | na | na | 80c |
| 50 | na | na | 175bc |
| Homeowner 1X 270 mg | na | na | na |
| Homeowner 2X 270 mg | na | na | na |
| 300 1X 3 gal pot | 6000 | 6000 | 1568bc |
| 600 2X 3 gal pot | 12000 | 12000 | 2950b |
| 300 21 days later | na | 20000 | na |
| 600 21 days later | na | 34000 | na |
| 1200 | na | na | 8337a |
| | F=25.86, (2,22) 0.001 | F=22.72, (2,6) 0.0016 | F=25.8, 0.0001 |

**Summary of bumblebee research**

**Result 1-2: Imidacloprid effects on bumblebee colony growth and survival**

**Figure 1. Queen mortality at weeks 1-11, A,** Imidacloprid, Week 6: Chi-square test = 9.26, DF = 4, 235, p<0.055, week 11: Chi-square test = 75.49, DF = 4,435, p<0.001. **B,** Clothianidin, Week 6: Chi-square test = 22.87, DF = 4, 247, p<0.001, week 11: Chi-square test = 102.78, DF = 4, 457, p<0.001, Kruskal-Wallis, Wilcoxon Test.

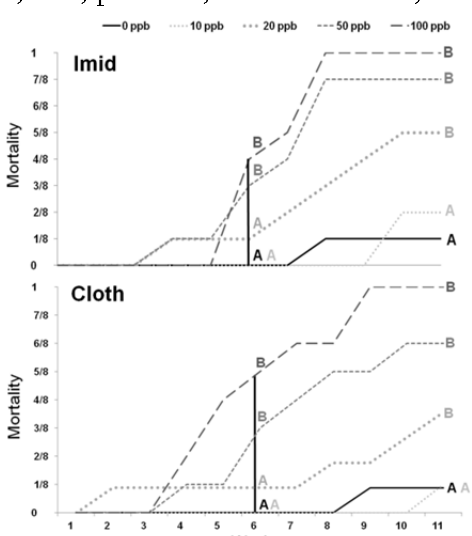


**Figure 2. Bee consumption**, **A,** Imidacloprid, Week 2: F = 30.97, DF = 4, 16, p<0.001, Week 4: F = 10.31, DF = 4, 33, p<0.001, Week 6: F = 0.89, DF = 4, 8, p = 0.513, Week 8: F = 2.51, DF = 3, 17, p = 0.093. **B,** Clothianidin, Week 2: F = 17.68, DF = 4, 17, p<0.001, Week 4: F = 32.73, DF = 4, 15, p<0.001, Week 6: F = 9.37, DF = 4, 28, p<0.001, Week 8: F = 4.32, DF = 4, 8, p = 0.035, Proc Mixed, Tukey-Kramrer HSD and ANOVA.

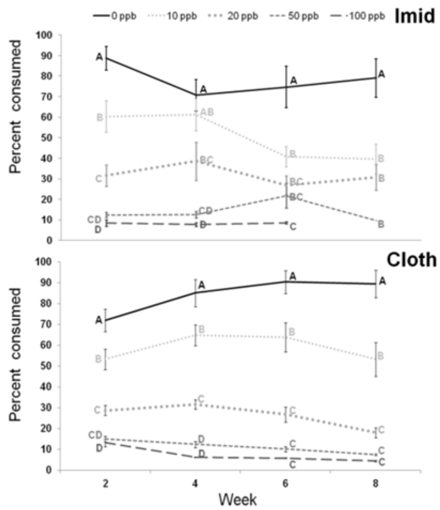



**Figure 3. Colony weight**, **A,** Imidacloprid, Week 0: F = 1.84, DF = 4, 16, p = 0.170, Week 11: F = 16.20, DF = 4, 35, p< 0.001. **B,** Clothianidin, Week 0: F = 0.87, DF = 4, 37, p = 0.492, Week 11: F = 16.10, DF = 4, 37, p<0.001, ANOVA.

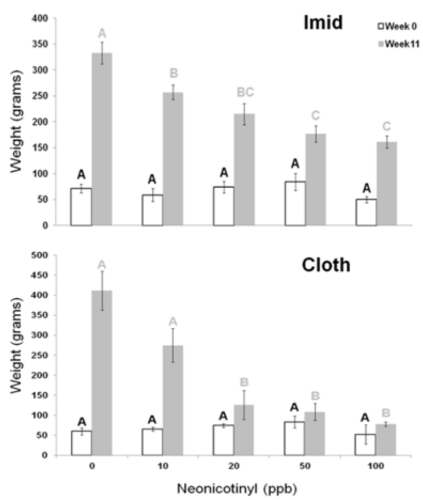


**Figure 4. Wax syrup pots added, A,** Imidacloprid, Chi-square test = 10.23, DF = 4, p = 0.0368. **B,** Clothianidin, Chi-square test, F = 21.54, DF = 4, p<0.0002, Kruskal-Wallis, Wilcoxon Test.

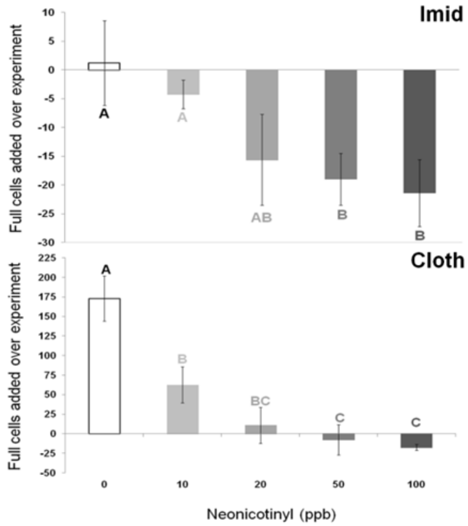
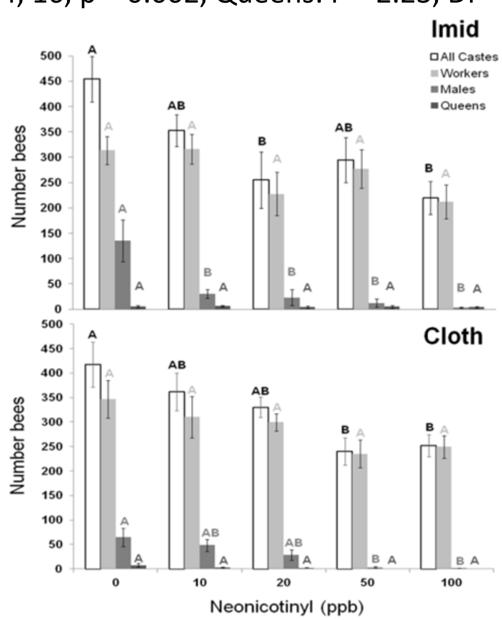



**Figure 5. Worker, male, and queen production,** Imidacloprid, Week 11: All Castes: F = 4.62, DF = 4, 35, p = 0.004, Workers: F = 1.92, DF = 4, 35, p = 0.129, Males: F = 4.59, DF = 4, 14, p = 0.014, Queens: F = 0.19, DF = 4, 35, p = 0.945. **B,** Clothianidin, Week 11: All Castes: F = 5.12, DF = 4, 37, p = 0.002, Workers: F = 2.15, DF = 4, 37, p = 0.094, Males: F = 7.44, DF = 4, 16, p = 0.002, Queens: F = 2.23, DF = 4, 37, p = 0.085, ANOVA.



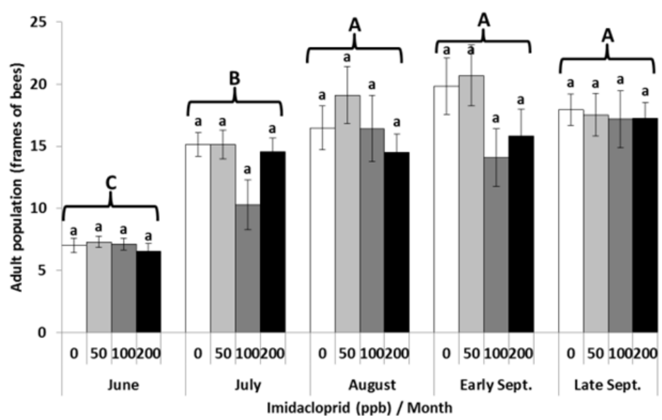
**Summary of honey bee research**
**Result 1-3: Imidacloprid effects on honey bee colony growth and survival**

| Table 1. Honeybee results 2010-2011 | | | |
|---|---|---|---|
| **June-Sept: 5 assessments June 8, July 6, Aug 3, Aug 31, Sept 22, 2011** | **F (df), P Month** | **F (df), P Treatment** | **F (df), P Interaction** |
| **Adult population** (frames of bees) | 53.38 (4, 129), <0.0001) | 1.43 (3, 34). 0.2362 | 1.69 (12, 129), 0.0752 |
| **Sealed brood area** (pupae) | 23.04 (4, 128), <0.0001) | 4.95 (3, 34), 0.0028 | 1.92 (12, 128), 0.0372 |
| **Open brood area** (1-5th instar larvae) | 33.32 (4, 129), <0.0001 | 0.59 (3, 34), 0.6224 | 0.96 (12, 129), 0.4881 |
| **Total brood area** (open + sealed) | 35.35 (4, 129), <0.0001) | 3.06 (3, 34), 0.0414 | 1.50 (12, 129), 0.1303 |
| **Pollen area** | 10.65 (4, 129), <0.0001 | 11.38 (3, 34), <0.0001) | 7.35 (12, 129), <0.0001 |
| **Mean missing cell area** | 6.98 (4, 118), <0.0001 | 3.06 (3, 34), 0.0412 | 1.51 (12, 118), 0.1309 |
| **Brood pattern** | 5.60 (4, 117), 0.0004 | 2.35 (3, 34), 0.0901 | 1.61 (12, 117), 0.0967 |
| ***Nosema* spp. levels** | 15.05 (4, 130), <0.0001 | 4.85 (3, 34), 0.0065 | 2.02, (12, 130), 0.0269 |
| ***Varroa destructor* levels** | 64.18, (3, 96), <0.0001 | 1.49 (3, 34), 0.2358 | 1.09 (9, 96), 0.3761 |
| **Total returning foragers** | 11.17 (2, 64), <0.0001) | 1.79 (3, 34), 0.1670 | 0.78 (6, 64), 0.5881 |
| **Percent returning pollen foragers** | 7.68 (2, 64), 0.0010 | 6.42 (3, 34), 0.0015 | 1.09 (6, 64), 0.3808 |
| **Dead bees** | 32.82 (3, 98), <0.0001 | 0.90 (3, 34), 0.4528 | 1.03 (9, 98), 0.4219 |
| **Consumption 48 hours** | 36.64 (3, 98), <0.0001 | 2.98 (3, 34), 0.0450 | 2.30 (9, 98), 0.0216 |
| **Consumption 1 week** | 29.06 (3, 98), <0.0001 | 3.07 (3, 34), 0.0407 | 2.74 (9, 98), 0.0068 |
| **Queen replacement (Date: ChiSquare, P value)** | Aug 31-Sept 22: 8.02, 0.0456 | Total June-Sept: 3.20, 0.3625 | Sept 22-Jan 12: 6.26, 0.0998 |

**Table 2. Imidacloprid residue (ppb) in sugar syrup and stored syrup in wax cells (USDA, AMS, Gastonia, NC).**

| | Sugar syrup (ppb) (% trt, syrup/trt) | | | Stored syrup in wax cells (ppb) (% syrup, cells/syrup) | | | | | **% changed in wax cells** |
|---|---|---|---|---|---|---|---|---|---|
| trt | 18July | 12Sept | mean | 18July | **18July** | **12Sept** | **12Sept** | **mean** | **mean cells/mean syrup** |
| 0 ppb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 ppb | 33 (66%) | 36 (72%) | 35 (70%) | 6 (18%) | 6 (18%) | 44 (122%) | 40 (111%) | 24 (66%) | 31% lower 24/35 |
| 100 ppb | 69 (69%) | 66 (66%) | 67 (68%) | 31 (45%) | 21 (32%) | 33 (50%) | 92 (140%) | 44 (67%) | 33% lower 44/67 |
| 200 ppb | 140 (70%) | 151 (75%) | 146 (73%) | 38 (27%) | 91 (65%) | 67 (44%) | 185 (123%) | 96 (65%) | 34% lower 96/146 |
| | 8/26/12 | 10/05/12 | mean | | | | | | |
| 100,000 ppb stock | 968000 (97) | 110000 (110) | 103400 (103) | - | - | - | - | - | - |

## Figure 1. Frames of bees




## Figure 2. Sealed brood

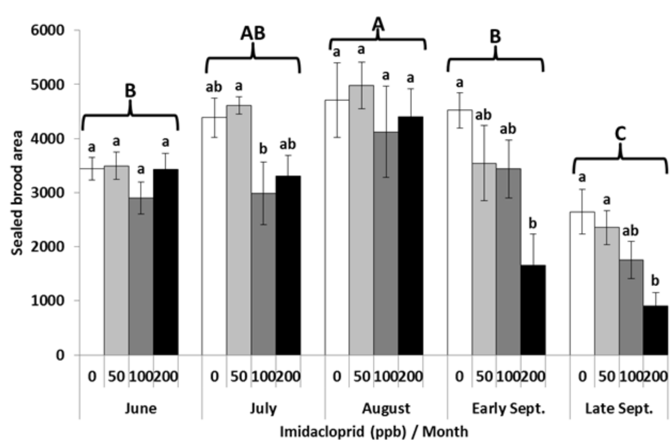


**Figure 3. Total brood area**

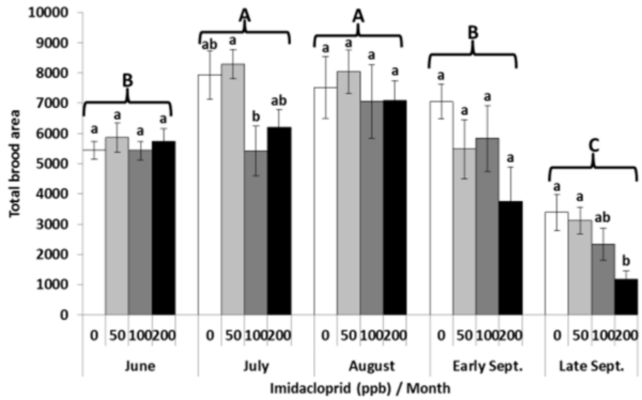



**Figure 4. Pollen area**

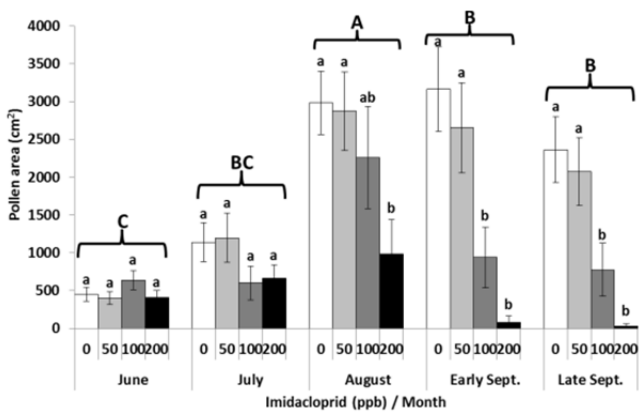
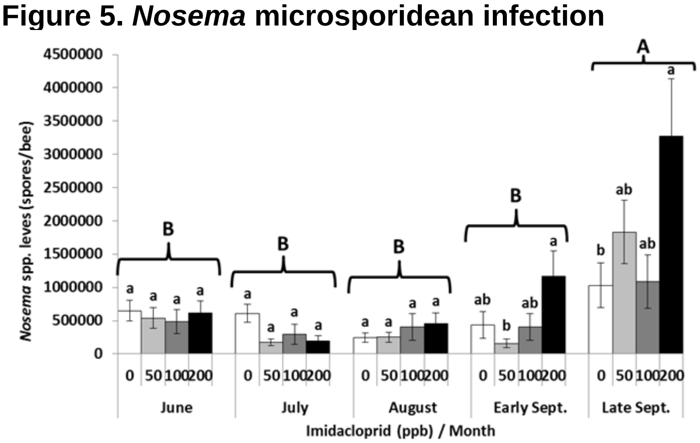
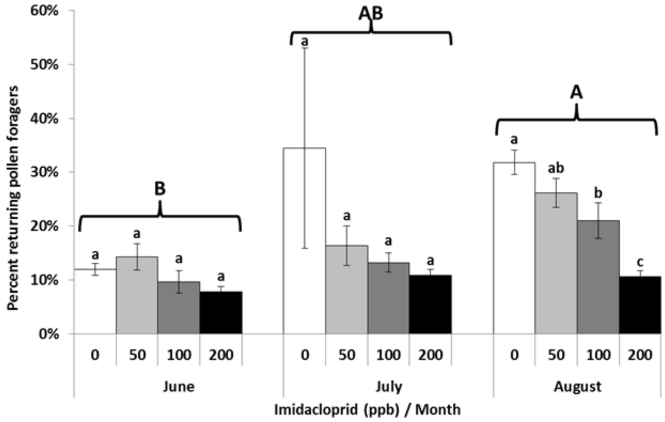


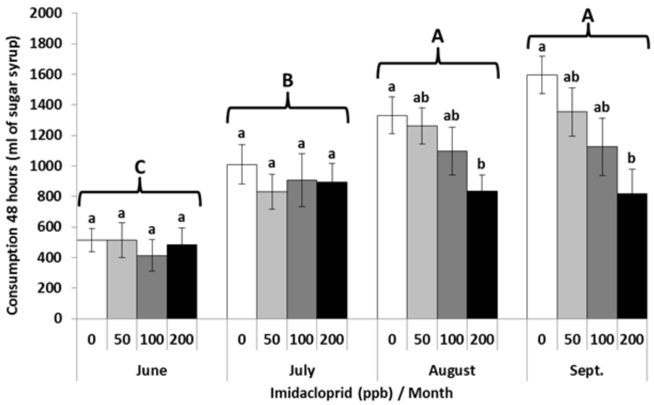

**Figure 5. *Nosema* microsporidean infection**



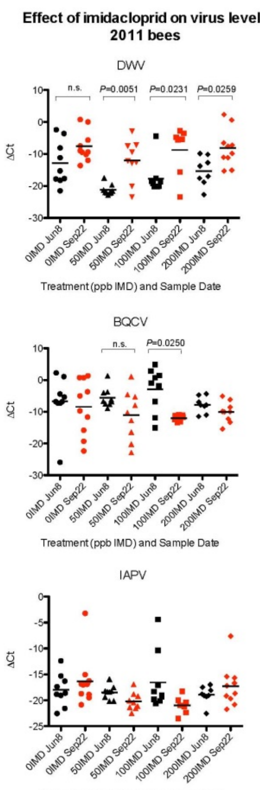
## Figure 6. Returning foragers




## Figure 7. Colony syrup consumption




## Figure 8. Virus levels



**Summary of best bee plants**
**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**

**Figure 1. Mean number of beneficial insects visiting native and bedding plants: Insect visits during 2 min of behavioral observation of flowers; sticky trap catches; and ant visits to bait stations under plants.**

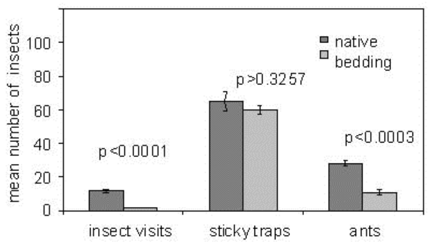



**Table 1. Behavioral observation: Mean and total number of beneficial insects found on flowers of native and bedding plants per two-minute interval**

| | **Native Sustainable Landscape** | | **Bedding Sustainable Landscape** | | **Statistics** |
|---|---|---|---|---|---|
| **Insect taxa** | **Total** | **Mean ± SEM** | **Total** | **Mean ± SEM** | **F, df, *P*** |
| **Cantharidae** | 4,8886 | 9.58 ± 0.72 | 221 | 0.39 ± 0.10 | 166.4, (1,364), .0001 |
| **Syrphidae** | 343 | 0.52 ± 0.12 | 359 | 0.62 ± 0.06 | 0.49, (1,364), 0.4845 |
| ***Bombus* spp.** | 336 | 0.68 ± 0.10 | 91 | 0.16 ± 0.02 | 26.95, (1,364), 0.0010 |
| **True bugs** | 275 | 0.52 ± 0.16 | 73 | 0.13 ± 0.03 | 5.8, (1,364), 0.0160 |
| **Other bees** | 185 | 0.38 ± 0.07 | 71 | 0.12 ± 0.02 | 12.1,(1,364), 0.0005 |
| **Odonata** | 51 | 0.10 ± 0.02 | 5 | 0.01 ± 0.01 | 20.4, (1,364), 0.0001 |

| | | | | | |
|---|---|---|---|---|---|
| **Parasitoids** | 43 | 0.08 ± 0.02 | 21 | 0.04 ± 0.01 | 2.2, (1,364), 0.1349 |
| ***Apis mellifera*** | 41 | 0.08 ± 0.03 | 115 | 0.20 ± 0.03 | 9.9, (1,364), 0.0017 |
| **Phymatidae** | 36 | 0.07 ± 0.02 | 0 | 0.00 ± 0.00 | 20.4,(1,364), 0.0001 |
| **Lepidoptera** | 33 | 0.06 ± 0.01 | 10 | 0.02 ± 0.01 | 9.97,(1,364), 0.0017 |
| **Pompilidae** | 19 | 0.03 ± 0.01 | 0 | 0.00 ± 0.00 | 10.0,(1,364), 0.0017 |
| **Coccinell-idae** | 4 | 0.08±0.004 | 5 | 0.10 ±0.004 | 0.02,(1,364), 0.8768 |
| **Totals** | 6,252 | | 971 | | 21.0,(1,364), 0.0001 |
| **Observations** | 189 | | 191 | | |

**Overall project summary of report 5. June 2012 to January 2013**

We have finished 99% of the research and 70% of the outreach outlined in the proposal with the remaining to be finished by June 30 2013. We are in the process of writing research into peer reviewed publications and outreach materials into online workshops for educational updates for professionals and consumers.

We demonstrated that a standard application rate of imidacloprid is translocated from the soil thru the plant to flower pollen and nectar at sufficient amounts to kill bees or affect their behavior. Floral residues greater than 20 ppb alter behavior and greater than 185 ppb kills bees on ingestion

We demonstrated that bumble bees are more sensitive to imidacloprid and clothianidin compared to honeybees. In imidacloprid-dosed sugar syrup studies in the greenhouse for 11 weeks, control (0 ppb) treatments, had the lowest queen mortality, the highest number of nest bees, the highest nest weight, the greatest production of drones, the greatest weight of stored syrup, the greatest syrup consumption, the greatest per capita sugar consumption, the most honeypots, and the highest worker movement in colonies.

Higher imidacloprid treatments of 50 and 100 ppb resulted in 30% and 50% queen mortality by week 6. These higher treatments of 50 and 100 ppb also had the lowest colony weights, the lowest amount of stored syrup, the lowest number of drones, the lowest number of alive brood at week 8, the lowest number of bees on the nest by week 8, the lowest colony consumption at week 2, 4, and 6 the lowest per capita consumption, and lower movement in colonies.

In 2011, honeybee colonies (n=10 colonies/trt) were provided 0, 50, 100, 200 ppb imidacloprid in sugar syrup for 15 weeks and colonies were assessed five times: June 8, July 6, August 3, August 31, and September 21 for 16 parameters of colony health: frames of bees, open brood, sealed brood, total brood, pollen stores, missing cell count, brood pattern, returning pollen foragers, percent returning foragers, sugar syrup consumption in 48 hrs and 1 week, dead bee counts, *Varroa* numbers, *Nosema* numbers, virus (distorted wing virus(DWV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV)) and queen mortality. *Nosema* fungus numbers and DWV were higher in 200 ppb treatments. Sealed and total brood, pollen stores, and proportion returning foragers were reduced in 200 ppb treatments. However, imidacloprid in stored nectar was 66 % less than that provided in sugar syrup at 50 (24, 66%), 100 (44, 67%), and 200 (96, 66%) ppb indicating detoxification by bees, heat, light, and microbes. The levels of imidacloprid found in nectar are below LD50 of 185-192 ppb found in studies (CA EPA 2009, Bayer, Fischer and Chalmers 2007) and should not kill bees on ingestion. There was no correlation of treatment with dead bee counts and queen replacement was higher in 100 but not 200 ppb treatments from only August 26-Septemeber 22, not the entire experimental period, indicating a nontoxic effect of imidacloprid.

Reduction in returning foragers and pollen stores indicate a sublethal effect of chronic exposure of imidacloprid on bee foraging. These data support the observation from bee keepers that nectar and brood remain in the hive, but foragers are missing. These data support accruing scientific data on the sublethal effects of imidacloprid on bee foraging. In addition, DWV was higher in 20, 50,100, and 200 ppb treatments compared to controls and BQV was higher in 100 ppb treatments.  One phorid fly, *Apocephalus borealis*, was found in the 200 ppb imidacloprid treatment. Microarray analyses of larvae and adult phorids and honey bees from phorid-infected hives revealed that bees are often infected with deformed wing virus and *Nosema ceranae* (Core et al. 2012). A metagenomic analysis of showed that honey bees from CCD-positive colonies had four pathogens: two viruses and two species of microsporidia, Nosema spp. (Cox-Foster et al. 2007).

Until June 30, 2013, for the remaining time of the grant, we will: 1. Finish greenhouse research on how imidacloprid reduces foraging in bumblebees, 2. Write the data into publications, 3. Develop outreach bulletin and poster, and 4. Convene an online workshop on “Mitigating pollinator decline” available for certification credit to Master Gardeners, Master Naturalists, Minnesota Nursery and Landscape Association, and International Society of Arboriculture members.

**Details**

For "Result 1-1: Residue analysis of imidacloprid in pollen and nectar" we determined that label rates of imidacloprid applied to the soil are translocated into pollen and nectar at levels of imidacloprid from 95-1973 ppb in 4 species of bee plants. The residue of imidacloprid in pollen and nectar is high enough to kill bees (greater than 158 ppb kills a bee according to Bayer) or affect their behavior (greater than 20 ppb according to Bayer). *Agastache* giant hyssop mint plants when treated with soil-applied imidacloprid killed bees on first sip on flowers at 2X soil treatments, which are permitted by the label.

For "Result 1-2: Imidacloprid effects on bumblebee colony growth and survival and bumblebee behavior (PERS)", we completed PERS studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory. We found that bumblebee in all imidacloprid treatments (20 ppb, 50 ppb, 100 ppb) except control (0ppb) and 20 ppb treatments had reduced movement in nest boxes. Similar bumblebee studies with clothianidin showed that workers had reduced movement at only 50 ppb and at all treatments except controls produced fewer nectar cells.

For "Result 1-3: Imidacloprid effects on honey bee colony growth and survival", we completed April to October studies on the effects of imidacloprid at 4 doses (0, 50, 100, 200 ppb) on honey bee colony health. In 2011, honeybee colonies (n=10 colonies/trt) were provided 0, 50, 100, 200 ppb imidacloprid in sugar syrup for 15 weeks and colonies were assessed five times: June 8, July 6, August 3, August 31, and September 21 for 16 parameters of colony health: frames of bees, open brood, sealed brood, total brood, pollen stores, missing cell count, brood pattern, returning pollen foragers, percent returning foragers, sugar syrup consumption in 48 hrs and 1 week, dead bee counts, Varroa numbers, Nosema numbers, virus (distorted wing virus(DWV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV)) and queen mortality. Nosema fungus numbers and DWV were higher in 200 ppb treatments. Sealed and total brood, pollen stores, and proportion returning foragers were reduced in 200 ppb treatments. However, imidacloprid in stored nectar was 66 % less than that provided in sugar syrup at 50 (24, 66%), 100 (44, 67%), and 200 (96, 66%) ppb indicating detoxification by bees, heat, light, and microbes. The levels of imidacloprid found in nectar are below LD50 of 185-192 ppb found in studies (CA EPA 2009, Bayer, Fischer and Chalmers 2007) and should not kill bees on ingestion. There was no correlation of treatment with dead bee counts and queen replacement was higher in 100 but not 200 ppb treatments from only August 26-Septemeber 22, not the entire experimental period, indicating a nontoxic effect of imidacloprid

For "Result 2: We will determine through research the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators", we have installed a demonstration plot on the Saint Paul campus. We will add 300 Agastache bee plants to that restoration in fall 2012. In spring 2013 we will develop bulletins and signage for the demonstration site to help people understand how to farm and garden to preserve bees and good bugs. We developed a section on the CUES on conserving bees thru proper plant selection, habitat restoration, and compatible pesticide use. We finished research on the effects of native and bedding plants on bees.

For "Result 3: Outreach talks and workshop", we created a “Pollinator Conservation website” at www.entomology.umn.edu/cues. We are in the process of developing online slide shows, bulletin, and poster for online workshops to be given in spring 2013 for certification credit to Master Gardeners, Master Naturalists, Minnesota Nursery and Landscape Association, and International Society of Arboriculture members.

**Overall project summary of report 4. January 2012 to June 2012**
We have finished 90% of the research outlined in the proposal with the remaining 10% to be done by December 2012. We are in the process of writing this research into peer reviewed publications and outreach materials for professionals and consumers.

We demonstrated that a standard application rate of imidacloprid is translocated from the soil thru the plant to flower pollen and nectar at sufficient amounts to kill bees or affect their behavior.

We demonstrated that bumble bees are more sensitive to imidacloprid and clothianidin compared to honeybees. Bumble bee nests showed reduced food consumption, nest weight and drone production in all concentrations (20, 50, 100 ppb) compared to controls (0 ppb) in 11 week studies. We completed PERS (proboscis extension reflex stimuli) studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory.

We determined that honeybee colonies flying freely in the field from April to October were only affected by imidacloprid at the highest rate of 200 ppb. However, the amount of imidacloprid in stored nectar was reduced by degradation (bees, heat, microbes) from 50 to 6 ppb, from 100 to 25 ppb, and from 200 to 65 ppb.

Until June 30, 2013, for the remaining time of the grant, we will: 1. Finish greenhouse research on how imidacloprid reduces foraging in bumblebees (December 2012), 2. Write the data into publications, 3. Develop outreach bulletins and website, and 4. Convene a workshop on “Mitigating pollinator decline”.

**Details**

For "Result 1-1: Residue analysis of imidacloprid in pollen and nectar" we determined that label rates of imidacloprid applied to the soil are translocated into pollen and nectar at levels of imidacloprid from 95-1973 ppb in 4 species of bee plants. The residue of imidacloprid in pollen and nectar is high enough to kill bees (greater than 158 ppb kills a bee according to Bayer) or affect their behavior (greater than 20 ppb according to Bayer). In our experiments, we were attempting to make a correlation between amount of imidacloprid applied to the soil and amount translocated to pollen and nectar. Levels of imidacloprid residue increase with increasing amount of imidacloprid applied to the soil, but the amount is highly variable between plant species and years. Dr. David Fischer, Bayer CropScience, acknowledges that Bayer cannot model that association either, due to the effects of application method, binding of imidacloprid to organic material in the soil, and other soil factors.

For "Result 1-2: Imidacloprid effects on bumblebee colony growth and survival and bumblebee behavior (PERS)", we completed PERS studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory. We have completed 4 long term studies on the effects of imidacloprid (ld 50=40 ppb) and clothianidin (ld 50=38 ppb) at 5 doses (0, 10, 20, 50, 100 ppb) on bumblebee colony health. We determined that 10, 20, 50, and 100 ppb imidacloprid and clothianidin in sugar syrup fed daily reduce colony growth and feeding in 11 week studies. We determined that imidacloprid and clothianidin had the same effect on bumblebee colonies.

For "Result 1-3: Imidacloprid effects on honey bee colony growth and survival", we completed 2-April to October studies on the effects of imidacloprid at 4 doses (0, 50, 100, 200 ppb) on honey bee colony health. In 2010, sealed brood and returning foragers were reduced at 200 ppb. In 2012, sealed brood was reduced, pollen stores were reduced, and amount of Nosema fungus increased at 200 ppb. We determined that there is a correlation between deformed wing virus (DWV) and imidacloprid (0 ppb, no virus and 50, 100, 200 ppb, significant amount virus). DWV is often found in colonies suffering from CCD (colony collapse disorder). In our research honey bee colonies in the field are fed imidacloprid sugar syrup in feeders attached to the colonies, but also, they can forage for other food. The nectar from stored sugar syrup contains significantly less imidacloprid due to dilution, degradation by the bees, heat, and microorganisms. Sugar syrup at 50 ppb is found as 6 ppb in nectar, 100 ppb as 25 ppb in nectar, and 200 ppb 65 ppb in nectar. These 3 levels should not kill a bee at consumption which is what we observed in the hives.

For "Result 2: We will determine through research the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators", we have installed a demonstration plot on the Saint Paul campus. We will add 300 Agastache bee plants to that restoration in fall 2012. In fall and spring 2012 we will develop bulletins and signage for the demonstration site to help people understand how to farm and garden to preserve bees and good bugs. We will develop a section on the CUES website that we will add reference materials on conserving bees thru proper plant selection, habitat restoration, and compatible pesticide use. We finished research on the effects of native and bedding plants on bees.

For "Result 3: Outreach talks and workshop", we will develop a workshop on protecting pollinators that will be given in spring 2013.

**Overall project summary as of January 2012, report 3**

Pollination of native plants and 35% of food crops require pollinators. In this grant we are focusing on the non-target effects on bees from higher rates of the systemic insecticide imidacloprid that is used in urban landscapes to protect plants from insects. Through research and a model we are determining the relationship between the amounts of insecticide that is applied to soil, how much is translocated to nectar and pollen of plants, and what levels (ppb) kill bees and beneficial insects. The goal of this project is to mitigate pollinator loss through research and education.

As of January 2012, our research progress is substantial. For "Result 1-1: Residue analysis of imidacloprid in pollen and nectar", we have grown 4 plant species *(Rosa*, *Agastache*, *Asclepias*, *Esperanza*) in the field, applied imidacloprid to the soil at 6 doses (0, 25, 50, 300 (1X landscape rate), 600 (2X), 1200 (3X) mg imidacloprid applied) and collected flowers for pollen and nectar. We sent samples to the USDA AMS lab in Gastonia, NC for residue analysis of imidacloprid and 2 metabolites (hydroxy, olefin). For those doses, we field collected dead bees on the flowers. Bees were killed from one sip when feeding on plants of dose 600 and 1200 mg imidacloprid applied to the soil. Residue analysis indicates that those plants contain 1000 ppb (300 mg applied to soil, 1X landscape rate) and 1700 ppb (600 mg applied to soil, can reapply during the season) imidacloprid.

For "Result 1-2: Imidacloprid effects on bumblebee colony growth and survival and bumblebee behavior (PERS)", we have completed PERS studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of .2 ng bee reduces learning and memory. We have completed 2 long terming studies on the effects of imidacloprid at 5 doses (0, 10, 20, 50, 100 ppb) on bumblebee colony health. We are finishing the last long term study on the related neonicotinyl insecticide clothianidin on bumblebee colony health. We have found that at 50 and 100 ppb imidacloprid in sugar syrup in 5-8 weeks bumblebee colonies die earlier and are statistically smaller. We are analyzing the data and preparing a publication.

"Result 1-3: Imidacloprid effects on honey bee colony growth and survival" we have completed 2 full summers of research. We have completed 2 long terming studies on the effects of imidacloprid at 4 doses (0, 50, 100, 200 ppb) on honey bee colony health. Only 200 ppb colonies demonstrated reduced colony health after 16 weeks. We are analyzing the data and preparing a publication.

In Table 1 we predict the relationship between the volume a bee consumes, the amount of imidacloprid in pollen or nectar, and the effect on mortality. Until now the link between these 3 factors was not available. Orange in the body of the table is the amount of insecticide inside a bee that causes mortality. The aqua highlights demonstrate that landscape rates of imidacloprid result in high enough dose of imidacloprid in pollen and nectar to kill bees. Table 2 demonstrates that most neonicotinyl insecticides used in landscape are not toxic to humans, but are highly toxic to bees

For "Result 2: We will determine through research the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators", we have installed a demonstration plot on the Saint Paul campus. In summer 2012 we will develop bulletins and signage for the demonstration site to help people understand how to farm and garden to preserve bees and good bugs.
We have a place on the CUES website that we will add reference materials on conserving bees thru proper plant selection, habitat restoration, and compatible pesticide use.

For "Result 3: Outreach talks and workshop" In summer 2012 we will develop a workshop on protecting pollinators.

**Overall project summary of report 4. January 2012 to June 2012**

We have finished 90% of the research outlined in the proposal with the remaining 10% to be done by December 2012. We are in the process of writing this research into peer reviewed publications and outreach materials for professionals and consumers.

We demonstrated that a standard application rate of imidacloprid is translocated from the soil thru the plant to flower pollen and nectar at sufficient amounts to kill bees or affect their behavior.

We demonstrated that bumble bees are more sensitive to imidacloprid and clothianidin compared to honeybees. Bumble bee nests showed reduced food consumption, nest weight and drone production in all concentrations (20, 50, 100 ppb) compared to controls (0 ppb) in 11 week studies. We completed PERS (proboscis extension reflex stimuli) studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory.

We determined that honeybee colonies flying freely in the field from April to October were only affected by imidacloprid at the highest rate of 200 ppb. However, the amount of imidacloprid in stored nectar was reduced by degradation (bees, heat, microbes) from 50 to 6 ppb, from 100 to 25 ppb, and from 200 to 65 ppb.

Until June 30, 2013, for the remaining time of the grant, we will: 1. Finish greenhouse research on how imidacloprid reduces foraging in bumblebees (December 2012), 2. Write the data into publications, 3. Develop outreach bulletins and website, and 4. Convene a workshop on “Mitigating pollinator decline”.

**Details**
For "Result 1-1: Residue analysis of imidacloprid in pollen and nectar" we determined that label rates of imidacloprid applied to the soil are translocated into pollen and nectar at levels of imidacloprid from 95-1973 ppb in 4 species of bee plants. The residue of imidacloprid in pollen and nectar is high enough to kill bees (greater than 158 ppb kills a bee according to Bayer) or affect their behavior (greater than 20 ppb according to Bayer). In our experiments, we were attempting to make a correlation between amount of imidacloprid applied to the soil and amount translocated to pollen and nectar. Levels of imidacloprid residue increase with increasing amount of imidacloprid applied to the soil, but the amount is highly variable between plant species and years. Dr. David Fischer, Bayer CropScience, acknowledges that Bayer cannot model that association either, due to the effects of application method, binding of imidacloprid to organic material in the soil, and other soil factors.

For "Result 1-2: Imidacloprid effects on bumblebee colony growth and survival and bumblebee behavior (PERS)", we completed PERS studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory. We have completed 4 long term studies on the effects of imidacloprid (ld 50=40 ppb) and clothianidin (ld 50=38 ppb) at 5 doses (0, 10, 20, 50, 100 ppb) on bumblebee colony health. We determined that 10, 20, 50, and 100 ppb imidacloprid and clothianidin in sugar syrup fed daily reduce colony growth and feeding in 11 week studies. We determined that imidacloprid and clothianidin had the same effect on bumblebee colonies.

For "Result 1-3: Imidacloprid effects on honey bee colony growth and survival", we completed 2-April to October studies on the effects of imidacloprid at 4 doses (0, 50, 100, 200 ppb) on honey bee colony health. In 2010, sealed brood and returning foragers were reduced at 200 ppb. In 2012, sealed brood was reduced, pollen stores were reduced, and amount of Nosema fungus increased at 200 ppb. We determined that there is a correlation between deformed wing virus (DWV) and imidacloprid (0 ppb, no virus and 50, 100, 200 ppb, significant amount virus). DWV is often found in colonies suffering from CCD (colony collapse disorder). In our research honey bee colonies in the field are fed imidacloprid sugar syrup in feeders attached to the colonies, but also, they can forage for other food. The nectar from stored sugar syrup contains significantly less imidacloprid due to dilution, degradation by the bees, heat, and microorganisms. Sugar syrup at 50 ppb is found as 6 ppb in nectar, 100 ppb as 25 ppb in nectar, and 200 ppb 65 ppb in nectar. These 3 levels should not kill a bee at consumption which is what we observed in the hives.

For "Result 2: We will determine through research the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators", we have installed a demonstration plot on the Saint Paul campus. We will add 300 Agastache bee plants to that restoration in fall 2012. In fall and spring 2012 we will develop bulletins and signage for the demonstration site to help people understand how to farm and garden to preserve bees and good bugs. We will develop a section on the CUES website that we will add reference materials on conserving bees thru proper plant selection, habitat restoration, and compatible pesticide use. We finished research on the effects of native and bedding plants on bees.

For "Result 3: Outreach talks and workshop", we will develop a workshop on protecting pollinators that will be given in spring 2013.

**Overall project summary as of January 2012, report 3**

Pollination of native plants and 35% of food crops require pollinators. In this grant we are focusing on the non-target effects on bees from higher rates of the systemic insecticide imidacloprid that is used in urban landscapes to protect plants from insects. Through research and a model we are determining the relationship between the amounts of insecticide that is applied to soil, how much is translocated to nectar and pollen of plants, and what levels (ppb) kill bees and beneficial insects. The goal of this project is to mitigate pollinator loss through research and education.

As of January 2012, our research progress is substantial. For "Result 1-1: Residue analysis of imidacloprid in pollen and nectar", we have grown 4 plant species *(Rosa*, *Agastache*, *Asclepias*, *Esperanza*) in the field, applied imidacloprid to the soil at 6 doses (0, 25, 50, 300 (1X landscape rate), 600 (2X), 1200 (3X) mg imidacloprid applied) and collected flowers for pollen and nectar. We sent samples to the USDA AMS lab in Gastonia, NC for residue analysis of imidacloprid and 2 metabolites (hydroxy, olefin). For those doses, we field collected dead bees on the flowers. Bees were killed from one sip when feeding on plants of dose 600 and 1200 mg imidacloprid applied to the soil. Residue analysis indicates that those plants contain 1000 ppb (300 mg applied to soil, 1X landscape rate) and 1700 ppb (600 mg applied to soil, can reapply during the season) imidacloprid.

For "Result 1-2: Imidacloprid effects on bumblebee colony growth and survival and bumblebee behavior (PERS)", we have completed PERS studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of .2 ng bee reduces learning and memory. We have completed 2 long terming studies on the effects of imidacloprid at 5 doses (0, 10, 20, 50, 100 ppb) on bumblebee colony health. We are finishing the last long term study on the related neonicotinyl insecticide clothianidin on bumblebee colony health. We have found that at 50 and 100 ppb imidacloprid in sugar syrup in 5-8 weeks bumblebee colonies die earlier and are statistically smaller. We are analyzing the data and preparing a publication.

"Result 1-3: Imidacloprid effects on honey bee colony growth and survival" we have completed 2 full summers of research. We have completed 2 long terming studies on the effects of imidacloprid at 4 doses (0, 50, 100, 200 ppb) on honey bee colony health. Only 200 ppb colonies demonstrated reduced colony health after 16 weeks. We are analyzing the data and preparing a publication.

In Table 1 we predict the relationship between the volume a bee consumes, the amount of imidacloprid in pollen or nectar, and the effect on mortality. Until now the link between these 3 factors was not available. Orange in the body of the table is the amount of insecticide inside a bee that causes mortality. The aqua highlights demonstrate that landscape rates of imidacloprid result in high enough dose of imidacloprid in pollen and nectar to kill bees. Table 2 demonstrates that most neonicotinyl insecticides used in landscape are not toxic to humans, but are highly toxic to bees

For "Result 2: We will determine through research the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators", we have installed a demonstration plot on the Saint Paul campus. In summer 2012 we will develop bulletins and signage for the demonstration site to help people understand how to farm and garden to preserve bees and good bugs.
We have a place on the CUES website that we will add reference materials on conserving bees thru proper plant selection, habitat restoration, and compatible pesticide use.

For "Result 3: Outreach talks and workshop" In summer 2012 we will develop a workshop on protecting pollinators.

**Table 1. The dose makes the poison: Understanding how much a bee consumes to ppb in nectar or pollen**

| **ppb nectar, pollen or solution** | **standard volume LD 50 studies 1microl** | **Frasier 2011 4 mg pollen/day** | **Frasier 2011 10 mg (=10microl) nectar/day** | **real world bee gut 10 microl** | **real world bee gut 100 microl** | **real world bbee gut 150 microl** | **real world bbee gut 250 microl** |
|---|---|---|---|---|---|---|---|
| | **ng (lab LD50 studies show that >4-40ng (orange) kills a bee)** | | | | | | |
| LD50 solution 40,000 | 40ng | 1600ppb=160 ng | 4000ppb=400ng | 400 | 4,000 | 6,000 | 10,000 |
| LD50 solution 4,000 | 4ng | 160ppb=16 ng | 400ppb=40ng | 40 | 400 | 600 | 1000 |
| LD50 solution | 0.4ng | 16ppb=1.6ng | 40ppb=4.0ng | 4 | 40 | 60 | 100 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 400 | | | | | | | |
| Krischik 12,000ppb 2X milkweed | 12ng | 480ppb =24ng | 1200ppb =120ng | 120 | 1200 | 1800 | 3000 |
| Krischik 6,000ppb 1X milkweed | 6ng | 240ppb =24ng | 600ppb=60ng | 60 | 600 | 900 | 1500 |
| 600ppb | 0.6ng | 24ppb =2.4ng | 60ppb=6ng | 6 | 60 | 90 | 150 |
| 500ppb | 0.5ng | 20 ppb=2ng | 50 ppb=5ng | 5 | 50 | 75 | 125 |
| 250ppb | 0.25ng | 10 ppb=1ng | 25 ppb=2.5ng | 2.5 | 25 | 37 | 62 |
| 200ppb 2011 bee | 0.2ng | 8 ppb=.8ng | 20 ppb=2ng | 2 | 20 | 30 | 50 |
| 100ppb 2011 bee | 0.1ng | 4 ppb=.4ng | 10 ppb=1ng | 1 | 10 | 15 | 25 |
| 50ppb 2011 bee | 0.05ng | 2 ppb=.2ng | 5 ppb=0.5ng | 0.5 | 5 | 7.5 | 12.5 |
| 20ppb Schmuck1999. Bayer alters bee behavior | 0.02ng | 0.8ppb= 0.08ng | 2 ppb= 0.2ng | 0.2 | 2 | 3 | 5 |
| 10ppb pollen loads France | 0.01ng | 0 .4ppb= 0.04ng | 1 ppb= 0.1ng | 0.1 | 1 | 1.5 | 2.5 |
| seed trt 5ppb | 0.005ng | 0.2ppb= 0.02ng | 0.5 ppb= 0.05ng | 0.05 | 0.5 | 0.75 | 0.12 |
| seed trt 2ppb | 0.002ng | 0.02ppb= 0.002ng | 0.05ppb= 0.005ng | 0.02 | 0.2 | 0.3 | 0.5 |
| seed trt 1ppb | 0.001ng | 0.04ppb= 0.004ng | 0.1 ppb=.01ng | 0.01 | 0.1 | 0.15 | 0.25 |

0.2ng/bee= alters memory in bumblebees
4,000 ppb imidacloprid in tree flowers
http://www.cdpr.ca.gov/docs/registration/canot/2009/ca2009-02.pdf ))

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**Table 2. Neonicotinyl insecticides are safer for people, but not bees**

| Active ingredient | Class* | Application method | Toxicity bees | LD50 (µg/bee) | LD 50 (mg/kg rats) higher number means safer for humans |
|---|---|---|---|---|---|
| Imidacloprid | Neo | Oral acute (24–48h) | Highly | 0.004 -.04 | 450 |
| Clothianidin | Neo | Oral acute | Highly | 0.004 | 2000 |
| | | Contact acute | Highly | 0.044 | 4000 |
| Thiamethoxam | Neo | Oral acute | Highly | 0.005 | 1563 |
| | | Contact acute | Highly | 0.024 | 2000 |
| Chlorpyrifos | OP | Acute oral | Highly | 0.36 | 155 |
| | | Acute contact | Highly | 0.070 | 202 |
| Coumaphos | OP | Acute oral | Moderately | 2.030 | 13 - 41 |
| Esfenvalerate | PYR | Acute contact | Highly | 0.21 | 88.5 |
| Fluvalinate | PYR | Acute contact | Highly | 0.2 | 2000 |

*Neo=neonicotinyl, OP=organophospahte, PYR=pyrethroid

**Overall project summary as of June 2011, report 2**

Pollination of native plants and 35% of food crops require pollinators. Colony Collapse Disorder (CCD) and the disappearance of native bees can be attributed to multiple factors such as poor nutrition, diseases, and insecticides. In this grant we are focusing on higher rates of the systemic neonicotinyl insecticide imidacloprid that is used in urban landscapes in flowers and turf.

Research determined that imidacloprid from a label rate of imidacloprid applied to the soil is translocated to nectar and pollen of "Mr. Lincoln" rose at levels of 821 to 1648 ng/g (ppb). This means that if a honey bee or bumblebee eats 4 mg of rose pollen, it will receive a dose of 3-7 ng which will alter bee behavior or kill the bee. We are in the second year of determining imidaclopird levels in rose pollen and the first year for 3 other flowers: hummingbird mint, milkweed, and Texas yellow bells. We are in the process of collecting these data.

For bumblebees in short term lethal dose studies (LD50) we found that bees are killed with a single dose of 4 ppb. In long term greenhouse rearing studies, we found that imidaclopird spiked sugar syrup (0, 10, 20, 50, 100 ppb) caused a significant reduction in brood and worker survival in colonies in all treatments compared to controls. We filmed the bees foraging in their containers to determine if imidacloprid decreases bee foraging. We are analyzing these data.

A standard way to identify the effects of a treatment on learning, is an experiment called PERS, proboscis reflex response stimulus, a study analogous to Pavlov's dogs. Bumblebees are conditioned to stick their tongues out to eat sugar syrup when a puff of odor is directed at them. In the next trial, the bees are given the odor and no food. If the bee remembers it will stick out its tongue and if not it will not stick out its tongue. We found that as little as 0.2 ng imidacloprid, clothianidin, and thiamethoxam (3 common neonicotinyl insecticides) decrease bumblebee memory. We are in the process of analyzing these data.

In long term rearing studies in summer 2010 and 2011, we found that imidaclopird spiked sugar syrup caused a significant reduction in honeybee colony health. In 2010, 5/6 honey bee colonies died in the 200 ppb treatments and had a significant reduction in brood, worker survival, and returning foragers. In 2011, there was no mortality of colonies, but there was a significant reduction in brood and returning foragers in 200 ppb treatments. We are in the process of analyzing these data.

We studied at the MN landscape Arboretum the differences in pollinator recruitment to native and bedding plants. Of the 10 best bee plants, 9 are native. We started to restore a native plant demonstration site on the St. Paul Campus and will add more plants to the site with a walking tour and poster. Recently published bulletins by the Xerces Society and US Golf Association contain lists of bee plants. We will develop from these resources a website for promoting pollinator protection in Minnesota. In 2010-2011, the PI and the graduate student gave talks at numerous meetings in Minnesota to help educate people on pollinator protection. Ms. Judy Wu, the graduate student on the grant, received a prestigious EPA STAR fellowship on the toxicology of imidacloprid to bee immune system function. Her graduate student position was filled by another student, Jamison Scholer from St. Cloud.

**Overall project summary as of January 2011, report 1**

Research will investigate the accumulation of systemic insecticides in nectar and pollen on mortality and behavior of pollinators. Systemic insecticides are applied to the soil, absorbed by the roots, and distributed throughout the plant. Recently, these insecticides were suggested as one factor behind Colony Collapse Disorder (CCD), which is causing enormous loss of honey bees. Also, bumble bees are in decline, which may be due to insecticides used in landscapes.

Systemic neonicotinyl insecticides, such as imidacloprid, are banned in Germany and France for use on corn and canola seed, since the chemical was translocated from seed to nectar and pollen and altered behavior and killed honey bees. In the US, imidacloprid is applied to landscape plants at 800 times higher rate and when the plant is flowering so more chemical is moved to nectar and pollen. Besides our preliminary work at the University of Minnesota, research has not investigated the contribution of these higher levels used in landscapes on pollinator decline.

Outcomes are to mitigate pollinator decline by the development of landscape management recommendations that use insecticides that do not kill pollinators for managing pest insects. Also, for urban landscapes a list of pollinator-friendly plants that provide food throughout the season will be developed through research. Talks, workshops, bulletins, and website on promoting pollinators will be delivered to homeowner and professional communities to help save pollinators. An email listserve to the"Outreach Committee" will disseminate information to change management practices to mitigate pollinator decline.

**Result 1**
**<u>Result 1-1: Residue analysis of imidacloprid in pollen and nectar</u>**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**

**Result status of report 5. June 2012 to January 2013**
**Finished in last report period.**
**A manuscript is being written for publication.**

**Result status of report 4. January 2012 to June 2012**

Data from the USDA AMS Lab in Gastonia, NC determined that samples analyzed by a different chemical method in previous research on imidacloprid residues in milkweed resulted in the same detection of residue levels. Now, these data can be added to our data set which improves the predictions of the research. The imidacloprid residue found in milkweed pollen for 1X (300 mg imidacloprid added to soil) is around 6, 000 ppb and for 2X is around 12,000 ppb (600 mg imidacloprid added to soil). See Table 1 and 2 in detailed section

We sent flower pollen and nectar samples for residue analysis to the USDA AMS lab in Gastonia, NC form 4 plant species (*Rosa-rose*, *Agastache-humingbird mint*, *Asclepias-mikweed, Esperanza-Texas yellowbells* ) grown in the field that had 6 doses of 0, 25, 50, 300 (1X landscape rate), 600 (2X), 1200 (3X) mg imidacloprid applied to the soil. For roses, we compared 2 products; a professional (Marathon 1%G, 300 mg) and homeowner (Bayer 3 in 1).

We determined that label rates (300 mg) of imidacloprid applied to the soil in 4 species of bee plants are translocated into pollen and nectar at levels from 95-1973 ppb. A second and third application of imidacloprid is permitted on the label, which results in a higher residue. The residue of imidacloprid in pollen and nectar is high enough to kill bees (greater than 158 ppb immediately kills a bee according to Bayer) or affect their behavior (greater than 20 ppb according to Bayer). According to the ld50 of 4-40ng/bee, 40–400 ppb should kill a bee. These levels are highlighted in orange in Table 3 and 4. For the experiment, we field collected dead bees on the flowers. Bees were killed from one sip when feeding on plants of dose 600 and 1200 mg imidacloprid applied to the soil (see column 1 in Table 3). These data are summarized in Table 3 and 4 in detailed section below.

In our experiments, we were attempting to make a correlation between amount of imidacloprid applied to the soil and amount translocated to pollen and nectar. Levels of imidacloprid residue should increase with increasing amount of imidacloprid applied to the soil, but the amount is highly variable between plant species and years. On July 18, 2012 Dr. David Fischer, Bayer CropScience, visited our lab. Dr. Fischer acknowledges that Bayer cannot model that association either, due to the effects of application method, binding of imidacloprid to organic material in the soil, and other soil factors.

Our research objectives are realized as we collect data on the relationship of imidacloprid dose applied to the soil, dose translocated to flower pollen and nectar, and mortality of insects feeding on the flowers.

**Result status of report 3. June 2011 to Jan 2012**
We sent flower pollen and nectar samples for residue analysis to the USDA AMS lab in Gastonia, NC form 4 plant species (*Rosa*, *Agastache*, *Asclepias, Esperanza* ) grown in the field that had 6 doses of 0, 25, 50, 300 (1X landscape rate), 600 (2X), 1200 (3X) mg imidacloprid applied to the soil. For those doses, we field collected dead bees on the flowers. Bees were killed from one sip when feeding on plants of dose 600 and 1200 mg imidacloprid applied to the soil. Residue analysis indicates that those plants contain 1000 ppb (300 mg applied to soil) and 1700 ppb (600 mg applied to soil) imidacloprid. Consequently, our research objectives are realized as we collect data on the relationship of imidacloprid dose applied to the soil, dose translocated to flower pollen and nectar, and mortality of insects feeding on the flowers.

Data from the USDA AMS Lab in Gastonia, NC determined that samples analyzed by a different chemical method in previous research on imidacloprid residues resulted in the same detection of residue levels as the methods used by the USDA AMS lab. This permits us to incorporate previous data in our models.

**Result status of report 2. Jan to June 2011**
We have been receiving the residue data from rose from the USDA AMS Lab in Gastonia, NC. The data is being processed using standards provided by the EPA so the data can used for the re-registration of the neonicotinyl insecticides. We found that imidacloprid is translocated to pollen (anthers) from a soil application and the ppb in pollen ranges from 0 to 1648 ppb/g pollen. We performed 2 experiments, one in the field in August 2010 (0, 270mg (1X) homeowner formulation, 540 mg (2X) homeowner formulation, and 300 mg professional formulation. The second experiment was in the GH in the fall (0, 25, 50 150, 300, 600, 1200 mg). The GH experiment showed much lower accumulation of imidacloprid in pollen and we are repeating the experiment in the field in summer 2011. Also, we will investigate the imidacloprid accumulation in pollen and nectar for 3 plants: Esperanza, *Tacomia statens*, Mexican milkweed *Asclepias currasavica*, and hummingbird mint, *Agastache foeniculum* in summer 2011. The plants are growing and we will treat them in mid August with the same doses as the rose experiment.

**Result status of report 1. July 2010 to Jan 2011**
Since August 2010, we investigated how much imidacloprid is translocated to rose anthers (pollen) from a soil treatment of imidacloprid (6 treatments: 0, .25x, .5x, 1x, 2x, 3x). We will plot application rates against residue to get a model for how much is translocated to flowers for different plant species.

I have made a model that demonstrates the relationship between amount of imidacloprid ingested and ppb in plants. The orange line indicates the LD50 and green highlighted areas above the orange indicate mortality. The first vertical column is the limited ppb/plant residue data that is available.

Table. Krischik, V and J. Wu. 2011. Understanding LD50 imidacloprid to bees in relation to ppb in nectar or pollen. In progress. Not yet for publication

| ppb nectar or solution | 1microl ld50 studies | 10 microl | 50 microl | 100 microl hb gut | 150 microl bb gut | 200 microl | 250 microl | 300 microl |
|---|---|---|---|---|---|---|---|---|
| LD50 40,000 | 40ng | 400 | 2,000 | 4,000 | 6,000 | 8,000 | 10,000 | 12,000 |
| LD50 4,000 | 4 ng | 40 | 200 | 400 | 600 | 800 | 1000 | 1200 |
| tree 400 | 0.4 | 4 | 20 | 40 | 60 | 80 | 100 | 120 |
| landscape 100 | 0.1 | 1 | 5 | 10 | 15 | 20 | 25 | 30 |
| landscape 50 | 0.05 | 0.5 | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 |
| landscape 20 | 0.02 | 0.2 | 1 | 2 | 3 | 4 | 5 | 6 |
| pollen loads France 10 | 0.01 | 0.1 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 |
| seed trt 2 | 0.002 | 0.02 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

0.2ng/bee= alters memory in bb PERS
oral LD50 HB imidacloprid 41 ng/bee to 81 ng/bee (Schmuck 1999)
oral LD50 HB imidacloprid 4 to 80 ng/bee (French)
oral LD50 HB imidacloprid 3.7 ng/bee to 41 ng/bee
oral LD50 HB imidacloprid 8 ng/bee
(EPA from http://npic.orst.edu/factsheets/imidacloprid.pdf)
4,000 ppb imidacloprid in tree flowers
(EPA from California, (http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/Imidclprdfate2.pdf
http://www.cdpr.ca.gov/docs/registration/canot/2009/ca2009-02.pdf ))

**Result 1**
**Result 1-2: Imidacloprid effects on bumble bee growth and survival**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**
**Result 1-2: Imidacloprid effects on bumble bee growth and survival**
**Result status of report 5. June 2012 to January 2013**
**We finished the data analysis of the long term studies on the effects of imidacloprid and clothianidin on bumblebee colonies. We are finished the last replicates on the effects of imidacloprid on foraging in large greenhouse cages. A manuscript is being written for publication.**

**Result status of report 4. January 2012 to June 2012**

We completed PERS studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory. We have completed 4 long term studies on the effects of imidacloprid (ld 50=40 ppb) and clothianidin (ld 50=38 ppb) at 5 doses (0, 10, 20, 50, 100 ppb) on bumblebee colony health. We determined that 10, 20, 50, and 100 ppb imidacloprid and clothianidin in sugar syrup fed daily reduce colony growth and feeding in 11 week studies. We determined that imidacloprid and clothianidin had the same effect on bumblebee colonies.

**Result status of report 3. June 2011 to Jan 2012**
We finished 2 replicate experiments with imidacloprid and bumblebee colonies in the greenhouse in fall 2011. We started the same experimental design with the neonicotinyl insecticide clothianidin in January 2012. We found that nest size was lower in 100 ppb and 50 ppb imidacloprid treatments compared to controls (0 ppb) and 10 ppb imidacloprid.

**Result status of report 2. Jan to June 2011**
We have worked out the issues with rearing *Bombus impatiens* in the GH; they need a flight box and must be ordered as small research A with no drones from Koppert, Howell, MI. Most of the research papers from France used *B. terrestris* and started them with single overwintered queens. We tried for 10 mos to get this method to work with *B. impatiens* and failed. We are investigating the effects of imidacloprid dose (0, 10, 20, 50, 100 ppb, 4 colonies each dose, 4 rep exp) on bumblebee colony health and foraging. We are analyzing the videos now. The experiments were started in June and will run for 11 weeks.

**Result status of report 1. July 2010 to Jan 2011**
In 2010, we performed LD 50 experiments for imidacloprid and found that 4 ng/bee or 40 ppb will kill 50% of the bees. Now we have a reference that our lab experiments are similar to published studies. Also, we found thru PERS that 0.2 ng/bee or 2 ppb affects bee memory.

**Result 1**
**<u>Result 1-3: Imidacloprid effects on honeybee growth and survival</u>**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**
**<u>Result 1-3: Imidacloprid effects on honey bee colony growth and survival</u>**

**Result status of report 5. June 2012 to January 2013**
**Finished in last report period. A manuscript is written for publication, submit by September 2013.**

**Result status of report 4. January 2012 to June 2012**
In 2011, the nectar from stored sugar syrup contains significantly less imidacloprid due to dilution, degradation by the bees, heat, and microorganisms. If we take the mean of the 2 samples, stored nectar at 50 ppb is found as 6 ppb in July and 42 ppb in Sept, 100 ppb as 25 ppb in July and 62 ppb in Sept, and 200 ppb as 65 ppb in July and 126 ppb in Sept. (See Table 5 below).

In our research honey bee colonies in the field are fed imidacloprid sugar syrup in feeders attached to the colonies, but also, they can forage for other food. We fed honey bee colonies sugar syrup spiked with 0, 50, 100, 200 ppb imidacloprid (7 colonies each dose) in summer 2010 (Aug 16-Sept 27) and 2011 (April to Sept 27).

The 2010 study is called the Quad, Sai study and the 2011 study is called the Cig, Donnelly study. The 2011 graphs are included below since the 2010 graphs were provided in a previous report. In 2010, we fed the bees pollen patties from April to Aug 15 at 0, 2.3, and 23 ppb. These low doses had no affects on any life history parameter. On August 16 we switched to sugar syrup with 0, 50 100, 200 ppb. See Table 6 below and the following graphs.

In 2010, sealed brood and returning foragers were reduced at 200 ppb. In 2011, sealed brood, total brood, stored pollen, and proportion returning forgers were reduced. The amount of Nosema fungus increased at 200 ppb. We determined that there is a correlation between deformed wing virus (DWV) and imidacloprid (0 ppb, no virus and 50, 100, 200 ppb, significant amount virus). DWV is often found in colonies suffering from CCD (colony collapse disorder).

**Result status of report 3. June 2011 to Jan 2012**
We fed honey bee colonies sugar syrup spiked with 0, 50, 100, 200 ppb (7 colonies each dose) imidacloprid in summer 2011. We can compare these results to our data from summer 2010. By the end of September 2011, sealed brood (SB), pollen area, and brood pattern were lower in 200 ppb colonies. However, there was no difference in colony mortality among treatments.

**Result status of report 2. Jan to June 2011**
We started feeding honey bee colonies sugar syrup spiked with 0, 50, 100, 200 ppb (7 colonies each dose) imidacloprid. Graphs through the second assessment on July 2, 2011 are enclosed. Frames of brood, sealed brood, open brood, and area of pollen are lower in the 100 and 200 ppb treatments compared to the 0 and 50 ppb treatments. As of early August, 3 - 200 ppb colonies died.

**Result status of report 1. July 2010 to Jan 2011**
In 2010, we demonstrated that 200 ppb imidacloprid given in sugar syrup for 3 weeks to a healthy honeybee colony killed the colony. Queen superscedures were 5 times higher than controls. Total brood, pollen stores, and individual worker mortality was higher than in control and 10 ppb treatments. Also, we caged small colonies of bees on 1x, 2x, 20x and 40x treated plants. We found that honeybees in the 20x and 40x treatments did not store nectar or pollen and had less foragers.

**Result 2**
**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**

**Result status of report 5. June 2012 to January 2013**
**We added 400 bee plants to the restoration on the corner of Gortner and the dirt road on the St. Paul Campus of the University of Minnesota. Finished in last report period.**
**A manuscript is being written for publication.**

**Result status of report 4. January 2012 to June 2012**
We studied established plantings at the MN Landscape Arboretum (Chaska, MN) and St. Paul Campus to determine the best plants for seasonal phenology of food for pollinators in MN. Twenty-four stations (divided into 3 plots) were chosen based on proximity to specific plant species in order to obtain replicated samples. These stations were used for data collection on behavioral observations of beneficial insects visiting flowers and sticky trap collection. Teams of 2 observed flowers at each of the 24 flagged station, 8 times each month. Observation duration

was for ten-2 minute intervals between 1000 and 1500 h and the number of insects visiting the flowers and taxa of insect were recorded. Standard yellow sticky traps (Gempler's No bait, length x width: 20.3 x 30.5 cm) were placed at the flagged stations for a 48 h period 4 times a month. From our observations, we will add bee plants to the present restoration on Gortner, north of the greenhouses. We have completed a manuscript that will be submitted soon, where we compared visits of beneficial insects to native and bedding plants.

**Result status of report 3. June 2011 to Jan 2012**
We overwintered 200 *Agastache* mint plants to add to the restoration demonstrate project on the Saint Paul campus.

**Result status of report 2. Jan to June 2011**
Discussion with partners began

**Result status of report 1. July 2010 to Jan 2011**
Discussion with partners began.

**Result 3**
**Result 3: Outreach talks and workshop**

**Result status of report final. January 2013 to June 2013**
**Website, poster, bulletin on bee conservation, and bulletin on insecticides are finished.**

**Result status of report 5. June 2012 to January 2013**
**The website is posted at www.entomology.umn.edu/cues . We are working on the online workshop, poster, and bulletin, which are all in progress.**
The PI and graduate student gave 6 talks on the research: 2 at MN Honey Producers annual Meeting, Duluth, MN; 2 at Minnetonka Naturalists, Ramsey County Master Gardeners, MN State Horticultural Society.

**Result status of report 4. January 2012 to June 2012**
The PI and graduate student gave 6 talks on the research: 2 at MN Honey Producers annual Meeting, Duluth, MN; 2 at Minnetonka Naturalists, Ramsey County Master Gardeners, MN State Horticultural Society.

We will create an updated section on the CUES website for information on the research and best management practices to conserve bees.

We will deliver a workshop in spring 2013 on the results of the grant. We will charge a small registration fee to cover advertising, room, and food. We will spend 5 hours with presentations and a visit to the demonstration project on the St. Paul campus. Travel funds are requested for Mr. Eric Mader, Xerces Insect Conservation Society (Portland, OR) to attend and provide talks at the meeting since he is active in developing legislation and literature on pollinators.

**Result status of report 3. June 2011 to Jan 2012**
The PI and graduate student gave 4 talks on the research at Roseville Garden Club, American Bee Keeping Meeting, National Honeybee Research Meeting, and Department of Entomology, UM.

**Result status of report 2. Jan 2011 to June 2011**

The PI and graduate student gave 2 talks on the research, MN Honey Producers summer meeting

**Result status of report 1. July 2010 to Jan 2011**
In 2010, the PI gave 6 talks on the research in 2010 and the graduate student 3 talks Wild Ones Native Plant Society, Minneapolis; Fruit and Vegetables Conference, St. Cloud; MN Rose Society, Minneapolis; Anoka County Master Gardeners Meeting, Maplewood; MN State Fair Bee keepers, St. Paul; MN Honey Producers summer meeting, Waconia (2 talks), ND bee keepers, Annual American Beekeeping meeting.

## IV. OUTLINE OF PROJECT RESULTS: Detailed data

**Result 1-1: Residue analysis of imidacloprid in pollen and nectar**
Recently, the translocation of systemic neonicotinyl insecticides from roots into nectar and pollen has been suggested as one of the factors behind Colony Collapse Disorder (CCD), which is causing an enormous loss of honey bee colonies. Also, native pollinators (bumble bees) and beneficial insects (lady beetles, lacewings, and wasps) are in decline, which may be due to systemic insecticides in nectar and pollen that the pollinators feed on when foraging. Consumers and professionals use these insecticides to manage pest insects, but the movement into pollen and nectar of these insecticides and effects on pollinators has not been evaluated by research.

Research in France on the seed treatment Gaucho used in corn, sunflower, and canola demonstrated that imidacloprid was translocated to nectar and pollen. The label of Gaucho states that 0.375 mg AI for corn and 0.11 mg AI for canola should be applied. The greenhouse rate used on perennial landscape plants states that 300 mg AI/ 3gallon be used. This is an 800 times higher rate than used on corn and 2700 times higher rate than used on canola. Consequently, greenhouse and urban landscapes use higher concentrations of imidacloprid, which are often reapplied and used at peak flowering, which results in higher concentration being translocated directly to flowers. Consequently, these levels have great potential to alter behavior or kill pollinators and beneficial insects

Pollinators include passive pollinators (lady beetles, lacewings, and parasitic wasps), native pollinators (bumble bees), and managed pollinators (honey bees). Pollinators need to feed on a sugar source, nectar, and a protein sources, pollen, to survive and lay eggs. Systemic insecticides are applied to soil and translocated from roots throughout the plant to nectar and pollen. Insecticide residues that are found in pollen and nectar from rates used on landscape plants based on EPA approved labels, is not known. The effects of these levels of chemicals on pollinator survival and behavior are not known.

**Growing plants and applying imidacloprid to the soil and then collecting flowers to detemine the amount of imidacloprid translocated to flower pollen and nectar**
For all research, we will always perform 2-3 experiments (replicated experiments). We will use 6-10 plants per treatment. These numbers increase the amount of plants used in the experiments, but are necessary for appropriate statistical analysis.

In field research on the St. Paul Campus of the University of Minnesota, imidacloprid will be applied at 3 rates: control, 1X label rate, and 2X label rates to dandelion, rose, and linden trees and clothianidin will be similarly applied to rose. Flowers will be collected from these plants and stored on dry ice and placed in an ultralow freezer to prevent decomposition. The amount of imidacloprid and clothianidin translocated to nectar and pollen will be measured through Liquid Chromatography-Mass Spectrometry residue analysis. The effects on pollinator behavior and mortality will be analyzed when flowers from treated plants are given to pollinators to feed on in controlled bioassay experiments in the lab and greenhouse.

**Residue analysis**

First, from the published literature, we will develop a table of published values of imidacloprid and its metabolites (olefin and hydroxy) and clothianidin translocated to nectar and pollen for different plant species. We will use this information as a reference to compare to the values that we obtain in this research.

We will determine the concentration of clothianidin, imidacloprid and its 2 metabolites (olefin and hydroxy) translocated to nectar and pollen in flowers. We will use Liquid Chromatography-Mass Spectrometry residue analysis as we did in our prior research (Krischik et al. 2007 and Krischik et al. 2009 submitted; and others, such as Laurent and Rathahao 2003). This residue analysis will be conducted by ALS Laboratory Group, Environmental Division, Edmonton, Alberta, Canada, which has performed our residue analysis on 2 plant species for imidacloprid. They can also perform residue work on clothianidin, which is similar to imidacloprid residue analysis.

For residue analysis, each sample of 1.0 g of pollen or nectar (approximately 200 flowers combined from at least 3 vials) will be placed in 15 ml of water in a 50 ml culture tube, followed by an ultrasonic bath for 2 min, then placed on a wrist shaker for 2 hr, filtered, partitioned with dichloromethane, filtered, and evaporated to dryness. The residue will be dissolved in 20% acetonitrile/0.1% acetic acid and brought to 1 ml, frozen, and then extracted with acetonitrile and concentrated with a rotovaporator. The samples will be analyzed by Liquid Chromatography-Mass Spectrometry LC/MS (PE Sciex API 3200 or 4000 Q-trap system) with variant solvent delivery system, and Agilent Automatic Sample Injector. The operating conditions are a YMC-ODS-AM column, 5 µm particle size, 40 ºC, mobile phase A 0.1% acetic acid in water and mobile phase B 0.1% acetic acid in acetonitrile, flow rate 0.5 ml/min, and injection volume 15 µl. Gradient is 0 min 90% A, 10% B: 6.5 min 30% A, 70% B; 8.0 min 50% A, 50% B; 13 min 90% A, 10% B.

The standards will be purchased from Bayer CropSciences (Research Triangle Park, NC) ( lot no. 0625200305, purity 99.2%; hydroxy lot no. 072620061 purity 96.8%; olefin lot no. 12192000301, purity 79.8%). The spiking standards were prepared in 20% acetonitrile/0.1% acetic acid. Samples were fortified with imidacloprid, hydroxy, and olefin at 0.05 and 0.10 ppm. Retention time was 7.75 min for imidacloprid (mass transition 256.6 to 209.0), 7.36 for hydroxy (mass transition 272.0 to 225.0) and 7.24 min for olefin (mass transition 254.0 to 207.0). The limit of quantification for imidacloprid, hydroxy, and olefin was 0.05 ppm based on a 1.0 g sample and final volume of 1.0 ml. The average recovery of imidacloprid, hydroxy, and olefin was 95%, 74%, and 96% respectively at 0.05, 0.10, and 15 ppm.

**Result 1-1: Residue analysis of imidacloprid in pollen and nectar**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**

**Result status of report 5. June 2012 to January 2013**
**Finished and results given in previous report.**

Result status of report 4. January 2012 to June 2012 Data from the USDA AMS Lab in Gastonia, NC determined that samples analyzed by a different chemical method in previous research on imidacloprid residues in milkweed resulted in the same detection of residue levels. Now, these data can be added to our data set which improves the predictions of the research. The imidacloprid residue found in milkweed pollen for 1X (300 mg imidacloprid added to soil) is around 6, 000 ppb and for 2X is around 12,000 ppb (600 mg imidacloprid added to soil). See Table 1 and 2 below.

Table 1

| | USDA-AMS-NSL results in PPM | | | Canadian lab results in PPM | | |
|---|---|---|---|---|---|---|
| Client ID | Imidacloprid | 5 -Hydroxy Imidacloprid | Imidacloprid Olefin | Imidacloprid | 5 -Hydroxy Imidacloprid | Imidacloprid Olefin |
| 1X.1.1-1.4 | 7.57 | 0.157 | 0.386 | 8.20 | 0.565 | 0.405 |
| 1X.1.5-1.7 | 10.4 | 0.177 | 0.495 | 9.4 | 0.565 | 0.390 |
| 1X.2.1-2.4 | 6.43 | 0.241 | 0.471 | 4.80 | 0.720 | 0.500 |
| 1X.2.5-2.7 | 5.44 | 0.198 | 0.410 | 5.10 | 0.510 | 0.360 |
| 2X.1.1-1.3 | 14.7 | 0.393 | 0.504 | 16.0 | 0.710 | 0.410 |
| 2X.1.4-1.8 | 15.6 | 0.436 | 0.609 | 18.0 | 0.940 | 0.710 |
| 2X.2.1-2.4 | 8.02 | 0.256 | 0.437 | 6.40 | 0.300 | 0.200 |
| 2X.2.5-2.9 | 6.93 | 0.192 | 0.460 | 4.60 | 0.250 | 0.180 |
| C.2.4-2.6 | 0.0121 | N.D. | N.D. | N.D. | N.D. | N.D. |
| C.2.7-2.10 | 0.0135 | N.D. | N.D. | N.D. | N.D. | N.D. |
| C.3.5-3.7 | 0.0216 | N.D. | N.D. | N.D. | N.D. | N.D. |
| C.3.8-3.10 | 5.46 | 0.208 | 0.453 | 3.63 | 0.338 | 0.186 |

Table 2

| | USDA-AMS-NSL results in PPM | | | Canadian lab results in PPM | | |
|---|---|---|---|---|---|---|
| Client ID | Imidaclopr id | 5 -Hydroxy Imidacloprid | Imidacloprid Olefin | Imidaclo prid | 5 -Hydroxy Imidacloprid | Imidacloprid Olefin |
| 200 1X | 14.6 | 0.908 | 1.47 | 18.0 | 2.200 | 3.70 |
| 201 1X | 8.80 | 0.527 | 0.946 | 24.00 | 3.400 | 2.900 |
| 202 1X | 22.5 | 1.47 | 2.54 | 26.0 | 3.50 | 2.70 |
| 203 1X | 32.5 | 1.59 | 2.34 | 34.0 | 4.60 | 2.70 |
| 2092X | 36.1 | 1.99 | 2.52 | 42.0 | 6.10 | 2.70 |
| 210 2X | 53.7 | 2.68 | 3.14 | 42.0 | 5.90 | 3.20 |
| 211 2X | 34.0 | 1.79 | 2.11 | 32.0 | 4.20 | 1.90 |
| 212 2X | 43.3 | 1.23 | 2.52 | 68.0 | 8.20 | 4.30 |
| 121 C | 0.0117 | N.D. | N.D. | N.D. | N.D. | N.D. |
| 122 C | 0.0060 | N.D. | N.D. | N.D. | N.D. | N.D. |
| 123 C | 0.0091 | N.D. | N.D. | N.D. | N.D. | N.D. |
| 125 C | 0.0184 | N.D. | N.D. | 0.0390 | N.D. | N.D. |

We sent flower pollen and nectar samples for residue analysis to the USDA AMS lab in Gastonia, NC form 4 plant species (*Rosa*, *Agastache*, *Asclepias, Esperanza* ) grown in the field that had 6 doses of 0, 25, 50, 300 (1X landscape rate), 600 (2X), 1200 (3X) mg imidacloprid applied to the soil. For roses, we compared 2 products; a professional (Marathon 1%G, 300 mg) and homeowner (Bayer 3 in 1).

We determined that label rates (300 mg) of imidacloprid applied to the soil in 4 species of bee plants are translocated into pollen and nectar at levels from 95-1973 ppb. A second and third application of imidacloprid is permitted on the label, which results in a higher residue. The residue of imidacloprid in pollen and nectar is high enough to kill bees (greater than 158 ppb immediately kills a bee according to Bayer) or affect their behavior (greater than 20 ppb according to Bayer). According to the ld50 of 4-40ng/bee, 40–400 ppb should kill a bee. These levels are highlighted in orange in Table 3 and 4. For the experiment, we field collected dead bees on the flowers.

Bees were killed from one sip when feeding on plants of dose 600 and 1200 mg imidacloprid applied to the soil (see column 2 in Table 3). These data are summarized in Table 3 and 4 below.

Table 3

## 2011 Imidacloprid residue plants

| Dose in mg/soil | Dead bees on Agasatche | *Agastache* spp. nectar ppb | *Asclepias* spp. nectar ppb | *Esperanza* spp. nectar ppb | *Rosa* spp. pollen ppb |
|---|---|---|---|---|---|
| 0 | 0.6b | 6b | 3c | 0c | 26b |
| 25 | 0.6b | 52b | 80c | 8c | 36b |
| 50 | 0.5b | 133b | 175bc | 21c | 30b |
| 300 1X 3 gal pot | 1.1ab | 1973b | 1568bc | 106c | 95b |
| 600 2X 3 gal pot | 2.4a | 5265ab | 2950b | 276b | 332b |
| 1200 | 2.4a | 9335a | 8337a | 9162a | 720a |
| | F=3.2, 0.01 | F=3.7, 0.017 | F=25.8, 0.0001 | F=166, 0.0001 | F=5.7, 0.0025 |

Table 4

## 2009, 2010, 2011 Imidacloprid residue rose

| Dose in mg/soil Marathon 1%G | Rose 2009 field | Rose 2010 GH | Rose 2011 field |
|---|---|---|---|
| 0 | 9d | 0c | 26b |
| 25 | na | 5c | 36b |
| 50 | na | 7c | 30b |
| Homeowner 1X 270 mg | 812c | na | na |
| Homeowner 2X 270 mg | 1648a | na | na |
| 300 1X 3 gal pot | 1175b | 32bc | 95b |
| 600 2X 3 gal pot | na | 161ab | 332b |
| 1200 | na | 268a | 720a |
| | F=256, 0.0001 | F=4.9, 0.0045 | F=5.7, 0.0025 |

In our experiments, we were attempting to make a correlation between amount of imidacloprid applied to the soil and amount translocated to pollen and nectar. Levels of imidacloprid residue should increase with increasing amount of imidacloprid applied to the soil, but the amount is highly variable between plant species and years. On July 18, 2012 Dr. David Fischer, Bayer CropScience, visited our lab. Dr. Fischer acknowledges that Bayer cannot model that association either, due to the effects of application method, binding of imidacloprid to organic material in the soil, and other soil factors.

Our research objectives are realized as we collect data on the relationship of imidacloprid dose applied to the soil, dose translocated to flower pollen and nectar, and mortality of insects feeding on the flowers.

**Result status of report 3. June 2011 to Jan 2012**

We are working with the USDA AMS Lab in Gastonia, NC on these data. We are statistically analyzing the data as it arrives. This Table demonstrates that the extraction methods of the Canadian lab used in previous research finds the same levels of imidacloprid in samples.

| | Detections (PPM) | | |
|---|---|---|---|
| Client ID | Imidacloprid | Imidacloprid Olefin | 5 -Hydroxy Imidacloprid |

| | | | | |
|---|---|---|---|---|
| Canadian method | 203 1X | 32.5 | 2.34 | 1.59 |
| USDA method - shaking only | 203 1X | 66.6 | 5.70 | 4.14 |
| USDA method - sample ground | 203 1X | 50.9 | 4.91 | 3.30 |
| Canadian method | 212 2X | 43.3 | 2.52 | 1.23 |
| USDA method - shaking only | 212 2X | 84.6 | 7.24 | 6.08 |
| USDA method - sample ground | 212 2X | 72.6 | 6.13 | 5.37 |

We sent flower pollen and nectar samples for residue analysis to the USDA AMS lab in Gastonia, NC form 4 plant species (Rosa, *Agastache*, *Asclepias, Esperanza* ) grown in the field that had 6 doses of 0, 25, 50, 300 (1X landscape rate), 600 (2X), 1200 (3X) mg imidacloprid applied to the soil. For those doses, we field collected dead bees on the flowers. Bees were killed from one sip when feeding on plants of dose 600 and 1200 mg imidacloprid applied to the soil. Residue analysis indicates that those plants contain 1000 ppb (300 mg applied to soil) and 1700 ppb (600 mg applied to soil) imidacloprid. Consequently, our research objectives are realized as we collect data on the relationship of imidacloprid dose applied to the soil, dose translocated to flower pollen and nectar, and mortality of insects feeding on the flowers.

**JMP ANOVA for dead bees found on field grown plants**

| **Level** | | | **Mean** |
|---|---|---|---|
| 1200 | A | | 2.4000000 |
| 600 | A | | 2.4000000 |
| 300 | A | B | 1.1000000 |
| 0 | | B | 0.6000000 |
| 50 | | B | 0.6000000 |
| 25 | | B | 0.5000000 |

**Result status of report 2. Jan to June 2011**.
We treated dandelions with 0, 1X, and 6X imidacloprid (Menards Grub Killer, 0 .2% imidacloprid). However, 2 weeks after the application in June it became very warm and the dandelion died. We will repeat this experiment next spring. The rose study is started. The linden study will began in spring 2012. Since April 2011, we have been receiving the residue data from rose from the USDA AMS Lab in Gastonia, NC. The data is being processed using standards provided by the EPA so the data can used for the re-registration of the neonicotinyl insecticides.

We found that imidacloprid does get translocated to pollen (anthers) from a soil application and the ppb in pollen ranges from 0 to 1648 ppb/g pollen. We performed 2 experiments, one in the field in August 2010 (0, 270mg (1x) homeowner formulation, 540 mg (2x) homeowner formulation, and 300 mg professional formulation. and one in the GH in the fall ( 0, 25, 50 150, 300, 600, 1200 mg). The GH experiment showed much lower accumulation of imidacloprid in pollen and we are repeating the experiment in the field in summer 2011. Also, we will investigate the imidacloprid accumulation in pollen and nectar for 3 plants: Esperanza, *Tacomia stans*, Mexican milkweed, *Asclepias currasavica*, and hummingbird mint *Agastache foeniculum* in summer 2011. The plants are growing and we will treat them in mid August with the same doses as the rose experiment.

**Result status of report 1. July 2010 to Jan 2011**
We investigated how much imidacloprid is translocated to rose anthers (pollen) from a soil treatment of imidacloprid (6 treatments: 0, .25x, .5x, 1x, 2x, 3x).
We will do this for the other plants in the study to determine the relationship between treatment amount and bioaccumulation of imidacloprid. We can then plot application rates against residue to get a model for how much is translocated to anthers for the different crops and plants.

Anthers were cut from rose flowers and frozen for the 6 0 treatments (0, .25x, .5x, 1x, 2x, 3x) and sent for residue analysis to ALS Lab in Edmonton, Canada and USDA AMS Gastonia, NC. Using 2 labs will permit us to compare results. The EPA has expressed interest in using data analyzed by the USDA lab. With funds from a USDA SARE grant we will have data on the bioaccumulation of imidacloprid in canola. Those pollen samples are currently being analyzed by both labs. These data will be available for the June 2011 report. We will compare these results to our published data as presented in the following table.

**Rose Exp 1 Field 2010**
**USDA analyzed July 27, 2011**

| **Imidacloprid (mg) soil** | **Number** | **Imidacloprid (ppb)** | **Std Error** |
|---|---|---|---|
| 0 | 5 | 10.98 | 62.004 |
| Bayer Homeowner 540 mg, 2X | 6 | 1648.33 | 56.601 |
| Marathon professional 300 mg | 7 | 1116.43 | 52.403 |
| Bayer Homeowner 270 mg 1X | 5 | 821.60 | 62.004 |

**Rose Exp 2 GH 2010**
**USDA analyzed May 3, 2011**

| **Imidacloprid (mg) soil** | **Number** | **Imidacloprid (ppb)** | **Std Error** |
|---|---|---|---|
| 0 | 3 | 0.100 | 1.55 |
| 50 | 2 | 11.350 | 6.86 |
| 300 | 3 | 34.200 | 16.17 |
| 600 | 3 | 88.567 | 24.32 |
| 1200 | 4 | 276.725 | 226.78 |

Table. Comparison of imidacloprid, hydroxy, and olefin levels in nectar of *Asclepias curassivica* (AC) and *Fagopyrum esculentum* (FE)* after a soil application of Marathon 1%G

| Plant species and day after application | C (no. flowers) ppb/fl (flower) | 1X (300 mg) (no. flowers) ppb/fl (flower) | 2X (600 mg) (no. flowers) ppb/fl (flower) |
|---|---|---|---|
| Imidacloprid | | | |
| FE: 21d ppb/ fl | 0 ppb | 16 ppb | 29 ppb |
| AC: 21d ppb/ fl | 0 ppb | 31 ppb | 61 ppb |
| AC: 21d + 7mo, ppb/fl | 0 ppb | 105 ppb | 218 ppb |
| Hydroxy metabolite | | | |
| FE: 21d ppb/ fl | 0 ppb | 2 ppb | 4 ppb |
| AC: 21d ppb/ fl | 0 ppb | 3.2 ppb | 3.5 ppb |
| AC: 21d + 7mo, ppb/fl | 0 ppb | 14.3 ppb | 27.6 ppb |
| Olefin metabolite | | | |
| FE: 21d ppb/ fl | 0 ppb | 0.5 ppb | 1 ppb |
| AC: 21d ppb/ fl | 0 ppb | 1.7 ppb | 2.4 ppb |
| AC: 21d + 7mo, ppb/fl | 0 ppb | 11 ppb | 16 ppb |

*FE data=Krischik, V. A., A. L. Landmark, and G. E. Heimpel. 2007. Soil-applied imidacloprid translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). Environ. Entomol. 36: 1238-1245. AC data in progress, not final report

## Result 1-2: Imidacloprid effects on bumblebee colony growth and survival

We will obtain commercially purchased bumble bee colonies from Koppert Biological Systems (Romulus, Michigan). Koppert supplies *Bombus impatiens* colonies for greenhouse pollination of tomatoes; therefore colonies in any stage of their annual life-cycle can be purchased year round. We can easily rear *B. impatiens*, but due to facility constraints, can only initiate colonies during their normal colony life cycle in MN, between June and late August.

We will follow published protocols to study the effects of the behavior and survivorship of bumble bees (Regali and Rasmont 1995, Tasei et al. 2000, Babendreier et al. 2008). Starting year one (Fall 2009) we will determine if bumble bees can detect  dissolved in sucrose solution, and we will quantify the number and duration of visits to the feeders as a correlate of effects of foraging behavior (Babendreier et al 2008). Thirty large (forager) bumble bee workers from each of four colonies will be individually tagged on the thorax (using commercially available tags for honey bees). The colonies with marked bees will be placed in cages within a greenhouse maintained at 25°C with a 16 light: 8 dark photoperiod. Sugar syrup (50% wt/vol) will be provided in feeders within the cage. After several days, the sucrose solution in the cages will be spiked with imidacloprid; one colony will be treated at 20 ppb (published concentration that affects bee behavior), a second colony with 40 ppb (concentration found in Mexican, *Asclepias currasavica,* nectar), and a third colony at 400 ppb (high dose) (Bayer Chemical Co, Analytical Grade). The fourth colony will serve as a control and the sucrose will not be spiked.  Food solutions will be provided *ad libitum* and feeders will be weighed and replaced daily. In addition, 3.5 g of mixed floral pollen (collected from honey bee colonies and stored frozen) will be provided daily in a Petri dish placed in front of the hive entrance.  Four observation periods will be conducted each day to record each visit and duration of a marked bumble bee at the feeder. The experiment will last for 5 days. The experiment will be repeated three times, using new hives for each replicate. Repeated measures ANOVA will be used to analyze differences in number and duration of bee visits to the feeders across the treatments. In year 2 and 3, these behavioral observations may be repeated using concentrations derived from field studies.

**Imidacloprid effects on bumblebee behavior**

One bioassay commonly used to study learning in bees, and the effects on learning from pesticides or immune challenges, is a classical conditioning paradigm based on the proboscis-extension reflex (Bitterman et al., 1983; Laloi et al., 1999; Masterman et al. 2001). In brief, an individual bee is harnessed in the laboratory and an odor is passed across the bees' antennae. While the odor is being presented, a drop of sucrose solution is touched to one antenna of the bee, which elicits an automatic proboscis-extension response, or PER. The sucrose is then fed to the bee as a reward.  After several presentations of the odor (the conditioned stimulus, CS) followed by the sucrose (unconditioned stimulus, US), the bee learns to anticipate the US upon presentation of the CS alone.  M. Spivak and students have published numerous studies on the use of PER learning in honey bees (e.g., Masterman et al., 2001) and all equipment is available in her lab. Here, we propose to use PER on *B. impatiens*, to study the effects of imidacloprid on learning in bumble bees, which will serve to quantify sub-lethal effects of imidacloprid on these bees.

After the experiments are finished on the colonies used in the greenhouses (above), tagged bumble bees known to have fed on the imidacloprid solutions, will be collected and harnessed in plastic tubes in the laboratory. Only bees that display a PER response to sucrose will be used in learning trials.  After the trials, the bees will be returned to their colonies and will not be tested again. We will compare the bee's acquisition (learning curve) to the presentation of linalool, a floral odor, as the CS over 8 presentations of the CS for 12 seconds (with a 15 minute inter-trial interval).  Depending on the results of the acquisition trials, we can continue with studies of extinction (to quantify memory) and discrimination. (Bitterman et al., 1983; Matserman et al., 2001).

**Behavioral observation of beneficial insects (passive pollinators)**

Insects need to have natural light to forage on flowers. Research bioassays on pollinators will be accomplished in the greenhouse. We will use levels of imidaclopird and clothianidin obtained in residue analysis to determine the effects of these levels found in nectar and pollen on different parameters of insect health (mortality, behavior, colony health, etc.). First, from the published literature, we will develop a table of published LD50 oral and contact values for all species of insects that were tested. We will use this information as a reference to compare the values that we obtain in this research.

Beneficial insects, green lacewing (*Chrysoperla carnea*, 1 species of wasp (*Anagyrus psuedococci*), and 3 species of lady beetles (*Harmonia axyridis*, *Hippodaemia convergens*, *Coleomegilla maculata*) will be ordered from Roncon Vitova Insectaries (Ventura, CA) or field-collected. Procedures developed by Krischik et al (2007, 2009) will be followed. Mesh cages (30 cm × 30 cm × 30 cm) (BioQuip, Rancho Dominquez, CA) will be daily supplied with cut flowers and water. When insects are received and prior to the study they will be conditioned with commercial artificial diet for lacewings and lady beetles (Rincon-Vitova) and 20% honey-water for all species (Aquatube, Syndicate Sales, Kokomo, IN). For 2 weeks, mortality and trembling will be observed 2X daily. Flowers from field studies will be used. At least 10 cages for each treatment will be used and the experiment will be replicated 3 times.

**Result 1-2: Imidacloprid effects on bumblebee colony growth and survival**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**

**Result status of report 5. June 2012 to January 2013**
**We finished the analysis of the effects of imidacloprid and clothianidin on bumblebees for 12 parameters of colony health. Both chemicals reduce colony weight, food consumption, food weight, worker movement, and increase queen mortality.**

**Imidacloprid Figures:**

**Fig. 1.** Queen mortality at week 1 - 11.
**Queen mortality by week**

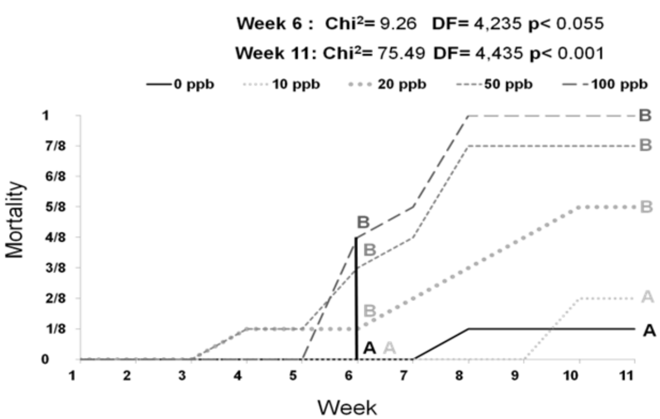


**Fig. 2.** Mean colony weight at week 1 and 11
**Colony weight**

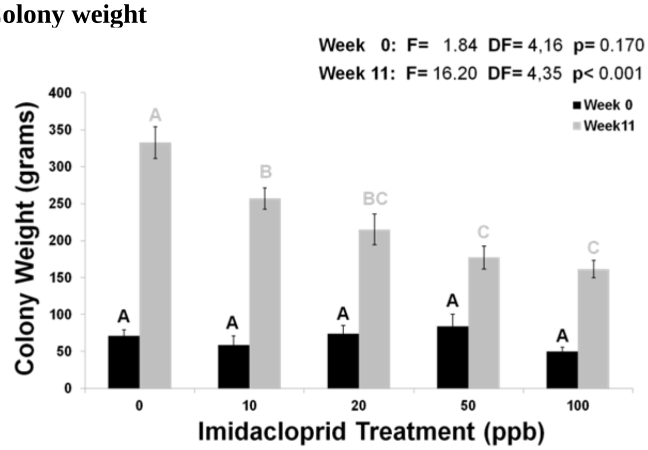


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**Fig. 3.** Stored. syrup (grams) at week 11
**Weight of stored syrup**

**Fig. 4.** Number of bees working on the nest at weeks 0, 2, 4, 6, 8 in an 11 week study.
**Number of bees on nest**

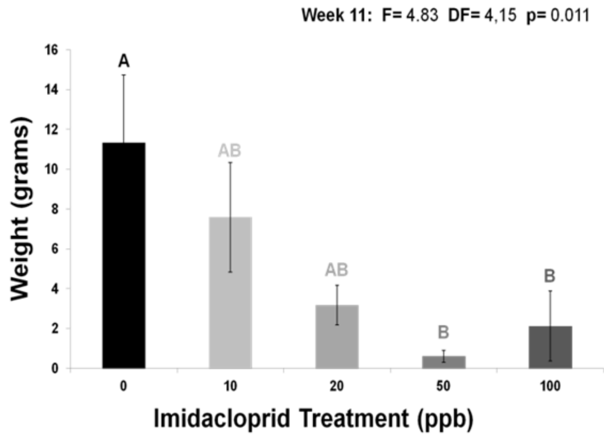



**Fig. 5.** Cumulative number of bees produced from week 1 - 11.**Cumulative bees by caste**

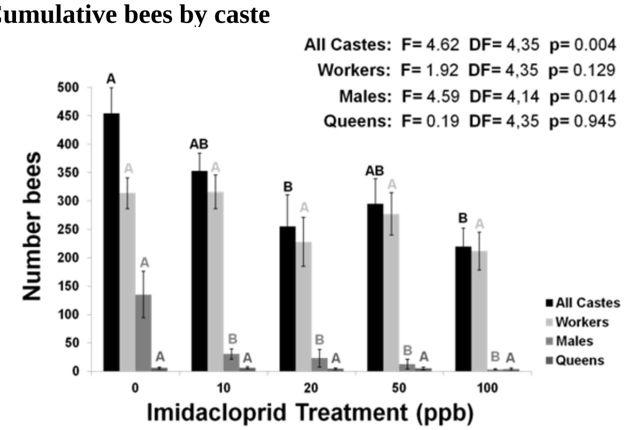
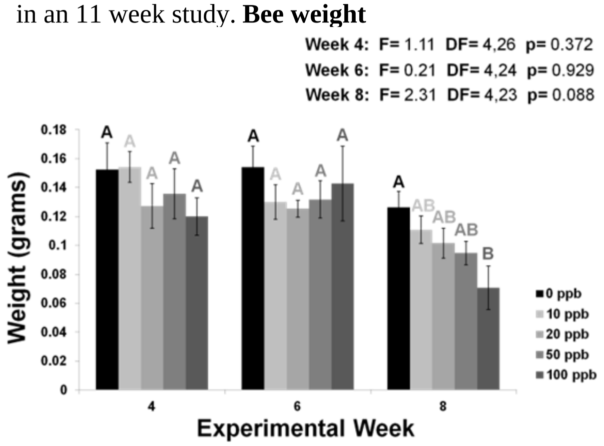



**Fig. 7.** Individual mean bee weights at 4, 6, and 8 weeks in an 11 week study. **Bee weight**




**Individual bee consumption**

**Fig. 9.** Individual bee consumption at 4, 6, and 8 weeks in an 11 week study.

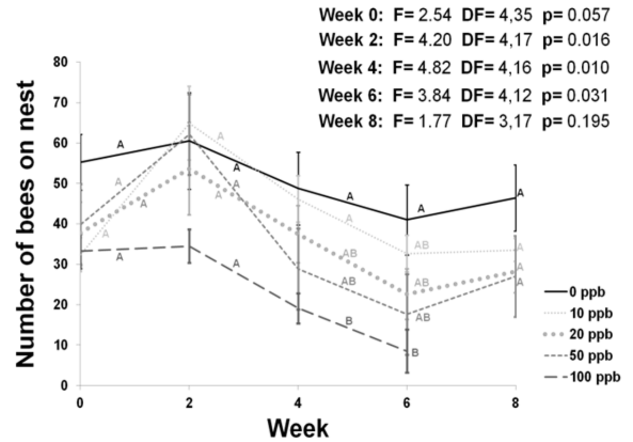



**Fig. 6.** Number of immature bees in nest, week 11 dissection.
**Cumulative total, dead and alive brood**

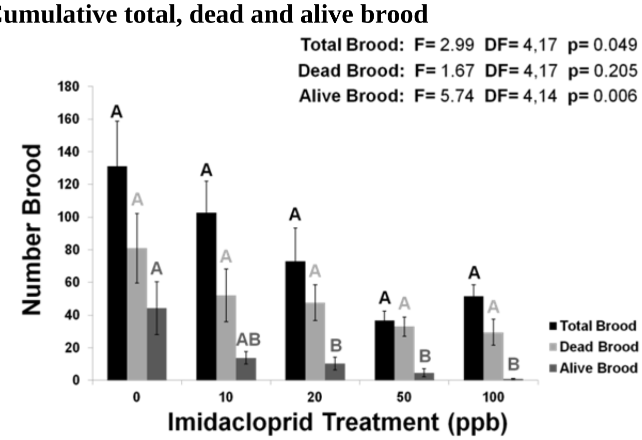
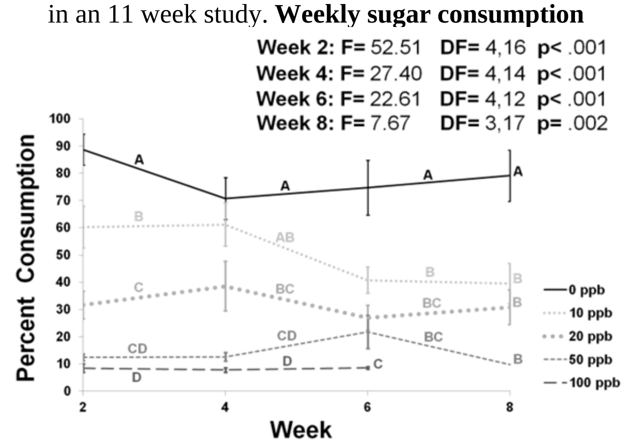



**Fig. 8.** Mean percent syrup consumption at 4, 6, and 8 weeks in an 11 week study. **Weekly sugar consumption**




**Queen movement / 300 seconds**

**Fig. 10.** Mean time queen moved / 300 seconds

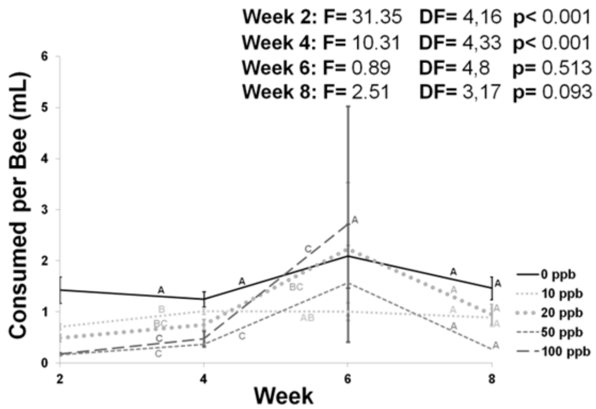
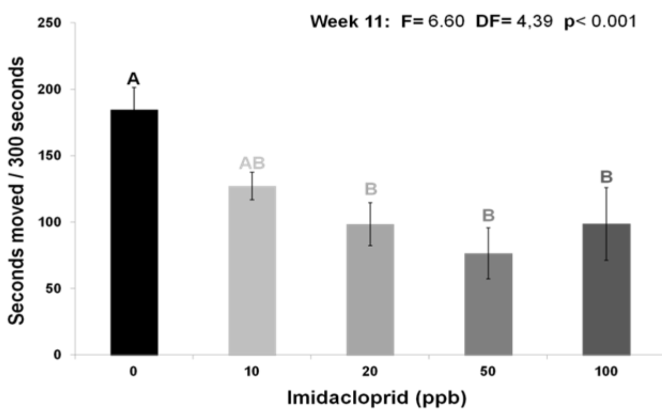



**Fig. 11.** Mean time worker moved / 300 seconds
**Worker movement / 300 seconds**




**Clothianidin Figures:**

**Fig. 1.** Queen mortality at week 1 - 11.
**Queen mortality by week**

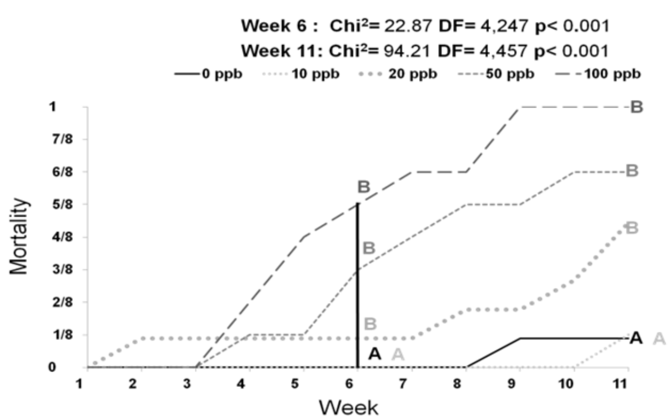



**Fig. 3.** Stored. syrup (grams) at week 11
**Weight of stored syrup**

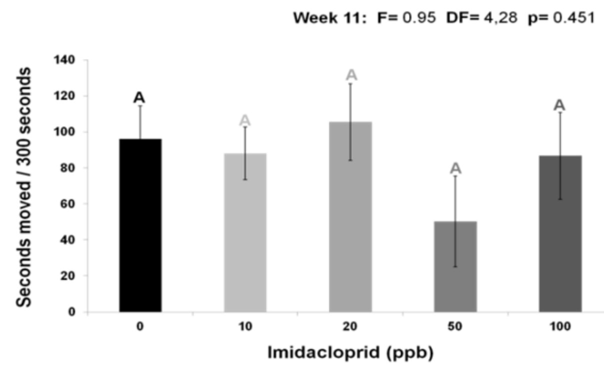



**Fig. 12.** Number of syrup cells present in week 11 - pretreated number of syrup cells
**Syrup cells added over experiment**

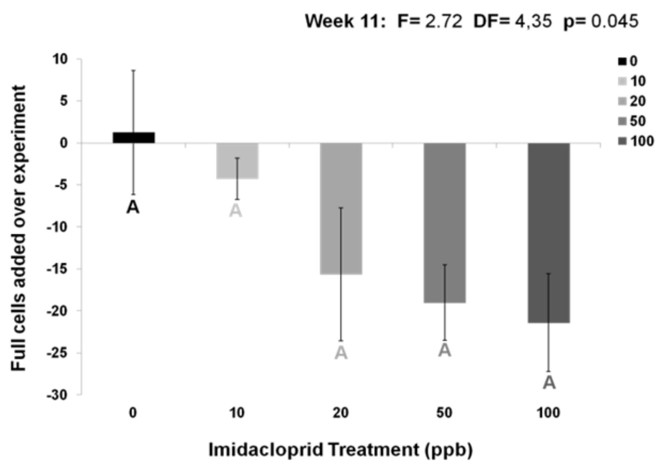



**Fig. 2.** Mean colony weight at week 1 and 11.
**Colony weight**

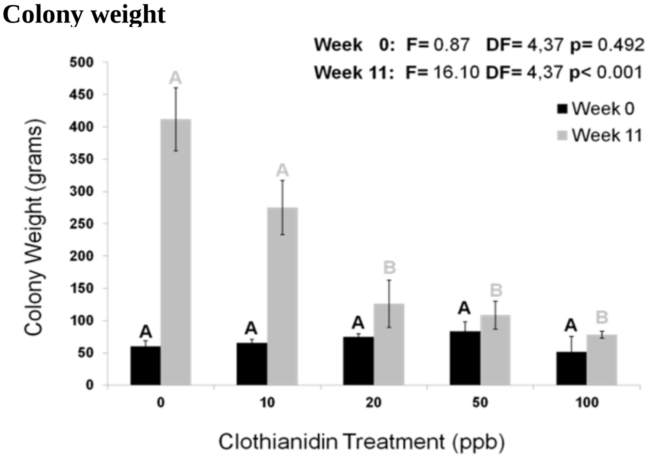
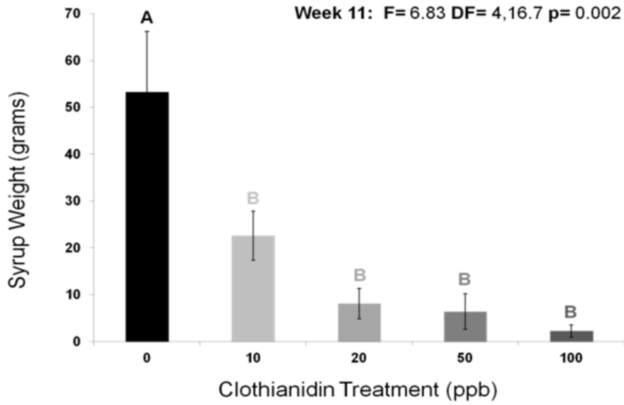



**Fig. 4.** Number of bees working on the nest at weeks 0, 2, 4, 6, 8 in an 11 week study.
**Number of bees on nest**



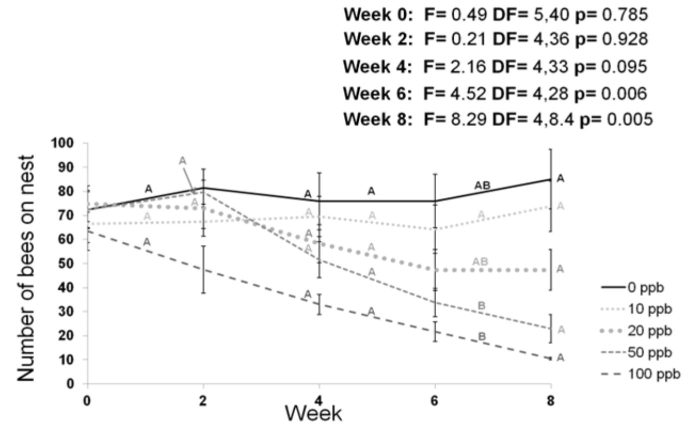




**Fig. 5.** Cumulative number of bees produced from week 1 - 11.**Cumulative bees by caste**

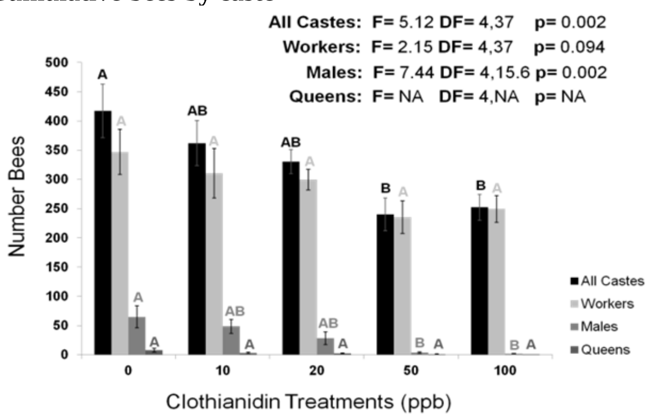



**Fig. 6.** Number of immature bees in nest, week 11 dissection.**Cumulative total, dead and alive brood**

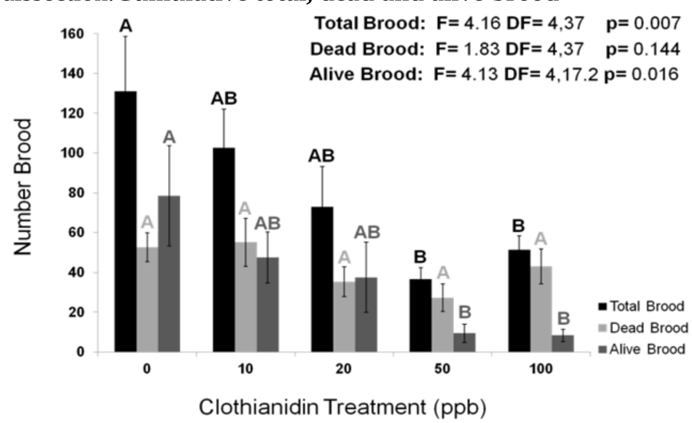



**Fig. 7.** Individual mean bee weights at 4, 6, and 8 weeks in an 11 week study. **Bee weight**

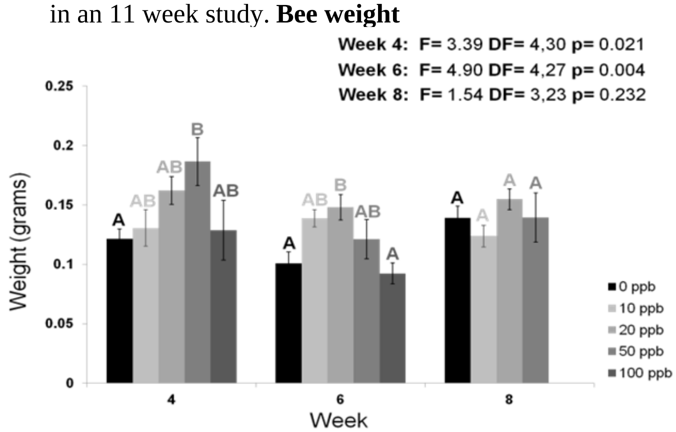



**Fig. 8.** Mean percent syrup consumption at 4, 6, and 8 weeks in an 11 week study.**Colony consumption by week**

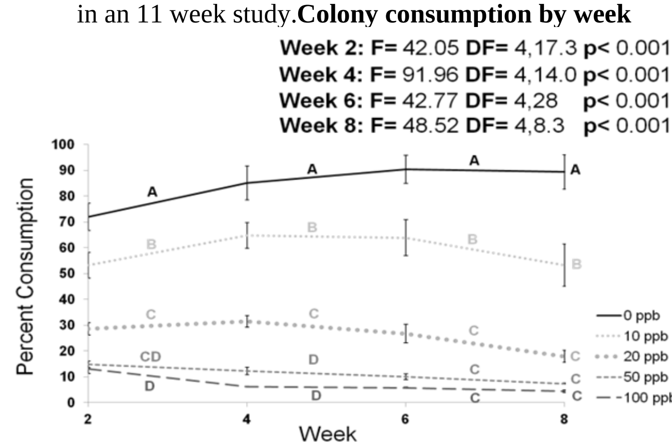



**Fig. 9.** Individual bee consumption at 4, 6, and 8 weeks
**Individual bee consumption**

in an 11 week

**Fig. 10.** Mean time queen moved / 300 seconds
**Queen movement / 300 seconds**

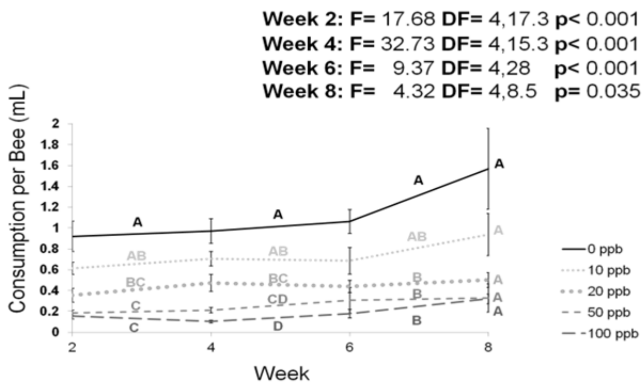
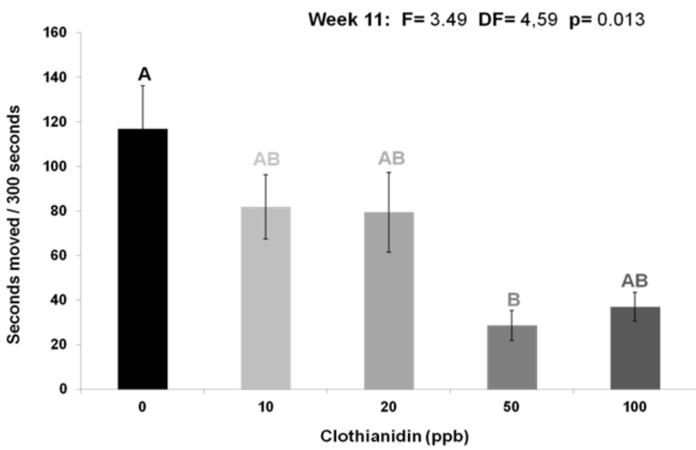



**Fig. 11.** Mean time worker moved / 300 seconds

**Worker movement / 300 seconds**



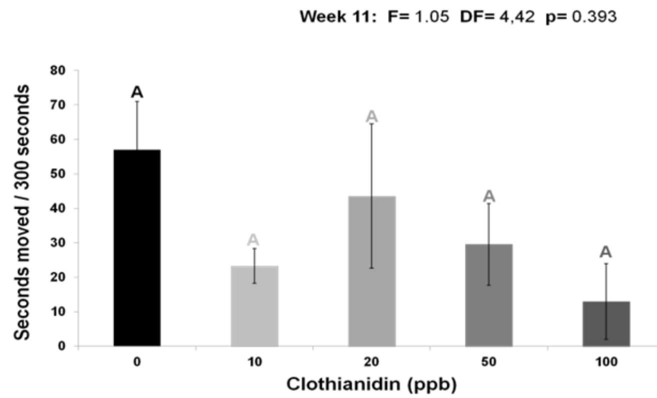
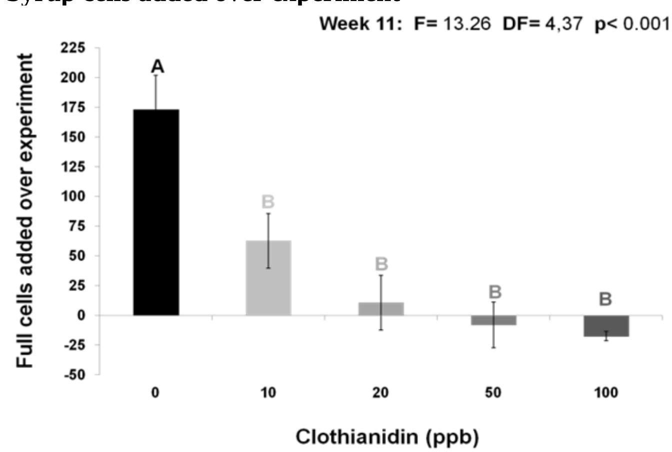




**Fig. 12.** Number of syrup cells present in week 11 - pretreated number of syrup cells

**Syrup cells added over experiment**



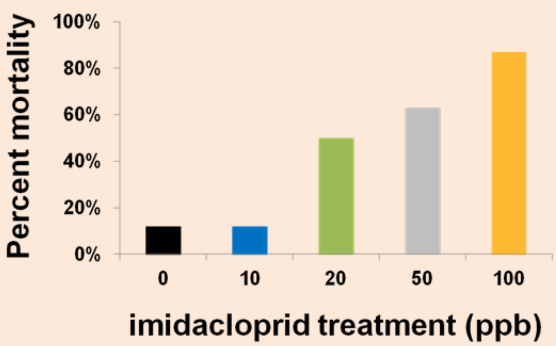

**Result status of report 4. January 2012 to June 2012**

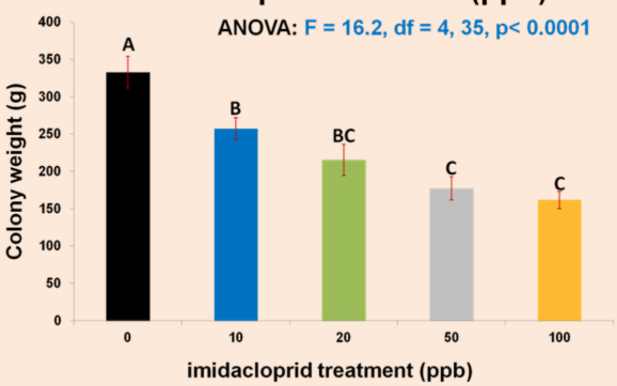
We completed PERS studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory.

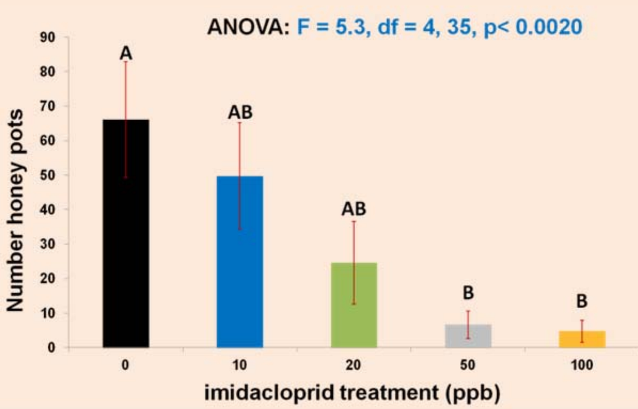
We have completed 4 long term studies (11 weeks) on the effects of imidacloprid (ld 50=40 ppb) and clothianidin (ld 50=38 ppb) at 5 doses (0, 10, 20, 50, 100 ppb) on bumblebee colony health. We determined that 10, 20, 50, and 100 ppb imidacloprid and clothianidin in sugar syrup fed daily reduce colony growth and sugar consumption. Higher doses of imidacloprid reduce colony weight, number of honey pots, number of adult bees, number of males, and sugar consumption. We determined that imidacloprid and clothianidin had the same effect on bumblebee colonies. We are still analyzing the results from clothianidin and will include those data in the next report. Please see Table 5 and the following graphs.

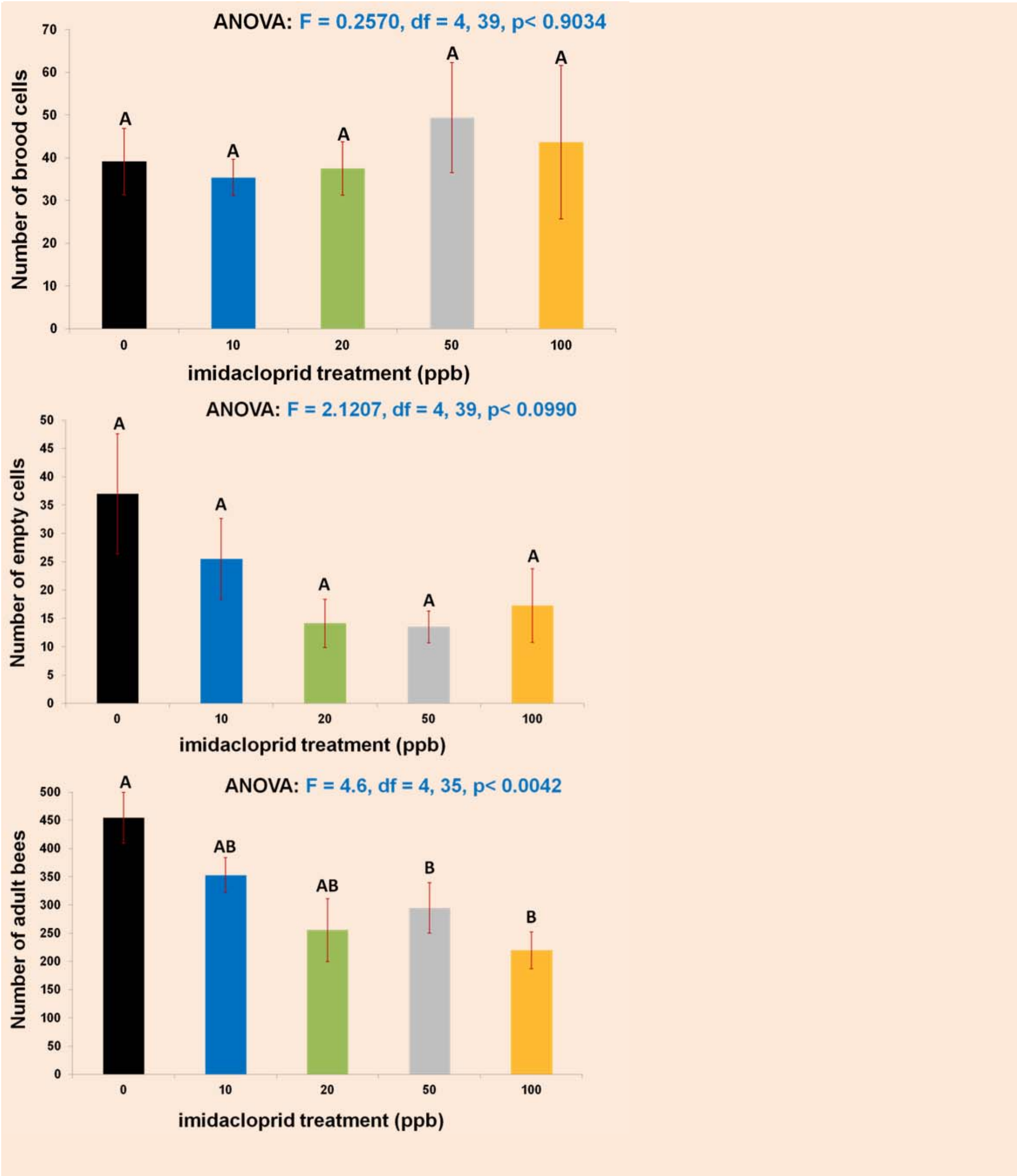
| Table 5 | 2010-2011 | 2011-2012 |
|---|---|---|
| Life history | imidacloprid | clothianidin, Finished, data in |

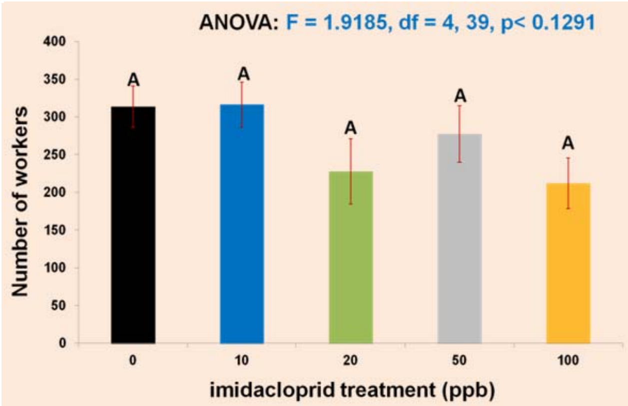
| parameters assessed | | January 2013 report |
|---|---|---|
| Percent mortality | Higher 20,50,100 ppb | |
| Colony weight | P=0.0001, less 10, 20, 50,100 ppb | |
| Number of honey pots | P=0.002, less, 50, 100 ppb, | |
| Number brood cells | NS | |
| Number of empty cells | NS | |
| Number of adult bees | P=0.004, less, 50, 100 ppb | |
| Number workers | NS | |
| Number males | P=0.0003, less 10, 20, 50, 100 ppb | |
| Number queens | NS | |
| Bee weight | P=0.05,less 100 ppb | |
| Percent consumption | P=0.0001, less 10, 20, 50, 100 ppb | |

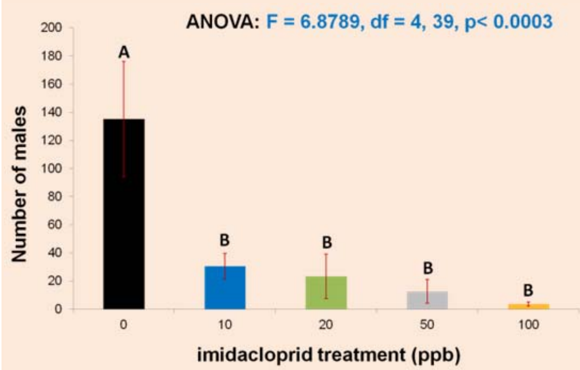


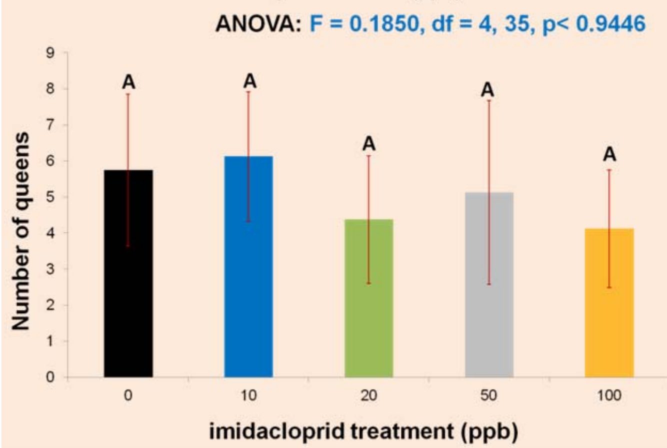



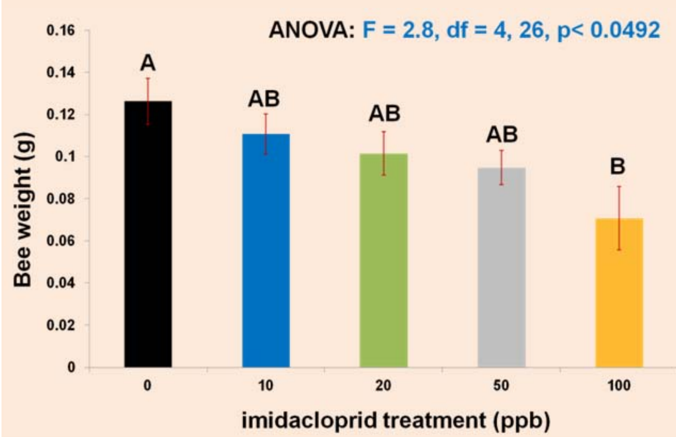



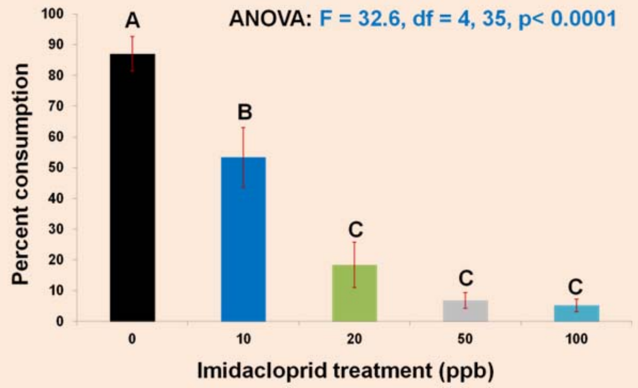

ANOVA: F = 0.2570, df = 4, 39, p< 0.9034
Number of brood cells
70
60
50
40
30
20
10
0
A
A
A
A
A
0
10
20
50
100
imidacloprid treatment (ppb)
ANOVA: F = 2.1207, df = 4, 39, p< 0.0990
Number of empty cells
50
45
40
35
30
25
20
15
10
5
0
A
A
A
A
A
0
10
20
50
100
imidacloprid treatment (ppb)
ANOVA: F = 4.6, df = 4, 35, p< 0.0042
Number of adult bees
500
450
400
350
300
250
200
150
100
50
0
A
AB
AB
B
B
0
10
20
50
100
imidacloprid treatment (ppb)


ANOVA: F = 1.9185, df = 4, 39, p< 0.1291
Number of workers
0
50
100
150
200
250
300
350
400
A
A
A
A
A
0
10
20
50
100
imidacloprid treatment (ppb)


ANOVA: F = 6.8789, df = 4, 39, p< 0.0003
Number of males
0
20
40
60
80
100
120
140
160
180
200
A
B
B
B
B
0
10
20
50
100
imidacloprid treatment (ppb)


ANOVA: F = 0.1850, df = 4, 35, p< 0.9446
Number of queens
0
1
2
3
4
5
6
7
8
9
A
A
A
A
A
0
10
20
50
100
imidacloprid treatment (ppb)


ANOVA: F = 2.8, df = 4, 26, p< 0.0492
Bee weight (g)
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
A
AB
AB
AB
B
0
10
20
50
100
imidacloprid treatment (ppb)




**Result status of report 3. June 2011 to Jan 2012**
**When bumblebee colonies are exposed to 5 concentrations of imidacloprid in 50% sugar water (0, 10, 20, 50, 100 ppb), colonies fed 50 and 100 ppb have lower weight than colonies fed 0 and 10 ppb.**

**JMP ANOVA  for colony weight**

F Ratio    4.4607

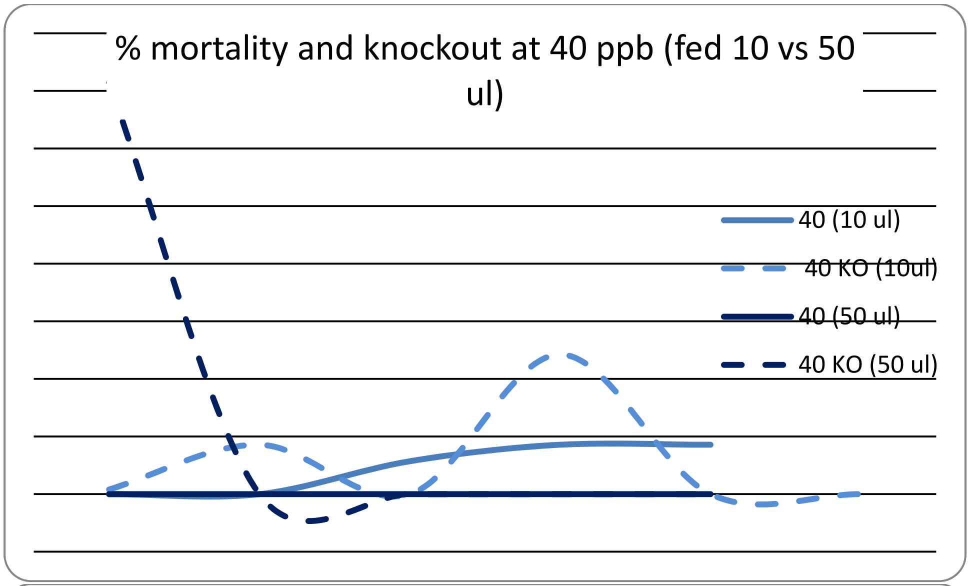
Prob = 0.0051, n=8 colonies

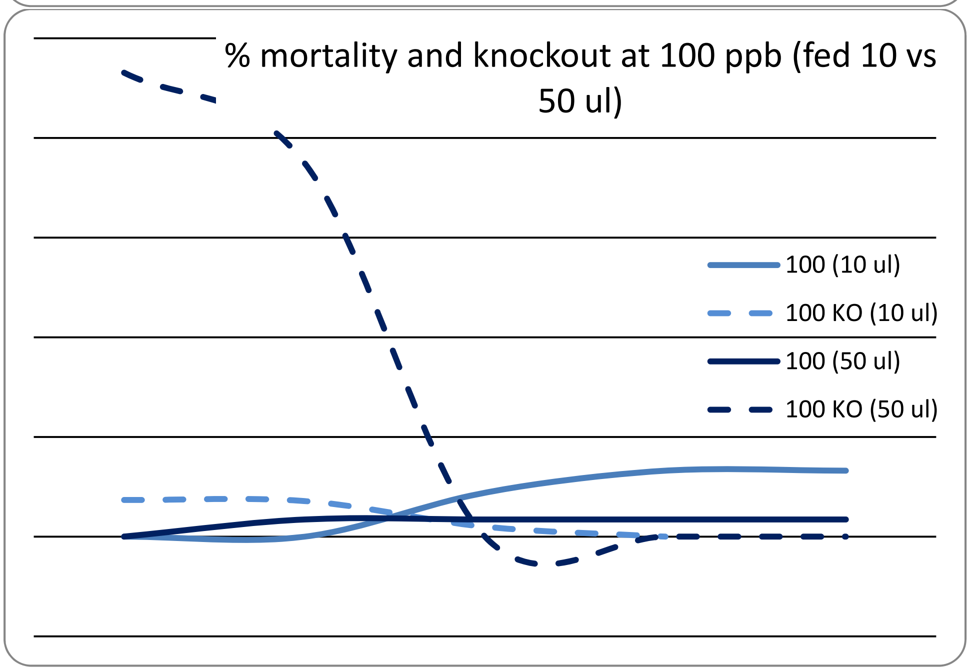
| | | | | Mean |
|---|---|---|---|---|
| 0 | A | | | 803 |
| 10 | A | B | | 769 |
| 20 | | B | C | 701 |
| 100 | | | C | 674 |
| 50 | | | C | 655 |

**Result status of report 2. Jan 2011 to June 2011**.
We worked out the issues with rearing *Bombus impatiens* in the GH; they need a flight box and must be ordered as small research A with no drones from Koppert, Howell, MI. Most of the research papers from France used *B. terrestris* and started them with single overwintered queens. We tried for 10 mos to get this method to work with B. impatiens and failed. We are looking at the effects of imidacloprid dose (0,10, 20, 50, 100 ppb, 4 colonies each dose, 4 rep exp) on bumblebee foraging. We are analyzing the videos now. The experiments were started in June and will run for 11 weeks.

**Result status of report 1. July 2010 to Jan 2011**
We have spent the last 6 months learning how to raise bbees in the GH. We started colonies with overwintering females put in diapause in the incubator at 5C and revived with CO2 for 2-30 min exposures. We placed them in small bee boxes. Exp 1 with 100 females failed. The grower provided 100 more females and 1 month later 20% have started to lay eggs. We decided this is too long a period to maintain the colonies. Now we have made new bee boxes and will use Research A colonies (30 bbee worker's and queen) from Koppert. We will begin this study in June 2011. We have determined the LD50 for imidacloprid and bbees. These data are presented in the following graphs.

Preliminary results with  form J. Wu grad student on the grant:
IMD (fed 10 microliters) Rep 1 & Rep 2
IMD (fed 50 microliters) Rep 1 (Rep 2 in progress)




**Result 1-2: Imidacloprid effects on bee behavior PERS**

**Result status of report 4. January 2012 to June 2012**

PERS studies for imidacloprid, clothianidin, and thiamethoxam, 3 closely related neonicotinyls are finished. Finished and results given in previous report.

**Result status of report 3. June 2011 to Jan 2012**

PERS studies for imidacloprid, clothianidin, and thiamethoxam, 3 closely related neonicotinyls are finished. Data awaits analysis.

**Result status of report 2. Jan 2011 to June 2011**

By Sept 2011, the PERS studies for imidacloprid, clothianidin, and thiamethoxam, 3 closely related neonicotinyls will be finished. So far the first 2 are done and we have 2 more weeks of research for the third chemical. Data show that all 3 chemicals alter bee learning at 0.2 ppb.

**Result status of report 1. July 2010 to Jan 2011**

This research is in the second replicate experiment of imidacloprid. Data will be presented in the next report. However, we have found that at 0.2ng/bee=2 ppb that bees stop learning. We will do PERS for imidacloprid, clothianidin, and thiamethoxam, 3 closely related neonicotinyls.

**Result 1-2: Imidacloprid effects on beneficial insects (passive pollinators)**
**Result status of report 5. June 2012 to January 2013**
Bioassays on beetles were finished and the data are in manuscript.
**Result 1-2: Imidacloprid effects on beneficial insects (passive pollinators)**
**Result status of report 4. January 2012 to June 2012**
Bioassays on beetles were finished and the data are in manuscript.
**Result status of report 3. June to Jan 2012**.
Bioassays on beetles were finished and the data are in manuscript.
**Result status of report 2. Jan 2011 to June 2011**.
These bioassays on beetles, lacewings, and wasps are to begin in fall 2012.
**Result status of report 1. July 2010 to Jan 2011**
These bioassays on beetles, lacewings, and wasps are to begin in fall 2012.

**Result 1-3: Imidacloprid effects on honey bee colony growth and survival**
Based on the residue levels in field, we will treat 36 colonies as follows: one set of 12 colonies will receive a low concentration of imidacloprid (1X, 40 ppb); another 12 colonies will receive a high concentration of imidacloprid (10x, 400 ppb), and the last 12 colonies will be untreated to serve as controls. In the first summer, the imidacloprid will be added to sugar syrup (50% wt/vol) and fed to the colonies. In the second summer, imidacloprid will be added to pollen patties (supplementary protein feed: Mann Lake Beekeeping Supply). The colonies will begin as packages or 3lbs of bees and a queen, and hived in new beekeeping equipment. They will be treated with the antibiotic Fumagillan to treat for *Nosema* sp (a microsporidian), and with ApiGuard to treat for *Varroa destructor* mites. In this way, we will minimize the primary confounding pathogens that negatively affect colony health so we can focus primarily on the effects of the insecticide.

Forty days after the new colonies are initiated, when the adult bees in the colonies have at least doubled in population and brood of all stages (eggs, larvae and pupae) is present, we will begin the sugar syrup or pollen treatments. We will place dead bee traps in front of all colonies to quantify daily mortality of adult bees (dead bees will be counted in the traps every 3 days). We will quantify egg laying rates of queens 3 days and 2 weeks after treatment by confining the queen to one comb within a screened cage for 24 hours and measuring the number of wax cells containing an egg. We will quantify brood viability by counting the number of $5^{th}$ (last) instar larvae, and 10 days later the number of pre-emergence pupae within 3 replicated 100 cell areas. By recording viability of larvae and pupae we can begin to determine if the imidacloprid affects either or both stages of development. We will measure short-term weight gain, an assay highly correlated with honey production. Finally, we will record queen supersedure attempts (rejection by the workers), and any clinical symptoms of disease or parasites.

**Result 1-3: Imidacloprid effects on honey bee colony growth and survival**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**
**Result status of report 5. June 2012 to January 2013**
**We finished the analysis of the effects of imidacloprid on honey bees for 15 parameters of colony health. Imidacloprid reduces returning foragers, pollen stores, colony weight, food consumption, food weight, worker movement, Nosema levels, and distorted wing virus in 200 ppb treatments. The graphs and statistics are 20 pages long and will not be included here. Only the summary table, below, is included.**

| **Summary of 2011 Cig results** Measurements | | | |
|---|---|---|---|
| **June-Sept: 5 assessments**<br>**June 8**<br>**July 6**<br>**Aug 3**<br>**Aug 31**<br>**Sept 22** | **F (df), P**<br>**Month** | **F (df), P**<br>**Treatment** | **F (df), P**<br>**Interaction** |
| **Adult population** (frames of bees) | 53.38 (4, 129), <0.0001) | 1.43 (3, 34). 0.2362 | 1.69 (12, 129), 0.0752 |
| **Sealed brood area** (pupae) | 23.04 (4, 128), <0.0001) | 4.95 (3, 34), 0.0028 | 1.92 (12, 128), 0.0372 |
| **Open brood area** (1-5th instar larvae) | 33.32 (4, 129), <0.0001 | 0.59 (3, 34), 0.6224 | 0.96 (12, 129), 0.4881 |
| **Total brood area** (open + sealed) | 35.35 (4, 129), <0.0001) | 3.06 (3, 34), 0.0307 | 1.50 (12, 129), 0.1303 |
| **Pollen area** | 10.65 (4, 129), <0.0001 | 11.38 (3, 34), <0.0001) | 7.35 (12, 129), <0.0001 |
| **Mean missing cell area** | 7.00 (4, 118), <0.0001 | 3.05 (3, 34), 0.0418 | 1.51 (12, 118), 0.1293 |
| **Brood pattern** | 5.60 (4, 117), 0.0004 | 2.35 (3, 34), 0.0901 | 1.61 (12, 117), 0.0967 |
| ***Nosema* spp. levels** | 15.05 (4, 130), <0.0001 | 4.85 (3, 34), 0.0065 | 2.02, (12, 130), 0.0269 |
| ***Varroa destructor* levels** | 64.18, (3, 96), <0.0001 | 1.49 (3, 34), 0.2358 | 1.09 (9, 96), 0.3761 |
| **Total returning foragers** | 11.17 (2, 64), <0.0001) | 1.79 (3, 34), 0.1670 | 0.78 (6, 64), 0.5881 |
| **Percent returning pollen foragers** | 7.68 (2, 64), 0.0010 | 6.64 (3, 34), 0.0015 | 5.17 (6, 64), 0.3808 |
| **Dead bees** | 32.82 (3, 98), <0.0001 | 0.90 (3, 34), 0.4528 | 1.09 (9, 98), 0.4219 |
| **Consumption 48 hours** | 36.64 (3, 98), <0.0001 | 2.98 (3, 34), 0.0450 | 2.30 (9, 98), 0.0216 |
| **Consumption 1 week** | 29.06 (3, 98), <0.0001 | 3.07 (3, 34), 0.0407 | 2.74 (9, 98), 0.0068 |
| **Queen replacement (Date: ChiSquare, P value)** | Aug 31-Sept 22: 8.02, 0.0456 | Total June-Sept: 3.20, 0.3625 | Sept 22-Jan 12: 6.26, 0.0998 |

**Result 1-3: Imidacloprid effects on honey bee colony growth and survival**

**Result status of report 4. January 2012 to June 2012**

In 2011, the nectar from stored sugar syrup contains significantly less imidacloprid due to dilution, degradation by the bees, heat, and microorganisms. If we take the mean of the 2 samples, stored nectar at 50 ppb is found as 6 ppb in July and 42 ppb in Sept, 100 ppb as 25 ppb in July and 62 ppb in Sept, and 200 ppb as 65 ppb in July and 126 ppb in Sept. (See Table 6 below).

In our research honey bee colonies in the field are fed imidacloprid sugar syrup in feeders attached to the colonies, but also, they can forage for other food. We fed honey bee colonies sugar syrup spiked with 0, 50, 100, 200 ppb imidacloprid (7 colonies each dose) in summer 2010 (Aug 16-Sept 27) and 2011(April to Sept 27).

The 2010 study is called the Quad, Sai study and the 2011 study is called the Cig, Donnelly study. The 2011 graphs are included below since the 2010 graphs were provided in a previous report. In 2010, we fed the bees pollen patties from April to Aug 15 at 0, 2.3, and 23 ppb. These low doses had no affects on any life history parameter. On August 16 we switched to sugar syrup with 0, 50 100, 200 ppb.

In 2010, sealed brood and returning foragers were reduced at 200 ppb. In 2011, sealed brood, total brood, stored pollen, and proportion returning forgers were reduced. The amount of Nosema fungus increased at 200 ppb. We determined that there is a correlation between deformed wing virus (DWV) and imidacloprid (0 ppb, no virus and 50, 100, 200 ppb, significant amount virus). DWV is often found in colonies suffering from CCD (colony collapse disorder). See Table 6 and 7 below and the following graphs.

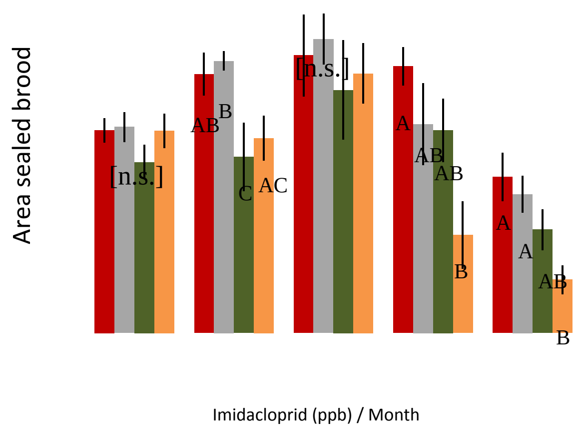
| Table 6 | **2011 Cig, Donelly** | | | | | |
|---|---|---|---|---|---|---|
| | June-Sept 30 | | July | | Sept | |
| | **SS** | **SS** | **nectar** | **nectar** | **nectar** | **nectar** |
| | **18 July imid 34** | **12 Sept imid 34** | **18 July imid 34** | **18 July imid 34** | **12 Sept imid 34B** | **12 Sept imid 34B** |
| 0 ppb SS | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 ppb SS | 33 | 36 | 6 | 6 | 44 | 40 |
| 100 ppb SS | 69 | 66 | 31 | 21 | 33 | 92 |
| 200 ppb SS | 141 | 151 | 38 | 91 | 67 | 185 |

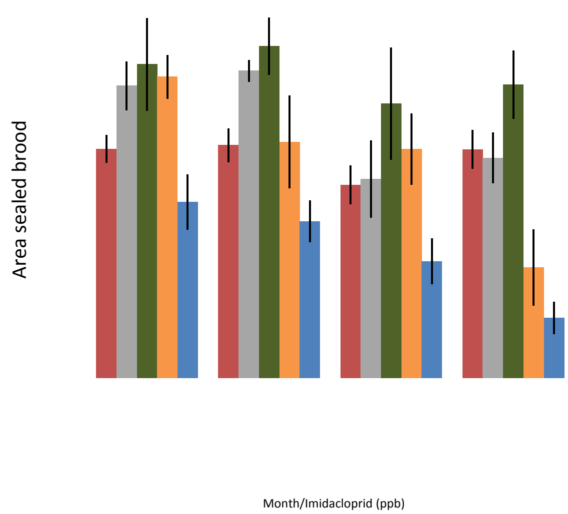
Table 7

| **Life history parameters assessed** | **2010** | **2011** |
|---|---|---|
| | **Sugar Syrup Aug16-Sept 27** | **Sugar Syrup Aug16-Sept 27** |
| Frames of bees | ns | ns |
| Open brood area (1-5$^{th}$ instar larvae) | ns. | ns |
| Area sealed brood area (pupae) | p=0.0301 | P=0.0028 (interaction) |
| Total brood area (open + sealed) | ns | P=0.0307 |
| Stored Pollen | ns | P=0.0001 (interaction) |
| Average missing cell count | Not done | ns |
| Brood pattern | ns | ns |
| *Varroa destructor* mite levels | ns | ns |

| | | |
|---|---|---|
| *Nosema spp.* levels | ns | P=0.0001 |
| Total returning foragers | p=0.0008 (interaction) | ns |
| Returning pollen foragers (proportion) | Not done | P=0.0001 (interaction) |
| Dead bee count | ns | ns |
| Virus | | |
| Israeli paralysis virus | not done | ns |
| Deformed wing virus (DWV) | not done | P=0.0001 |
| Black queen virus | not done | ns |

**Sealed brood area**



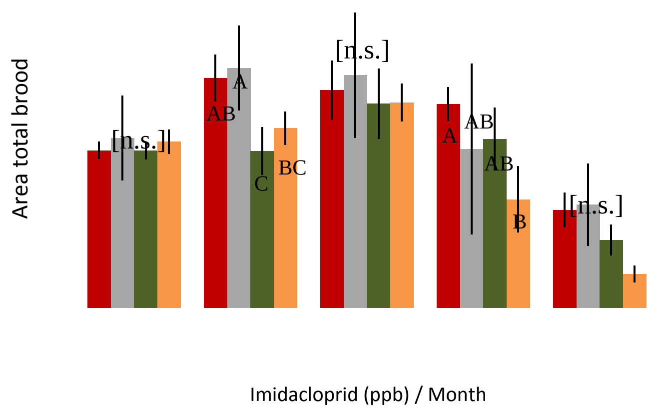

| SB area (cm^2) | Num df | Den df | P value | F value |
|---|---|---|---|---|
| month | 4 | 128 | <0.0001 | 23.04 |
| trt | 3 | 34 | ***0.0028*** | 4.95 |
| month*trt | 12 | 128 | ***0.0372*** | 1.92 |

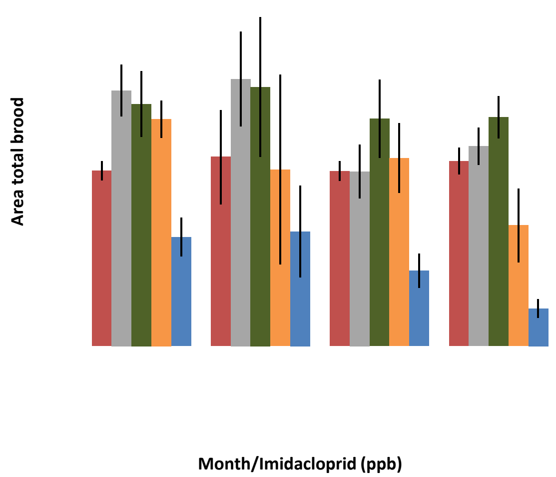
***Interaction: main effect not ok***

**SEALED BROOD AREA**

| | June | | | | July | * p=0.026 | | | August | | | | early September | | | *p<.0001 | late September | | | *p=0.021 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ppb | n | mean | ± st err | | n | mean | ± st err | | n | mean | ± st err | | n | mean | ± st err | | n | mean | ± st err | |
| 0 | 10 | 3439 | ± 430 | A | 10 | 4387 | ± 430 | AB | 10 | 4710 | ± 430 | A | 10 | 4521 | ± 430 | A | 10 | 2645 | ± 430 | A |
| 50 | 9 | 3495 | ± 454 | A | 9 | 4614 | ± 454 | B | 9 | 4984 | ± 454 | A | 9 | 3544 | ± 454 | AB | 9 | 2354 | ± 454 | A |
| 100 | 9 | 2900 | ± 454 | A | 9 | 2986 | ± 454 | C | 7 | 4013 | ± 510 | A | 7 | 3331 | ± 510 | AB | 7 | 1646 | ± 510 | AB |
| 200 | 10 | 3430 | ± 430 | A | 10 | 3306 | ± 430 | AC | 10 | 4403 | ± 430 | A | 10 | 1662 | ± 430 | B | 9 | 807 | ± 430 | B |

## Total brood area

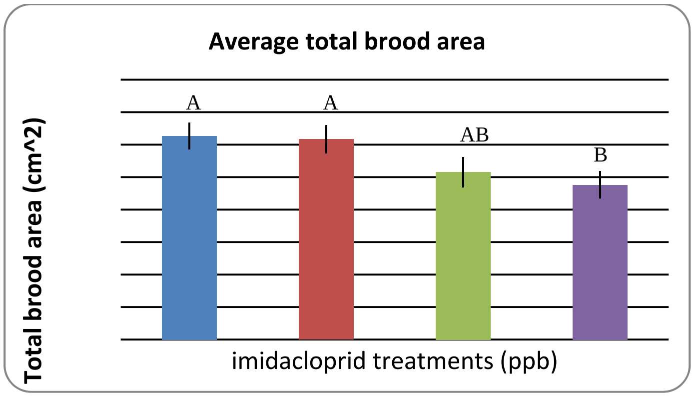



| Total brood area (cm^2) | Num df | Den df | P value | F value |
|---|---|---|---|---|
| month | 4 | 129 | <0.0001 | 35.35 |
| trt | 3 | 34 | ***0.0307*** | 3.06 |
| month*trt | 12 | 129 | 0.1303 | 1.5 |

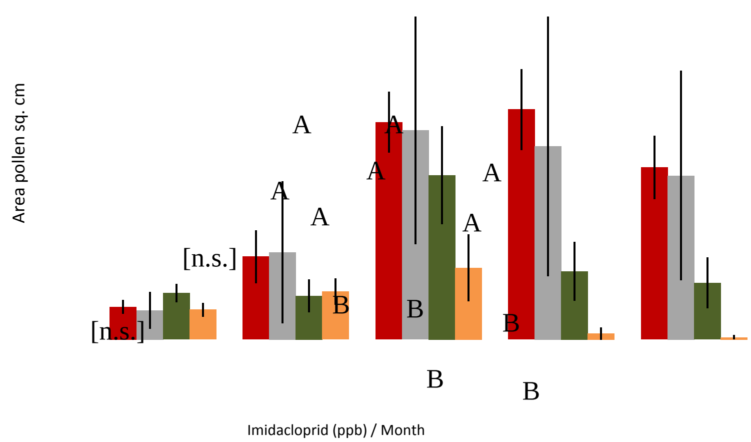
| TOTAL BROOD AREA | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | | | | July | | | *p=0.0134 | August | | | | early September | | | *p=0.0106 | late September | | | |
| ppb | n | mean | ± st err | | **n** | **mean** | **± st err** | | n | mean | ± st err | | **n** | **mean** | **± st err** | | n | mean | ± st err | |
| 0 | 10 | 5441 | ± 687 | A | **10** | **7937** | ± 687 | **AB** | 10 | 7517 | ± 687 | A | **10** | **7050** | ± 687 | **A** | 10 | 3383 | ± 687 | A |
| 50 | 9 | 5872 | ± 724 | A | **9** | **8292** | ± 724 | **A** | 9 | 8043 | ± 724 | A | **9** | **5482** | ± 724 | **AB** | 9 | 3117 | ± 724 | A |
| 100 | 9 | 5432 | ± 724 | A | **9** | **5422** | ± 724 | **C** | 7 | 6959 | ± 724 | A | **7** | **5732** | ± 816 | **AB** | 7 | 2241 | ± 816 | A |
| 200 | 10 | 5747 | ± 687 | A | **10** | **6206** | ± 687 | **BC** | 10 | 7099 | ± 687 | A | **10** | **3750** | ± 687 | **B** | 9 | 993 | ± 687 | A |

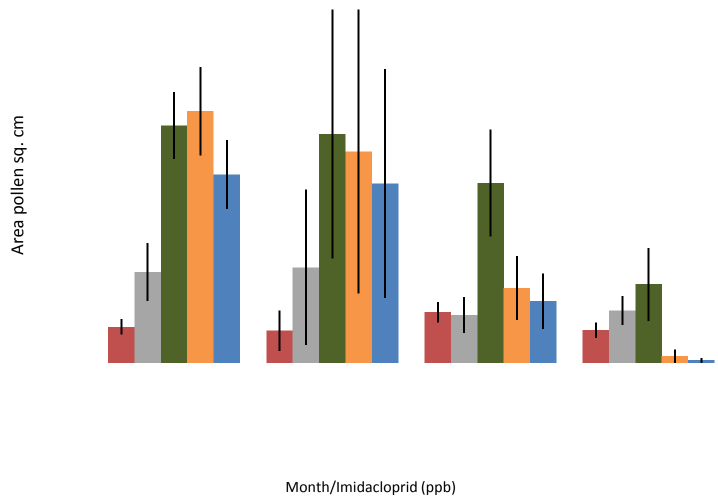
***No interaction: main effect ok***




| Treatment | n | mean | ± st err | Tukey's HSD |
|---|---|---|---|---|
| Control | 10 | 6265.74 | 415.79 | A |
| 50 ppb IMD | 9 | 6161.28 | 438.28 | A |
| 100 ppb IMD | 9 | 5157.31 | 462.26 | AB |
| 200 ppb IMD | 10 | 4758.48 | 418.04 | B |

**Pollen stores**



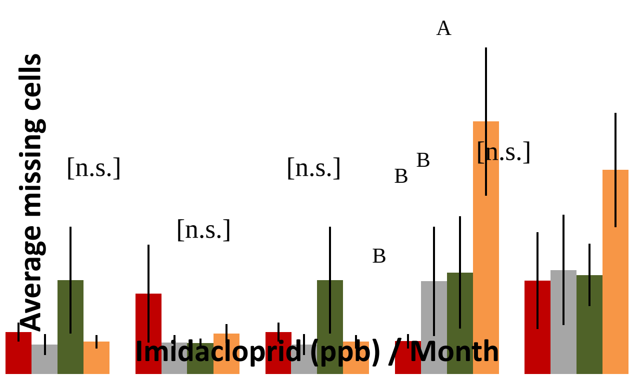


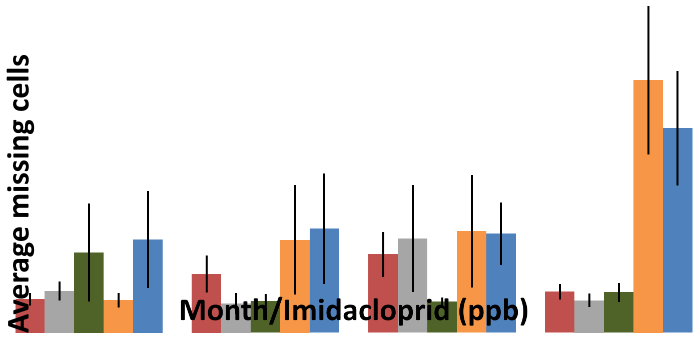

| Pollen area (cm^2) | Num df | Den df | P value | F value |
|---|---|---|---|---|
| month | 4 | 128 | <0.0001 | 10.65 |
| trt | 3 | 34 | ***0.0001*** | 11.33 |
| month*trt | 12 | 128 | ***0.0001*** | 7.35 |

***Interaction: main effect not ok***

| POLLEN AREA | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | | | | July | | | | August *p=0.0002 | | | | early September *p<0.0001 | | | | late September *p<0.0001 | | | |
| ppb | n | mean | ± st err | | n | mean | ± st err | | n | mean | ± st err | | n | mean | ± st err | | n | mean | ± st err | |
| 0 | 10 | 453 | ± 343 | A | 10 | 1141 | ± 343 | A | 10 | 2986 | ± 343 | A | 10 | 3163 | ± 343 | A | 10 | 2366 | ± 343 | A |
| 50 | 9 | 404 | ± 361 | A | 9 | 1200 | ± 361 | A | 9 | 2876 | ± 361 | A | 9 | 2657 | ± 361 | A | 9 | 2077 | ± 361 | A |
| 100 | 9 | 638 | ± 361 | A | 9 | 601 | ± 361 | A | 7 | 2208 | ± 395 | A | 7 | 888 | ± 395 | B | 7 | 726 | ± 395 | B |
| 200 | 10 | 411 | ± 343 | A | 10 | 661 | ± 343 | A | 10 | 988 | ± 343 | B | 10 | 84 | ± 343 | B | 9 | 1 | ± 354 | B |

**Missing cell count (out of 100 brood cells)**



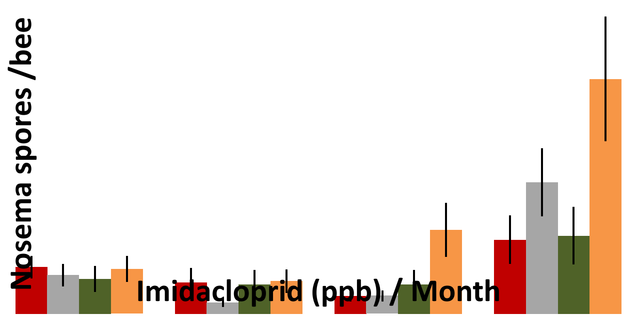


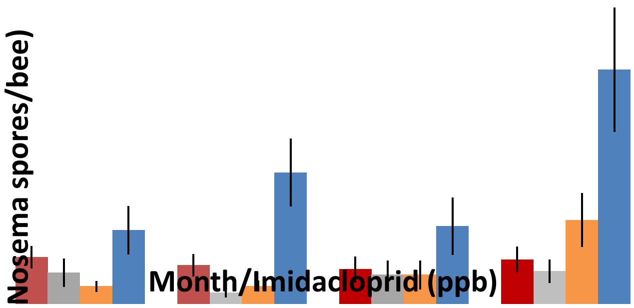

| Missing cells | Num df | Den df | P value | F value |
|---|---|---|---|---|
| month | 4 | 128 | <0.0001 | 7.02 |
| trt | 3 | 34 | ***0.071*** | 2.4 |
| month*trt | 12 | 128 | ***0.0094*** | 2.35 |

***Interaction: main effect not ok***

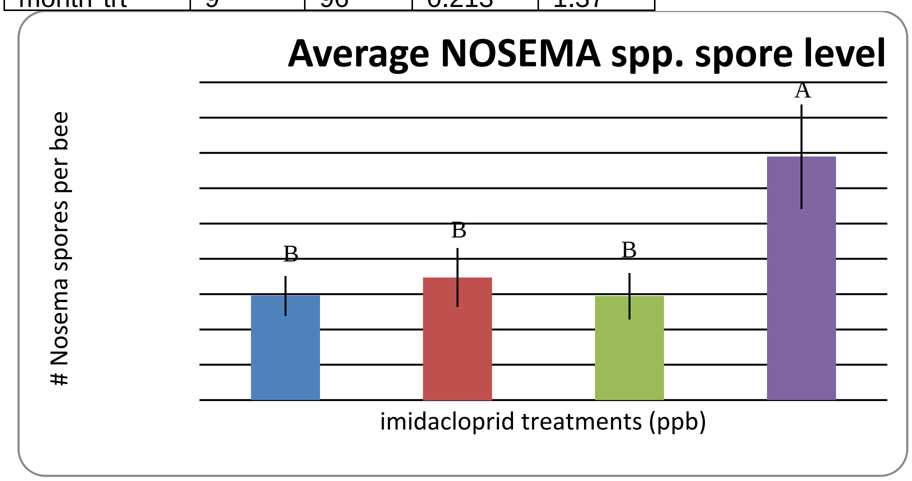
| AVERAGE MISSING CELLS (out of 100) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | | | | July | | | | August | | | | **early September** | | | **p=0.0003** | late September | | | |
| ppb | n | mean | ± st err | | n | mean | ± st err | | n | mean | ± st err | | **n** | **mean** | **± st err** | | n | mean | ± st err | |
| 0 | 10.0 | 6.5 | ± 6.9 | A | 10.0 | 8.1 | ± 6.9 | A | 10.0 | 15.5 | ± 6.9 | A | **10.0** | **6.3** | **± 6.9** | **B** | 10.0 | 18.0 | ± 6.9 | A |
| 50 | 9.0 | 11.3 | ± 7.3 | A | 9.0 | 5.6 | ± 7.3 | A | 9.0 | 6.0 | ± 7.3 | A | **9.0** | **17.9** | **± 7.3** | **B** | 9.0 | 20.1 | ± 7.3 | A |
| 100 | 9.0 | 15.1 | ± 7.3 | A | 9.0 | 18.1 | ± 7.3 | A | 7.0 | 7.3 | ± 8.2 | A | **7.0** | **20.9** | **± 8.2** | **B** | 7.0 | 20.4 | ± 8.2 | A |
| 200 | 10.0 | 7.6 | ± 7.3 | A | 10.0 | 6.2 | ± 7.3 | A | 10.0 | 7.8 | ± 6.9 | A | **10.0** | **48.7** | **± 6.9** | **A** | 9.0 | 40.0 | ± 7.3 | A |

***Nosema spp.* spore levels**





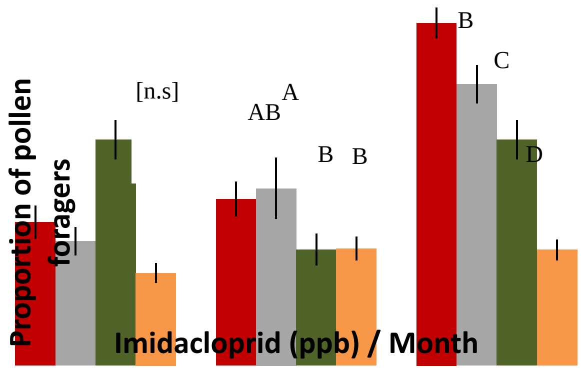

| *Nosema spp.* | Num df | Den df | P value | F value |
|---|---|---|---|---|
| month | 3 | 96 | <0.0001 | 14.33 |
| trt | 3 | 34 | ***<0.0001*** | 7.85 |
| month*trt | 9 | 96 | 0.213 | 1.37 |

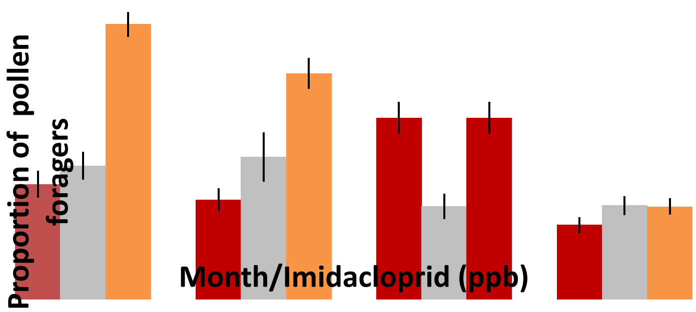



| Treatment | n | mean | ± st err | Tukey's HSD |
|---|---|---|---|---|
| Control | 10 | 590000 | 112876 | B |
| 50 ppb IMD | 9 | 694444 | 166462 | B |
| 100 ppb | 9 | 588333 | 130333 | B |
| 200 ppb | 10 | 1378750 | 295250 | A |

**Proportion of returning pollen foragers**

A



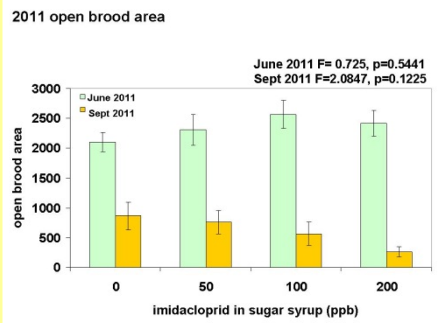



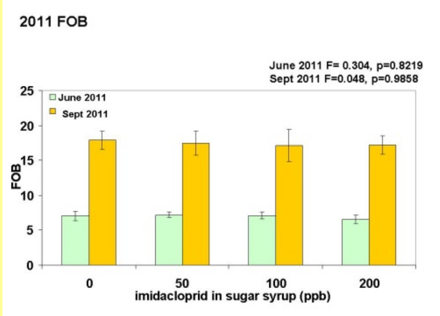

| Proportion pollen foragers | Num df | Den df | P value | F value |
|---|---|---|---|---|
| month | 3 | 96 | <0.0001 | 14.33 |
| trt | 3 | 34 | ***0.0001*** | 7.85 |
| month*trt | 9 | 96 | ***<0.0001*** | 1.37 |

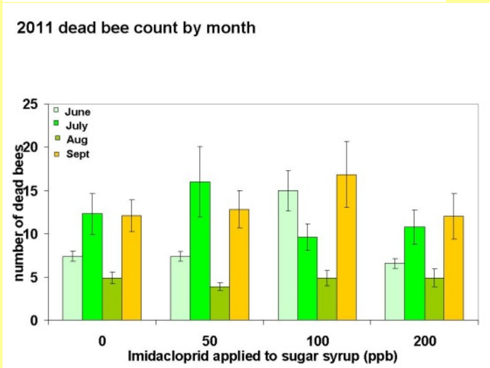
***Interaction: main effect not ok***

| **Pollen foragers (proportional)** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | June | | | | July | | **p=0.081** | | **August** | | **p<0.0001** |
| ppb | n | mean | ± st err | | n | mean | ± st err | | **n** | **mean** | **± st err** | |
| 0 | 10 | 0.133 | 0.021 | A | 10 | 0.155 | 0.0193 | AB | **10** | **0.318** | **0.018** | **A** |
| 50 | 9 | 0.116 | 0.021 | A | 9 | 0.165 | 0.049 | A | **9** | **0.261** | **0.0185** | **B** |
| 100 | 9 | 0.0961 | 0.022 | A | 9 | 0.107 | 0.05 | B | **7** | **0.202** | **0.021** | **C** |
| 200 | 10 | 0.0861 | 0.021 | A | 10 | 0.109 | 0.046 | B | **10** | **0.107** | **0.018** | **D** |

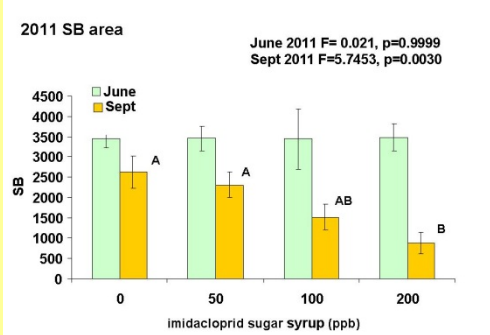
**Result status of report 3. June 2011 to Jan 2012**
We fed honey bee colonies sugar syrup spiked with 0, 50, 100, 200 ppb (7 colonies each dose) imidacloprid in summer 2011. We can compare these results to our data from summer 2010. By the end of the September 2011, sealed brood (SB) area, pollen area, and brood pattern was lower in 200 ppb colonies. However, there was no difference in colony mortality among treatments.

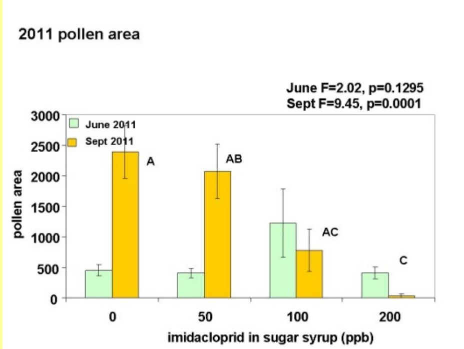


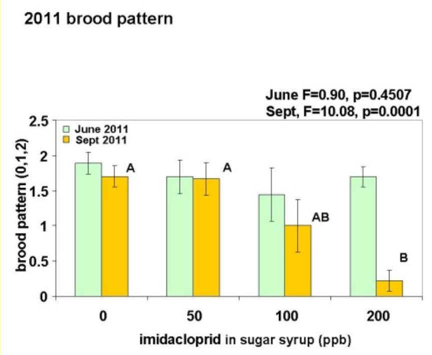




| | June | | July | | Aug | | Sept | |
|---|---|---|---|---|---|---|---|---|
| 0 | A | 7.4±0.60 | A | 12.3±2.33 | A | 4.9±0.71 | A | 12.1±1.83 |
| 50 | B | 7.4±0.56 | A | 16.0±4.10 | A | 3.9±0.47 | A | 12.8±2.17 |
| 100 | B | 15.0±2.29 | A | 9.6±1.5 | A | 4.9±0.88 | A | 16.9±3.80 |
| 200 | B | 6.6±0.53 | A | 10.8±1.94 | A | 4.9±1.04 | A | 12.1±2.64 |
| | | F=10.5 p=0.5698 | | F=1.07 p=3642 | | F=0.4165 p=7413 | | F=0.6654 p=5762 |

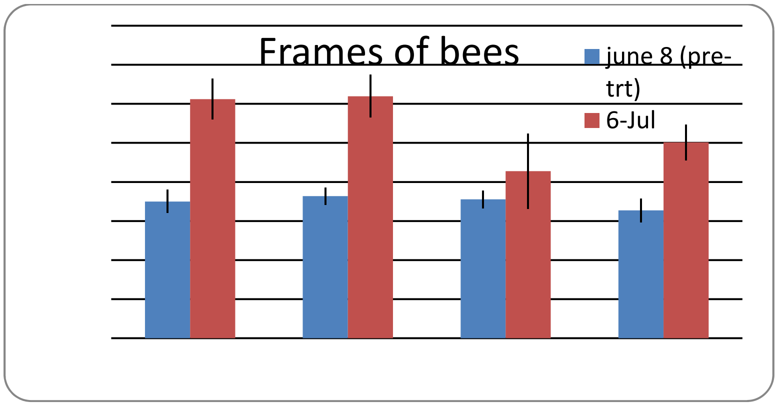


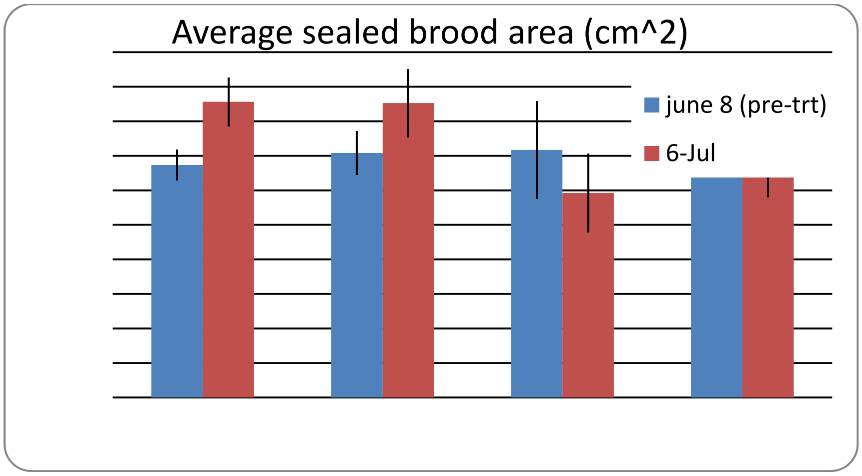



**Period 2. From Jan 2011 to June 2011.** We started feeding honey bee colonies sugar syrup spiked with 0, 50, 100, 200 ppb (7 colonies each dose) imidacloprid. Graphs through the second assessment on July 2, 2011 are enclosed. Frames of brood, sealed brood, open brood, and area of pollen are lower in the 100 and 200 ppb treatments compared to the 0 and 50 ppb treatments. As of early August, 3 - 200 ppb colonies died.

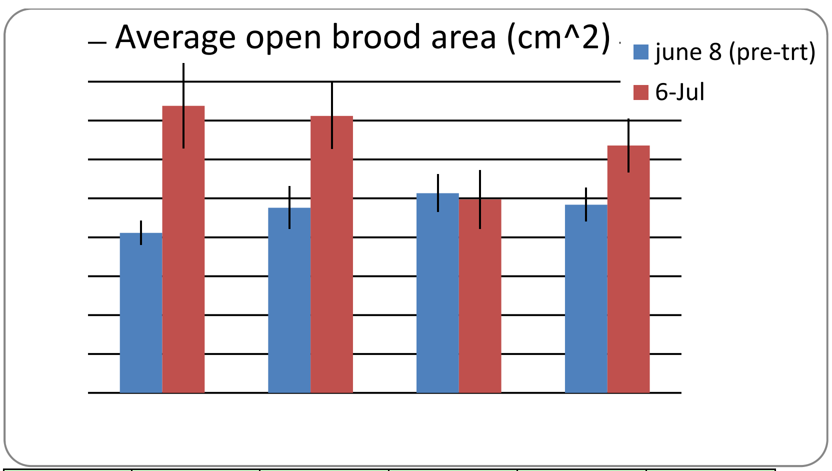
| FOB | Trt (ppb) | june 8 (pre-trt) | st err | 6-Jul | st err |
|---|---|---|---|---|---|
| | | | | | |
| | Control | 7.00 | 0.59 | 12.24 | 1.04 |
| | 50.00 | 7.28 | 0.44 | 12.39 | 1.09 |
| | 100.00 | 7.11 | 0.45 | 8.56 | 1.93 |
| | 200.00 | 6.55 | 0.61 | 10.03 | 0.91 |
| | | | | | |
| | | | | | |



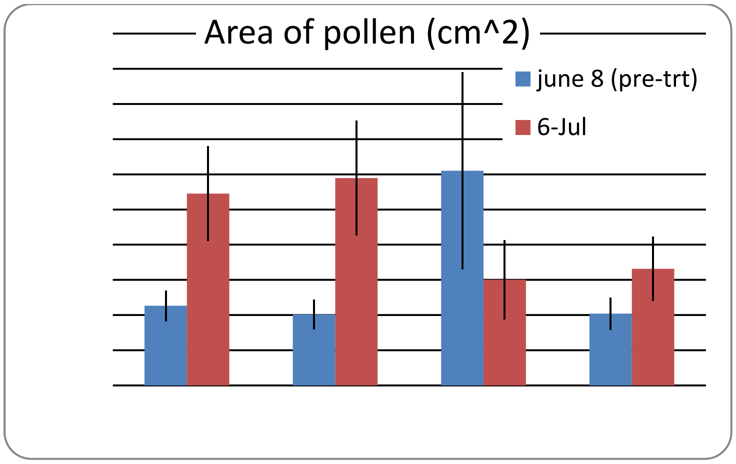

| Area Sealed Brood | Trt (ppb) | june 8 (pre-trt) | st err | 6-Jul | st err |
|---|---|---|---|---|---|
| | Control | 3366.961 | 222.5488 | 4283.114 | 351.9381 |
| | 50 | 3542.186 | 318.98 | 4263.848 | 496.3836 |
| | 100 | 3585.971 | 706.7897 | 2963.263 | 570.9836 |
| | 200 | 3482.264 | 0.187083 | 3268.974 | 366.6617 |
| | | | | | |



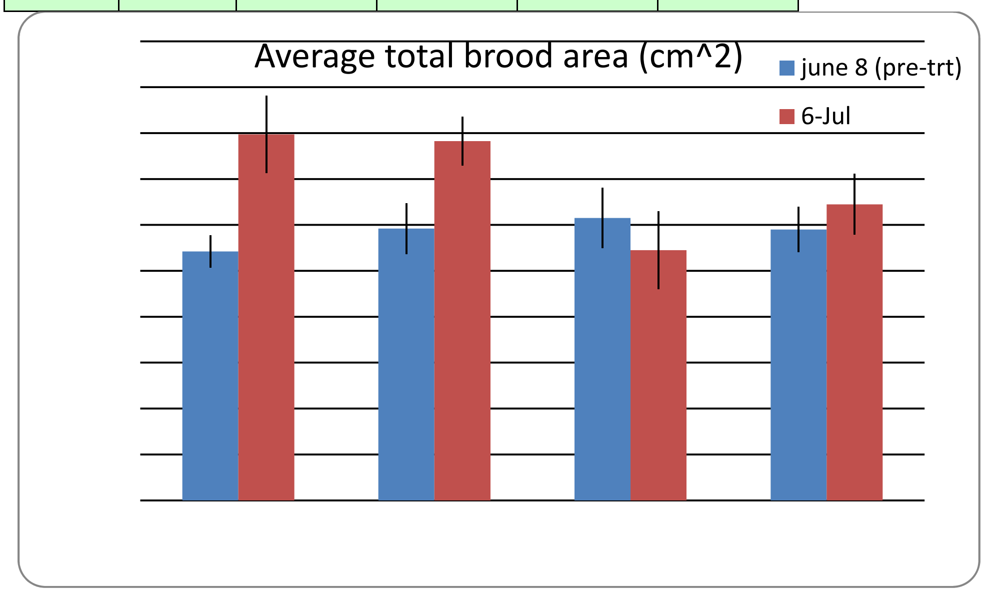

| Area Open Brood | Trt (ppb) | june 8 (pre-trt) | st err | 6-Jul | st err |
|---|---|---|---|---|---|
| | Control | 2056.228 | 155.6669 | 3689.696 | 546.7482 |
| | 50 | 2380.554 | 273.8598 | 3560.136 | 424.6895 |
| | 100 | 2567.077 | 242.5198 | 2487.508 | 378.2132 |
| | 200 | 2419.20 | 217.07 | 3180.2 | 344.3649 |




| Area Pollen | Trt (ppb) | june 8 (pre-trt) | st err | 6-Jul | st err |
|---|---|---|---|---|---|
| | | | | | |
| | Control | 452.5152 | 86.77905 | 1090.243 | 269.4161 |
| | 50 | 403.8415 | 84.82 | 1178.779 | 325.5042 |
| | 100 | 1220.299 | 560.6939 | 600.7156 | 225.5301 |
| | 200 | 408.6185 | 92.73725 | 663.3277 | 182.4588 |




| Area Total Brood | Trt (ppb) | june 8 (pre-trt) | st err | 6-Jul | st err |
|---|---|---|---|---|---|
| | Control | 5423.189 | 354.116 | 7972.81 | 841.11 |
| | 50 | 5922.741 | 551.40 | 7823.984 | 531.0561 |
| | 100 | 6153.049 | 656.5686 | 5450.77 | 849.6654 |
| | 200 | 5901.459 | 494.1774 | 6449.174 | 661.4216 |
| | | | | | |




**Result status of report 1. July 2010 to Jan 2011**
We found that 200 ppb in sugar syrup kills honeybee colonies, reduces number of foragers, and reduces mobility. The data is presented in the table copied below.

**Study in summer 2010 with 5 treatments(n=6 colonies each)**
Conclusion: 1/6 200 ppb colonies alive compared to 5/6 controls by Sept 17 2011

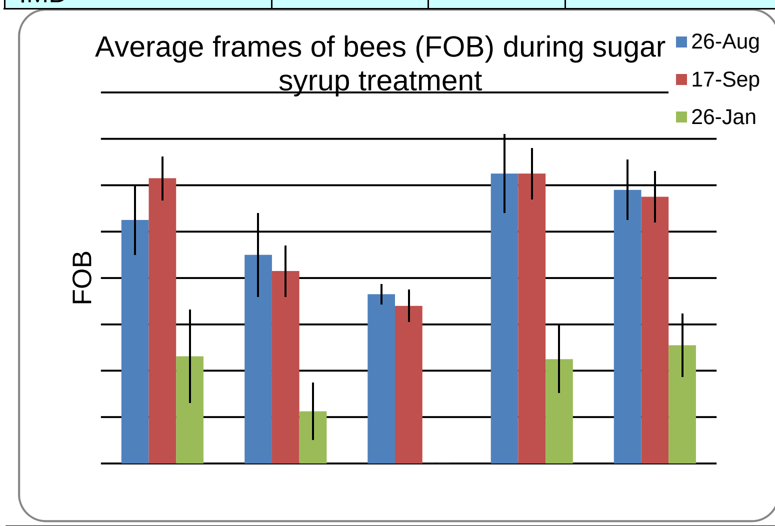
| Treatment | n=6 colonies | Colony ID |
|---|---|---|
| Untreated | 5 | 51, 56, 64, 70, 7 |
| 10 ppb IMD | 3 | 52, 53, 79 |
| 200 ppb IMD | 1 | 55 |
| 15 ppm Fungicides | 6 | 61, 68, 71, 75, 84, 87 |
| 10 ppb IMD +15 ppm Fung | 5 | 60, 69, 72, 76, 80 |

**Pollen patty treatment**

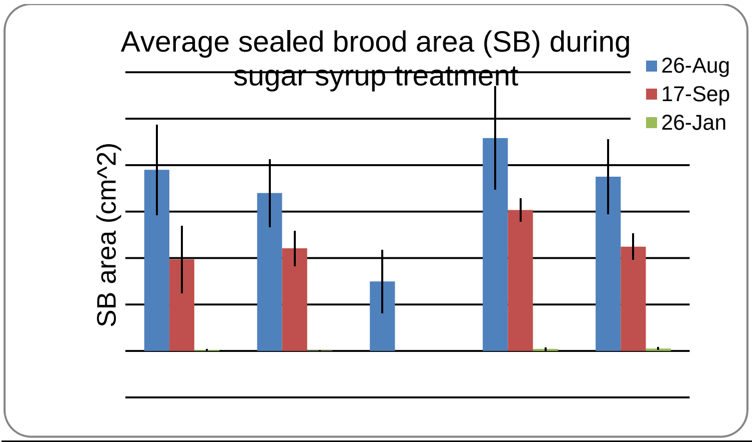
| | July1 | Aug 3 | **Total Queen Replacement or Loss** |
|---|---|---|---|
| Untreated | 0 | 1 | **1** |
| Low IMD | 1 | 0 | **1** |
| High IMD | 1 | 0 | **1** |
| Fungicide | 0 | 0 | **0** |
| Fungicide = Low IMD | 1 | 1 | **2** |

**Syrup treatment**

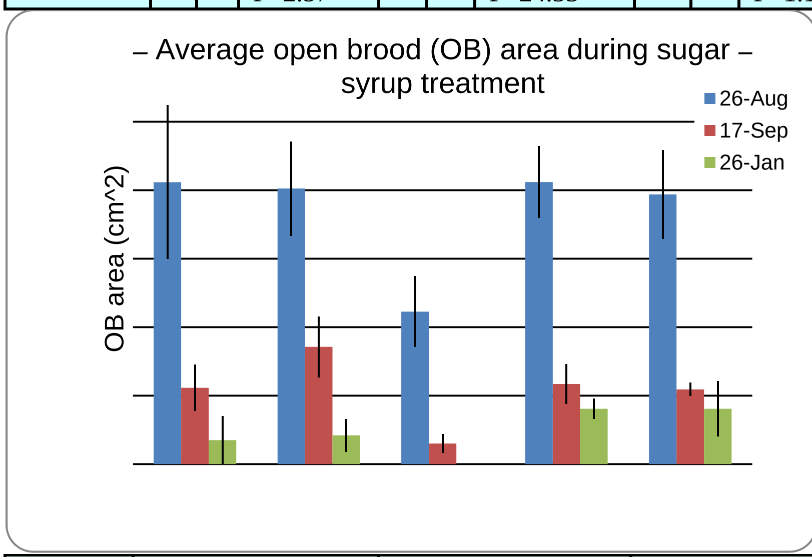
| | Aug 26 | Sept 17 | **Total Queen Replacement or Loss** |
|---|---|---|---|
| Untreated | 0 | 0 | **0** |
| Low IMD | 0 | 0 | **0** |
| High IMD | 3 | 2 | **5** |
| Fungicide | 1 | 0 | **1** |
| Fungicide = Low IMD | 0 | 0 | **0** |



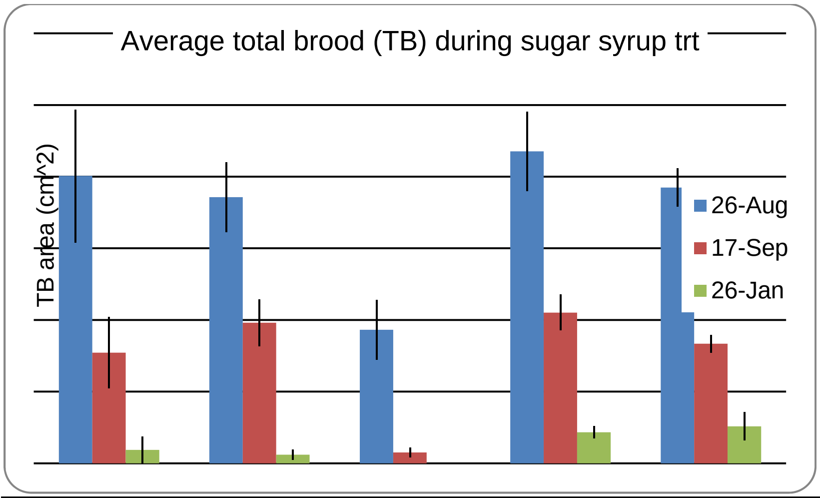

| **FOB** | 26-Aug | | | 17-Sep | | | 26-Jan | | |
|---|---|---|---|---|---|---|---|---|---|
| CON | A | 5 | 10.5±1.5 | AB | 5 | 12.3±0.94 | A | 5 | 4.63±2.01 |
| 10 IMD | A | 6 | 9±1.8 | BC | 6 | 8.3±1.1 | AB | 3 | 2.25±1.24 |
| 200 IMD | A | 6 | 7.3±0.44 | C | 6 | 6.8±0.70 | - | 1 | 0.008±0.008 |
| 15 F | A | 6 | 12.5±1.7 | A | 6 | 12.5±1.1 | A | 6 | 4.5±1.45 |
| 15F + 10 IMD | A | 6 | 11.8±1.3<br>p=0.082<br>F=2.36 | AB | 6 | 11.5±1.1<br>p=0.0009*<br>F=6.74 | A | 5 | 5.1±1.36<br>p=0.0020*<br>F=6.00 |



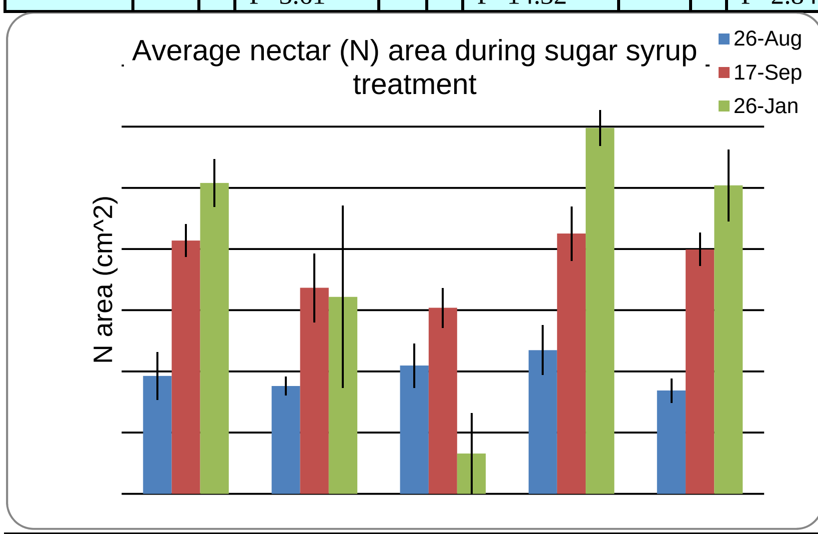

| SB | 26-Aug | | | 17-Sep | | | 26-Jan | | |
|---|---|---|---|---|---|---|---|---|---|
| CON | A | 5 | 1951±485 | A | 5 | 986±362 | A | 5 | 10.3±10.3 |
| 10 IMD | A | 6 | 1701±364 | A | 6 | 1105±189 | A | 3 | 4.3±4.3 |
| 200 IMD | A | 6 | 748±341 | - | 6 | 0±0 | - | 1 | 0±0 |
| 15 F | A | 6 | 2292±557 | A | 6 | 1518±124 | A | 6 | 21.5±16.9 |
| 15F + 10 IMD | A | 6 | 1877±403 p=0.064 F=2.57 | A | 6 | 1122±142 p<0.0001* F=24.33 | A | 5 | 25.8±13.3 p=0.346 F=1.17 |



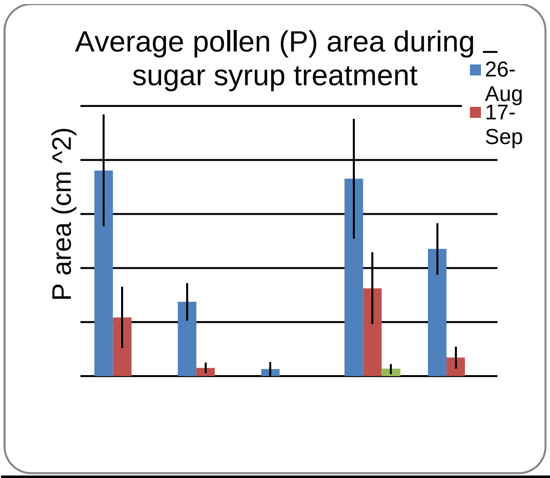

| OB | 26-Aug | | | 17-Sep | | | 26-Jan | | |
|---|---|---|---|---|---|---|---|---|---|
| CON | A | 5 | 2059±561 | AB | 5 | 557±169 | AB | 5 | 175±175 |
| 10 IMD | A | 6 | 2013±344 | A | 6 | 856±222 | AB | 3 | 211±119 |
| 200 IMD | A | 6 | 1114±259 | B | 6 | 151±67.8 | - | 1 | 0±0 |
| 15 F | A | 6 | 2060±263 | AB | 6 | 585±144 | A | 6 | 404±75 |
| 15F + 10 IMD | A | 6 | 1970±325 p=0.42 F=1.01 | AB | 6 | 546±47.8 p=0.06* F=3.35 | AB | 5 | 404±202 p=0.44* F=2.89 |



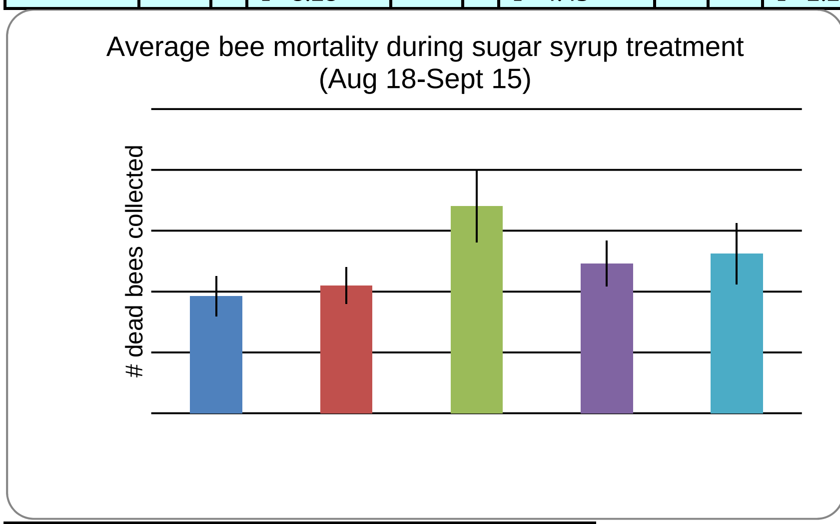

| TB | 26-Aug | | | 17-Sep | | | 26-Jan | | |
|---|---|---|---|---|---|---|---|---|---|
| CON | AB | 5 | 4010±928 | A | 5 | 1543±497 | AB | 5 | 185±185 |
| 10 IMD | AB | 6 | 3714±488 | A | 6 | 1961±327 | AB | 3 | 215±121 |
| 200 IMD | B | 6 | 1862±418 | B | 6 | 151±68 | - | 1 | 0±0 |
| 15 F | A | 6 | 4353±552 | A | 6 | 2103±250 | A | 6 | 426±88 |
| 15F + 10 IMD | AB | 6 | 3847±266 p=0.019* F=3.61 | A | 6 | 1669±124 p<0.0001* F=14.32 | AB | 5 | 430±215 p=0.046* F=2.84 |



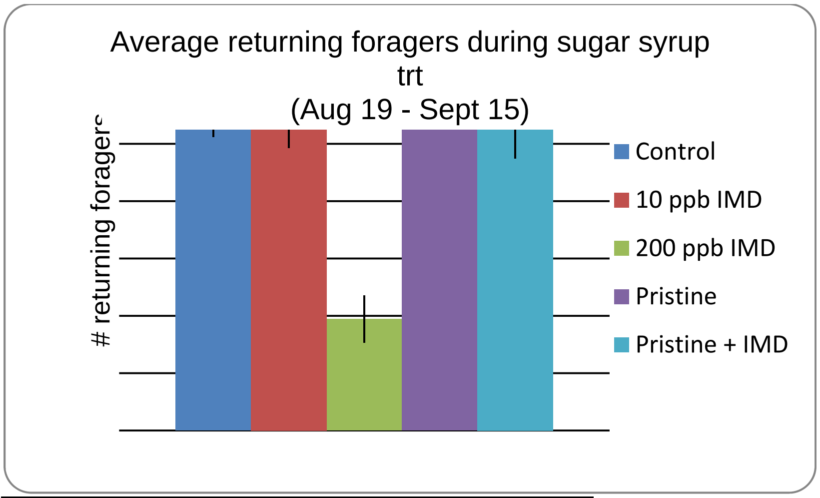

| Nectar | 26-Aug | | | 17-Sep | | | 26-Jan | | |
|---|---|---|---|---|---|---|---|---|---|
| CON | A | 5 | 3850±782 | A | 5 | 8279±468 | A | 5 | 10163±782 |
| 10 IMD | A | 6 | 3523±308 | A | 6 | 6735±888 | AB | 3 | 6439±2980 |
| 200 IMD | A | 6 | 4193±726 | A | 6 | 6082±653 | - | 1 | 1316±1316 |
| 15 F | A | 6 | 4697±811 | A | 6 | 8507±1123 | A | 6 | 11961±584 |
| 15F + 10 IMD | A | 6 | 3374±398 p=0.655 F=0.617 | A | 6 | 7987±540 p=0.153 F=1.84 | A | 5 | 10082±1177 p=0.0003* F=8.15 |




| **Pollen** | 26-Aug | | | 17-Sep | | | 26-Jan | | |
|---|---|---|---|---|---|---|---|---|---|
| CON | A | 5 | 761±207 | AB | 5 | 217±113 | A | 5 | 0±0 |
| 10 IMD | AB | 6 | 275±69 | AB | 6 | 30±19.3 | A | 3 | 0±0 |
| 200 IMD | B | 6 | 258±258 | - | 6 | 0±0 | - | 1 | 0±0 |
| 15 F | A | 6 | 731±222 | A | 6 | 325±133 | A | 6 | 26±18 |
| 15F + 10 IMD | A | 6 | 471±95<br>p=0.0002*<br>F=8.28 | AB | 6 | 688±39.8<br>p=0.0078*<br>F=4.45 | A | 5 | 0±0<br>p=0.09<br>F=2.28 |




| **Dead count** | ANOVA | mean ±st err |
|---|---|---|
| CON | A | 9.63±1.90 |
| 10 IMD | AB | 10.5±1.74 |
| 200 IMD | B | 17.04±1.74 |
| 15 F | AB | 12.23±1.74 |
| 15F + 10 IMD | AB | 13.12±1.74 |
| F =2.797 p=0.0169* | | |




| Foragers | ANOVA | mean ±st err |
|---|---|---|
| CON | A | 29.4±3.86 |
| 10 IMD | A | 27.2±2.64 |
| 200 IMD | B | 9.7±2.07 |
| 15 F | A | 18.9±2.58 |
| 15F + 10 IMD | A | 27.3±3.61 |
| $F = 8.19$ $p < 0.0001$* | | |

**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators.**

**Result status of report final. January 2013 to June 2013**
**Finished in last report period.**
**Result status of report 5. June2012 to January 2013**
**Finished and results given in previous report.**

**Result status of report 4. January 2012 to June 2012**
We studied established plantings at the MN Landscape Arboretum (Chaska, MN) and St. Paul Campus to determine the best plants for seasonal phenology of food for pollinators in MN. Twenty-four stations (divided into 3 plots) were chosen based on proximity to specific plant species in order to obtain replicated samples. These stations were used for data collection on behavioral observations of beneficial insects visiting flowers and sticky trap collection. Teams of 2 observed flowers at each of the 24 flagged station, 8 times each month. Observation duration was for ten-2 minute intervals between 1000 and 1500 h and the number of insects visiting the flowers and taxa of insect were recorded. Standard yellow sticky traps (Gempler's No bait, length x width: 20.3 x 30.5 cm) were placed at the flagged stations for a 48 h period 4 times a month. From our observations, we will add bee plants to the present restoration on Gortner, north of the greenhouses. We completed a manuscript that will be submitted soon, where we compared visits of beneficial insects to native and bedding plants.

**Result 3: Outreach talks and workshop**

**Result status of report final. January 2013 to June 2013**
**Finished website, poster, bulletin, online workshop**
A new pollinator conservation site was posted on the CUES website that contains 11 sections on education and research: value of bees, types of bees, bees and pesticides, colony collapse disorder, conservation and habitat, plants for pollinators, bumble bee conservation, US EPA and

pollinators, European Union (EU EFSA) and pollinators, online 5 part workshop for Master Gardeners, Krischik's research on bees, beneficial insects, and butterflies. We developed a bulletin on ready to use consumer insecticides and their toxicity to bees, poster on best bee plants, and a pollinator conservation bulletin with agricultural and consumer insecticides and effects on bees See CUES website on Pollinator Conservation http://www.entomology.umn.edu/cues/pollinators/index.html

1. LCCMR Ready to use consumer insecticides and their toxicity to bees
2. LCCMR Best Bee Plant Poster
3. LCCMR Pollinator Conservation Bulletin
4. LCCMR Online Pollinator Conservation Workshop.
5 LCCMR Research and outreach products
6. LCCMR Website with research papers, EPA and EFSA EU documents and discussions on bee conservation

The PI gave discussed her research results with Steve Ellis and Jeff Anderson, two Minnesota beekeepers and presented talks at the annual meeting of the Minnesota Honey Producers. The funds on the grant supported a Master Student who worked on the bumble bees and is graduating in August 2013. Numerous talks were provide on the research: Wild Ones Native Plant Society, Minneapolis; Fruit and Vegetables Conference, St. Cloud; MN Rose Society, Minneapolis; Anoka County Master Gardeners Meeting, Maplewood; MN State Fair Bee keepers, St. Paul; MN Honey Producers summer meeting, Minnesota Green Expo, MNLA (MN Nursery and Landscape Association)

**Result status of report 5. June 2012 to January 2013**
**We are developing an online training workshop with the University of Minnesota Master Gardener program, the Master Naturalist Program, the Minnesota Nursery and Landscape Association, and the International Society of Arboriculture for certification credit. We finished the "Pollinator Conservation" website is at www.entomology.umn.edu/cues with extensive educational materials on bee plants, bee identification, bee conservation, and pesticide use compatible with bees.**

**Result 3: Outreach talks and workshop**

**Result status of report 4. January 2012 to June 2012**

We will create an updated section on the CUES website for information on the research and best management practices to conserve bees.

The PI and graduate student will provide talks in established meetings around the state

We will deliver a workshop in spring 2013 on the results of the grant. We will charge a small registration fee to cover advertising, room, and food. We will spend 5 hours with presentations and a visit to the demonstration project on the St. Paul campus. Travel funds are requested for Mr. Eric Mader, Xerces Insect Conservation Society (Portland, OR) to attend and provide talks at the meeting since he is active in developing legislation and literature on pollinator conservation.

**IV. OUTLINE OF PROJECT RESULTS:**
**Budget description**

**Result 1: Residue analysis of imidacloprid in pollen and the effects on bee colony survival and behavior**

**Result 1-1: Residue analysis of imidacloprid in pollen and nectar**
We determined that label rates of imidacloprid applied to the soil are translocated into pollen and nectar at levels of imidacloprid from 95-1973 ppb in 4 species of bee plants. We completed studies on the amount of imidacloprid residue in flowers from soil applications. *Agastache foeniculum* mint and *Asclepias curassavica* milkweed contained significant imidacloprid residue in flowers from an imidacloprid soil application. A standard 1X imidacloprid rate to the soil produced flowers residue of 1973 ppb in mint and 1568 ppb in milkweed. The residue needed to kill a bee when feeding is an estimated oral LC50 for honey bees of 185 ppb (CA EPA 2009) or 192 ppb (Bayer, Fischer and Chalmers 2007). Consequently, a 1X label rate provides enough imidacloprid to the soil for sufficient imidacloprid residue to be found in flowers that would kill a bee. The woody native plant *Esperanza* yellowbells (YB) and Rose (R) had a residue of 106 ppb (YB) and 95 ppb (R) imidacloprid in the flowers, which should be a sublethal dose, but a 2X label rate produced 276 ppb (YB) and 332 ppb (R), a residue amount that would kill a bee. Previous research demonstrated 6000 ppb in *Fagopyrum esculentum* buckwheat and *Asclepias curassavica* milkweed flowers. In 2009 after a 1X label rate of imidacloprid to the soil, a homeowner's formulation of imidacloprid produced 812 ppb in rose flowers and a professional formulation 1175 ppb in rose flowers. All residues above 185 ppb are sufficient to kill a bee after a few seconds of feeding.

Mint flowers treated with an imidacloprid label rate had dead bees in the flowers, but a statistically significant number of dead bees were found on 2X label treatments. We found many bees dead on the ground but could not identify them to treatment. So, at 2X dose, which is permitted according to the EPA label, bees are killed while feeding on the flowers. Consequently, standard EPA registered doses of imidacloprid in homeowner and professional products translocate sufficient imidacloprid from the soil to the flowers to kill a bee (Table 1-3).

**Result 1-2: Imidacloprid effects on bumblebee colony growth and survival**
We completed in 2011-2012 an 11-week greenhouse study on caged queenright colonies of *Bombus impatiens* Cresson (n=9 colonies/trt) that were fed 0, 10, 20, 50 and 100 ppb imidacloprid or clothianidin in sugar syrup. Neonicotinyl treatments used in this study ranged from the highest amount found in seed-treatments (corn, canola and sunflower) (10 ppb) to levels found in landscape plants (20-100 ppb). Our study demonstrates that 20 ppb imidacloprid or clothianidin fed to queenright colonies of *B*. *impatiens* for 11 weeks increased queen mortality and reduced colony consumption, colony weight, wax syrup pots that were added, and male production. Neither neonicotinyl decreased worker and queen production. Starting at 6 weeks, queen mortality was significantly higher in 50-100 ppb and by 11 weeks in 20-100 ppb imidacloprid- and clothinaidin-treated colonies. As queens started to die at week 6, workers in 20-100 ppb treatments produced fewer males, but did continue to invest in new queen production.

We completed studies on the effects of memory and learning on bumblebees. We found that a very low amount of imidacloprid of 0.2 ng/ bee reduces learning and memory.

**Result 1-3: Imidacloprid effects on honey bee colony growth and survival**
We completed in 2011 a 16 week field study, honeybee colonies (n=10 colonies/trt) that were fed 0, 50, 100, 200 ppb imidacloprid in sugar syrup and colonies and were assessed five times: June 8, July 6, August 3, August 31, and September 21 for 16 parameters of colony health:

frames of bees, open brood, sealed brood, total brood, pollen stores, missing cell count, brood pattern, returning pollen foragers, percent returning foragers, sugar syrup consumption in 48 hrs and 1 week, dead bee counts, *Varroa* numbers, *Nosema* numbers, virus (distorted wing virus(DWV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV)) and queen mortality.

Data from the USDA AMS Lab in Gastonia, NC determined that samples analyzed by a different chemical method in previous research on imidacloprid residues in milkweed resulted in the same detection of residue levels. Now, these data can be added to our data set which improves the predictions of the research. The imidacloprid residue found in milkweed pollen for 1X (300 mg imidacloprid added to soil) is around 6, 000 ppb and for 2X is around 12,000 ppb (600 mg imidacloprid added to soil).

**Deliverable 1-1 to 1-3.** We worked with state agencies and landscape-related associations that use insecticides to make them aware of the potential damage to pollinators. We discussed the data at the National level with the EPA. We will publish research papers in peer reviewed journals on the amount of imidacloprid and clothianidin translocated to nectar and pollen and effects on pollinators.

**Deliverable 1-4.** We developed a landscape pest management bulletin and insecticide list using pollinator-friendly insecticides and EPA approved low risk insecticides. A new pollinator conservation site was posted on the CUES website that contains 11 sections on education and research: value of bees, types of bees, bees and pesticides, colony collapse disorder, conservation and habitat, plants for pollinators, bumble bee conservation, US EPA and pollinators, European Union and pollinators, online 5 part workshop for UMN Master Gardeners, Krischik research on bees, beneficial insects, and butterflies. We developed a bulletin on ready to use consumer insecticides and their toxicity to bees, poster on best bee plants, and a pollinator conservation bulletin with agricultural and consumer insecticides and effects on bees See CUES website on Pollinator Conservation
http://www.entomology.umn.edu/cues/pollinators/index.html

1. LCCMR Ready to use consumer insecticides and their toxicity to bees
2. LCCMR Best Bee Plant Poster
3. LCCMR Pollinator Conservation Bulletin
4. LCCMR Online Pollinator Conservation Workshop.
5 LCCMR Research and outreach products
6. LCCMR Website with research papers, EPA and EFSA EU documents and discussions on bee conservation

**Summary Budget Information**
**Result 1: Residue analysis of imidacloprid in pollen and nectar and the effects on bee colony and survival**
**Result status of report final. January 2013 to June 2013.**
**Work finished**

**ENRTF Budget: $296,000**
**Amount Spent: $296,000**
**Balance: $0**

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Research papers (3) finished and available online** | June 2013 | **$213,362 labor<br>$81,893 supplies** |
| **2. Bulletin on pollinator friendly insecticide** | July 2012 | |

| | | |
|---|---|---|
| **recommendations, addendum to "IPM of Midwest landscapes", finished and available online** | | ---------------- |
| **3. "Mitigating Pollinator Decline" section of the CUES website, finished and available online** | July 2012 | ---------------- |
| **4. Color poster on bees, bee plants, and links to pollinator website** | June 2012 | **$755 printing** |

**<u>Result 1: Residue analysis of imidacloprid in pollen and nectar and the effects on bee colony, survival, and behavior</u>**
**Result status of report 5. June 2012 to January 2013**
**Work started**

**ENRTF Budget: $215,000**
**Amount Spent: $187,773**
**Balance: $25,227**

**Amendment approved by the LCCMR February 25, 2013**
**Explain reason for the amendment: We have finished 99% of the research and 70% of the outreach outlined in the proposal with the remaining to be finished by June 30 2013. We are requesting a one month extension of the end date until June 30, 2013. The reason for the requested extension is to provide more time to finish the greenhouse bumblebee foraging research and to provide dissemination of the outreach activates on the grant in the spring when people are interested in learning about management in landscapes and gardens. We have generated a lot of data and will have 5 research papers. We posted an online website. We are currently working on the educational materials. These proposed budgetary changes do not change the work scope of this project.**

**Funds need to be added to the personnel category for salary support for the 3 people ($3187) as it is taking longer to develop the online workshop and educational materials than we anticipated and we need help to finish research activities 1, 2, and 3. Currently we are 50% finished with a greenhouse bumblebee foraging experiment that shows at 100 ppb in 3 weeks bumblebee colonies weigh less, have fewer workers, and less stored food. We will be done with that research by end of April. Additional research supplies ($4718) are needed such as 40 bumblebee colonies to finish the greenhouse foraging experiment at $80 each ($3200), biocontrol agents to kill pests on GH plants used in greenhouse foraging experiments ($325), 2-terabite storage media to store and access videos used in foraging experiments ($400), storage media to store data to move between computers ($60).**

**<u>Result 1: Residue analysis of imidacloprid in pollen and nectar and the effects on bee colony, survival, and behavior</u>**
**Result status of report 4. January 2012 to June 2012**
**Work started**

**ENRTF Budget: $222,000**
**Amount Spent: $164,749**
**Balance: $57,251**

**Explain reason for the amendment:** As of August 2012 we finished 90% of the research on bumble bees, honey bees, and residue in plants. However, the research took more undergraduate labor than what was in the original budget. Consequently, we needed to add more funds to undergraduate labor

We spent more on undergraduate labor to maintain the bees, extract the pollen and nectar from flowers, care and grow for the flowering plants, and add the data to Excel spreadsheets. Also, it took much labor than anticipated to freeze the residue samples, store them in an 80 below freezer, inventory the samples, pack them for chemical analysis at the USADA lab in NC, and add the resulting residue data to Excel spreadsheets. Also, the experiments took ten times longer than anticipated due to issues with designing the best method to rear bumble bees in the greenhouse. Most studies are on a European species of bumblebee. We could not get the rearing containers used in European studies to work for the American bumblebee species. We had to design a different rearing system in the greenhouse. The experiment on honeybees needed one undergraduate to perform the daily research and additional students to help with the 5 monthly assessments. Consequently, undergraduate labor increased over what we originally anticipated for the project.

The research took more supplies than what was in the original budget. We needed larger flight boxes for the American bumblebee species and had to order 2 types of cages, a rearing box and flight box for 40 colonies. Initially we designed 4 types of wood nest boxes based on the European designs. We decided to increase the number of honeybee colonies in each treatment. Also, we needed many more bumblebee colonies than anticipated. Other research supplies were needed to protect students from pesticides. We needed disposable paper coveralls for field experiments, purple nitrile disposable gloves for field and greenhouse experiments and field shoes as pesticides were added to the soil that we walked upon. We needed more supplies for the field than anticipated: pots, soil, ground cloth, fertilizers, and pesticides. In the lab we needed protective bench paper, storage containers, scales, and storage vials. All of these needs increased the amount spent on research supplies.

We will did not need as much funds in field space as we were charged only $100 for space for growing residue plants and $100 for spraying plants for Japanese beetles over the summer.

Costs for residue analysis decreased from our original estimate. Fees for residue analysis were only $124 for samples at the USDA AMS lab in Gastonia, NC. Compared to $300 at ALS Labs in Edmonton, Alberta. We switched to the USDA lab from the original Canadian residue lab as the USDA lab is approved by the EPA.

We decided to reduce printing costs of the updates to the IPM manual and the reduce risk insecticide bulletin by placing the bulletins on the CUES website for free downloads by the users. We will deliver limited amounts of printed material to workshops and talks.

**Amendment Approved:**___________________

**Result 1: Residue analysis of imidacloprid in pollen and nectar and the effects on bee colony, survival, and behavior**

**Result status of report 3. June 2011 to Jan 2012**

**Work started**

**ENRTF Budget: $73,500**
**Amount Spent: $64,402**
**Balance: $9,098**

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Research paper (at least 3 papers)** | June 2013 | $146,984 labor<br>$110,478 supplies |
| **2. Bulletin and table on pollinator friendly insecticide recommendations** | July 2012 | $1,000 printing |
| **3. "Mitigating Pollinator Decline" section of the CUES website with pollinator-friendly insecticides.** | July 2012 | ---------------- |
| **4. "IPM of Midwest landscapes" revision** | June 2012 | $00 printing |

**Result 1: Residue analysis of imidacloprid in pollen and nectar and the effects on bee colony, survival, and behavior**
**Result status of report 2. Jan 2011 to June 2011**
**Work started**

**ENRTF Budget: $73,500**
**Amount Spent: $64,402**
**Balance: $9,098**

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Research paper (at least 3 papers)** | June 2013 | $146,984 labor<br>$110,478 supplies |
| **2. Bulletin and table on pollinator friendly insecticide recommendations** | July 2012 | $2,000 printing |
| **3. "Mitigating Pollinator Decline" section of the CUES website with pollinator-friendly insecticides.** | July 2012 | ---------------- |
| **4. "IPM of Midwest landscapes" revision** | June 2012 | $2,000 printing |

**Comment:** Ms. Judy Wu, the graduate student on the grant from summer 2010 to Aug 2011, received a prestigious EPA STAR fellowship on the toxicology of imidacloprid to bee immune system function. We used data generated in the first year of the LCCMR research to provide background for her proposal and her new research direction. Since her salary is covered for 3 years by the EPA grant, the undergraduate on the bumblebee project, Jameson Scholer, will now use the graduate student funds for his MS degree on imidacloprid effects on bumblebee colony health. Also, we have spent more funds that we anticipated on personnel. The bumblebee rearing pilot studies took 1 year before we developed a way to rear the bumblebees and start our research. Consequently, I will be submitting an application to move funds from residue analysis to personnel for result 1. The USDA lab in Gastonia NC performs residue work for $121.50 /sample and we listed $300/sample in the budget.

**Result Completion Date:** June 2013
**Result Status as of report 1:** Jan 2011
**Result Status as of report 2:** June 2011
**Result Status as of report 3:** Jan 2012
**Result Status as of report 4:** August 8, 2012
**Result Status as of report 5:** Jan 2013
**Final Report Summary:** June 2013

**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**

We demonstrated that native plants have more beneficial insects visiting them than bedding plants and goldenrods *Solidago* and Joe-pyeweed *Eupatorium* were some of the best bee plants. We installed a demonstration plot on the Saint Paul campus in Fall 2012 added 300 *Agastache* mint bee plants. We developed a section on the CUES on conserving bees thru proper plant selection, habitat restoration, and compatible pesticide use. We finished research on the effects of native and bedding plants on bees. We developed a bulletin and poster on best bee plants, identifying bees, and insecticides and bees.

**Deliverable 2-1.** We will publish a research paper in peer reviewed journals on the preferred native plants for pollinators. We presented these data at research meetings and to our electronic "Outreach Committee" by creating the pollinator conservation website

**Deliverable 2-2.** We developed a bulletin and posters on the best pollinator plants.

**Deliverable 2- 3.** We developed a website on pollinator conservation with research results, online workshop, bulletins, and posters.

**Deliverable 2-4.** We added plants to the native plant restoration at the UMinnesota St. Paul Campus, on the corner of Gortner, north of the Horticulture Greenhouses.

**Summary Budget Information**
**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**
**Result status of report final. January 2013 to June 2013**
**Work finished**

**ENRTF Budget: $1,000**
**Amount Spent: $1,000**
**Balance: $0**

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Research paper, finished and available online** | June 2013 | $1,000 labor |
| **2. Bulletin and poster on best pollinator plants, finished and available online** | July 2012 | ---------- |
| **3. "Mitigating Pollinator Decline" section on the CUES website, finished and available online** | July 2012 | --------- |
| **4. Demonstration restoration on the UMinnesota St. Paul Campus, finished pictures online** | June 2013 | -------- |

**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**
**Result status of report 5. June 2012 to January 2013**
**Work finished**

**ENRTF Budget: $82,000**
**Amount Spent: $81,000**
**Balance: $1,000**

**Amendment approved by the LCCMR February 25, 2013**
**Explain reason for the amendment: We finished research result 2, except for poster and bulletin. We can reduce the printing costs to $1,000 for the colored poster on identifying bee plants, pollinator species, and best management pesticide practices.**

**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**
**Result status of report 4. January 2012 to June 2012**
**Work finshed**

**ENRTF Budget: $82,000**
**Amount Spent: $64,402**
**Balance: $11,598**

**Explain reason for the amendment:** As of August 2012 we finished research on the best bee plants. We have a manuscript on the plants most visited by bees at a restoration site. However, the research took more undergraduate labor than what was in the original budget. Consequently, we needed to add more funds to undergraduate labor. Due to the added time to perform this research we decided not to travel to another restoration. Travel for the research was done with cost sharing commitments from the P.I.

We decided to reduce printing costs of the best bee plants bulletin, updates to the IPM manual, and reduced risk insecticides bulletin by placing the bulletins on the CUES website for free downloads by the users. We will deliver limited amounts of printed material at workshops and talks.

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**Amendment Approved:_______________**

**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**

**Result status of report 3. June 2011 to Jan 2012**

**Work started**

**ENRTF Budget: $33,000**
**Amount Spent: $20,018**
**Balance: $12,982**

**Result 2: Determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators**

**Result status of report 2. Jan 2011 to June 2011**

**Work started**

**ENRTF Budget: $33,000**
**Amount Spent: $20,018**
**Balance: $12,982**

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Research paper (at least 1 paper)** | June 2013 | $24,000 labor<br>$3,000 travel |
| **2. Bulletin and table and poster on best pollinator plants** | July 2012 | |
| **3. "Mitigating Pollinator Decline" section on the CUES website with best pollinator plants.** | July 2012 | ------ |
| **4. Demonstration restoration on the UMinnesota St. Paul Campus with best pollinator plants.** | | $3,000 supplies |

Comment: I did an extensive research project where I compared native plants and bedding plants for insect visits and determined the best bee plants in MN The paper is in manuscript. On the St. Paul campus I planted a restoration of best bee plants. In 2011 I started to remove the invasive exotic species, fertilize, and weed the site. I will use the limited funds for purchasing and planting bee plants on this grant to upgrade the restoration. I will use add a poster and handouts in a mailbox to educate the public on our research. I will use the St. Paul restoration to collect more data to demonstrate the best bee plants. I ordered recent updated publications on the best plants from the Xerces Society, Golf Course Association, and others that were published since this grant was submitted. I am working on developing a website with these materials. Since I maintain the CUES website myself, I can do this myself. Money in this result has been used for labor to clean up the restoration and grow native plants for the research. We need to water daily 300 pots of plants which takes 1.5 hrs, maintain/repot the plants, fertilize weekly, and spray for Japanese beetle and disease. This has taken many undergraduate student hours so we already have spent much money on undergraduate student help. I discussed in a comment under result 1 that I will submit an application to rebudget the grant to accommodate the unanticipated need for student workers.

**Result Completion Date:** June 2013

**Result Status as of report 1:** Jan 2011

**Result Status as of report 2:** June 2011

**Result Status as of report 3:** Jan 2012

**Result Status as of report 4:** August 8, 2012

**Result Status as of report 5:** Jan 2013
**Final Report Summary:** June 2013

**Result 3: Outreach talks and workshop**

We created a "Pollinator Conservation" section on the CUES website for information on the research and best management practices to conserve bees. These research data have been requested by groups that need to understand more about the risk of neonicotinyl insecticides to bees: US EPA, Center for Food Safety, Pesticide Action Network (PANNA), Xerces Society for Invertebrate Conservation, Washington State Department of Agriculture, Pesticide Research Institute, MN Honey Producers, Boulder County Bee Keepers, and Colorado State Beekeepers.

The website was used to educate media representatives when they did reviews of our research: MN Public Radio (3), Harvest Public Media, Iowa Public Radio, WCCO, Kare 11 News, KSTP, Pioneer Press, Star Tribune, and the Minnesota State Fair. Krischik has provided her research results to the US EPA twice: an online slide show webinar to EPA scientists and a visit to UM by the US EPA Administrator for the Office of Chemical Safety and Pollution Prevention (OCSPP).

We created a 5 part online workshop with slides, quiz at the end of the slides, and reading materials. The MN Department of Agriculture and the UMN Master Gardener program was informed of the site and it is being actively used.

**Summary Budget Information**
**Result 3: Outreach talks and workshop**
**Result status of report final. January 2013 to June 2013**
**Work finished.** **ENRTF Budget: $0**
**Amount Spent: $0**
**Balance: $0**
**Summary Budget Information for Deliverable 3: ENRTF Budget:**

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Website** | June 2013 | $0 |
| **2. PI to deliver at least 8 talks around Minnesota** | June 2013 | $0 |
| **3. Workshop delivered** | June 2013 | $0 |

**Result 3: Outreach talks and workshop**
**Result status of report 5. June 2012 to January 2013**
**Work not started** **ENRTF Budget: $0**
**Amount Spent: $0**
**Balance: $0**
**Amendment approved by the LCCMR February 25, 2013**
**Explain reason for the amendment: We are actively working on research activity 3, the outreach component. We have developed a great "Pollinator conservation" website (www.entomology.umn.edu/cuesscroll down to pollinator conservation). The grant partners requested a website and online workshop; we do not need travel funds or, speaker funds. Our cooperators asked for an online workshop since it is easier for working people to use the computer to update their educational knowledge and not have to miss a work day.**
**Result 3: Outreach talks and workshop**
**Result status of report 4. January 2012 to June 2012**
**Work not started** **ENRTF Budget: $1,500**
**Amount Spent: $0**
**Balance: $1,500**

**Explain reason for the amendment:** We reduced the cost of the workshop as we are going to charge a small fee for attendance to cover costs as room and food. We will hold the workshop on the Saint Paul campus in Borlaug Hall during spring break so we do not have to pay room fees. We will put the slides from each speaker on the CUES website so outstate participants can access the slides and information.

We decided to reduce printing costs by placing the bulletins on the CUES website for free downloads by the users. We will deliver limited amounts of printed material at workshops and talks.

**Amendment Approved:__________________**

**Result 3: Outreach talks and workshop**
**Result status of report 3. June 2011 to Jan 2012**
**Work not started**
**ENRTF Budget: $2,538**
**Amount Spent: $0**
**Balance: $2,538**

**Result 3: Outreach talks and workshop**
**Result status of report 2. Jan 2011 to June 2011**
**Work not started**
**ENRTF Budget: $2,538**
**Amount Spent: $0**
**Balance: $2,538**

**Summary Budget Information for Deliverable 3: ENRTF Budget:**

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Website to share information with "Outreach Committee"** | June 2013 | $0 |
| **2. PI to deliver at least 8 talks around Minnesota** | June 2013 | $1,038 travel |
| **3. Workshop delivered** | June 2013 | $1,500 travel |

**Result Completion Date:** June 2013
**Result Status as of report 1:** Jan 2011
**Result Status as of report 2:** June 2011
**Result Status as of report 3:** Jan 2012
**Result Status as of report 4:** August 8, 2012
**Result Status as of report 5:** Jan 2013
**Final Report Summary:** June 2013

**V. TOTAL ENRTF PROJECT BUDGET:**
**Personnel:** $ 170,984
**Contracts:** $ 0
**Equipment/Tools/Supplies**: $ 113,478
**Printing:** $ 7,000
**Acquisition (Fee Title or Permanent Easements):** $ 0
**Travel:** $ $5,538
**TOTAL ENRTF PROJECT BUDGET: $297,000**

**VI. PROJECT STRATEGY**

## A. Project Partners

no salary, in-kind: Krischik 30%/yr X 3 yr = $89,022
Electronic listserve "Outreach Committee**"**

1. National Honey Bee Advisory Board, Clint Walker, co-chair and Darren Cox, co-chair
2. MN Honey Bee producers, Dan Whitney, President
3. Old Mill Honey Co, Steve Ellis
4. California-Minnesota Honey Farms, Jeff Anderson
5. MN Hobby Beekeepers Association, Dan Malmgren
6. MNLA, MN Landscape Association
7. MNTIF, MN Turf and Grounds Foundation

## B. Project Impact and Long-term Strategy

The purpose of this research was to determine if neonicotinyl insecticides had an impact on bee foraging and colony health. The research showed that rates of imidacloprid used in landscapes reduced foraging and colony health of bees. This information was discussed with consumers, master gardeners, state agencies, commodity groups, and the US EPA.

We created a “Pollinator Conservation” section on the CUES website for information on the research and best management practices to conserve bees. These research data have been requested by groups that need to understand more about the risk of neonicotinyl insecticides to bees: US EPA, Center for Food Safety, Pesticide Action Network (PANNA), and Xerces Society for Invertebrate Conservation, Washington State Department of Agriculture, Pesticide Research Institute, MN Honey Producers, Boulder County Bee Keepers, and Colorado State Beekeepers. The lab was interviewed by TV and radio many times: MN Public Radio (3), Harvest Public Media, Iowa Public Radio, WCCO, Kare 11 News, KSTP, Pioneer Press, Star Tribune, and the Minnesota State Fair. Krischik has provided her research results to the US EPA twice: an online slide show webinar to EPA scientists and a visit to UM by the US EPA Administrator for the Office of Chemical Safety and Pollution Prevention (OCSPP). Krischik’s expertise from this research has made her a reviewer for 2 white papers from the Xerces Society of Invertebrate Conservation and another from the Friends of the Earth as well as per reviewer on related scientific manuscripts.

Neonicotinyl insecticides are neurotoxins that affect vision, olfaction, learning, and memory and bind to mushroom bodies in bee brains which are particularly large in social bees compared to other insects. Bees fed 13 ppb or 23 ppb imidacloprid were less likely to form long-term memory and had reduced learning and at 24 ppb imidacloprid performed fewer communicative waggle dances. **Our research data confirmed this as bumblebees fed 20 ppb imidacloprid foraged poorly and more colonies died compared to controls**.

The ubiquitous use of neonicotinyl insecticides on crops and landscape plants throughout the season will lead to chronic sublethal and lethal effects on worker foraging and colony health. Social bee colonies, such as bumblebees and honey bees, rely on division of labor and need foragers to return nectar to the hive for the queen and brood. Native, annual bee colonies or bumblebee queens in spring and fall are even more vulnerable to neonicotinyl insecticides since the solitary queens can be impaired when foraging. Since most studies show reduction in foraging behavior below 10 ppb and residues in crop and landscape flowers are higher than 10 ppb, bees are likely to be experiencing chronic, sublethal doses with consequences on queen and colony health.

## C. Other Funds Proposed to be Spent during the Project Period

For the research outlined in this proposal there are no funds from other sources.

## D. Spending HIstory

No previous LCCMR

## VII. DISSEMINATION

Research results was disseminated through 3 research papers, pollinator conservation website, talks, workshops, 2 bulletins, and poster.

## VIII. REPORTING REQUIREMENTS

Periodic work program progress reports will be submitted not later than Jan 2011, June 2011, Jan 2012, June 2012, Jan 2013, June 2013. A final work program report and associated products will be submitted between June 30 and August 1, 2013 as requested by the LCCMR.

## IX. RESEARCH PROJECTS

Peer review document submitted separately as LCCMR 221GPeerReview.doc

**Timetable of research and deliverables:**

| | July 2010 -June 30 2011 | | | | July 2011-June 30 2012 | | | | 2012 July-June 30 2013 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Su | Fall | Wi | Sp | Su | Fall | Wi | Sp | Su | Fall | Wi | Sp |
| **Research result 1: Perform residue analysis on insecticide treatments, bioassay with insects** | | | | | | | | | | | | |
| **Establish plants** | x | x | | x | X | X | | X | | | | |
| **Collect flowers** | x | x | | | X | X | | X | X | X | | |
| **Residue analysis** | | x | x | x | | X | X | X | X | X | | |
| **Bioassay with insects** | x | x | | x | X | X | | X | X | X | | |
| **Deliverable: 1. Research paper (at least 3 papers). 2. Bulletin and table on pollinator friendly insecticide recommendations. 3. "Mitigating Pollinator Decline" section of the CUES website with pollinator-friendly insecticides. 4. "IPM of Midwest landscapes" revision** | | | | | | | | | | | | |
| **Research paper** | | | | x | | X | X | X | | X | X | X |
| **Bulletin, table** | | x | x | x | | X | X | X | | X | | |
| **Website** | | x | x | x | | X | X | X | | X | | |
| **Update IPM Manual** | | x | x | x | X | X | X | X | X | X | X | X |
| **Research result 2: Field observation on best plants for pollinators, develop demonstration plots on St. Paul Campus** | | | | | | | | | | | | |
| **Field observation** | **x** | **x** | | | X | X | | | | | | |
| **Deliverable: 1. Research paper (at least 1 paper). 2. Bulletin and table and poster on best pollinator plants. 3. "Mitigating Pollinator Decline" section on the CUES website with best pollinator plants. 4. Demonstration restoration on the UMinnesota St. Paul Campus with best pollinator plants.** | | | | | | | | | | | | |
| **Research paper** | | x | x | x | X | X | X | X | X | X | X | X |
| **Bulletin, table, poster** | | | x | x | X | X | X | X | X | X | | |
| **Website** | | x | x | x | X | X | X | X | X | X | X | X |
| **Develop demo plots** | | | | | X | X | | | X | X | | |
| **Result 3 and deliverable. 1. Listserve, 2. PI to deliver at least 8 talks around Minnesota. 3. Workshop delivered 2 times, travel funds for speaker, Eric Mader, Xerces Society, Portland, OR** | | | | | | | | | | | | |
| **Listserve** | x | x | x | x | X | X | X | X | X | X | X | X |
| **PI trael to deliver talks** | | x | x | x | | X | X | X | | X | X | X |
| **Workshops** | | | | | | | | | | | | X |

**Attachment A: Budget Detail for 2010 Projects**

**Project Title: 221-G Mitigating Pollinator Decline**

**Project Manager Name: Vera Krischik, UMinnesota**

**Trust Fund Appropriation: $ 297,000**

Proposed revised budget - August 8, 2012

**2010 Trust Fund Budget**

| **BUDGET ITEM** | **Result 1 Residue analysis, bioassays (revised as of Aug. 2012)** | **Amount Spent 6/30/13** | **Balance 6/30/13** | **Result 2 Best bee plants (revised as of Aug. 2012)** | **Amount Spent 6/30/13** | **Balance 6/30/13** | **Result 3 Workshop (revised as of Aug. 2012)** | **Amount Spent 6/30/13** | **Balance 6/30/13** | **TOTAL BUDGET** | **TOTAL BALANCE** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **PERSONNEL:**<br>**Graduate Student, research** $35,000/yr, fringe is calculated at 17.14%. Increase of 3.25% is included for year 2 and 3=$104,984 | 104,984 | 104,984 | **0** | 0 | 0 | **0** | | 0 | **0** | 104,984 | **0** |
| **PERSONNEL:**<br>**Undergraduate Student, research residue and bioassay , best bee plants**<br>$14,000/yr, fringe is calculated at 9% =$42,000 **increase** | 33,168 | 35,330 | **0** | 20,000 | 20,000 | **0** | | 0 | **0** | 53,168 | **0** |
| **PERSONNEL:**<br>**Undergraduate Student, research best bee plants, development of website, outreach bulletins (update IPM manual, bee-friendly insecticide bulletin and table, and best bee plant bulletin and table)** $8,000/yr, fringe is calculated at 9% =$24,000 **increase** | 0 | 0 | **0** | 52,000 | 48,943 | **0** | | 0 | **0** | 52,000 | **0** |
| **Research supplies: Purchase bumble bees, honey bees, and beneficial insects from insectaries, rearing supplies, cages, plants, bioassay containers, analytical grade imidacloprid/clothianidin**<br>Bumble bee colonies w/queen (Koppert),<br>$120 x 48 colonies =$4,800 Bumblebees boxes 30 x $100=$3,000<br>Honey bee packages (70 pkgs x $65/pkg) =$4,500<br>beekeeping equipment (boxes, covers, wooden frames) =$1,500<br>Beneficial insects from Rincon-Vitova Insectaries<br>lady beetles 500 for $10 = 5 cages 5 trts X 2 reps X 8 reps/tr =80/5 X $10=$160 X 3 species lady beetles= $500<br>**increase** | 32,146 | 39,593 | **0** | | 0 | **0** | | 0 | **0** | 32,146 | **0** |
| green lacewings 240 for $52 = 2 cages<br>5 trts X 2 reps X 8 reps/trts=80/2 x $52=$2,100<br>Anagyrus 250 for $50 = 5cages<br>5 trts X 2 reps X 8 reps/trts=80/5= 16 X 50= $800<br>Bioquip cages $200 x 26cages= $5,200<br>Misc: netting replacement, diet, petri dishes, cotton, plastic film, wipes, postage, analytical grade imidacloprid and clothianidin, etc=$1,078 | | | **0** | | | **0** | | | **0** | 0 | **0** |
| **Research field space for demonstration project on best bee plants:** Purchasing young native plants, soil amendments, mulch, planting tools, irrigation, permanent display =$2,700<br>Land rental<br>$100 per plot (60 ft x30 ft) X 3yr = $300 **Remove** | 0 | 0 | **0** | 0 | 0 | **0** | | 0 | **0** | 0 | **0** |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Research field space for growing dandelion, rose, linden for residue analysis and bioassays:**<br>**Field space:** Dandelion and linden field space: $100 per plot (60 ft x30 ft), 1 plot dandelion and 2 plots linden=$300/yr X 3 years= $900<br>purchasing linden whips and planting = $1,000<br>**Rose field space:**<br>more expense since irrigation is needed. Each block is 960 sq ft. = $600/mo X 5 mo =$3,000 X 2 yr=$6,000<br>Insecticides for treating lindens, dandelion, rose = $100<br>**Reduce** | 500 | 500 | **0** | | 0 | **0** | | 0 | **0** | 500 | **0** |
| **Research greenhouse space for bioassays:**<br>8 treatments (= 3 passive (ladybeetle, lacewing, wasp) + 3 bee + 2 plant rearing) x 100 sg ft x $0.64/ sg ft x 12 mo=$6,600 /yr X 3 yr =$20,000 Reduce | 13,000 | 14,597 | **0** | | 0 | **0** | | 0 | **0** | 13,000 | **0** |
| **Residue analysis:** Measure amount of imidacloprid/clothianidin in pollen and nectar of dandelion, rose, linden with HPLC-mass spec.<br>ALS Laboratory Group, Environmental Division<br>Residue analysis $300/sample for preparing samples and HPLC-MS analysis imidacloprid, olefin, hydroxy and analysis for pollen and nectar<br>imidacloprid X 3 plant species (dandelion, rose, linden) X4 plants/species X 2 plant parts (pollen, nectar) X 2 rep exper X 3 trts =146 samples X $300=$43,000<br>clothianidin on rose X 5 plants/species X 2 plant parts (pollen, nectar) X 2 rep exper X 3 trts =60 samples X $300=$17,000<br>Total = $60,000 **Reduce** | 36,202 | 32,298 | **0** | | 0 | **0** | | 0 | **0** | 36,202 | **0** |
| **Travel to sites for research on best bee plants :** In Minnesota mileage @$0.55 X 800 mi =$400 mileage + $600 food (40 days X $15/day)= $1,000 yr X 3yr= $3,000. **Remove** | | 0 | **0** | 0 | **0** | **0** | | 0 | **0** | 0 | **0** |
| **Travel to outreach, workshops:** In Minnesota mileage @$0.55 x 200 mi=$400 mileage + $120 food (8 days X $15/day)= $520 x 2yr=$1,037. **Remove** | | 0 | **0** | | **0** | **0** | 0 | 0 | **0** | 0 | **0** |
| **Printing best bee plants bulletin, table, and poster:**<br>1. Bulletin with best bee plant table: available at demonstration site , for outreach, and for workshops $0.09/pg x 10 pg + cost paper = $1.00 @ 1000 copies=$1,000<br>2. Best bee plant poster 500 copies=$2,000 **Reduce** | | 0 | **0** | 1,500 | **755** | **0** | | 0 | **0** | 1,500 | **0** |
| **Printing updated IPM Manual:** 350 pages x $0.10/pg=$35 = colored pages/cover=$40 x 50 copies=$2,000<br>**Reduce** | 1,000 | 0 | **0** | | **0** | **0** | | 0 | **0** | 1,000 | **0** |
| **Printing reduced risk insecticides bulletin and table:**<br>Bulletin and table on reduced risk insecticides: $0.09/pg x 10 pg = $1.00 @ 1000 copies=$1,000 /yr<br>Green Expo = 300 copies/yr Pesticide Certification Workshops =200copies/yr<br>Master Gardener Training = 200 copies/yr<br>State agencies, landscapers = 100 copies/yr Workshop = 200 copies/yr **Reduce** | 1,000 | 0 | **0** | | **0** | **0** | | 0 | **0** | 1,000 | **0** |
| **Workshop costs:** invited speaker airfare, hotel, per diem, and honorarium **Reduce** | | 0 | **0** | | 0 | **0** | 1,500 | 0 | **0** | 1,500 | **0** |
| **COLUMN TOTAL** | **222,000** | **227,302** | **0** | **73,500** | **69,698** | **0** | **1,500** | **0** | **0** | **297,000** | **0** |