M.L. 2014 Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE: Understanding Systemic Insecticides as Protection Strategy for Bees
Project Manager: Vera Krischik
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 06b

APPROPRIATION AMOUNT: $326,000
AMOUNT SPENT: $325,534
AMOUNT REMAINING: $466

Overall Project Outcomes and Results

Our objectives were to understand how to protect pollinators. We wanted to understand if bees were affected when feeding on pollen from ornamental plants that were treated with imidacloprid, a neonicotinoid insecticide. Neonicotinoids are systemic and are applied to the soil or injected into trees. Both native bees, *Bombus impatiens*, and managed bees, *Apis meliifera,* are affected in similar ways by imidacloprid. The imidacloprid dose in flower pollen that kills bees is 40 ppb and below 25 ppb imidacloprid causes sublethal effects on behavior.

Objective 1-1, 1-2, 1-3.. Determine imidacloprid residue in leaves, flowers, soil, and pollen from a soil drench and trunk injection.
We studied imidacloprid residue in linden trees, bee friendly flowers, blueberries, and greenhouse plants grown to be installed in the landscape. Also, we investigated the effects on the EPA NOEL or sub-lethal limit of imidacloprid (20 ppb) on bumblebee colony health in the field.

Our data showed that trunk injections of imidacloprid caused very high levels of imidacloprid in flowers and pollen that would kill foraging bees. Soil drenches produced lower amounts in flower that are below the EPA sublethal level. However, dogwoods growing under the trees to which a soil drench was applied contained sufficient imidacloprid residue to kill a foraging bee. These same flowers would not kill a house sparrow that fed on the dogwood berries. However, recent papers say these sublethal levels will affect bird movement and feeding. Bee friendly plants in landscapes did not accumulate enough residue after 1 application to kill a foraging bee. However, greenhouse applications to flowering baskets and pots resulted in sufficient residues to kill foraging bees.

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee.
A tier 3 EPA research field study with replicate plots was performed on the St Paul UM Campus. The bumblebee colonies were free flying and were fed 20 ppb imidacloprid in sugar syrup. The EPA NOEL (Not Effective Adverse Level or sub lethal dose) is 25 ppb imidacloprid. The bees in the treated colonies showed decreased movement, decreased sugar consumption, decreased brood, deceased queen

production, and decreased hygenic behavior. Bumblebee colonies are negatively affected by 20 ppb imidacloprid. So the NOEL identified by the EPA in March 2016 as 25 ppb is incorrect.

Our residue data and our bumblebee study tells us that imidacloprid residue in flowers from a trunk injection or flowering plants growing under trees treated with soil drenches or greenhouse treated flowering plants would contain sufficient residue to kill or negatively affect native bumblebee colonies

Imidacloprid residue in plant parts after a standard imidacloprid EPA approved label rate application							
Species /application type	Applied	Leaves (ppb)	Soil (ppb)	Flowers (ppb)	Pollen (ppb)	Sub Lethal <25 ppb	Lethal >40 ppb
Ratio of imidacloprid in whole flowers to pollen							
13 EPA docs submitted by industry					25% of residue in flowers		
Prairie petunia, *Ruella humilis*	300 mg			1,100	267		X
Yellow bells, *Tecoma stans*	300 mg			109	109		X
Landscape trees: Imidacloprid residue ***Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection.***							
Linden 20 in DBH, soil drench	48 g	Yr1 July: 727 Aug: 1,023 Yr2 July 706 Aug: 429	Yr 1 July:15,430 Aug: 5,956 Yr 2 July:1,634 Aug: 534	34 No flow 81 No flow	9 No flow 20 No flow	X X	
Linden 8 in DBH, soil drench	14 g	July: 13,675 Aug: 25,250	July: 290 Aug: 385	34 No flow	9 No flow	X	
Linden 8 in DBH, trunk injection	3 g	July: 848 Aug: 36,283	July: 14 Aug: 14	1,340 No flow	335 No flow		X
Landscape trees: Imidacloprid residue ***Objective 1-2. Determine imidacloprid residue in native plants around imidacloprid-treated trees***							
Dogwoods under soil drench		July: 21,061	Aug: 16,787	762 Fruit: 425 will not kill house sparrows eating fruit	190		X
Landscape Bee plants: Imidacloprid residue ***Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers and blueberry from imidacloprid soil drenches.***							

Agastace foeniculum, anise hyssop	25 g	561		94	24	X	
Asclepias currassavica, tropical milkweed	25 g	132		87	22	X	
Commercial blueberries Collaboration with Koppert				residue in 5/6 flower samples (220, 136, 42, 10, 12 ppb), mean 84 ppb	Bumblee bee colonies in these fields declined.		X
Greenhouse Bee plants: Imidacloprid residue ***Objective 1-3. Determine if greenhouse grown plants in hanging baskets contained sufficient residue to harm foraging bees.***							
Prairie petunia, *Ruella humilis*	120 mg	July: 14,400 Aug: 2,086		July: 1,100 Aug: 502	July: 267 Aug: 126		X X
Million bells, *Calibrachoa*	200 mg	July: 67,266 Aug: 34,166		July: 1,972 Aug: 333	July: 615 Aug: 83		X X
Greenhouse Bee plants: Imidacloprid residue ***Objective 1-3. Determine if greenhouse grown plants in pots contained sufficient residue to harm foraging bees.***							
Agastace foeniculum, anise hyssop	300 mg			1,973	493		X
Asclepias currassavica, tropical milkweed	300 mg			1,568	392		X
Yellow bells, *Tecoma stans*	300 mg			106	106		X
Canola	300 mg			4,144	1,036		X
Rose Consumer label	300 mg			1,175	293		X
Rose Greenhouse label	240 mg			812	203		X
Landscape experiment on bumblebees at 20 ppb imidacloprid below EPA NOEL of 25 ppb ***Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee.***							
Imidacloprid at the EPA sublethal rate of 20 ppb caused fewer queens to be produced, lower nest weight, and less hygenic behavior compared to controls.							

Project Results Use and Dissemination

Dissemination: Objective 1-4. **Share the research results through outreach with talks, workshops, pollinator website, and interviews.**

We talked to the public and other researchers about the effects of pesticides on bees, the data from this research, and what municipalities and consumers could do in their green space to conserve bees. We held 3 workshops at the MN Landscape Arboretum, produced 2 websites on native bee conservation, spoke about the research in 10 talks/yr, and gave over 6 interviews/yr to radio, television, and print media.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2014 Work Plan

Date of Report: August 31, 2017
Date of Next Status Update Report: none
Date of Work Plan Approval: February 10, 2014
Project Completion Date: June 30, 2017
Does this submission include an amendment request? no

PROJECT TITLE: **Understanding Systemic Insecticides as Protection Strategy for Bees**

Project Manager: Vera Krischik
Affiliation: University of Minnesota
Mailing Address: 1980 Folwell Ave # 219
City / State / Zip: St. Paul, MN 55108
Telephone Number: 612-625-7044
Email Address: krisc001@umn.edu
Web Site Address: www.entomology.umn.edu/cues

Location: statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$326,000**
	Amount Spent:	**$325,534**
	Balance:	**$466**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 06b

Appropriation Language:
$326,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to continue research on how native bee and honey bee colonies are impacted by systemic, neonicotinyl insecticides in pollen and nectar of plants growing in fields and landscapes. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: **Understanding Systemic Insecticides as Protection Strategy for Bees**

II. PROJECT STATEMENT:
Honey bees and bumblebees pollinate 1,000's of native plants and crops that produce the seeds, fruits, and nuts that we consume and bees contribute approximately $15 billion worth of crop yields. Since 2007 managed honey bee colony mortality was estimated as 30% and also, native North American bumblebee species are in decline. Bee loss is due to a combination of factors, such as insecticides, habitat loss, and disease. Neonicotinyl insecticides are systemic, which means they are applied to the soil or on seeds and move from the soil to roots, leaves, pollen, and nectar. In the U.S., one-third of all crop (143 million acres / total 442 million acres) are treated with over 2 million pounds of neonicotinyl insecticides. In 2009 in Minnesota, corn, soybeans, potatoes and canola used 46,766 pounds and landscapes used 6,000 pounds of imidacloprid and 19,347 pounds of clothianidin, two of the chemicals that are classified as neonicotinyl insecticides. The high use of neonicotinyl

insecticides makes it probable that a foraging bee will eat nectar and pollen from a neonicotinyl-treated plant, which can reduce foraging, reduce colony health, and kill the bees. Bee loss will contribute to reduced pollination, seeds, and fruits of native plants and crops.

One of the major deficits in knowledge is how much neonicotinyl insecticide is found in pollen and nectar of neonicotinyl–treated plants, besides seed-treated crops. A canola seed is covered with 0.11 mg active imidacloprid (neonicotinyl chemical) that results in 7.6 ppb imidacloprid pollen. In urban landscapes, where bees forage for pollen and nectar, a soil surface application of imidacloprid can be applied to a native plant (300 mg) and basswood tree (67 g) from which basswood honey is produced. We calculate that a 609,000 times greater amount of imidacloprid is applied to basswood trees compared to a canola seed. We do not know how much imidacloprid accumulates in pollen and nectar from these applications in the landscape and field. The proposed research is performed in the field, which represents actual conditions.

The purpose of this research is:

1. Determine imidacloprid residue in pollen and nectar of basswood trees from an imidacloprid soil drench and trunk injection.
2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees.
3. Determine imidacloprid residue in pollen and nectar of native flowers, squash, and blueberry from imidacloprid soil drenches.
4. Determine the impacts of these imidacloprid residues on colony health of native bumblebee colonies.

This research is different from our 2010 LCCMR grant as all studies are done in the field and the previous study was done in the greenhouse. For the research and outreach products from the 2010 LCCMR grant visit "Pollinator conservation" (www.entomology.umn.edu/cues/pollinators/index.html).
We have letters of support from the Department of Agriculture in the State of Washington, Colorado State Beekeepers, Boulder County Beekeepers, Minnesota Honey Producers Association, and two Minnesota commercial beekeepers.

III. PROJECT STATUS UPDATES:

Amendment request August 15, 2016
1. We are requesting to move $70,000 from "Personnel" Activity 1 into "Professional Technical Contracts" to pay for USDA AMS pesticide residue charges. A graduate student ($42,251/yr) was to be hired on the grant, but departmental delays prevented this. However, additional funds are needed for pesticide residue, as charges have increased and we are analyzing more samples than previously anticipated so we can answer some technical questions (the relationship of residue in flowers to residue in pollen and residue in field collected plants). Funds will remain in "Personnel" in Activity 1 and 2 to pay for 2 research technicians. Increased costs in "Professional Technical Contracts" were not anticipated in the last progress report and are a retroactive request.
2. We are requesting to move $5,410 from 'Professional Service Contracts" Activity 1 into "Professional Technical Contracts" Activity 2. More funds are needed for USDA AMS pesticide residue charges (residue in bumblebee hives and sugar syrup treatments). Charges have increased and we are analyzing more samples than previously anticipated. The need for services by the Tree Care Company is finished.
3. We are requesting to move $5,300 from "Travel" into "Equipment/Tools/Supplies" Activity 2 ($3,000) to pay for supplies, since our costs for purchasing bumblebees and supplies to maintain bees was more than we expected. Travel is finished for Activity 1 and travel is not necessary for Activity 2 as the bee research is on the St. Paul campus. Amendment approved: [09/09/2016]

Amendment request March 12, 2015
1. We are requesting to change the report dates to match the UM SPA generation of budget reports. The new report dates are February 15 instead of January 30 and August 15 instead of June 30.
2. We are requesting to add two personnel types to existing personnel categories in the budget.

We are adding under the category “students” undergraduate students and retaining graduate students. We are adding under the category “non-students” partime employees and retaining lab supervisor. This does not change the work plan activities or the budgeted amount for personnel. Amendment approved: [3/18/2015]

Final report summary August 30 2017
Overall Project Outcomes and Results:
We have performed all the research and outreach objectives outlined in the grant proposal.

Our objectives are to understand how to protect pollinators. Part of the research is to understand if ornamental plants, after systemic neonicotinoid, imidacloprid application (Marathon, LD50=40 ppb, highly toxic to bees), contained sufficient residues to have sub lethal affects (20-40 ppb in pollen and nectar) or lethal affects (>40 ppb in pollen and nectar) on bees. In the field we studied imidacloprid residue in linden flowers, leaves, and soil; imidacloprid levels in flowers and leaves of bee -friendly plants; imidacloprid levels in flowers of commercial greenhouse flowers; and imidacloprid levels in commercial blueberries. Also, we did research to determine if greenhouse grown hanging baskets and pots contained sufficient residue of imidacloprid 10 weeks after application at the time of sale to harm foraging bumblebees. We investigated the effects on the EPA NOEL or sub-lethal limit of imidacloprid (20 ppb) on bumblebee colony health in the field. We held three workshops, spoke about the research in20 talks/yr, and gave over 30 interviews to radio, television, and print media.

Objective 1-1, 1-2, 1-3.. Determine imidacloprid residue in leaves, flowers, soil, and pollen from a soil drench and trunk injection.
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Our data showed that trunk injections of imidacloprid caused very high levels of imidacloprid in flowers and pollen that would kill foraging bees. Soil drenches produced lower amounts in flower that are below the EPA sublethal level. However, dogwoods growing under the trees to which a soil drench was applied contained sufficient imidacloprid residue to kill a foraging bee. These same flowers would not kill a house sparrow that fed on the dogwood berries. However, recent papers say these sublethal levels will affect bird movement and feeding. Bee friendly plants in landscapes did not accumulate enough residue after 1 application to kill a foraging bee. However, greenhouse applications to flowering baskets and pots resulted in sufficient residues to kill foraging bees.

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee.
A tier 3 EPA research field study with replicate plots was performed on the St Paul UM Campus. The bumblebee colonies were free flying and were fed 20 ppb imidacloprid in sugar syrup. The EPA NOEL (Not Effective Adverse Level or sub lethal dose) is 25 ppb imidacloprid. The bees in the treated colonies showed decreased movement, decreased sugar consumption, decreased brood, deceased queen production, and decreased hygenic behavior. Bumblebee colonies are negatively affected by 20 ppb imidacloprid. So the NOEL identified by the EPA in March 2016 as 25 ppb is incorrect.

Our residue data and our bumblebee study tells us that imidacloprid residue in flowers from a trunk injection or flowering plants growing under trees treated with soil drenches or greenhouse treated flowering plants would contain sufficient residue to kill or negatively affect native bumblebee colonies

Dissemination: Objective 1-4. **Share the research results through outreach with talks, workshops, pollinator website, and interviews.**
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workshops at the MN Landscape Arboretum, produced 2 websites on native bee conservation, spoke about the research in 10 talks/yr, and gave over 6 interviews/yr to radio, television, and print media.

Imidacloprid residue in plant parts after a standard imidacloprid EPA approved label rate application							
Species /application type	Applied	Leaves (ppb)	Soil (ppb)	Flowers (ppb)	Pollen (ppb)	Sub Lethal <25 ppb	Lethal >40 ppb
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13 EPA docs submitted by industry					25% of residue in flowers		
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Asclepias currassavica, tropical milkweed	25 g	132		87	22	X	
Commercial blueberries				residue in 5/6 flower	Bumblee		X

Collaboration with Koppert				samples (220, 136, 42, 10, 12 ppb), mean 84 ppb	bee colonies in these fields declined.		
Greenhouse Bee plants: Imidacloprid residue ***Objective 1-3. Determine if greenhouse grown plants in hanging baskets contained sufficient residue to harm foraging bees.***							
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Canola	300 mg			4,144	1,036		X
Rose Consumer label	300 mg			1,175	293		X
Rose Greenhouse label	240 mg			812	203		X
Landscape experiment on bumblebees at 20 ppb imidacloprid below EPA NOEL of 25 ppb ***Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee.***							
Imidacloprid at the EPA sublethal rate of 20 ppb caused fewer queens to be produced, lower nest weight, and less hygenic behavior compared to controls.							

Project status as of June 30 2017

Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection.

A technical issue needed some research. The USDA method uses whole flowers to determine residue levels. However, pollen and nectar levels may be higher or lower than whole flowers. Nectar is only produced in bright light at specific times of days and it is more difficult to collect nectar for residue analysis. In addition the current USDA method needs at least 3-1g samples of nectar for one injection into the HPLC for residue analysis, which cannot be collected in sufficient quantity from flowers. Data from our experiments and review of 13 reports submitted to the EPA by chemical companies indicate that 25% of the residue in whole flowers was found in pollen. In order to determine the ratio of residue in pollen and whole flowers we performed two studies. Prairie petunia, *Ruella*, had 267 ppb imidacloprid in pollen and 1,100 ppb in whole flowers, or residue in pollen is 25% of residue in whole flowers. In another species, yellow bells, *Tecoma stans*, imidacloprid in pollen and whole flowers was the same. To be very conservative we will take 25% of the residue in flowers to estimate residue in pollen.

At three locations in the Twin Cities large 20 in and small 8 in DBH (diameter breast height) linden trees were treated with soil drenches of imidacloprid. USDA generated residue data from an imidacloprid soil drench (48 g) of large 20 in DBH trees showed that flowers in yr2 had around 80 ppb (20 ppb) imidacloprid. Residue of imidacloprid in the soil under the tree were 15,430 (yr 1, June); 5,956(yr 1, August); 1634 (yr 2, June); and 534 (yr 2, August) ppb which would result in high levels in flowers growing under the trees. In small 8 in linden trees soil drenches (14 g) caused very high levels of imidacloprid in the soil (2 mo after treatment 21,061 ppb) that is easily transported into small dogwoods growing under the treated trees and result in 762 ppb (190 ppb at 25% reduction) in dogwood flowers and 672 ppb in dogwood fruits

In 8in DBH trees our data show that trunk injections of imidacloprid (3g) resulted in 1,340 ppb imidacloprid residue in flowers (335 ppb at 25% reduction; 40 ppb kill bumblebee) 2 months after treatment which will kill foraging pollinators. Imidacloprid trunk injections caused very small amounts of imidacloprid to accumulate in the soil (14 ppb) which would not cause sufficient amounts in flowers of plants growing under the trees.

Objective 1-2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees

Trunk injections resulted in low 14 ppb of imidacloprid in the soil. However, soil drenches caused very high levels of imidacloprid in the soil (2 mo after treatment 21,061 ppb) that is easily transported into small dogwoods growing under the treated trees and resulted in 762 ppb (190 ppb at 25% reduction) in dogwood flowers and 672 ppb in dogwood fruits. These volunteer plants growing under treated trees accumulated sufficient amount of imidacloprid in foliage and flowers to kill pollinators.

However, fruits may not contain high enough residue to kill birds. The LD50 for a house sparrow is 0.041mg/g and a mean sparrow weight is 24g, so 0.98 mg of imidacloprid will kill a house sparrow. A house sparrow would need to eat 5,800-10 g fruits to reach the LD50. The NOEL (no observable effect level) is 0.003 mg/g, which would be 0.072 mg of imidacloprid. A house sparrow would need to eat 428-10 g fruits to reach the NOEL and for sub lethal behavioral effects to be observed.

Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers, and blueberry from imidacloprid soil drenches. Determine if greenhouse grown plants in hanging baskets and pots contained sufficient residue to harm foraging bees.

Soil drenches of imidacloprid (57 g /plant applied to the soil, Bayer consumer product) were done in each summer for three years. Residue in flowers and leaves were determined two months after application. The imidacloprid soil drench in Tropical Milkweed, *Ascelpius curasavica*, and Anise Hyssop, *Agastache foeniculum,* resulted in 90 ppb in flowers (25 ppb at 25% reduction) and 350 ppb in leaves (86 ppb), which is the NOEL for imidacloprid. However, out bumblebee study in objective 2-1 demonstrated that 20 ppb resulted in reduced colony health and queen production.

In addition, label rates of imidacloprid (300 mg) were applied to plants growing in 3 gallon pots every summer for three summers. Residue in flowers and leaves were determined two months after application. Very high imidacloprid residue was found in flowers (1973 (493) ppb hyssop and 1568 (392) ppb milkweed. These levels of residue killed honey bees foraging on the flowers every summer (P=0.0285).

Koppert Biological sells the bumblebees used in the research and used to augment pollinators in various crops. Koppert and their growers experienced mortality of bumblebee colonies in blueberry farms that use imidacloprid. Kristine Blum from Koppert bumblebee production collected samples of flowers in two grower's field. Imidacloprid residue was found in 5/6 flower samples (220, 136, 42, 10, 12 ppb). The pollen contained a mean 84 (21) ppb imidacloprid, which in our experiments reduced bumblebee foraging and colony health.

We performed an experiment to determine if plants purchased at garden centers by consumers may contain neonicotinoid residue. Residue in Prairie petunia (*Ruella humilis*, native) growing in hanging baskets were treated with a label rate of imidacloprid. Imidacloprid residues in flowers were 1,100 ppb at wk5 (267 ppb actual in pollen at 25% flower to pollen ratios) and 502 ppb at wk10 (125 ppb estimated in pollen). These residue levels of imidacloprid will alter behavior and kill bees.

Small pots (4 in) containing *Calibrachoa* (million bells, annual plant) were treated with foliar applied pymetrozine (Endeavor, LD50=1580 ppb, nontoxic to bees), soil applied imidacloprid (Marathon, LD50=40 ppb, highly toxic to bees) and soil applied dinotefuran (Safari, LD50=230 ppb, highly toxic to bees) and sampled at 5 and 10 wk post application.

For all neonicotinoid insecticides, residue in leaves and flowers decreased from 5 to 10 wk. By 10 wk, flowers in imidacloprid and dinotefuran treatments contained similar amounts of residue in sufficient amount that would kill foraging bees. Pymetrozine is a good alternative to neonicotinoid insecticides for managing aphids, since no aphids returned and no residue was found at 10 wk .

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee. A tier 3 EPA research field study with replicate plots (n=6-8 colonies/plot ,repeated June and August, total= 28 colonies) were performed on the St Paul UM Campus. The bumble colonies were free flying and were fed 20 ppb imidacloprid in sugar syrup. Around 120 pollinator plants in 3 gallon pots were placed around the nests to ensure that the bees were not nectar or pollen limited. The EPA NOEL (Not Effective Adverse Level or sub lethal dose) is 25 ppb imidacloprid. The bees in the treated colonies showed decreased movement, decreased sugar consumption, decreased brood, deceased queen production, and increased growth of fungus compared to control colonies. Bumblebee colonies are negatively affected by 20 ppb imidacloprid. So the NOEL identified by the EPA in March 2016 as 25 ppb is incorrect. Our residue data tells us that residue in flowers from a trunk injection or soil drench would be sufficient to negatively affect native bumblebee colonies.

Objective 1-4. **Share the research results through outreach with talks, workshops, pollinator website, and interviews.** Various radio and television interviews were given to promote pollinators and disseminate the research results from January to August 2017.

11 radio/television interviews in 2016 and 2017
63 talks were provided
2 websites on native bee conservation were created
http://ncipmhort.cfans.umn.edu/
https://campus.extension.org/enrol/index.php?id=1244
6 outreach products were posted at the UM Extension Nursery website
http://www.extension.umn.edu/garden/plant-nursery-health/
http://www.extension.umn.edu/garden/plant-nursery-health/toxicity-pollinators-insecticides/index.html).
A 3 part workshop on November 6, 2014, March 26, 2017, and May 21, 2017 on pollinator issues called "Pollinator cubed" was held at the University of Minnesota Landscape Arboretum and was attended by over 200 people; www.arboretum.umn.edu/Pollinators3.aspx .

Project status as of February 15 2017
Our objectives are to understand how to protect pollinators and beneficial insects. Our goal is to understand if ornamental plants, after application of the systemic, neonicotinoid imidacloprid to the soil or injected into trees, accumulate residue that potentially can alter behavior or increase mortality in beneficial insects.

Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection ; Objective 1-2. Determine the imidacloprid residue in native plants around imidacloprid-

treated trees; Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers, squash, and blueberry from imidacloprid soil drenches.

The USDA AMS Gastonia NC Lab performs the residue analysis for this research. Currently, we are waiting for the residue analysis to be finished.

The USDA method uses whole flowers to determine residue levels. However, pollen and nectar levels may be higher or lower than whole flowers. Data from our experiments and review of 13 reports submitted to the EPA by chemical companies indicate that 25% of the residue in whole flowers may be a conservative estimate of residue levels found in pollen and nectar. Our data show that trunk injections of imidacloprid result in 1,340 ppb imidacloprid residue in flowers (335 ppb at 25% reduction; 100 ppb kill bumblebee) 2 months after treatment which will kill foraging pollinators. Imidacloprid trunk injections cause very small amounts of imidacloprid to accumulate in the soil (14 ppb) which would not cause sufficient amounts in flowers of plants growing under the trees.

However, soil drenches cause very high levels of imidacloprid in the soil (2mo after treatment 21,061 ppb) that is easily transported into small dogwoods growing under the treated trees and result in 762 ppb (190 ppb at 25% reduction) in dogwood flowers and 672 ppb in dogwood fruits. These volunteer plants growing under treated trees accumulated sufficient amount of imidacloprid in foliage and flowers to kill pollinators.

However, fruits may not contain high enough residue to kill birds. The LD50 for a house sparrow is 0.041mg/g and a mean sparrow weight is 24g, so 0.98 mg of imidacloprid will kill a house sparrow. A house sparrow would need to eat 128-10 g fruits to reach the LD50. The NOEL (no observable effect level) is 0.003 mg/g, which would be 0.072 mg of imidacloprid. A house sparrow would need to eat 10-10 g fruits to reach the NOEL.

Kristine Blum from Koppert bumblebee production collected samples of blueberry flowers in grower's field. In 5/6 flower samples imidacloprid residue was found (220, 136, 42, 10, 12 ppb, mean = 84 ppb). Again, if we take 25% of 84 ppb, the pollen and nectar contains 21 ppb, which in our experiments reduced bumblebee foraging and colony health. Ms. Blum sent the samples to the USDA as her growers were experiencing bumblebee colony death in many of the blueberry fields that ordered colonies form Koppert. She was concerned that it was imidacloprid causing the bumble bee colonies to die.

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee
A field study with 26 bumble colonies that were free flying and were fed 25 ppb imidacloprid in sugar syrup, showed decreased movement, decreased sugar consumption, decreased brood, deceased queen production, and increased growth of fungus in the treated compared to control colonies. So the NOEL (no observable effect concentration) identified by the EPA in March 2016 as 25 ppb is incorrect. Bumblebee colonies are negatively affected by 25 ppb imidacloprid. Our residue data tells us that residue in flowers from a trunk injection or soil drench would be sufficient to negatively affect native bumblebee colonies.

The public has expressed interest in this research and over 26 talks were provided in 2016. Also a 3 part workshop at the University of Minnesota Landscape Arboretum was created and attended by over 200 people in 2014 - 2015 www.arboretum.umn.edu/Pollinators3.aspx . In addition, 6 outreach products were posted on the UMN extension website www.extension.umn.edu/garden/plant-nursery-health/.

Another UM website was created with videos and webinars on IPM, pollination, native bees, and invasive species management at ncipmhort.cfans.umn.edu/ cues.cfans.umn.edu/ .

Project status as of August 15 2016
Our objectives are to understand how to protect pollinators. Part of the research is to understand if ornamental plants, after application of the systemic, neonicotinoid imidacloprid to the soil, contained sufficient residues in

flowers to have sub lethal affects (sub lethal effects on behavior at >25 ppb in pollen and nectar, EPA, March 2016) or lethal affects (>150 ppb in pollen and nectar) on bees. In summary, soil drenches of imidacloprid to trees, blueberries, and flowers result in sufficient residue to alter behavior and cause decline of bumble bee colonies in the field.

Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection and Objective 1-2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees. Data showed residues in flowers of trunk injected trees were high enough to kill foraging bees (>150 ppb). We found that imidacloprid trunk injections in May resulted in June in residues of 1,340 ppb in basswood flowers (335 ppb actual in pollen at a conversion of 25% less residue in pollen compared to whole flowers), 14 ppb in soil, and 848 pp in leaves. Imidacloprid soil drenches in May resulted in 34 ppb (25 ppb (actual), >25 EPA value that alters behavior) in flowers and 762 ppb (180 ppb (actual),>150 ppb causes mortality) in dogwood flowers growing under the treated trees, while leaves had 13,675 ppb, and soil 21,061ppb. Much of the imidacloprid that would have been in the soil was taken up by the 2 ft high dogwoods growing under the basswood trees.

Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers, squash, and blueberry from imidacloprid soil drenches. Koppert Biological sells the bumblebees used in the research and they experienced mortality of bumblebee colonies in blueberry farms that use imidacloprid. In two separate farms, imidacloprid residues in blueberry flowers was 103 ppb and 136 ppb, while in three other farms the residue of imidacloprid was 9.6-41.7 ppb. Residue in Prairie petunia (*Ruella humilis*) from a greenhouse application resulted in imidacloprid residues in flowers of 1,100 ppb (267 ppb actual in pollen) at wk5 and 502 ppb (125 ppb estimated in pollen at 25% flower to pollen) at wk10, which will alter behavior and kill bees.

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee colonies. Two replicate plots on the St. Paul campus contained bumblebee colonies that were provided 25 ppb imidacloprid in nectar (sub lethal effects on behavior at >25 ppb in pollen and nectar, EPA, March 2016). Treated colonies started to decline in the field wk2 after imidacloprid treatment , due to lower feeding (nectar consumption), lower nectar storage in honey pots, reduced egg production, and reduced movement. In treated colonies the queen died in 2/6 colonies, while no queens died in controls (0/5). We are in the processing of performing the second replicate of this study.

In summary, soil drenches of imidacloprid to trees, blueberries, and flowers result in sufficient residue to alter behavior and cause decline of in bumble bee colonies in the field.

Project status as of February 15 2016

Our objectives are to understand how to protect pollinators. Part of the research is to understand if ornamental plants, after systemic insecticide application, contained sufficient residues to have sub lethal affects (20-40 ppb in pollen and nectar) or lethal affects (>40 ppb in pollen and nectar) on bees. In the field we studied residue in trees (see August 15 2015 data) and flowers (residue analysis in progress at USDA, AMS Gastonia).

We performed an experiment to determine if plants purchased at garden centers by consumers may contain neonicotinoid residue. Small pots (4 in) containing *Calibrachoa* (million bells, annual plant) were treated with foliar applied pymetrozine (Endeavor, LD50=1580 ppb, nontoxic to bees), soil –applied imidacloprid (Marathon, LD50=40 ppb, highly toxic to bees) and soil –applied dinotefuran (Safari, LD50=230 ppb, highly toxic to bees) and sampled at 5 and 10 wk post application.

For all neonicotinoid insecticides, residue in leaves and flowers decreased from 5 to 10 wk. By 10 wk, flowers in imidacloprid and dinotefuran treatments contained similar amounts of residue, that would kill foraging bees. The plants contained imidacloprid at purchase, before we applied any insecticide. Pymetrozine is a good alternative to neonicotinoid insecticides for managing aphids, since no aphids returned and no residue was found at 10 wk .

A new website http://ncipmhort.cfans.umn.edu/was created on “Mitigating pollinator decline” with a pre-recorded webinar, videos on bee-friendly plants, plant lists, videos on native bee foraging, and identification information and pictures of 26 bee families.

Figure1. **Flowers:** *Calibrachoa* purchased in 4in sg pots were treated on June 28 2015 with pymetrozine and July1 2015 with imidacloprid and dinotefuran and sampled on August 3 2015 (5wk) and Sept 8 2015 (10wk). Leaves and flowers were analyzed at the USDA AMS Gastonia lab in Jan 2016.

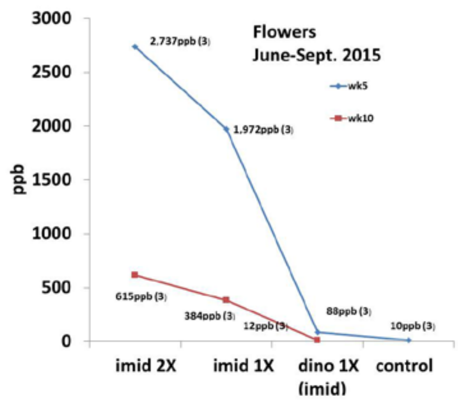
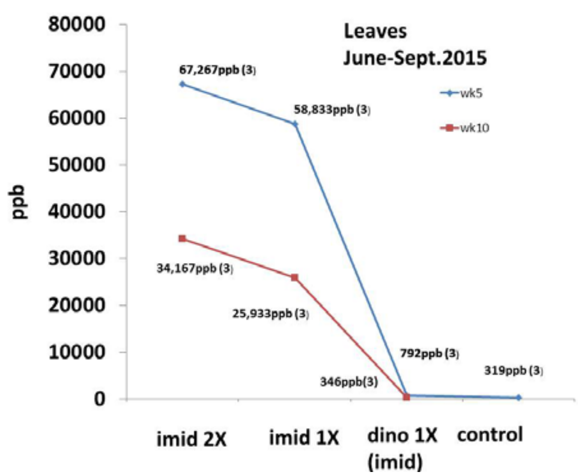


Figure 2. Leaves: *Calibrachoa* purchased in 4in sg pots. Pots were treated on June 28 2015 with pymetrozine and July1 2015 with imidacloprid and dinotefuran and sampled on August 3 2015 (5wk) and Sept 8 2015 (10wk). Leaves and flowers were analyzed at the USDA AMS Gastonia lab in Jan 2016.



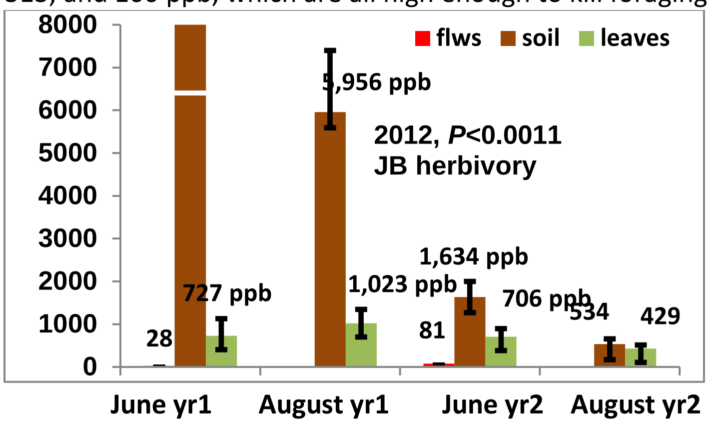
Project status as of August 15 2015

Two replicate basswood/linden plots were established on the Minneapolis Park and Recreation Board (MPRB) land on the west side of the Mississippi River across from the UM campus and 2 sites on the west bank of the UM campus. In June both sites were treated with 3 treatments (trt) by S&S trees: soil drench (10 trees/trt/plot), soil injection, and trunk injection. Leaves, soil, flowers, and dogwood flowers growing under the soil drench trees were collected in June and August for imidacloprid residue analysis.

Landscape plots (13) were established on the St. Paul campus containing milkweed, giant anise hyssop, rose, blueberry, and pussy willows. In August a consumer-landscape rate of imidacloprid was applied to 2 species (milkweed and giant anise hyssop). In September flowers and leaves were collected for imidacloprid residue analysis.

In the greenhouse, Mexican petunia samples were collected to determine the imidacloprid residue levels in flower, leaf, and pollen. This is a technique issue that needs some data and discussion, the relationship between whole flower residue and pollen residue. Samples from the above experiments were mailed on dry ice to the USDA AMS lab in Gastonia, NC for imidacloprid analysis.

USDA generated residue data from an imidacloprid soil drench (48g) of large 20in dbh (diameter breast height) trees showed that flowers in yr2 had around 80 ppb imidacloprid, which was high enough to kill foraging bees. Levels in the soil under the tree were 15,430; 5,956; 1634; and 534 ppb which would result in high levels in flowers growing under the trees. Based on data from other experiments, flowers would have around 3130, 900, 313, and 100 ppb, which are all high enough to kill foraging bees.



An extension bulletin was peer-reviewed and posted on my extension website on the LD50 to bees of all insecticides registered for use on plants in landscape and greenhouse. (http://www.extension.umn.edu/garden/plant-nursery-health/toxicity-pollinators-insecticides/index.html).

Project status as of January 30 2015

Research plants were grown to install in the landscape (roses, Mexican petunia, *Ruellia hirtus*, Mexican milkweeds, *Ascelpius curasavica*, and giant anise hyssop, *Agastache foeniculum*).

The native Mexican petunia produces copious amounts of nectar. Research with this plant will permit us to measure the imidacloprid residue in nectar and whole flowers and then make a regression between imidacloprid residue in nectar and whole flowers. In this way, we can estimate the amount of imidacloprid residue in nectar of species of plants that produce too little nectar to collect. Currently, from these plants we collect residues from whole flowers, which overestimate the amount of imidacloprid residue. In early September, the Mexican petunia plants went dormant and stopped flowering so we placed them into coolers to overwinter. We planned to return the petunias to the greenhouse in January 2015 to make flowers and start the experiment.

Overall Project Outcomes and Results:

The outcome and results of this project are to understand how much residue of imidacloprid is found in pollen and nectar of flowering plants from a soil drench and trunk injection of imidacloprid and investigate the effects on bumblebee colony health in the field.

1V. PROJECT ACTIVITIES AND OUTCOMES

ACTIVITY 1: Determine imidacloprid residue in pollen and nectar of flowers
Description: We will determine the amount of imidacloprid in nectar and pollen of flowering plants after a soil drench and trunk injection of imidacloprid. The USDA AMS Lab in Gastonia, NC performed the residue analysis as the results will be accepted by the EPA and other regulatory agencies interested in the effects of imidacloprid on bees and beneficial insects.

Summary Budget Information for Activity 1: **ENRTF Budget $268,590**
Amount Spent: $268,124
Balance: $466

Activity Completion Date: June 30 2017

Outcome 1. Determine imidacloprid in flowers.	**Completion Date**	**Budget**
1-1. Determine imidacloprid residue in pollen and nectar of basswood trees from a soil drench and trunk injection.	2017	$120,000
1-2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees.	2017	$43,610
1-3. Determine imidacloprid residue in pollen and nectar of native flowers, squash, and blueberry from imidacloprid soil drenches.	2017	$76,300
1-4. Share the research results with collaborators through talks, additions to the pollinator website, and emails.	2017	$0

Final report summary August 30 2017
We have performed all the research and outreach objectives outlined in the grant proposal.

Our objectives were to understand how to protect pollinators. We wanted to understand if bees were affected when feeding on pollen from ornamental plants that were treated with imidacloprid, a neonicotinoid insecticide. Neonicotinoids are systemic and are applied to the soil or injected into trees. Both native bees, *Bombus impatiens*, and managed bees, *Apis meliifera,* are affected in similar ways by imidacloprid. The imidacloprid dose in flower pollen that kills bees is 40 ppb and below 25 ppb imidacloprid causes sublethal effects on behavior.

Objective 1-1, 1-2, 1-3.. Determine imidacloprid residue in leaves, flowers, soil, and pollen from a soil drench and trunk injection.
We studied imidacloprid residue in linden trees, bee friendly flowers, blueberries, and greenhouse plants grown to be installed in the landscape. Also, we investigated the effects on the EPA NOEL or sub-lethal limit of imidacloprid (20 ppb) on bumblebee colony health in the field.

Our data showed that trunk injections of imidacloprid caused very high levels of imidacloprid in flowers and pollen that would kill foraging bees. Soil drenches produced lower amounts in flower that are below the EPA sublethal level. However, dogwoods growing under the trees to which a soil drench was applied contained sufficient imidacloprid residue to kill a foraging bee. These same flowers would not kill a house sparrow that fed on the dogwood berries. However, recent papers say these sublethal levels will affect bird movement and feeding. Bee friendly plants in landscapes did not accumulate enough residue after 1 application to kill a foraging bee. However, greenhouse applications to flowering baskets and pots resulted in sufficient residues to kill foraging bees.

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee.
A tier 3 EPA research field study with replicate plots was performed on the St Paul UM Campus. The bumblebee colonies were free flying and were fed 20 ppb imidacloprid in sugar syrup. The EPA NOEL (Not Effective

Adverse Level or sub lethal dose) is 25 ppb imidacloprid. The bees in the treated colonies showed decreased movement, decreased sugar consumption, decreased brood, deceased queen production, and decreased hygenic behavior. Bumblebee colonies are negatively affected by 20 ppb imidacloprid. So the NOEL identified by the EPA in March 2016 as 25 ppb is incorrect.

Our residue data and our bumblebee study tells us that imidacloprid residue in flowers from a trunk injection or flowering plants growing under trees treated with soil drenches or greenhouse treated flowering plants would contain sufficient residue to kill or negatively affect native bumblebee colonies

Dissemination: Objective 1-4. **Share the research results through outreach with talks, workshops, pollinator website, and interviews.**
We talked to the public and other researchers about the effects of pesticides on bees, the data from this research, and what municipalities and consumers could do in their green space to conserve bees. We held 3 workshops at the MN Landscape Arboretum, produced 2 websites on native bee conservation, spoke about the research in 10 talks/yr, and gave over 6 interviews/yr to radio, television, and print media.

Imidacloprid residue in plant parts after a standard imidacloprid EPA approved label rate application							
Species /application type	Applied	Leaves (ppb)	Soil (ppb)	Flowers (ppb)	Pollen (ppb)	Sub Lethal <25 ppb	Lethal >40 ppb
Ratio of imidacloprid in whole flowers to pollen							
13 EPA docs submitted by industry					25% of residue in flowers		
Prairie petunia, *Ruella humilis*	300 mg			1,100	267		X
Yellow bells, *Tecoma stans*	300 mg			109	109		X
Landscape trees: Imidacloprid residue ***Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection.***							
Linden 20 in DBH, soil drench	48 g	Yr1 July: 727 Aug: 1,023 Yr2 July 706 Aug: 429	Yr 1 July:15,430 Aug: 5,956 Yr 2 July:1,634 Aug: 534	34 No flow 81 No flow	9 No flow 20 No flow	X X	
Linden 8 in DBH, soil drench	14 g	July: 13,675 Aug: 25,250	July: 290 Aug: 385	34 No flow	9 No flow	X	
Linden 8 in DBH, trunk injection	3 g	July: 848 Aug: 36,283	July: 14 Aug: 14	1,340 No flow	335 No flow		X
Landscape trees: Imidacloprid residue ***Objective 1-2. Determine imidacloprid residue in native plants around imidacloprid-treated trees***							
Dogwoods under soil drench		July: 21,061	Aug: 16,787	762 Fruit: 425 will not kill house	190		X

				sparrows eating fruit			
Landscape Bee plants: Imidacloprid residue ***Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers and blueberry from imidacloprid soil drenches.***							
Agastace foeniculum, anise hyssop	25 g	561		94	24	X	
Asclepias currassavica, tropical milkweed	25 g	132		87	22	X	
Commercial blueberries Collaboration with Koppert				residue in 5/6 flower samples (220, 136, 42, 10, 12 ppb), mean 84 ppb	Bumblee bee colonies in these fields declined.		X
Greenhouse Bee plants: Imidacloprid residue ***Objective 1-3. Determine if greenhouse grown plants in hanging baskets contained sufficient residue to harm foraging bees.***							
Prairie petunia, *Ruella humilis*	120 mg	July: 14,400 Aug: 2,086		July: 1,100 Aug: 502	July: 267 Aug: 126		X X
Million bells, *Calibrachoa*	200 mg	July: 67,266 Aug: 34,166		July: 1,972 Aug: 333	July: 615 Aug: 83		X X
Greenhouse Bee plants: Imidacloprid residue ***Objective 1-3. Determine if greenhouse grown plants in pots contained sufficient residue to harm foraging bees.***							
Agastace foeniculum, anise hyssop	300 mg			1,973	493		X
Asclepias currassavica, tropical milkweed	300 mg			1,568	392		X
Yellow bells, *Tecoma stans*	300 mg			106	106		X
Canola	300 mg			4,144	1,036		X
Rose Consumer label	300 mg			1,175	293		X
Rose Greenhouse label	240 mg			812	203		X

Project status as of June 30 2017

Our objectives are to understand how to protect pollinators. Part of the research is to understand if ornamental plants, after systemic neonicotinoid, imidacloprid application (Marathon, LD50=40 ppb, highly toxic to bees), contained sufficient residues to have sub lethal affects (20-40 ppb in pollen and nectar) or lethal affects (>40 ppb in pollen and nectar) on bees. In the field we studied imidacloprid residue in linden flowers, leaves, and soil; imidacloprid levels in flowers and leaves of bee -friendly plants; imidacloprid levels in flowers of commercial greenhouse flowers; and imidacloprid levels in commercial blueberries. Also, we did research to determine if greenhouse grown hanging baskets and pots contained sufficient residue of imidacloprid 10 weeks after application at the time of sale to harm foraging bumblebees. We investigated the effects on the EPA NOEL or sub-lethal limit of imidacloprid (20 ppb) on bumblebee colony health in the field. We held three workshops, spoke about the research in20 talks/yr, and gave over 30 interviews to radio, television, and print media.

Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection.

A technical issue needed some research. The USDA method uses whole flowers to determine residue levels. However, pollen and nectar levels may be higher or lower than whole flowers. Nectar is only produced in bright light at specific times of days and it is more difficult to collect nectar for residue analysis. In addition the current USDA method needs at least 3-1g samples of nectar for one injection into the HPLC for residue analysis, which cannot be collected in sufficient quantity from flowers. Data from our experiments and review of 13 reports submitted to the EPA by chemical companies indicate that 25% of the residue in whole flowers was found in pollen. In order to determine the ratio of residue in pollen and whole flowers we performed two studies. Prairie petunia, *Ruella*, had 267 ppb imidacloprid in pollen and 1,100 ppb in whole flowers, or residue in pollen is 25% of residue in whole flowers. In another species, yellow bells, *Tecoma stans*, imidacloprid in pollen and whole flowers was the same. To be very conservative we will take 25% of the residue in flowers to estimate residue in pollen.

At three locations in the Twin Cities large 20 in and small 8 in DBH (diameter breast height) linden trees were treated with soil drenches of imidacloprid. USDA generated residue data from an imidacloprid soil drench (48 g) of large 20 in DBH trees showed that flowers in yr2 had around 80 ppb (20 ppb) imidacloprid. Residue of imidacloprid in the soil under the tree were 15,430 (yr 1, June); 5,956(yr 1, August); 1634 (yr 2, June); and 534 (yr 2, August) ppb which would result in high levels in flowers growing under the trees. In small 8 in linden trees soil drenches (14 g) caused very high levels of imidacloprid in the soil (2 mo after treatment 21,061 ppb) that is easily transported into small dogwoods growing under the treated trees and result in 762 ppb (190 ppb at 25% reduction) in dogwood flowers and 672 ppb in dogwood fruits

In 8in DBH trees our data show that trunk injections of imidacloprid (3g) resulted in 1,340 ppb imidacloprid residue in flowers (335 ppb at 25% reduction; 40 ppb kill bumblebee) 2 months after treatment which will kill foraging pollinators. Imidacloprid trunk injections caused very small amounts of imidacloprid to accumulate in the soil (14 ppb) which would not cause sufficient amounts in flowers of plants growing under the trees.

Objective 1-2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees

Trunk injections resulted in low 14 ppb of imidacloprid in the soil. However, soil drenches caused very high levels of imidacloprid in the soil (2 mo after treatment 21,061 ppb) that is easily transported into small dogwoods growing under the treated trees and resulted in 762 ppb (190 ppb at 25% reduction) in dogwood flowers and 672 ppb in dogwood fruits. These volunteer plants growing under treated trees accumulated sufficient amount of imidacloprid in foliage and flowers to kill pollinators.

However, fruits may not contain high enough residue to kill birds. The LD50 for a house sparrow is 0.041mg/g and a mean sparrow weight is 24g, so 0.98 mg of imidacloprid will kill a house sparrow. A house sparrow would need to eat 5,800-10 g fruits to reach the LD50. The NOEL (no observable effect level) is 0.003 mg/g, which would be 0.072 mg of imidacloprid. A house sparrow would need to eat 428-10 g fruits to reach the NOEL and for sub lethal behavioral effects to be observed.

Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers, and blueberry from imidacloprid soil drenches. Determine if greenhouse grown plants in hanging baskets and pots contained sufficient residue to harm foraging bees.

Soil drenches of imidacloprid (57 g /plant applied to the soil, Bayer consumer product) were done in each summer for three years. Residue in flowers and leaves were determined two months after application. The imidacloprid soil drench in Tropical Milkweed, *Ascelpius curasavica*, and Anise Hyssop, *Agastache foeniculum,* resulted in 90 ppb in flowers (23 ppb at 25% reduction) and 350 ppb in leaves (86 ppb), which is the NOEL for imidacloprid. However, out bumblebee study in objective 2-1 demonstrated that 20 ppb resulted in reduced colony health and queen production.

In addition, label rates of imidacloprid (300 mg) were applied to plants growing in 3 gallon pots every summer for three summers. Residue in flowers and leaves were determined two months after application. Very high imidacloprid residue was found in flowers (1973 (493) ppb hyssop and 1568 (392) ppb milkweed. These levels of residue killed honey bees foraging on the flowers every summer (P=0.0285).

Koppert Biological sells the bumblebees used in the research and used to augment pollinators in various crops. Koppert and their growers experienced mortality of bumblebee colonies in blueberry farms that use imidacloprid. Kristine Blum from Koppert bumblebee production collected samples of flowers in two grower's field. Imidacloprid residue was found in 5/6 flower samples (220, 136, 42, 10, 12 ppb). The pollen contained a mean 84 (21) ppb imidacloprid, which in our experiments reduced bumblebee foraging and colony health.

We performed an experiment to determine if plants purchased at garden centers by consumers may contain neonicotinoid residue. Residue in Prairie petunia (*Ruella humilis*, native) growing in hanging baskets were treated with a label rate of imidacloprid. Imidacloprid residues in flowers were 1,100 ppb at wk5 (267 ppb actual in pollen at 25% flower to pollen ratios) and 502 ppb at wk10 (125 ppb estimated in pollen). These residue levels of imidacloprid will alter behavior and kill bees.

Small pots (4 in) containing *Calibrachoa* (million bells, annual plant) were treated with foliar applied pymetrozine (Endeavor, LD50=1580 ppb, nontoxic to bees), soil applied imidacloprid (Marathon, LD50=40 ppb, highly toxic to bees) and soil applied dinotefuran (Safari, LD50=230 ppb, highly toxic to bees) and sampled at 5 and 10 wk post application.

For all neonicotinoid insecticides, residue in leaves and flowers decreased from 5 to 10 wk. By 10 wk, flowers in imidacloprid and dinotefuran treatments contained similar amounts of residue in sufficient amount that would kill foraging bees. Pymetrozine is a good alternative to neonicotinoid insecticides for managing aphids, since no aphids returned and no residue was found at 10 wk .

Objective 1-4. **Share the research results through outreach with talks, workshops, pollinator website, and interviews.** Various radio and television interviews were given to promote pollinators and disseminate the research results from January to August 2017.

Interviews on radio in 2016 ad 2017

Krischik, MN PBS TV Almanac, Friday night news show, August 4, 2017:
http://www.tpt.org/almanac/video/The-Wrap--Top--Minnesota-Favorite-Bugs-30524/
http://www.tpt.org/almanac/video/Japanese-Beetles-Swarm-Minnesota-Gardens-30517/
Krischik, MN public radio, Friday July 28, 2017
Krischik, MN WCCO radio, Friday July 28, 2017
Krischik, MN WCCO television, Monday July 31, 2017

Krischik, Tonka Gardens Pollinator Festival, Sunday July 30 2017
Krischik, Radio Interview, Food Seuth Radio, June 7, 2017, https://beta.prx.org/stories/210290
Krischik, "Pollinator Week Beyond pesticides program for Washington, DC", June 2, 2017
http://beyondpesticides.org/dailynewsblog/2017/06/restaurants-nations-capital-feature-foods-reliant-pollinators-national-pollinator-week/
Krischik, video Symposium Keynote speaker, 22 mins, Beyond Pesticides 35TH National Pesticide Forum, April 29, 2017, Bees, Pollinators, and Biodiversity
Krischik radio interview, 1 hr, Pollination festival in Stillwater, MN, September 11, 2016
https://www.spreaker.com/user/backroomstewdios/jewbalations-at-the-polli-nation-event
Krischik radio talk show, 1 hr, MN Broadcasters Association, Jim du Bois, August 6, 2016
http://www.accessminnesotaonline.com/2016/08/03/the-decline-of-insect-pollinators/

Various outreach bulletins, videos, and talks were created to promote pollinators and disseminate the research results from 2014 to 2017.

Talks
The public has expressed interest in this research and over 63 talks were provided from 2014 to 2017. Each year I give talks to at least 5 large nursery and landscape industry workshops. At each meeting for the 3 year grant period I spoke of different ways to protect pollinators in greenhouses and landscapes.

I provided talks at meetings on the research to NCERA 224, USDA Nursery and Landscape Group; USDA SARE NCIPM Stakeholders meeting; ESA (entomology Society National Meeting); and SETAC (Society of Environmental Toxicology and Chemistry).

Bulletins on websites
In June 2017 in cooperation with the USFWS, Michigan DNR, Wisconsin, DNR, Xerces Society for Invertebrate Conservation, and UMinnesota, two bulletins on protecting Rusty patch bumble bee (RPBB) were created and are posted online at the UM Extension Nursery website. The bulletins discussed how to reduce pesticide use in urban landscapes and farmlands to bring back RPBB http://www.extension.umn.edu/garden/plant-nursery-health/

An extension bulletin was peer-reviewed and posted in August 2015 on the UM Extension Nursery Website on the LD50 to bees of all insecticides registered for use on plants in landscape and greenhouse. (http://www.extension.umn.edu/garden/plant-nursery-health/toxicity-pollinators-insecticides/index.html).

In addition, 6 outreach products were posted at the UM Extension Nursery website http://www.extension.umn.edu/garden/plant-nursery-health/

Two new websites were created to promote pollinators and disseminate the research results.
A new website http://ncipmhort.cfans.umn.edu/was created in February 2016 called “Mitigating pollinator decline” with a pre-recorded webinar, videos on bee-friendly plants, plant lists, videos on native bee foraging, and identification information for 26 bee families.

Also, a video on protecting pollinators was created on the USDA National Extension Website. https://campus.extension.org/enrol/index.php?id=1244

Articles in commodity journals
Also, 6 articles were published in 3 commodity journals (MNLA, MN Nursery and Landscape Industry, Scoop; MTGF, MN Turf and Grounds Foundation Superintendents, Hole Notes; and MNCTA, MN Christmas Tre Association bulletin) to promote pollinators and encourage planting of crop crops in Christmas tree plantations to support bees.

Three workshops were created to promote pollinators and disseminate the research results.
A 3 part workshop on November 6, 2014, March 26, 2017, and May 21, 2017 on pollinator issues called "Pollinator cubed" was held at the University of Minnesota Landscape Arboretum and was attended by over 200 people; www.arboretum.umn.edu/Pollinators3.aspx .

Project status as of February 15 2017

Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection; Objective 1-2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees
The USDA AMS Gastonia NC Lab performs the residue analysis for this research. Currently, we are waiting for the residue analysis to be finished. We have data for year 1 for soil drench and trunk injection at the UM site and are waiting for data from year 2. At the second MPRB site, we are waiting for data from year 1 and 2.

The USDA method uses whole flowers to determine residue levels. However, pollen and nectar levels may be higher or lower than whole flowers. Data from our experiments and review of 13 reports submitted to the EPA by chemical companies indicate that 25% of the residue in whole flowers may be a conservative estimate of residue levels found in pollen and nectar. Our data show that trunk injections of imidacloprid result in 1,340 ppb imidacloprid residue in flowers (335 ppb at 25% reduction; 100 ppb kill bumblebee) 2 months after treatment which will kill foraging pollinators. Imidacloprid trunk injections cause very small amounts of imidacloprid to accumulate in the soil (14 ppb) which would not cause sufficient amounts in flowers of plants growing under the trees.

However, soil drenches cause very high levels of imidacloprid in the soil (2mo after treatment 21,061 ppb) that is easily transported into small dogwoods growing under the treated trees and result in 762 ppb (190 ppb at 25% reduction) in dogwood flowers and 672 ppb in dogwood fruits. These volunteer plants growing under treated trees accumulated sufficient amount of imidacloprid in foliage and flowers to kill pollinators.

However, fruits may not contain high enough residue to kill birds. The LD50 for a house sparrow is 0.041mg/g and a mean sparrow weight is 24g, so 0.98 mg of imidacloprid will kill a house sparrow. A house sparrow would need to eat 128-10 g fruits to reach the LD50. The NOEL (no observable effect level) is 0.003 mg/g, which would be 0.072 mg of imidacloprid. A house sparrow would need to eat 10-10 g fruits to reach the NOEL.

Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers, squash, and blueberry from imidacloprid soil drenches
Kristine Blum from Koppert bumblebee production collected samples of blueberry flowers in grower's field. In 5/6 flower samples imidacloprid residue was found (220, 136, 42, 10, 12 ppb, mean = 84 ppb). Again, if we take 25% of 84 ppb, the pollen and nectar contains 21 ppb, which in our experiments reduced bumblebee foraging and colony health. Ms. Blum sent the samples to the USDA as her qrower's were experiencing bumblebee colony death in many of the blueberry fields that ordered colonies form Koppert. She was concerned that it was imidacloprid causing the bumble bee colonies to die.

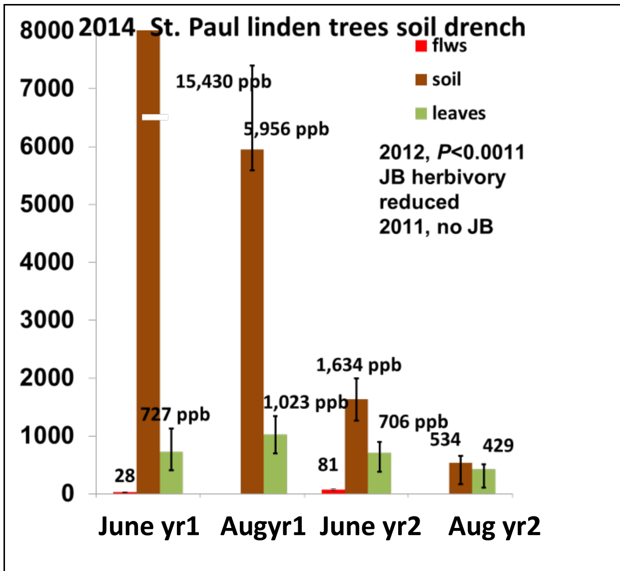
Objective 1-4. Share the research results with collaborators through talks, additions to the pollinator website, and emails. The public has expressed interest in this research and over 26 talks were provided in 2016. Also a 3 part workshop at the University of Minnesota Landscape Arboretum was created and attended by over 200 people in 2014 - 2015 www.arboretum.umn.edu/Pollinators3.aspx . In addition, 6 outreach products were posted on the UMN extension website www.extension.umn.edu/garden/plant-nursery-health/.

Another UM website was created with videos and webinars on IPM, pollination, native bees, and invasive species management at ncipmhort.cfans.umn.edu/ cues.cfans.umn.edu/ .

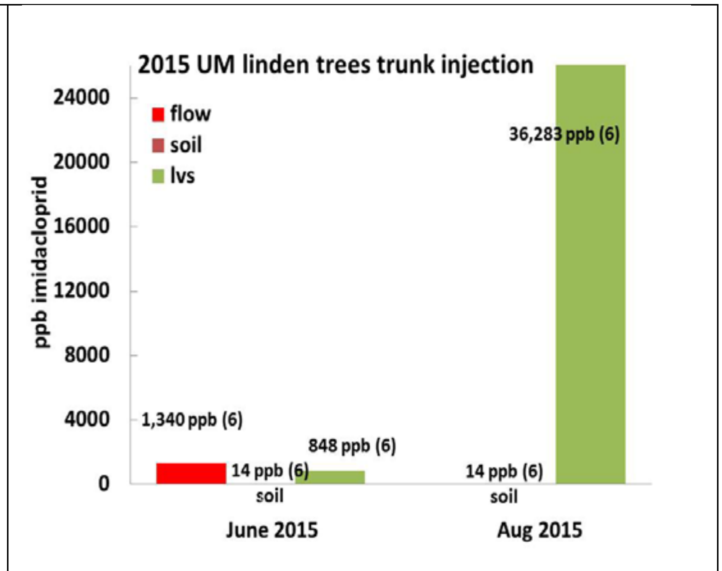
Project status as of August 15 2016

Our objectives are to understand how to protect pollinators. Part of the research is to understand if ornamental plants, after application of the systemic, neonicotinoid imidacloprid to the soil, contained sufficient residues in flowers to have sub lethal affects (>25 ppb in pollen and nectar alters behavior, EPA, March 2016) or lethal affects (>150 ppb in pollen and nectar) on bees. In summary, soil drenches of imidacloprid to trees, blueberries, and flowers result in sufficient residue to alter behavior and cause decline of in bumble bee colonies in the field.

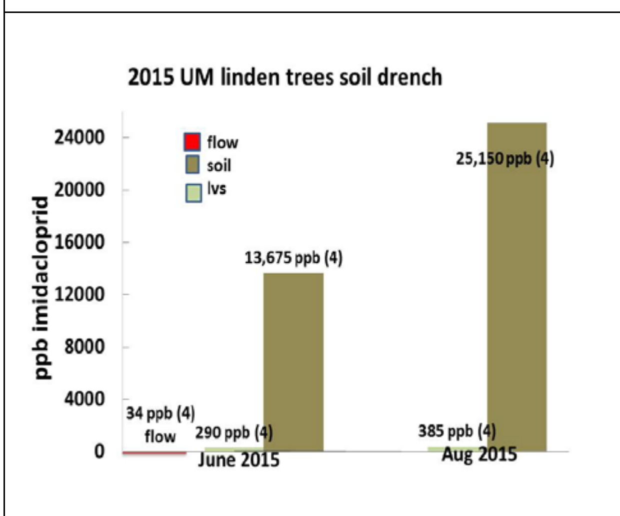
Objective 1-1. Determine imidacloprid residue in pollen and nectar of basswood (linden) trees from a soil drench and trunk injection and Objective 1-2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees. Data showed residues in flowers of trunk injected trees were high enough to kill foraging bees (>150 ppb). We found that imidacloprid trunk injections in May resulted in June in residues of 1,340 ppb in basswood flowers (335 ppb estimated (est)in pollen at a conversion of 25% less residue in pollen compared to whole flowers), 14 ppb in soil, and 848 pp in leaves. Imidacloprid soil drenches in May resulted in 34 ppb (25 ppb (est), >25 EPA value that alters behavior) in flowers and 762 ppb (180 ppb (est),>150 ppb causes mortality) in dogwood flowers growing under the treated trees, while leaves had 13,675 ppb, and soil 21,061ppb. Much of the imidacloprid that would have been in the soil was taken up by the 2 ft high dogwoods growing under the basswood trees.

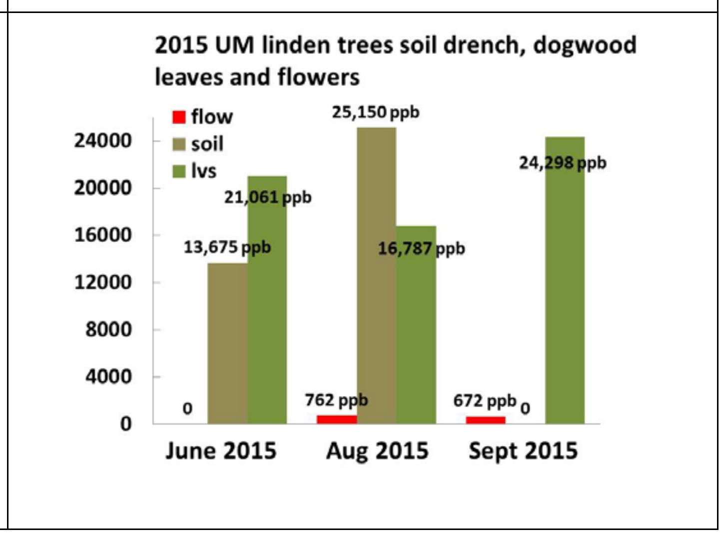


Imidacloprid ppb in basswood leaves, soil, and flowers from an imidacloprid soil drench.



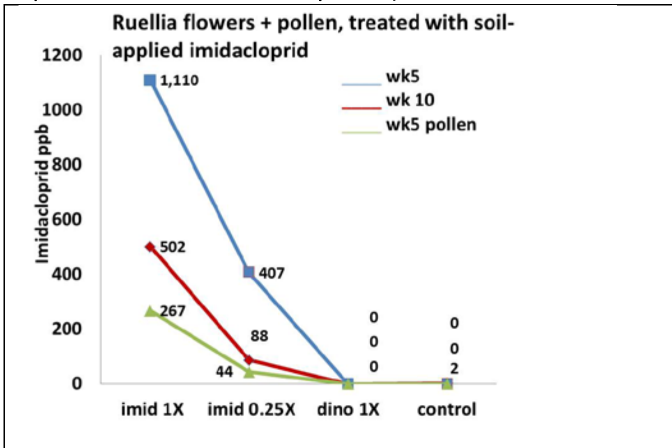
Imidacloprid ppb in basswood leaves, soil, and flowers from an imidacloprid trunk injection, 2015.



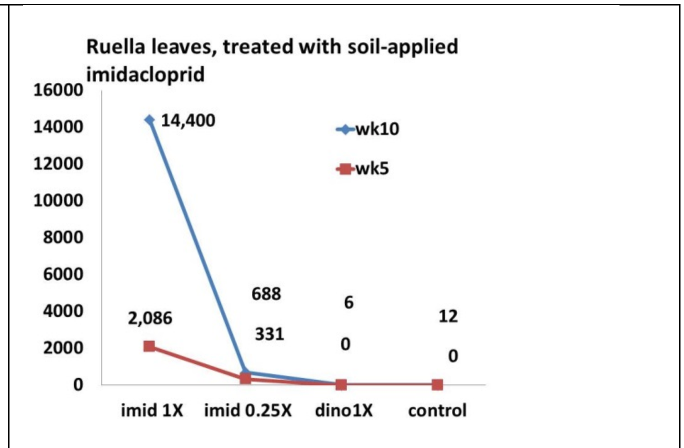


Imidacloprid ppb in basswood leaves, soil, and flowers from an imidacloprid soil drench, 2015.	Imidacloprid ppb in dogwood flowers and leaves growing underbasswood trees treated with an imidacloprid soil drench, 2015.

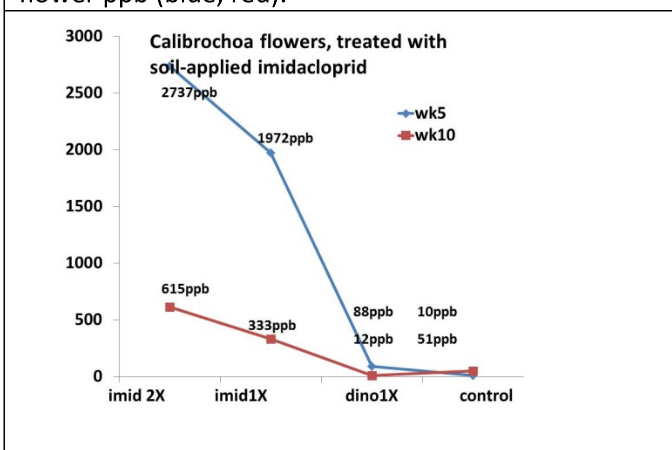
Objective 1-3. Determine imidacloprid residue in pollen and nectar of native flowers, squash, and blueberry from imidacloprid soil drenches. Koppert Biological sells the bumblebees used in the research and they experienced mortality of bumblebee colonies in blueberry farms that use imidacloprid. In two separate farms, imidacloprid residues in blueberry flowers was 103 ppb and 136 ppb, while in three other farms the residue of imidacloprid was 9.6-41.7 ppb. Residue in Prairie petunia (*Ruella humilis*) from a greenhouse application resulted in imidacloprid residues in flowers of 1,100 ppb (267 ppb actual in pollen) at wk5 and 502 ppb (125 ppb estimated in pollen at 25% flower to pollen) at wk10, which will alter behavior and kill bees.



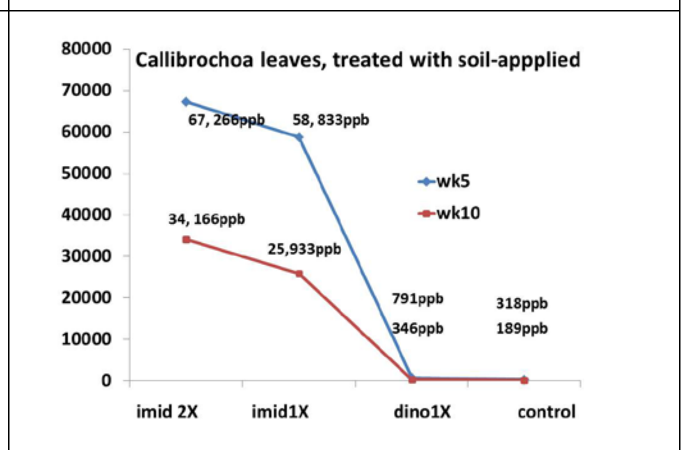
Imidacloprid ppb in *Ruella*, native petunia pollen (green line) is 25% lower than imidacloprid whole flower ppb (blue, red).



Imidacloprid ppb in *Ruella*, native petunia leaves.



Imidacloprid ppb in *Calibrachoa*, million bells flowers.



Imidacloprid ppb in *Calibrachoa*, million bells leaves.

Project status as of February 15 2016

Our objectives are to understand how to protect pollinators. Part of the research is to understand if ornamental plants after systemic insecticide application contained sufficient residues to have sublethal affects (20-40 ppb in pollen and nectar) or lethal affects (>40 ppb in pollen and nectar) on bees. We have treated plants in the field with landscape rates for trees (see August 15 2015 data) and flowers (residue analysis in progress at USDA, AMS Gastonia).

Plants purchased at garden centers may contain neonicotinoid residue. Small pots (4 in) with *Calibrachoa,* million bells, were treated with foliar applied pymetrozine (Endeavor, LD50=1580 ppb, nontoxic to bees), soil –

applied imidacloprid (Marathon, LD50=40 ppb, highly toxic to bees) and soil –applied dinotefuran (Safari, LD50=230 ppb, highly toxic to bees) and sampled at 5 and 10 wk post application.

For all neonicotinoid insecticides, residue in leaves and flowers decreased from 5 to 10 wk. Imidacloprid residues in flowers was around 4.5 times lower by 10 wk (5 wk, 1X=1,971, 2X=2,736 ppb; 10 wk, 1X=383, 2X=615 ppb). Dinotefuran residues in flowers was around 8 times lower by 10 wk (5 wk, 2,993 ppb; 10 wk, 386 ppb). By 10 wk, flowers in imidacloprid (383 ppb) and dinotefuran (386 ppb) treatments contained similar amounts of residue. All residue levels found in flowers at 5 or 10 wk after treatment would kill foraging bees. Imidacloprid and dinotefuran leaves contained 24 to 147 times more insecticide compared to flowers. Our data showed that the small plants that we purchased contained imidacloprid (control plants).

At 5 wk only1/9 samples (126 ppb) and by 10 wk 0/9 samples contained pymetrozine insecticide. Pymetrozine kills insects with sucking mouthparts, such as aphids, but conserves beneficial insects such as bees, lacewings and lady beetles. Pymetrozine was not shown to be highly systemic at either 5 or 10 wk and the data support its use on plants that may be visited by bees. Pymetrozine is a good alternative to neonicotinoid insecticides for managing aphids , since after treatment no aphids returned for 10 wk.

These data support the report by the Friends of the Earth, Gardeners Beware 2013 and 2014, that surveyed small plants in garden centers and found neonicotinoid residues that may kill bees.
http://libcloud.s3.amazonaws.com/93/07/d/3118/Gardeners_beware_report_8-13-13-acknts.pdf ,
http://www.foe.org/system/storage/877/3a/3/4738/GardenersBewareReport_2014.pdf

A new website http://ncipmhort.cfans.umn.edu/was created on “Mitigating pollinator decline” with videos on plants for bees, a plant list, videos of native bee foraging, and identification information and pictures for 26 bee families. Also, the website has a pre-recorded webinar containing talks by four MN researchers, Dr. Dan Cariveau, Dr. Vera Krischik, Dr. Karl Foord, and Ms. Health Holm.

Figure1. **Flowers:** *Calibrachoa* purchased in 4in sg pots were treated on June 28 2015 with pymetrozine and July1 2015 with imidacloprid and dinotefuran and sampled on August 3 2015 (5wk) and Sept 8 2015 (10wk). Leaves and flowers were analyzed at the USDA AMS Gastonia lab in Jan 2016.

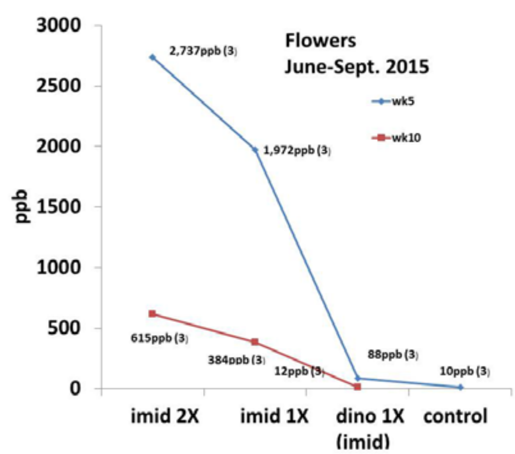


Figure 2. Leaves: *Calibrachoa* purchased in 4in sg pots. Pots were treated on June 28 2015 with pymetrozine and July1 2015 with imidacloprid and dinotefuran and sampled on August 3 2015 (5wk) and Sept 8 2015 (10wk). Leaves and flowers were analyzed at the USDA AMS Gastonia lab in Jan 2016.

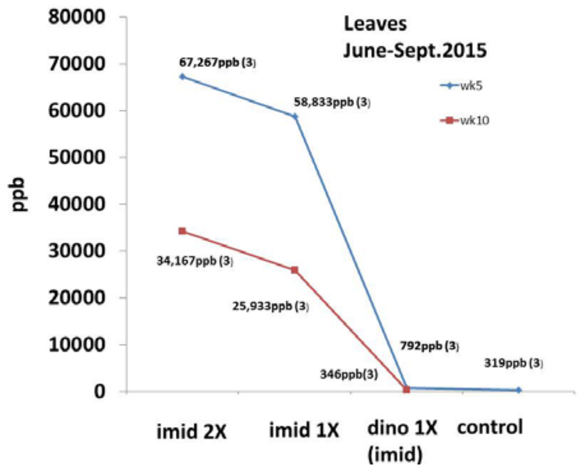


Table 1. *Calibrachoa* purchased in 4in sg pots were treated on June 28 2015 with pymetrozine and July1 2015 with imidacloprid/dinotefuran and flowers and leaves were sampled on August 3 2015 (5wk) and Sept 8 2015 (10wk) and analyzed at the USDA AMS Gastonia lab in Jan 2016.			
Treatment	**in pot**	**no**	**Mean**
flowers			
imidacloprid			**imidacloprid ppb**
5 wk control	0	3	10.05±0.95
5 wk imid 2x	22mg (0.5 tsp)	3	2,736.67±446.63
5 wk imid 1x	14mg (0.33 tsp)	3	1,971.67±554.23
5 wk dino(imid) 1x	0	3	87.60±40.64
10 wk control	0	3	na
10 wk imid 2x	22mg (0.5 tsp)	3	615.33±51.36
10 wk imid 1x	14mg (0.33 tsp)	3	383.67±53.64
10 wk dino(imid) 1x	0	3	11.60±3.09
dinotefuran			**dinotefuran ppb**
5 wk dino 1x control	0	3	0
5 wk dino 1x	22mg	3	2,993.3±364
10 wk dino control	0	3	na
10 wk dino 1x	22mg	3	386.3±167
pymetrozine			**pymetrozine ppb**
5 wk pym 1x control		3	0
5 wk pym 1x		9	1/9;126
10 wk pym control		3	na
10 wk pym 1x		9	0
leaves			
imidacloprid			**imidacloprid ppb**
5 wk control	0	3	319.0±67
5 wk imid 2x	22mg (0.5 tsp)	3	67,266.7±4672
5 wk imid 1x	14mg (0.33 tsp)	3	58,833.3±10841
5 wk dino(imid) 1x	0	3	791.7±161
10 wk control	0	3	na
10 wk imid 2x	22mg (0.5 tsp)	3	34,166.7±2801
10 wk imid 1x	14mg (0.33 tsp)	3	25,933.3±1364

10 wk dino(imid) 1x	0	3	346.3±53
dinotefuran			**Dinotefuran ppb**
5 wk dino control	0	3	0
5 wk dino1x	22mg	3	83,866.7±19629
10 wk dino control	0	3	na
10 wk dino1x	22mg	3	56,566.7±4420
pymetrozine			**pymetrozine ppb**
5 wk pym control		3	0
5 wk pym1x		9	2/9; 21.5, 21.7
10 wk pym control		3	na
10 wk pym1x		9	1/9; 45.2

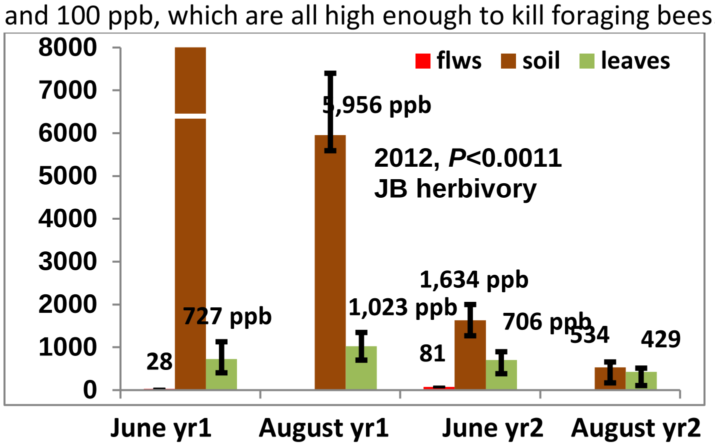
Project status as of August 15 2015

Two replicate basswood/linden plots were established on the Minneapolis Park and Recreation Board (MPRB) land on the west side of the Mississippi River across from the UM campus and 2 sites on the west bank of the UM campus. In June both sites were treated with 3 treatments (trt) by S&S trees: soil drench (10 trees/trt/plot), soil injection, and trunk injection. Leaves, soil, flowers, and dogwood flowers growing under the soil drench trees were collected in June and August for imidacloprid residue analysis.

Landscape plots (13) were established on the St. Paul campus containing milkweed, giant anise hyssop, rose, blueberry, and pussy willows. In August a consumer-landscape rate of imidacloprid was applied to 2 species (milkweed and giant anise hyssop). In September flowers and leaves were collected for imidacloprid residue analysis.

In the greenhouse, Mexican petunia samples were collected to determine the imidacloprid residue levels in flower, leaf, and pollen. This is a technique issue that needs some data and discussion, the relationship between whole flower residue and pollen residue. Samples from the above experiments were mailed on dry ice to the USDA AMS lab in Gastonia, NC for imidacloprid analysis.

USDA generated residue data from an imidacloprid soil drench of large 20in dbh (diameter breast height) trees showed that flowers in yr2 had around 80 ppb imidacloprid, which was high enough to kill foraging bees. Levels in the soil under the tree were 15,430; 5,956; 1634; and 534 ppb which would result in high levels in flowers growing under the trees. Based on data from other experiments, flowers would have around 3130, 900, 313, and 100 ppb, which are all high enough to kill foraging bees.



An extension bulletin was peer-reviewed and posted on my extension website on the LD50 to bees of all insecticides registered for use on plants in landscape and greenhouse. (http://www.extension.umn.edu/garden/plant-nursery-health/toxicity-pollinators-insecticides/index.html).

Project status as of January 30 2015

Research plants were grown to install in the landscape (roses, Mexican petunia, *Ruellia hirtus*, Mexican milkweeds, *Ascelpius curasavica*, and giant anise hyssop, *Agastache foeniculum*).

The native Mexican petunia produces copious amounts of nectar. Research with this plant will permit us to measure the imidacloprid residue in nectar and whole flowers and then make a regression between imidacloprid residue in nectar and whole flowers. In this way, we can estimate the amount of imidacloprid residue in nectar of species of plants that produce too little nectar to collect. Currently, from these plants we collect residues from whole flowers, which overestimate the amount of imidacloprid residue. In early September, the Mexican petunia plants went dormant and stopped flowering so we placed them into coolers to overwinter. We planned to return the petunias to the greenhouse in January 2015 to make flowers and start the experiment.

ACTIVITY 2:

2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee colonies.
Description: We will determine if bumblebees colonies established in the field near flowering plants that were treated with imidacloprid have reduced colony health.

Summary Budget Information for Activity 2: **ENRTF Budget: $57,410**
Amount Spent: $57,410
Balance: $0

Activity Completion Date: June 30, 2017

Outcome 2 Determine effects on bees.	**Completion Date**	**Budget**
2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee colonies.	2017	$49,700
2-2. Share the research results with collaborators through talks, additions to the pollinator website, and emails.	2017	$0

Final report summary August 30 2017

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee.

A tier 3 EPA research field study with replicate plots (n=6-8 colonies/plot ,repeated June and August, total= 28 colonies) were performed on the St Paul UM Campus. The bumble colonies were free flying and were fed 20 ppb imidacloprid in sugar syrup. Around 120 pollinator plants in 3 gallon pots were placed around the nests to ensure that the bees were not nectar or pollen limited. The EPA NOEL (Not Effective Adverse Level or sub lethal dose) is 25 ppb imidacloprid. The bees in the treated colonies showed decreased movement, decreased sugar consumption, decreased brood, deceased queen production, and increased growth of fungus compared to control colonies. Bumblebee colonies are negatively affected by 20 ppb imidacloprid. So the NOEL identified by the EPA in March 2016 as 25 ppb is incorrect. Our residue data tells us that residue in flowers from a trunk injection or soil drench would be sufficient to negatively affect native bumblebee colonies.

Project status as of June 30 2017

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee.

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Project status as of February 15 2017

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee colonies.

Bumblebee colonies are negatively affected by 25 ppb imidacloprid. Our residue data tells us that residue in flowers from a trunk injection or soil drench would be sufficient to negatively affect native bumblebee colonies.

In 2016, 2 replicate plots on the St. Paul campus contained six bumblebee colonies (n=12) and was repeated for a total of 26 colonies. The colonies were provided 25 ppb imidacloprid in nectar (>25 ppb in pollen and nectar alters behavior, EPA, March 2016) and the bees were allowed to freely fly. Imidacloprid 25ppb-treated colonies started to decline in the field at wk3, due to lower feeding (nectar consumption), lower nectar storage in honey pots, reduced egg production, reduced movement. In imidacloprid treated 50% of the next years queens were produced compared to controls. These data are being analyzed .

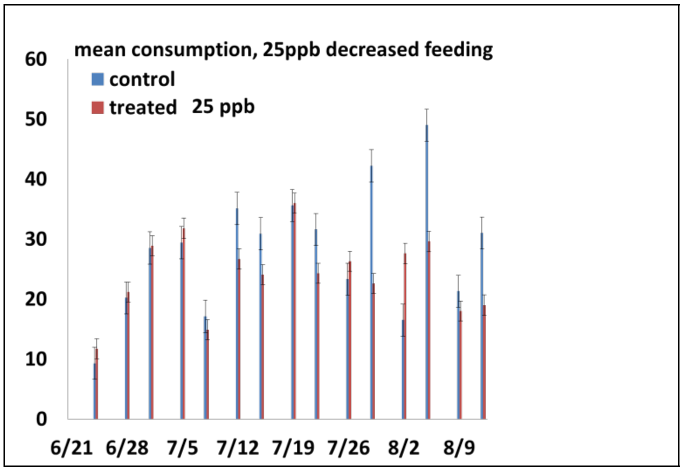
Objective 2-2. Share the research results with collaborators through talks, additions to the pollinator website, and emails.

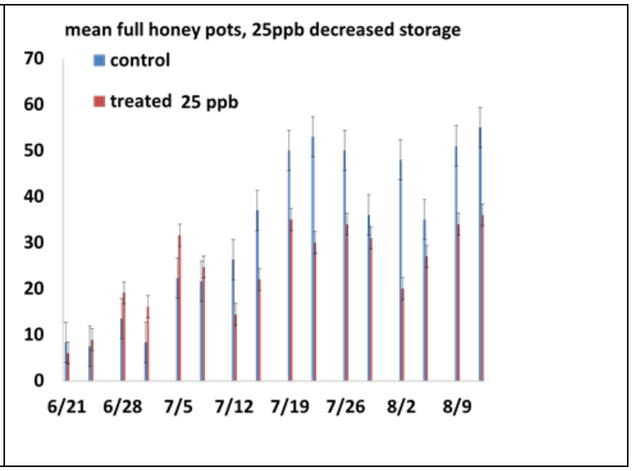
The public has expressed interest in this research and over 26 talks were provided in 2016. Also a 3 part workshop at the University of Minnesota Landscape Arboretum was created and attended by over 200 people in 2014 - 2015 www.arboretum.umn.edu/Pollinators3.aspx . In addition, 6 outreach products were posted on the UMN extension website www.extension.umn.edu/garden/plant-nursery-health/.

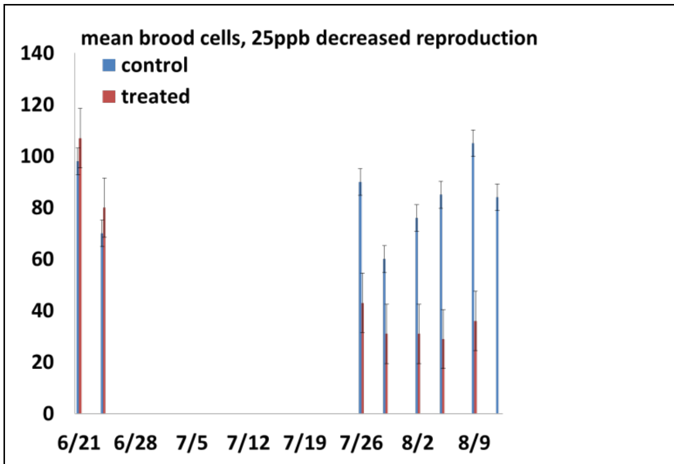
Another UM website was created with videos and webinars on IPM, pollination, native bees, and invasive species management at ncipmhort.cfans.umn.edu/ cues.cfans.umn.edu/ .

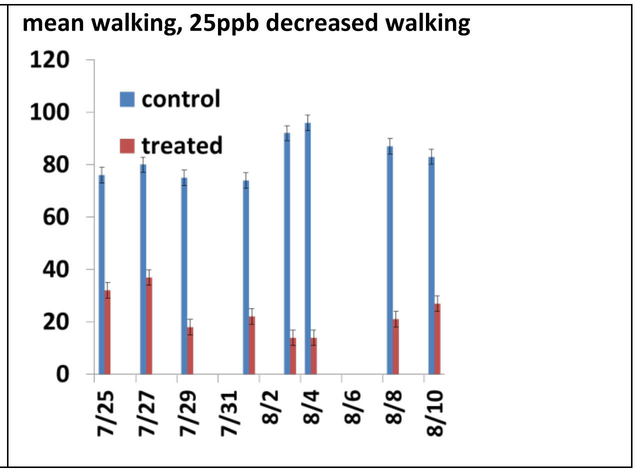
Project status as of August 15 2016

Objective 2-1. Determine the impacts of these imidacloprid residues on colony health of native bumblebee colonies. In 2016, 2 replicate plots on the St. Paul campus contained six bumblebee colonies (n=12) that were provided 25 ppb imidacloprid in nectar (>25 ppb in pollen and nectar alters behavior, EPA, March 2016). Imidacloprid treated colonies started to decline in the field at wk3, due to lower feeding (nectar consumption), lower nectar storage in honey pots, reduced egg production, and reduced movement. In imidacloprid treated colonies the queen died in 2/6 colonies, while no queens died in controls (0/5). We are in the processing of performing the second replicate of this study.









In summary, soil drenches of imidacloprid to trees, blueberries, and flowers result in sufficient residue to alter behavior and cause decline of in bumble bee colonies in the field.

V. DISSEMINATION:

Description: From the research, we will develop peer reviewed publications, websites, outreach bulletins, and outreach talks.

Final report summary August 30 2017

Dissemination: Objective 1-4. **Share the research results through outreach with talks, workshops, pollinator website, and interviews.**

We talked to the public and other researchers about the effects of pesticides on bees, the data from this research, and what municipalities and consumers could do in their green space to conserve bees. We held 3 workshops at the MN Landscape Arboretum, produced 2 websites on native bee conservation, spoke about the research in 10 talks/yr, and gave over 6 interviews/yr to radio, television, and print media.

Project status as of June 30 2017

Objective 1-4. **Share the research results through outreach with talks, workshops, pollinator website, and interviews.** Various radio and television interviews were given to promote pollinators and disseminate the research results from January to August 2017.

Interviews on radio in 2016 ad 2017

Krischik, MN PBS TV Almanac, Friday night news show, August 4, 2017:
http://www.tpt.org/almanac/video/The-Wrap--Top--Minnesota-Favorite-Bugs-30524/
http://www.tpt.org/almanac/video/Japanese-Beetles-Swarm-Minnesota-Gardens-30517/
Krischik, MN public radio, Friday July 28, 2017
Krischik, MN WCCO radio, Friday July 28, 2017
Krischik, MN WCCO television, Monday July 31, 2017
Krischik, Tonka Gardens Pollinator Festival, Sunday July 30 2017
Krischik, Radio Interview, Food Seuth Radio, June 7, 2017, https://beta.prx.org/stories/210290
Krischik, "Pollinator Week Beyond pesticides program for Washington, DC", June 2, 2017
http://beyondpesticides.org/dailynewsblog/2017/06/restaurants-nations-capital-feature-foods-reliant-pollinators-national-pollinator-week/
Krischik, video Symposium Keynote speaker, 22 mins, Beyond Pesticides 35TH National Pesticide Forum, April 29, 2017, Bees, Pollinators, and Biodiversity
Krischik radio interview, 1 hr, Pollination festival in Stillwater, MN, September 11, 2016
https://www.spreaker.com/user/backroomstewdios/jewbalations-at-the-polli-nation-event
Krischik radio talk show, 1 hr, MN Broadcasters Association, Jim du Bois, August 6, 2016

http://www.accessminnesotaonline.com/2016/08/03/the-decline-of-insect-pollinators/

Various outreach bulletins, videos, and talks were created to promote pollinators and disseminate the research results from 2014 to 2017.

Talks
The public has expressed interest in this research and over 63 talks were provided from 2014 to 2017. Each year I give talks to at least 5 large nursery and landscape industry workshops. At each meeting for the 3 year grant period I spoke of different ways to protect pollinators in greenhouses and landscapes.

I provided talks at meetings on the research to NCERA 224, USDA Nursery and Landscape Group; USDA SARE NCIPM Stakeholders meeting; ESA (entomology Society National Meeting); and SETAC (Society of Environmental Toxicology and Chemistry).

Bulletins on websites
In June 2017 in cooperation with the USFWS, Michigan DNR, Wisconsin, DNR, Xerces Society for Invertebrate Conservation, and UMinnesota, two bulletins on protecting Rusty patch bumble bee (RPBB) were created and are posted online at the UM Extension Nursery website. The bulletins discussed how to reduce pesticide use in urban landscapes and farmlands to bring back RPBB http://www.extension.umn.edu/garden/plant-nursery-health/

An extension bulletin was peer-reviewed and posted in August 2015 on the UM Extension Nursery Website on the LD50 to bees of all insecticides registered for use on plants in landscape and greenhouse. (http://www.extension.umn.edu/garden/plant-nursery-health/toxicity-pollinators-insecticides/index.html).

In addition, 6 outreach products were posted at the UM Extension Nursery website http://www.extension.umn.edu/garden/plant-nursery-health/

Two new websites were created to promote pollinators and disseminate the research results.
A new website http://ncipmhort.cfans.umn.edu/was created in February 2016 called “Mitigating pollinator decline” with a pre-recorded webinar, videos on bee-friendly plants, plant lists, videos on native bee foraging, and identification information for 26 bee families.

Also, a video on protecting pollinators was created on the USDA National Extension Website. https://campus.extension.org/enrol/index.php?id=1244

Articles in commodity journals
Also, 6 articles were published in 3 commodity journals (MNLA, MN Nursery and Landscape Industry, Scoop; MTGF, MN Turf and Grounds Foundation Superintendents, Hole Notes; and MNCTA, MN Christmas Tre Association bulletin) to promote pollinators and encourage planting of crop crops in Christmas tree plantations to support bees.

Three workshops were created to promote pollinators and disseminate the research results.
A 3 part workshop on November 6, 2014, March 26, 2017, and May 21, 2017 on pollinator issues called "Pollinator cubed" was held at the University of Minnesota Landscape Arboretum and was attended by over 200 people; www.arboretum.umn.edu/Pollinators3.aspx

Project status as of February 15 2017
The PI gave talks on insecticides and pollinators to over 1,000 people at the MNLA Green Expo, 3-MDA/MNLA/UM pesticide recertification workshops, and 3-MDA/MNKA/UM certification workshops.

Project status as of August 15 2016

The PI Krischik has provided 12 talks in 2016 on the subject of neonicotinoids and bees. Krischik will give a talk to the International Entomological Society meeting in Sept 2016, the NC IPM USDA Landscape group in October, and SETAC (Society of Environmental Toxicology and Chemistry) in November. A website on pollinator identification with a webinar, videos on bees, and videos on food plants was created at http://ncipmhort.cfans.umn.edu/

Project status as of February 15 2016
An extension bulletin was peer-reviewed and posted on my extension website on the LD50 to bees of all insecticides registered for use on plants in landscape and greenhouse. (http://www.extension.umn.edu/garden/plant-nursery-health/toxicity-pollinators-insecticides/index.html).

The PI Krischik created, moderated, and gave talks in a 3 part workshop series (http://www.arboretum.umn.edu/pollinators3.aspx) at the Minnesota Landscape Arboretum on pollinators: Nov 6 2014, March 26 2015, and May 21 2015.

Project status as of August 15 2015
A paper from the research was published in March 2015 in in PlosOne entitled, " Soil-applied imidacloprid is translocated to ornamental flowers and reduces survival of adult *Coleomegilla maculata, Harmonia axyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui*", accepted PONE-D-14-18550R1

The PI Krischik has provided 33 talks in 2015 on the subject of neonicotinoids and bees and advised 2 white papers by the Friends of the Earth and the MN League of Women Voters. The MN Extension website that I maintain on nursery and landscape has 6 extension bulletin/posters that I created on pollinator conservation.

Project status as of January 30 2015
PlosOne entitled, " Soil-applied imidacloprid is translocated to ornamental flowers and reduces survival of adult *Coleomegilla maculata, Harmonia axyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui*", accepted PONE-D-14-18550R1

Six extension publications were developed and posted on my websites in 2014
1. UMKrischik CFANS extension bee poster
2. UMKrischik consumer protecting bees
3. UMKrischik garden centers bee labelling
4. UMKrischik insecticides used in greenhouse
5. UMKrischik nursery greenhouse bee labelling
6. UMKrischik pollinator conservation bulletin

Also I created a 3 part series at the Minnesota landscape Arboretum on pollinators.
Here is the url, www.arboretum.umn.edu/Pollinators3.aspx.
I manage 3 websites that contain pollinator and pesticide information.
1. CFANS CUES website, cues.cfans.umn.edu/
2. UM extension greenhouse, nursery, and landscape website, www.extension.umn.edu/garden/plant-nursery-health/
3. Original CUES Website, www.entomology.umn.edu/cues/

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$220,700	Grad student , technicians
Professional/Technical/Service Contracts Tree care company to apply imidacloprid to soil and to inject basswood trees Residue analysis of imidacloprid performed at USDA AMS Lab in Gastonia, NC, EPA approved lab, cost $166/sample, 20 trees x 2 samples x 2 months x 2 yrs= 160 samples x $166 = $26,560; and 4 flowering plant species x 12 individuals x 2 samples x 2 yrs = 192 samples x$166 = $31,872; total 352 samples x $166 = $58,432 + $1568 shipping samples overnight express on dry ice	$7,000 $60,000	Licensed MDA arborists for trunk injections Residue analysis must be done at the EPA approved USDA AMS, Gastonia, NC lab to be valid
Equipment/Tools/Supplies: Research supplies: Bumblebee colonies, greenhouse space, insecticides, research landscapes to be planted	$30,300	Equipment to ready bumblebee colonies to be established and monitored in the field; insecticides and plants to set up trial gardens for determining imidacloprid residue in flowers and the effects of imidacloprid on bumblebee colony health
Printing: Reports and fact sheets for distribution at meetings	$2,000$	Cost for duplicating management recommendations, factsheets, handouts for use at meetings and talks.
Travel Expenses in MN: Instate travel to research sites	$6,000	Instate travel to research
TOTAL ENRTF BUDGET:	**$326,000**	

Explanation of Use of Classified Staff: none

Explanation of Capital Expenditures Greater Than $5,000: none

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 1.5 FTE for a graduate student, 1.5 FTE for a Post Doc, and 0.68 FTE for a technician, = total of 3.68 FTE.

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: Tree Arborist Service for trunk injections and soil drenches of basswood trees, 0.05 FTE (5 weeks each year for 2 years). USDA AMS NC residue lab to quantify imidacloprid, 0.5 FTE, = total 0.55 FTE

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state 2015 MNLA, MN Nursery Association Grant	$20,000	$20,000	Research and extension
2015 USDANCIPM grant develop webinar and website on pollinators	$10,000	$10,000	Extension
In-kind Services: 1% PI cost share	$3,205	$0	
TOTAL OTHER FUNDS:	**$3,205**	**$0**	

VII. PROJECT STRATEGY:

A. Project Partners:

The research will be performed in the lab of Dr. Vera Krischik (Landscape Plant Pest Management), Department of Entomology at the University of Minnesota, St. Paul Campus. Interested parties will be sent email reports every 6 mo. We have letters of support from some of our project interested parties: 1. Minnesota Honey Producers (President Dan Whitney), 2. and 3. MN Beekeepers (Steve Ellis and Jeff Anderson), 4.Colorado State Beekeepers (President Beth Conrey), 5.Boulder County Beekeepers (President Miles McGaughey), and 6. And 7. Washington Department of Agriculture (Director Bud Hoover and Chief Erik Johansen). Other interested parties are:

8. Sarah Rudolf, Minnesota Pollution Control Agency,
9. and 10. Crystal Boyd and Dana Robert, Minnesota Department of Natural Resources,
11. Lois Eberhart, City of Minneapolis Surface Water & Sewers Administrator, Department of Public Works,
12. Gail Nozal, certified arborist, S & S Tree Service,
13. Ralph Siefert, MPRB, Minneapolis Park and Recreation Board,
14. Les Potts, Supervisor, Landcare, UMinnesota,
15. and 16. Eric Mader and Mathew Shepard, Xerces Society and adjunct extension educator, UMinnesota
17. Larissa Walker, Center for Food Safety, Washington DC
18. Lex Horan, Pesticide Action Network NA, PANNA, Minneapolis, MN
19. Erik Runquist, MN Zoo

B. Project Impact and Long-term Strategy:

The purpose of this research is to determine if systemic, neonicotinyl insecticides are translocated to pollen and nectar in flowers and what impact these insecticides have on bee foraging and colony health. 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.

The ubiquitous use of neonicotinyl insecticides on crops and landscape plants throughout the season may 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 probably higher than 10 ppb, bees are likely to be experiencing chronic, sublethal doses with consequences on queen and colony health.

The research will be posted on our outreach center the CUES website (www.entomology.umn.edu/cues) and updated every 6 mo. This information will be discussed with consumers, master gardeners, commodity groups, state agencies in Washington, Colorado, and Minnesota, and the US EPA. So far, 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.

VII. Spending History:

Funding Source	**M.L. 2009 or FY10**	**M.L. 2011 or FY12-13**	**M.L. 2015 or FY15**
USDA SARE grant	$175,000	Finished	
LCCMR 2010 221G, Mitigating bee decline	$297,000	Finished	
UMN MAES project	$4,000	$4,000	

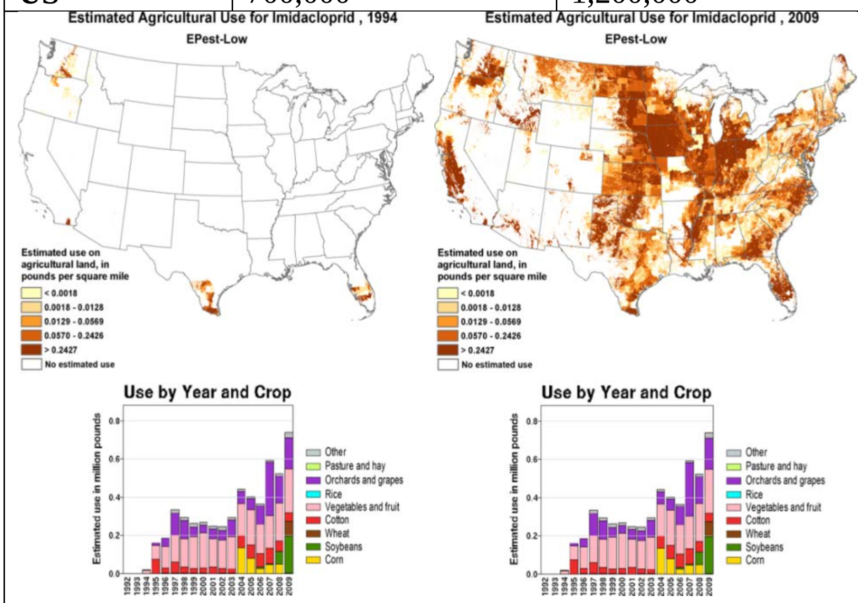
VIII. ACQUISITION/RESTORATION LIST: none

IX. VISUAL ELEMENT or MAP(S):

2014 LCCMR proposal
Project title: Understanding Systemic Insecticides as Protection Strategy for Bees
Project PI: Vera Krischik, Department of Entomology, U of Minnesota

In 2009 143/442 million acres in the US use a neonicotinyl insecticide, 83 million acres of corn have seed treatments of neonicotinyls, and honeybees rely on corn for pollen

	Imidacloprid lbs (ai)	Clothianidin lbs (ai)	Thiamethoxam lbs (ai)
MN	52,048	43,663	68,876
CA	348,247	3,182	30,687
US	700,000	1,200,000	990,000



The purpose of this research is:
1. Determine imidacloprid residue in pollen and nectar of basswood trees from an imidacloprid soil drench and trunk injection.
2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees.
3. Determine imidacloprid residue in pollen and nectar of native flowers, squash, and blueberry from imidacloprid soil drenches.
4. Determine the impacts of these imidacloprid residues on colony health of native bumblebee colonies.

Bees feed on pollen and nectar which results in pollination and the production of fruits and seeds. Both native bumblebees and managed honey bees have been in decline since neonicotinyl insecticides were registered in 1990. Loss of habitat, new pathogens, and lack of native plants for food also contribute to reduced bee health. Recent papers show that pesticide exposure to bees makes them more vulnerable to pathogens.

The majority of insecticides are called contact insecticides as the insect, by walking on the leaf or eating the leaf, absorbs the insecticide from the surface of the plant
for 1-3 weeks. A flower that opens after a contact insecticide is sprayed has no insecticide in the pollen and nectar. Systemic insecticides move from the soil to the leaves and pollen and nectar of the plant and can remain in the plant for a year. Every flower that opens has neonicotinyl insecticides in it. Every time an insect feeds on the pollen and nectar the bee consumes the systemic insecticide.

Systemic neonicotinyl insecticides (imidacloprid, clothianidin, dinotefuran, and thiamethoxam) are widely used due to low toxicity to humans, but they are very toxic to bees and birds as addressed in two new review papers by the Xerces Society (2012) and American Bird Conservatory (2013). To understand how little kills a bee, let us think of a heart healthy aspirin that is 80 milligrams = 80,000 micrograms= 80,000,000 nanograms (ng). A bee that eats 4-40 ng imidacloprid can be killed and 1- 3 ng reduces the bee's ability to forage, navigate, and return to the hive. Research showed that bee brains have 40x more nicotinic receptors compared to other insects, as bees perform higher brain functions dealing with memory, spatial orientation, and learning.

Soil drench or trunk injection of trees is very commonly practiced, but little data on neonicotinyl residue in tree flowers is published. On June 18 2013, 25,000 bumblebees were killed at a Target store in Wilsonville, Oregon when the bees fed on nectar from linden trees treated with the neonicotinyl insecticide dinotefuran (label Safari). The incident was documented by the Oregon Department of Agriculture which covered the treated trees with netting and a 6 mo. ban on dinotefuran was initiated.

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: none

XI. RESEARCH ADDENDUM: A research addendum was submitted to the LCCMR staff on January 15, 2014.

XII. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted around August 15 2015; February 15 2016; August 15 2016; February 15 2017; August 15 2017. A final report and associated products will be submitted by August 30, 2017.

Environment and Natural Resources Trust Fund

M.L. 2014 Project Budget

Project Title: Understanding Systemic Insecticides as Protection Strategy for Bees

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 06b

Project Manager: *Vera Krischik*

Organization: *University of Minnesota*

M.L. 2014 ENRTF Appropriation: $ *326,000*

Project Length and Completion Date: 3*Years, June 30, 2014-June 30, 2017*

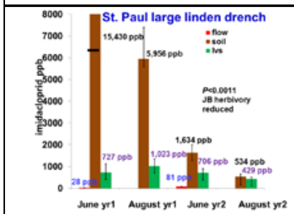
Date of Report: *August 30, 2017*

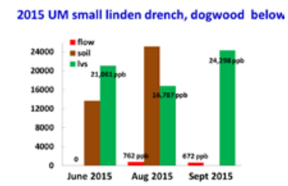
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	**Revised Activity1 Budget**	**Activity1 Amount Spent**	**Activity1 Balance**	**Revised Activity2 Budget**	**Activity 2 Amount Spent**	**Activity 2 Balance**	**Aug 30 2017 TOTALSPENT**	**Aug 30 2017 TOTAL BALANCE**
BUDGET ITEM								
Personnel (Wages and Benefits):	$118,000	$118,000	$0	$32,700	$26,202	$6,498	$150,700	$467
***Students: A** ug 15 redudget, move $70,000 to"Professional Technical Contracts (line 17)". Research assistant 1 (also a graduate student), FT until Aug 31, $700 wk x 8 wk=$5,600, then 50% time $1,600 + fringe $400 =$2,000/mo x 4.5mo=$9,000 until Jan 15th =$15,000*								
***Non-students:** Research assistant 2, $3,189.86 + fringe $829 =$ 4,180/mo X 5 mo = $22,260 + vacation time $2,483=$25,483, Aug 15 rebudget, move $70,000 to"Professional Technical Contracts (line 17)". TOTAL=$40,483*								
***Professional Service Contracts:** Aug 15 rebudget, move $5,410 to "Professional Technical Conrtacts (line 17)"* Tree care company to apply imidacloprid to soil and to inject basswood trees.	$1,590	$1,590	$0	$0	$0	$0	1,590	0
***Professional Technical Contracts:** Aug 15 rebudget, add $70,000 and add $5,410 from above* Residue analysis of imidacloprid performed at USDA AMS Lab in Gastonia, NC, EPA approved lab, cost $166/sample, 20 trees x 2 samples x 2 months x 2 yrs= 160 samples x $166 = $26,560; and 4 flowering plant species x 12 individuals x 2 samples x 2 yrs = 192 samples x$166 = $31,872; total 352 samples x $166 = $58,432 + $1568 shipping samples overnight express on dry ice.	$130,000	$83,199	$46,784	$5,410	$0	$5,410	$135,410	$0
***Equipment/Tools/Supplies: A** ug 15 rebudget , add $5,300 from "Travel",* Research supplies Bumblebee colonies 120 (40/yr) @$100 each =$12,000; bee food $1,000; greenhouse space for preparing bees $3,300; flowers and trees to apply insecticides need 20 linden trees, 400 each Mexican milkweed, hummingbird mint, rugosa rose=$7,000, insecticides $1,000; field charges=$1,000; misc supplies to perform research, dry ice, storage vials, small scale $6,000	$16,300	$16,300	$0	$19,300	$7,537	$2,485	$35,600	$0
Printing: *Reports and fact sheets for distribution at meetings*	$2,000	$1,562	$0	$0	$0	$0	$1,562	$0
Travel: *Aug 15 rebudget, move $5,300 to" Equipment, tools, supplies (line 18)"* **,** Instate travel to research sites, mileage for travel to and from research sites.	$700	$666	$0	$0	$0	$0	$700	$0
COLUMN TOTAL	**$268,590**	**$221,806**	**$46,784**	**$57,410**	**$43,000**	**$14,410**	$325,562	$466

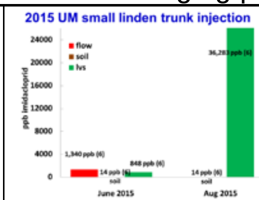
2014 -2017 LCCMR proposal, Final report August 2017
Project title: Protecting bees by understanding systemic insecticides
Project PI: Vera Krischik, Department of Entomology, University of Minnesota

Our research showed that the systemic neonicotinoid insecticide, imidacloprid, created residue in in flowers and pollen that will kill foraging bees.

Objective 1-1. Determine imidacloprid residue in pollen and nectar of linden trees from an imidacloprid soil drench and trunk injection. Large 20 in DBH (diameter breast height) linden trees treated with an imidacloprid soil drench (48 g) had residue in flowers in yr2 of 80 ppb (20 ppb), which is a sub-lethal amount that will affect bumblebee colony health. See objective 2-1. In small 8 in DBH linden trees trunk injections of imidacloprid (3 g) resulted in 1,340 ppb imidacloprid in flowers (335 ppb, actual= 25% reduction for the amount in the pollen not the whole flower) which will kill foraging pollinators.



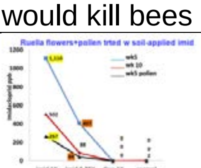




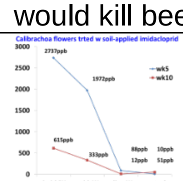
Objective 1-2. Determine the imidacloprid residue in native plants around imidacloprid-treated trees. Trunk injections (3 g) resulted in low 14 ppb of imidacloprid in the soil. Soil drenches (12 g) caused very high levels of imidacloprid in the soil (2 mo after treatment 21,061 ppb) that is easily transported into small dogwoods growing under the treated trees and resulted in 762 ppb (190 ppb actual) in dogwood flowers and 672 ppb in dogwood fruits that would kill pollinators. However, fruits do not contain high enough residue to kill birds.

Objectiive 1-3. Determine imidacloprid residue in pollen of native flowers and blueberry from imidacloprid soil drenches. Greenhouse pots treated with label rates of imidacloprid had residue in leaves and flowers that decreased from 5 to 10 wk. By 10 wk, flowers in imidacloprid treatments (60, 200 mg) contained residue in an amount sufficient to kill foraging bees. In addition, label rates of imidacloprid (300 mg) were applied to plants growing in 3 gallon pots. Residue in flowers at 10 wk after application was high enough to kill bees, 1973 ppb (493 actual) in hyssop and 1568 ppb (392 actual) in milkweed. In 5/6 fields sampled for blueberry flowers, imidacloprid residue was found (mean = 84 ppb). Bumble bee colonies declined in these fields.

Ruella, native petunia, flowers in GH: Imidacloprid was present in flowers at 10 wk after application at 502 ppb (125 ppb actual) which would kill bees



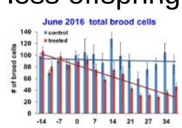
Calibrachoa, million bells, flowers in hanging baskets in GH: Imidacloprid was present in flowers at 10 wk after application at 333 ppb (84 ppb actual) which would kill bees



Objective 2-1. Determine the impacts of imidacloprid (20 ppb) on colony health of bumblebees.

2016 bee movement: Imidacloprid caused bees to move less.

2016 total brood cells: Imidacloprid colonies made less offspring.



Objective 1-4. Research results were discussed at talks, workshops, websites, and interviews on radio, television, and print media.