Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 08b
Project Abstract
For the Period Ending June 30, 2020

PROJECT TITLE: Promoting conversation biocontorol of beneficial insects
Project Manager: Vera Krischik
Organization: University of Minnesota
Mailing Address: 1980 Folwell Ave #219
City/State/Zip Code: Saint Paul, MN 55108
Telephone Number: (612) 625-7044
Email Address: krisc001umn.edu
Web Address: www.entomology.umn.edu/cues; https://ncipmhort.cfans.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 08b
APPROPRIATION AMOUNT: $400,000
AMOUNT SPENT: $0
AMOUNT REMAINING: $400,000

Summary Project Outcomes and Results
All objectives on integrated pest management (IPM) and cultural methods for conserving beneficial insects were completed. The insecticide chlorantraniliprole was toxic to butterflies and cannot be used near butterfly habitat but is safe for bees. Pesticide residue was highest on wildflowers near potato fields and demonstrates the need for buffer strips. Download outreach/research products.

Overall Project Outcome and Results
Research investigated the best insecticides to conserve beneficial insects that can be used in green space. The new bee friendly insecticide chlorotraniliprole was highly toxic to butterflies and should not be used near butterfly habitat. Chlorotraniliprole did not kill bumblebees at 4 ppm, however Monarch butterfly larvae were killed at 0.2 ppm, while Painted lady butterfly larvae were killed at 0.03 ppm and adults were killed at 0.05 ppm chlorotraniliprole. This new and highly popular bee friendly insecticide is not butterfly friendly.

In contrast, the neonicotinoid insecticide chlothianidin that was commonly used as a seed treatment and foliar applied insecticide in agriculture, is highly toxic to bees, but not butterflies. Monarch butterfly larvae were killed at 4 ppm clothianidin, while Painted lady butterflies were killed at 96 ppm clothianidin, and adults were killed at 13 ppm clothianidin. At 20 ppb clothianidin bumblebees colonies had reduced nest weight and brood production. Bumblebees are more sensitive to the neonicotinoid clothianidin (40 ppb lethal dose, 20 ppb sublethal dose) compared to two species of butterfly (4, 96 ppm lethal dose).

Pesticide residue on wildflowers near potato fields showed that 100% of 36 samples tested contained at least 2 and up to 15 different pesticides. Research on pesticide residue on flowers near corn fields showed that of 40% of 32 samples tested contained only 1 pesticide and it was atrazine. Pesticide residue was highest on wildflowers near potatoes and demonstrates the need for buffer strips.

Beetle banks are 4 ft piles of mulch that were created at 3 park sites in Washington County. At a citizen science field day, beetle banks were found to a mean of 131 insects compared to control plots with 1 insect. Research on reed nests as habitat for native stem nesting bees showed that there were 236 occupied reeds or 95% of the nests were occupied. Both beetle banks and stem nests increased insect abundance and are cultural methods to increase insect numbers.

Project Results Use and Dissemination
The grant produced 8 new outreach bulletins, 1 new poster, and research results which are presented at a new website. These outreach bulletins are attached to the work plan.

Our lab has provided 4 workshops per year and 28 talks per year to professionals and consumers on issues related to the grant's research. The bulletins, poster, and research summaries were handed out at outreach events. After 2020 we will continue to use these bulletins at outreach events to educate consumers on IPM programs to protect bees, butterflies, and beneficial insects, such as the parasitoids of the emerald ash borer.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2017 LCCMR Work Plan Final Report

Date of Submission: **August 15, 2020**

Final Report

Date of Work Plan Approval: October 26, 2017

Project Completion Date: **June 30, 2020**

PROJECT TITLE: Promoting conversation biocontorol of beneficial insects

Project Manager: Vera Krischik

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Email Address: krisc001umn.edu

Web Address: www.entomology.umn.edu/cues; https://ncipmhort.cfans.umn.edu/

Location: Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$400,000**
	Amount Spent:	**$400,000**
	Balance:	**$0**

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 08b

Appropriation Language:
$400,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to research integrated pest management strategies, including insecticide alternatives, and overwintering habitat sites to conserve beneficial insects, including bees, butterflies, and predator insects. The integrated pest management strategies will be used to develop best management practices to increase pollinator and beneficial insect diversity and abundance in various restored habitats. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Promoting conversation biocontorol of beneficial insects

II. PROJECT STATEMENT:
The project's goals are to research ways to conserve beneficial insects (bees, butterflies, predators, and parasitoids) in landscapes and restoration projects thru conservation biocontrol, cultural management, use of EPA registered biorational insecticides, and proper conventional insecticide use.

Numerous local and state programs are involved with habitat restoration, which require bee-friendly plants to conserve beneficial insects. Beneficial insects include bees, butterflies, predators, and parasitoids. More than 99.9% of all insects are beneficial and these insects rely on pollen and nectar from plants to complete their life cycle. These restorations must be managed thru the principles of Integrated Pest Management (IPM). IPM promotes multiple tactics to manage, including biocontrol, conservation biocontrol, and using biorational insecticides friendly to beneficial insects, and proper use of conventional insecticides. Conservation biocontrol is the use of sustainable, cultural tactics, such as overwintering sites, conservation of ground nesting sites, mulch piles, and appropriate mowing practices. IPM promotes proper use of conventional insecticides including application practices, such as avoiding drift, spraying when bees are not active, using thresholds of pest abundance, and monitoring to see if the application was successful. Research and educational outreach are needed to demonstrate that these tactics increase beneficial insect abundance. We will promote the research results thru peer-reviewed publications, websites, webinars, bulletins, workshops, and talks.

This outreach program and applied research is different from our 2014 LCCMR grant as we are investigating ways to manage restorations so that they are supportive of beneficial insects. We will produce outreach products (website, bulletins, webinars, workshop) and work with university and community groups to disseminate the research results and outreach programs. For products from previous work visit the pollinator conservation website(http://cues.cfans.umn.edu/old/) ; UMinnesota Extension website http://www.extension.umn.edu/garden/plant-nursery-health/ ; NCIPM webinars website+pollinator+plant videos http://ncipmhort.cfans.umn.edu/ ; and a list of pollinator publications at http://www.entomology.umn.edu/faculty-staff/vera-krischik ; MN Arboretum Pollinator Cubed Workshops http://www.arboretum.umn.edu/P3Pesticides2.aspx

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of February 15, 2018:
Research: A site for the restoration research has been selected. The research will be done in Stillwater, MN at St Croix Bluffs Park with the help of Dan MacSwain, Natural Resources Coordinator, Parks Division, Washington County Parks; Carpenter Nature Center with the help of Jennifer Vieth, Executive Director and Manager; and Laurie Schneider, Pollinator Friendly Alliance.

In Feb 2018 I am hiring a supervisor PhD to run the program. A set of 3 plants (replicated 3 times) around a corn field were tested for residue in August 2018 and no substantial residue was found. However, the USDA Gastonia Lab does not have a group pesticide screen that is sensitive down to 1ppb. A search is under way to find another lab to perform the pesticide research.

Outreach: A website has been created that identifies the 24 families of bees that pollinator plants, as well as videos on important nectar plants and videos on bee life histories. USDA NCIPM webinars website+pollinator+plant videos
http://ncipmhort.cfans.umn.edu/

Since August 2017, 10 talks to commodity groups, pesticide applicators, pollinator groups, and Master Gardeners have been given.

Activity 1-2. A review of residue from insecticides in residues has been started and is 43 pages long and contains 60 papers. We plan to publish this as a peer review meta-analysis in a scientific journal. This preliminary document is attached in the appendix.

Project Status as of August 15, 2018:
Research on effects of insecticides on bumblebees was initiated. In the field, research on beetle bumps and stem nesting bees was initiated. Research on the effects of a bee friendly and conventional insecticide on bumblebees was initiated. Since August 2017, 20 talks to commodity groups, pesticide applicators, pollinator groups, and Master Gardeners have been given on conserving pollinators and other good bugs..

Project Status as of February 15, 2019:
Research on effects of insecticides on bumblebees are in the final 3 rd replicate experiment and data shows that the neonicotinoid insecticides provided in sugar water at a field relevant dose of 20 ppb alters colony behavior and health compared to the bumble bee friendly insecticide Acelepryn. We stated the experiments on the LD50 of 2 species of butterflies to 4 insecticides in acute short 96 hr tests and chronic 30 day tests.

Amendment request:
In the Feb 15 2019 project status report, I am requesting a re-budget to reduce funds from contracts ($46,000, pesticide residue analysis) and travel ($5,000) and add to supplies, since the supplies funds have been exhausted. Supply costs have increased substantially due to need for 2.5 yrs of greenhouse (2 GH) rental space, increased cost of BB (bumblebee) colonies and shipping, and cost of beneficial insects from insectaries. Travel funds can be reduced as we are not traveling to BB colonies in the field. In summer 2018, 40 field colonies were killed by honeybees. Since summer 2018 research on BB colonies was moved into the GH in 3 large (12x16x6ft) cages. GH fees have now substantial increased from around $2,400 to $6,000/yr since July 2018 as we need 2 GH for rearing and performing insecticide bioassays for bees and other beneficial insects. In addition, shipping costs of BB and their food (sugar syrup in boxes) has increased 200%. The quality of BB colonies was poor in Spring 2019, causing us to order 100% more colonies so we can choose the healthy ones for research. Residue analysis fees from the USDA Gastonia Lab were reduced for the insecticides that we study, so a reduction in funds in residue analysis will not affect the objectives of this study. Funds allocated for salary are unchanged. I was waiting for another grant to be processed to balance the budget, but those funds were not available until July 2019. In discussion with my UM business manager it was decided that a re-budget was the best way to deal with the deficit in supply funds.

Amendment approved by LCCMR on 8/29/19.

Project Status as of August 15, 2019:
The project is making progress on all its objectives. In the field we have collected samples of 150 plants near agricultural fields to investigate drift. We have created beetle bumps (bbumps) at 3 sites in parks in Washington Co . We sampled the bbumps for beneficial insects and found 100 times more good bugs in the bbumps than in grass. We put out 36 stem nesting bee boxes and found 20% occupancy of the stems after the first year. We performed 3 replicate experiments research on the new bee friendly insecticide Acelepryn and found it does not affect bumblebee health like the neonicotinoid clothianidin.

We are 75% completed on the research on monarch and painted lady butterflies. The LD50 for 4 insecticides were many time higher than levels of these insecticides found in the field. In chronic long term studies we found that these insecticides do not affect the 2 species of butterflies at lower field relevant doses.

Project Status as of February 15, 2020:
The project has made significant progress on all objectives. Data was collected from beetle banks to understand how this new habitat resulted in numerical increases in the abundance and diversity of insects. Beetle banks were installed at 3 sites in Washington County. At a citizen science field day, 36 beetle banks at 3 sites had a

mean of 131 insects in a sample of 10% of each beetle bank compared to control plots with 1 insect. Research on best habitat for native stem nesting bees called reed huts were installed at 3 sites in Washington County. We collected 36 nests to identify stem nesting bees to species and emergence time. At a citizen science field day, 36 reed bee huts were inspected at 3 sites which contained 236 occupied reeds or 95% of the huts occupied by nesting bees.

Research on pesticide residue on flowers near potato fields showed that 100% of 36 samples tested contained at least 2 and up to 15 different pesticides. Research on pesticide residue on flowers near corn fields showed that of 40% of 32 samples tested contained only 1 pesticide and it was atrazine

Most insecticide experiments were completed. We determined the LD50 for Painted Lady and Monarch butterflly larvae, which has not been researched previously. Monarch butterfly larvae had an LD50 of 3.7 ppm clothianidin, while painted lady butterflies could tolerate 96 ppm clothianidin. LD50 studies showed that Monarch and Painted lady butterflies tolerated higher doses of bifenthrin, imidacloprid, and clothianidin than bumble bees. We are finishing LD50 studies for Painted Lady and Monarch butterflies adults. Monarch adults were not able to fly at 5 ppm and had reduced flight at 1ppm clothianidin.

However, bumblebees at 100 times lower dose of 20 ppb clothianidin had reduction in nest weight and brood production. These data show that bumblebees are more sensitive to the neonicotinoid clothianidin compared to two species of butterfly.

The new bee friendly insecticide chlorotraniliprole did not kill bumblebees at 4 ppm. However, Monarch butterfly larvae had an LC50 of 0.2 ppm chlorotraniliprole, while painted lady butterflies could tolerate 0.03 ppm chlorotraniliprole. This new and highly popular bee friendly insecticide is not butterfly friendly. This is important research as this insecticide is used in urban landscapes and it is was not known that it is very toxic to butterfly larvae.

A new graphic for the LCCMR grant was attached at the end of the work plan.

We wrote 7 new outreach bulletins and developed 1 new website. The bulletin ”2020 toxicity of pesticides to pollinators” is a valuable tool to help IPM mangers identify and use bee friendly insecticides. Our lab has provided 4 workshops/year and 28 talks/year to professionals and consumers on issues related to the grant’s research.

Activity 1: Conservation biocontrol in restorations, new outreach bulletins

1-1. Pollinator lawn IPM, 2 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Pollinator Friendly Alliance
1-2. Conservation guide: pollinators, plants, pesticides, 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Emily Tenczar
1-3. Best practices for pollinators: conserving biodiversity in open spaces, 2 page color bulletin, by Dr. Vera Krischik
1-4. Conserving the endangered rusty patched bumble bee, 2 page color bulletin, by Dr. Vera Krischik, Xerces Society
1-5. New IPM and pollinator website at https://ncipmhort.dl.umn.edu

Activity 2: Beneficial insect friendly pesticides that do not kill bees, new outreach bulletins

2-1. Guide to integrated pest management (IPM), 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider
2-2. Think IPM for pollinator conservation, 12x16 color poster, by Dr. Vera Krischik, Laurie Schneider
2-3. 2020 Toxicity of pesticides to pollinators, 4 pages by Dr. Vera Krischik

Amendment Request as of June 15, 2020:

We request the following amendments to the project budget:

Personnel from $289,000 to $294,000
Professional Technical Contracts from $ 29,000 to $35,872
Equipment/Tools/Supplies from $76,000 to $67,542
Printing: from $4,000 to $1,736
Travel from $2,000 to $850

The reason for these changes are:
Supplies cost more than anticipated. Confusion between the three reviewers, LCCMR, UM CFANS accounting, and Krischik lab made us change this budget.

Reconciled budget approved by LCCMR June 17, 2020.

Project Status as of August 15, 2020:
<u>Overall Project Outcome and Results</u>
Research investigated the best insecticides to conserve beneficial insects that can be used in green space. The new bee friendly insecticide chlorotraniliprole was highly toxic to butterflies and should not be used near butterfly habitat. Chlorotraniliprole did not kill bumblebees at 4 ppm, however Monarch butterfly larvae were killed at 0.2 ppm, while Painted lady butterfly larvae were killed at 0.03 ppm and adults were killed at 0.05 ppm chlorotraniliprole. This new and highly popular bee friendly insecticide is not butterfly friendly.

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Pesticide residue on wild flowers near potato fields showed that 100% of 36 samples tested contained at least 2 and up to 15 different pesticides. Research on pesticide residue on flowers near corn fields showed that of 40% of 32 samples tested contained only 1 pesticide and it was atrazine. Pesticide residue was highest on wildflowers near potatoes and demonstrates the need for buffer strips.

Beetle banks are 4 ft piles of mulch that were created at 3 park sites in Washington County. At a citizen science field day, beetle banks were found to a mean of 131 insects compared to control plots with 1 insect. Research on reed nests as habitat for native stem nesting bees showed that there were 236 occupied reeds or 95% of the nests were occupied. Both beetle banks and stem nests increased insect abundance and are cultural methods to increase insect numbers.

IV. PROJECT ACTIVITIES AND OUTCOMES:
The project's goals are to research ways to conserve beneficial insects (bees, butterflies, predators, and parasitoids) in landscapes and restoration projects thru conservation biocontrol, cultural management, and proper conventional and biorational insecticides, using the principles of integrated pest management (IPM).

Activity 1: *Conservation biocontrol in restorations.* We will develop research and outreach educational programs on conservation biocontrol of beneficial insects by promoting cultural management, such as overwintering sites, native bee nesting sites, mowing times, and proper plant choice in small restorations. We plan to research the benefits of 4 types of cultural management:

1-1. Install mulch piles or overwintering banks to conserve predatory insects and bees during the winter.

1-2. Understand the duration of standing plant stems in spring so native bees can leave the stems as adults and not die if the stems are removed early. Best seasonal times to mow to encourage new flowering.

1-3. Identify the best larval host plants in MN for butterflies and bees and the best pollen and nectar producing plants for adult beneficial insect feeding in restorations.

1-4. Investigate the current levels of pesticide residue in plants in 4 areas that are considered good habitat for restoration.

Milkweed plants growing in roadsides and in restorations near high pesticide use areas may contain residues that affect monarch adults and larvae. Pesticide may be present from road deicers, mosquito abatement programs, and runoff (Repeated application of deicing salts test at 960 ppb imidacloprid has the potential to create residues that harm pollinators, since 40 ppb is the LD50 for bees, Niagara bee, August 2016; Krischik V, M Rogers, G Gupta, A Varshney. 2015. 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*, PLoS ONE March 23, 2015, DOI: 10.1371/journal.pone.0119133).

Basswood trees no longer create a good honey flow, according to MN bee keepers. We will investigate whether basswood trees near ditches contain pesticide residues in flowers.

Ground covers in agricultural fields are important for conserving soil and moisture, as well as providing pollen and nectar to beneficial insects. We will investigate whether these ground covers accumulate pesticides in sufficient amounts to harm beneficial insects.

LCCMR goals: We will work with small scale restorations to develop guidelines to protect beneficial insects and pollinators thru conservation biocontrol and IPM. We will determine conservation biocontrol management practices and determine pesticide residue can influence beneficial insect survival and behavior. We will work with the MN listserv team, local community groups, and MN Extension Service to disseminate these results.

Activity 2: *Beneficial insect friendly pesticides.* We will develop research and outreach educational programs on conservation biocontrol of beneficial insects by understanding what chemical management used in landscapes/ restorations/greenhouse conserve beneficial insects. We will identify to consumers and professional land managers the appropriate pesticide and application methods to use to reduce drift, reduce exposure to bees foraging, and reduce nontarget effects on other species. For instance use of insecticides to control Japanese beetle adults on roses or Japanese beetle grubs in turf may negatively affect leaf cutter bees and other bees. In addition, we will research what plants can be exempt from MN bee labeling laws, since these plants do not provide sufficient pollen and nectar for beneficial insects.

Plant establishment in landscapes/restorations and the production of bee-friendly plants usually require the use of an insecticide during propagation and after installation. The EPA has registered insecticides that are compatible with biocontrol, but are not widely used due to lack of data and relevant information accessible to the grower. We need to determine if biorational insecticides, such as**,** chlorantraniliprole (very high LD_{50}), s-kinoprene (prevents the growth of immature insects) and pymetrozine (stops the mouthparts of aphids from working) produce no residue and conserve beneficial insects compared to conventional insecticides. We need to determine if current management practices produce residues in leaves that may harm native bees when they collect leaves for their nest cavities.

We will promote the proper use of EPA registered insecticides that are compatible with bees.

LCCMR goals: The project's goals are to research ways to conserve beneficial insects (bees, butterflies, predators, and parasitoids) in landscapes and restoration projects thru the proper use of conventional and biorational insecticides. Plants not visited by bees will be identified, so they are exempt from special propagation practices. We will work with the MN listserv team, local community groups, and MN Extension Service to disseminate these results.

Activity 1: *Conservation biocontrol in restorations*
Description: Research and promote thru outreach website and bulletins ways to increase beneficial insect abundance in restorations

Summary Budget Information for Activity 1: **ENRTF Budget $210,000**
Balance: $0

Outcome	Completion Date
1-1 to 1-3. Research the use of four tactics: overwintering sites, mulch piles, standing plants, and moving for conserving beneficial insects.	Oct 1, 2019
1-4. Research pesticide residue and if the levels found alter behavior or survival of beneficial insects.	March 1, 2020
Create outreach website, webinars, and bulletins for information dissemination.	March 1, 2020

Activity 1 Status as of February 15, 2018
A site for the restoration research was selected: Stillwater, MN at St Croix Bluffs Regional Park with the help of Dan MacSwain, Natural Resources Coordinator, Parks Division, Washington County Parks; Carpenter Nature Center with the help of Jennifer Vieth, Executive Director and Manager; and Laurie Schneider, Pollinator Friendly Alliance. Mulch piles were added to the restorations so we can study their ability to increase overwintering sites for bumblebees and other beneficial insects. These will be sampled throughout 2019.

A new version of the "MN Butterfly Gardening" Bulletin and Plant List is under development and some suggestions were implemented in small restorations.

A plant list has been created and is being edited at http://cues.cfans.umn.edu/old/gervais/keytable.htm and http://ncipmhort.cfans.umn.edu/plant-lists-restoration-tactics-and-videos.

USDA NCIPM webinars website+pollinator+plant videos http://ncipmhort.cfans.umn.edu/
We have developed and maintained other websites about proper insecticide use to conserve bees for professional IPM managers in greenhouse and landscape.
UM/MNLA/MDA Pesticide Certification Training turf and ornamentals
http://pesticidecert.cfans.umn.edu/
UM CFANS CUES website
cues.cfans.umn.edu/
Activity 1 Status as of August 15, 2018:

Activity 1 Status as of February15, 2019:

Activity 1 Status as of August 15, 2019:
Research: *Conservation biocontrol in restorations: management practices and research the use of mulch piles or overwintering banks to conserve predatory insects and bees during the winter.*

Activity 1-1: Research the use of four tactics: overwintering sites, mulch piles, standing plants, and moving for conserving beneficial insects.

In 2019 Beetle bumps, which are overwintering ground areas for bees and beneficial insects, and hanging bee nests, were installed at three sites: two in Stillwater, MN at St Croix Bluffs Regional Park with the help of Dan MacSwain, Natural Resources Coordinator and Washington County Parks; and one site in Hastings, MN at Laurie Schneider, Pollinator Friendly Alliance. The first insect count in Feb 2019 revealed that the BEB contained more beneficial insects than on the bare ground. Beetle bumps need to be added to restorations, parks, backyards and gardens to provide habitat for good bugs and bees.
Washington County Park System serves 40,000 visitors each year. Their park and naturalist educators are now including beetle bumps in their public education programming. Xerces Society is promoting beetle bumps as a tool for rural and urban spaces. This research will support their outreach efforts with farmers, rural, and urban people. Pollinator Friendly Alliance, Washington Conservation District and Board of Soil & Water Resources is including beetle bump suggestions in information tailored for Minnesota's Lawns to Legumes incentive program to convert turf to biodiverse pollinator habitat.

Three beetle banks have been placed to date: St. Croix Bluffs Regional Park East, Hastings; St. Croix Bluffs Regional Park West, Hastings; and 9503 Norell Avenue, Stillwater. The two straw bales are placed on one side in a V shape to help contain the 2.5 yards of mulch which is piled high inside the V. The t-post is pounded into the ground at the tip of the V alongside the snow flag and a bee hut is placed on the t-post. Each bee hut has an identifier sticker 1-36.

Figure 1: Straw bales are placed on one side in a V shape to help contain the 2.5 yards of mulch which is piled high inside the V.

Figure 2: Beetle bump with bee hut

Figure 3: Bee hut closeup

Figure 4: Beetle bump construction

Figure 5: Sign on bee hut saying "Please do not disturb"

Activities 1-2. Research the duration of standing plant stems in spring so native bees can leave the stems as adults and not die if the stems are removed in fall mowing, which are usually prescribed. In winter 2019 native bee nest sites called "bee huts" were added to each beetle bump to determine if they would increase the number of native bees. As of July 2019, 90% of the bee huts were occupied. The number and type of bee will be counted in late October 2019 with the help pf citizen science groups. The data collection will continue until June 2020.

Activities 1-3. *Identify the best larval host plants in MN for butterflies, which is rarely part of plant lists. Research* and outreach: A new version of the "MN Butterfly Gardening" Bulletin and Plant List is under development in cooperation with the MN Lepidiopterists Society. Butterflies have regional host plants that differ from state to state so that the correct larval host plants need to be included with seed mixes for restorations

Activity 1-4. *Collect field samples near agricultural fields to determine drift residue.* Sites were identified for collecting flower samples near three soybean and three potato fields. Sampling will continue from June 2020. Samples will be evaluated for residue of insecticide, herbicides, and fungicides. Activities 1-2 Research pesticide residue and if the levels found alter behavior or survival of beneficial insects. Create outreach website and bulletins for information dissemination.

Figure 6: Potato field adjacent to habitat for drift study.

Figure 7: Bumblebee on flower adjacent to potato field.

Activity 1 Status as of February 15, 2020:

Data was collected from beetle banks to understand how this new habitat resulted in numerical increases in the abundance and diversity of insects. Beetle banks were installed at 3 sites in Washington County. At a citizen science field day, 36 beetle banks at 3 sites had a mean of 131 insects in a sample of 10% of each beetle bank compared to control plots with 1 insect. Research on best habitat for native stem nesting bees called reed huts were installed at 3 sites in Washington County. We collected 36 nests to identify stem nesting bees to species and emergence time. At a citizen science field day, 36 reed bee huts were inspected at 3 sites which contained 236 occupied reeds or 95% of the huts occupied by nesting bees.

Research on pesticide residue on flowers near potato fields showed that 100% of 36 samples tested contained at least 2 and up to 15 different pesticides. Research on pesticide residue on flowers near corn fields showed that of 40% of 32 samples tested contained only 1 pesticide and it was atrazine.

A new graphic for the LCCMR grant was attached at the end of the work plan.

For Activity 1, we wrote 4 new outreach bulletins and developed 1 new website.
1-1. Pollinator lawn IPM, 2 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Pollinator Friendly Alliance
1-2. Conservation guide: pollinators, plants, pesticides, 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Emily Tenczar
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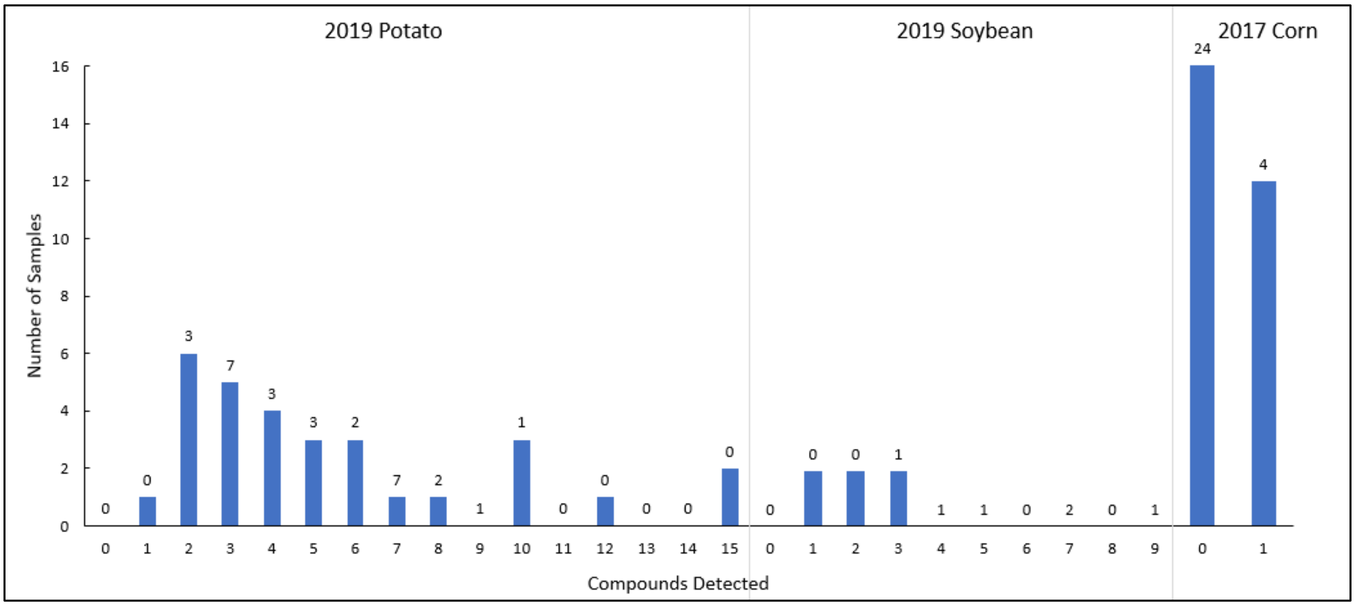
1-5. New IPM and pollinator website at https://ncipmhort.dl.umn.edu

Our lab has provided 4 workshops/year and 28 talks/year to professionals and consumers on issues related to the grant's research.



Number of wild plant samples possessing a given number of pesticide compound residues. Data labels indicate the number of additional samples for which trace amounts of pesticides were detected. Plant samples were collected from habitat adjacent to potato (leaves), soybean (leaves), and corn (leaves and flowers) fields, and were analyzed for the presence of pesticide residues. Potato samples (n = 30) were collected around one potato field in Hastings, MN and two fields in Big Lake, MN (2019). Soybean samples (n = 6) were collected around one soybean field in North Branch, MN (2019). Corn samples (n = 28) were collected around four corn fields in Alexandria, MN (2017). All samples processed by the U.S. Department of Agriculture (2020).

Residue in wild flowers 100 ft from ag fields July and August 2019, 4 sites, 3 plants/site

Insecticide	Soybean ppb	Soybean trace	Potato ppb	Soybean trace	Corn ppb	Corn trace	Comment
clothianidin*	0	2	7/3, 3, 4, 4, 4, 4, 4 **3.7 (+0.17)**	8	0	0	Insecticide Neonic
coumaphos	0	1	0	0	0	0	Insecticide OP
lambda cyhalothrin	0	0	0	17	0	0	Insecticide Pyrethroid
DDE p,p’	0	0	0	9	0	0	Insecticide chlorinated hydrocarbon
DEET	0	0	2/16, 21 **18.5 (+1.77)**	10	0	0	Insecticide
diflubenzuron	0	0	6/4,4,4,4,4,4 **4.0 (+0)**	0	0	0	insecticide growth regulator

Insecticide	Soybean ppb	Soybean trace	Potato ppb	Soybean trace	Corn ppb	Corn trace	Comment
malathion	0	0	11/2040, 2550, 27, 3000, 34, 37, 383, 39, 4130, 49, 543 **1166.55 (+428.34)**	4	0	0	insecticide
novaluron	0	6	18/1050, 1110, 116, 1190, 1430, 155, 184, 1850, 215, 219, 258, 3540, 399, 448, 68, 739, 923, 931 **823.61 (+195.64)**	4	0	0	insecticide growth regulator
methidathion	0	2	0	0	0	0	insecticide
permethrin	0	0	6/1020, 1550, 2310, 2900, 4030, 5850 **2943.33 (+659.82)**	0	0	0	insecticide
thiamethoxam*	0	0	3/6, 7, 7 **6.67 (+0.27)**	11	0	0	insecticide
phorate	0	2	0	3	0	0	insecticide OP
propargite	0	0	0	1	0	0	insecticide/miticide organosulphite
Insecticide total		**13**	**53**	**67**	**0**	**0**	

Herbicide	Soybean ppb	Soybean trace	Potato ppb	Soybean trace	Corn ppb	Corn trace	Comment
acetochlor	2/116, 133 **124.5 (+6.01)**	4	0	16	0	0	herbicide
atrazine	4/12, 7, 7, 8 **8.5 (+1.03)**	2	23/62, 50, 65, 64, 10, 10, 3, 37, 38, 6, 7, 7, 7, 12, 8, 19, 6, 8, 8, 3, 3, 6, 4, 13, 9, 3, 4 **19.04 (+4.41)**	2	11/8, 10, 19, 5, 7, 8, 16, 7, 12, 11, 11 **10.36 (+1.19)**	3	herbicide

Herbicide	Soybean ppb	Soybean trace	Potato ppb	Soybean trace	Corn ppb	Corn trace	Comment
carbenzadim	0	0	0	2	0	0	herbicide
chlorthal-dimethyl	0	1	0	2	0	0	herbicide
dimethenamid	0	0	0	2	0	0	herbcide
metribuzin	0	0	6/14, 14, 16, 17, 21, 24 **17.67 (+1.50)**	10	0	0	herbicide
pendimethalin	0	0	4/25, 26, 26, 28 **26.25 (+0.54)**	3	0	0	herbicide
thymol	0	0	0	0	1/83	thymol	0
Herbicide total	**6**	**7**	**33**	**37**	**12**	**3**	

Fungicide	Soybean ppb	Soybean trace	Potato ppb	Soybean trace	Corn ppb	Corn trace	Comment
azoxystrobin	0	5	7/13, 13, 14, 14, 16, 3, 8 **11.57 (+1.58)**	3	0	0	fungicide
chlorothalonil	6/30, 31, 34, 35, 35, 36 33.5 **33.5 (+0.91)**	0	28/108000, 129000, 136, 186, 267, 29, 30, 30, 30, 30, 31, 31, 31, 33, 34, 35, 35, 37, 39, 40, 51700, 54, 54, 58, 67300, 73900, 82, 91200 **18658.29 (+7101.51)**	0	0	0	fungicide
difenoconazole	0	0	2/99, 119 109 **109 (+7.07)**	0	0	0	fungicide
diphenylamine	0	4	1/3 **3 (+0)**	17	0	0	fungicide

Fungicide	Soybean ppb	Soybean trace	Potato ppb	Soybean trace	Corn ppb	Corn trace	Comment
famoxadone	0	3	16/1180, 1410, 151, 171, 172, 179, 199, 216, 323, 425, 469, 555, 60, 68, 85, 87 **359.38 (+95.81)**	6	0	0	fungicide
fluopyram	0	0	10/185, 2, 210, 220, 331, 4, 4, 41, 5, 65 **106.7 (+35.83)**	5	0	0	fungicide
fluxaproxad	0	0	0	2	0	0	fungicide
mandipropamide	0	0	2/129, 135 **132 (+2.12)**	0	0	1	
metalaxyl	0	0	0	2	0	0	fungicide
metconazole	0	0	1/12 **12 (+0)**	3	0	0	fungicide
					0	0	
metolachlor	0	5	0	14	0	0	herbicide
propiconazole	0	0	6/11, 8, 15, 13, 11, 15 **12.17 (+1.01)**		0	0	fungicide
pyraclostrobin	0	0	6/10, 14, 15, 21, 27, 8 **15.83 (+2.64)**	1	0	0	fungicide
pyrimethanil	0	0	6/1150, 925, 512, 524, 37, 76 **537 (+165.80)**		0	0	fungicide
trifloxystrobin	0	0	0	4	0	0	fungicide
Fungicide	**6**	**17**	**85**	**57**	**0**	**1**	
total	**12**	**37**	**171**	**161**			

* The neonicotinoid insecticides were measured at a lower LOD clothianidin (3 ppb), imidacloprid (2 ppb), dinotefuran (10 ppb), thiamethoxam (4 ppb) Mean ppb is in **bold**.
N= 38 different composite samples of wildflowers, 3 species/site; 4 sites; July14-August 1

Status as of August 15, 2020:
Activity 1 Final Report Summary:

All objectives on IPM and cultural methods for conserving beneficial insects were completed. Research identified ways that IPM programs for green space and restorations might improve the conservation bees, butterflies, and beneficial insects, such as the parasitoids of the emerald ash borer.

Pesticide residue on wild flowers near potato fields showed that 100% of 36 samples tested contained at least 2 and up to 15 different pesticides. Research on pesticide residue on flowers near corn fields showed that of 40% of 32 samples tested contained only 1 pesticide and it was atrazine. Buffer strips and drift education programs would help reduce pesticide drift near potato fields. See attached bulletin 18. Pesticide residue wildflowers research

Beetle banks are 4 ft piles of mulch that were created at 3 park sites in Washington County. At a citizen science field day, beetle banks were found to a mean of 131 insects compared to control plots with 1 insect. Research on reed nests as habitat for native stem nesting bees showed that there were 236 occupied reeds or 95% of the nests were occupied. Among the 3 sites there was no significant difference in total number of insects in the stems. However, there were significantly more wasps in stem nests in the sun compared to shade, but no difference in the total number of insects in sun and shade. Both beetle banks and stem nests increased insect abundance and are cultural methods to increase insect numbers. See attached bulletin 17. Beetle bumps research summary

Outreach/research products are found at https://ncipmhort.cfans.umn.edu/

ACTIVITY 2: ***Beneficial insect friendly pesticides that do not kill bees***
Description:
In order to reduce risk to pollinators from pesticides, IPM need to promote pest management that reduces risk to honey bees and other beneficial insects (lady beetles, lacewings, bumblebees, solitary bees, and monarch butterflies). We need to determine if other biorational insecticides (7 and pymetrozine) cause less impact on bee and beneficial insect behavior and survivorship, compared to neonicotinoids (imidacloprid and clothianidin; Appendix1, Table 5).

Summary Budget Information for Activity 2: **ENRTF Budget: $190,000**
Balance: $0

Outcome	Completion Date
1 Research the efficacy of conventional and biorational insecticides (chlorantraniliprole, s-kinoprene, pymetrozine) compared to neonicotinoids (imidacloprid, clothianidin)) on pests and whether the insecticide conserves beneficial insects (lady beetles, lacewings, bumblebees, solitary bees) in propogation and restorations.	Oct 1 2019
2. Research what bedding plants do not provide food (nectar or pollen) for beneficial insects and make plant lists of these non-pollinator friendly plants.	March 1 2020
Create outreach website, webinars, and bulletins for information dissemination.	March 1 2020

Activity 2 Status as of February 15, 2018:
Activity 2-1: In order to reduce risk to pollinators from pesticides, IPM need to promote pest management that reduces risk to honey bees and other beneficial insects (lady beetles, lacewings, bumblebees, solitary bees, and monarch butterflies). We need to determine if other biorational insecticides (chlorantraniliprole and pymetrozine) cause less impact on bee and beneficial insect behavior and survivorship, compared to neonicotinoids (imidacloprid and clothianidin; Appendix1, Table 5).

For greenhouse IPM programs, we demonstrated in experiments with two plant species (*Ruellia*, Prairie petunia, and bedding plant, *Calibrachoa*, Million Bells), that pymetrozine (bee friendly Endeavor) was not detected on leaves or flowers from five to 10 weeks after planting. Pymetrozine (Endeavor) controls pests with sucking mouthparts. Pymetrozine residue at five weeks (1/9) decreased 100 % by 10 weeks and its use will reduce insecticide exposure to bees. These data support the FOE Gardeners Beware reports that showed 62% of purchased plants can contain neonicotinoid residue (2 to 879 ppb).

We performed 3 field tests on the effects of control, 20 ppb clothianidin and 4 ppm Acelepryn (bee friendly chloratraniliprole) on bumblebees, *Bombus impatens*. Data showed that the bee friendly insecticide Acelepryn had no effects on colony weight, consumption, brood production or movement, when clothianidin affected all of these parameters within three weeks after exposure. A new paper by Crall et al., 2018 (Science 362, 683–686 with *Bombus impatiens* found reduction in thermoregulation and wax curtains at 6 ppb imidacloprid. We did not observe any difference in wax curtain production among the three treatments.

Activity status 2-2: Beneficial insect friendly pesticides may not be needed on bedding plants that bees do not visit. We will research what plants can be exempt from MN bee labeling laws, since these plants do not provide sufficient pollen and nectar for beneficial insects. A yearly renewable grant of $20,000 was submitted to the USDA NC IPM Center to fund a working group in Midwest states to collaborate on bulletins on protecting bees from pesticides in greenhouse production.

Activity 2 Status as of August 15, 2018:
Activity 2-1. Beneficial insect friendly pesticides that do not kill bees.
We performed 3 field tests on the effects of 20 ppb clothianidin (neonicotinoid) and 4 ppm Acelepryn (bee friendly clorantraniliprole) on bumblebees, *Bombus impatens*. Data showed that the bee friendly insecticide Acelepryn had no effects on colony weight, consumption, brood production or movement, when the neonicotinoid clothianidin affected all of these parameters. We have initiated research on the effects of bee friendly and neonicotinid insecticides on monarch butterflies, *Danaus plexippus.*

In order to reduce risk to pollinators from pesticides, IPM need to promote pest management that reduces risk to honeybees and other beneficial insects (lady beetles, lacewings, bumblebees, solitary bees, and monarch butterflies). We need to determine if other biorational insecticides (chlorantraniliprole and pymetrozine) cause less impact on bee and beneficial insect behavior and survivorship, compared to neonicotinoids (imidacloprid and clothianidin; Appendix1, Table 5).

Research on butterflies: We have initiated research on the effects of bee friendly and neonicotinoid insecticides on monarch butterflies, *Danaus plexippus.* and painted lady butterflies*, Vanessa cardui.* Lab experiments will start in May 2019 on the effects on the 3 classes of insecticides, clothianidin and imidacloprid (neonicotinoid), bifenthrin (pyrethroid), and bee friendly insecticide (chlorantraniliprole) on 2 species of butterflies.

Activity 2-2: We will research what plants can be exempt from MN bee labeling laws, since these plants do not provide sufficient pollen and nectar for beneficial insects. A list was created to identify plants that are not considered to be visited by bees. This will help growers target bee friendly plant BMP.

Activity 2 Status as of February15, 2019:
Research on butterflies: We have initiated research on the effects of bee friendly and neonicotinid insecticides on monarch butterflies, *Danaus plexippus.* and painted lady butterflies*, Vanessa cardui.* Lab experiments will start in May 2019 on the effects on the 3 classes of insecticides, clothianidin and imidacloprid (neonicotinoid), bifenthrin (pyrethroid), and bee friendly insecticide (clorantraniliprole) on 2 species of butterflies.

Activity 2 Status as of August 15, 2019:
Beneficial insect friendly pesticides that do not kill bees. In order to reduce risk to pollinators from pesticides, IPM need to promote pest management that reduces risk to honeybees and other beneficial insects (lady beetles, lacewings, bumblebees, solitary bees, and monarch butterflies). We need to determine if other biorational insecticides (chlorantraniliprole and pymetrozine) cause less impact on bee and beneficial insect behavior and survivorship, compared to neonicotinoids (imidacloprid and clothianidin; see Appendix 1, Table 5).

Research on bumblebees: Eastern bumblebee, *Bombus impatiens*, demonstrated that 20 ppb clothianidin (neonicotinoid) and 4 ppm Acelepryn (bee friendly chlorantraniliprole) had different effects on BB colony health. Data showed that the bee friendly insecticide Acelepryn had no effects on colony weight, consumption, brood production or movement, when the neonicotinoid clothianidin affected all of these parameters. Another research project demonstrates that use of clothianidin and chlorantraniliprole at field rates of foliar application killed foraging bumblebees.

Research on butterflies: We have initiated research on the effects of bee friendly and neonicotinoid insecticides on monarch butterflies, *Danaus plexippus.* and painted lady butterflies*, Vanessa cardui.* Lab experiments started in May 2019 on the effects on the 3 classes of insecticides, clothianidin and imidacloprid (neonicotinoid), bifenthrin (pyrethroid), and bee friendly insecticide (chlorantraniliprole) on 2 species of butterflies. We have found in short term bioassays that larvae of both butterfly species are killed by neonicotinoid, pyrethroid, and birational, friendly insecticides. However, the dose for killing adults is higher than what is found in nectar. Larvae feeding on leaves were killed by the bee friendly biorational insecticide. So contrary to what is now promoted, the new chemical chlorantraniliprole may be bee friendly, but not monarch friendly. Currently we are studying what sublethal doses can do to larvae of both species of butterflies and effect behavior.

Figure 8: Mallow leaves in water pix for bioassay study on Painted lady butterflies (#1)

Figure 9: Mallow leaves in water pix for bioassay study on Painted lady butterflies. (#2)

Figure 10: Monarch and painted lady butterflies are fed sugar syrup from wells on watermelons.

Figure 11: Over 300 potted host plants for butterflies and bees are grown outside and inside the UM greenhouses

Activity 2-2. *Beneficial insect friendly pesticides may not be needed on bedding plants that bees do not visit.* We will research what plants can be exempt from MN bee labeling laws, since these plants do not provide sufficient pollen and nectar for beneficial insects.
A list was created to identify plants that are not considered to be visited by bees. This will help growers target bee friendly plant BMP.
Details
We are 75% completed on the research on monarch and painted lady butterflies. The LD50 for 4 insecticides were many time higher than levels of these insecticides found in the field. In chronic long term studies we found that these insecticides do not affect the 2 species of butterflies at lower field relevant doses.

We are finishing research on the effects of bee friendly and neonicotinid insecticides on monarch butterflies, *Danaus plexippus.* and painted lady butterflies*, Vanessa cardui.* Lab experiments on LD50 demonstrated that butterflies can tolerate mush higher amounts of these the 3 classes of insecticides, clothianidin and imidacloprid (neonicotinoid), bifenthrin (pyrethroid) in the field than is what is commonly found.

Residue of imidacloprid in potted plants and landscapes

Plant	Type	Imidacloprid ppb – 5 weeks	Imidacloprid ppb – 10 weeks
Calibrochoa leaves	pot	58,883	25,993
Ruella/prairie petunia leaves	pot	14,400	2,086
Dogwood leaves	landscape	70	na
Agastache leaves	landscape	528	na
Swamp milkweed leaves	landscape	1000	na
Residue near ag fields	landscape	7	
		Dinotefuran ppb	
Calibrochoa leaves	pot	83,866	56,566
Ruella/prairie petunia leaves	pot	30,200	15,400
		Clothianidin	na
Residue near ag fields		4	na

2019 Acute 96 hr bioassay larval painted lady butterfly data compared to monarch data. Larvae were fed treated leaves every day for 4 days

chemical	ppm	microg/ul	ng/ul ppm	ppm	microg/ul	ng/ul ppm
	2019 UM	Krishnan, ISU 2018	Krishnan, ISU 2018	2019 UM	Krishnan, ISU 2018	Krishnan, ISU 2018
	PLB 3rd instar	Monarch 3rd instar	Monarch 3rd instar	PLB 5rd instar	Monarch 5TH instar	Monarch 5TH instar
bifenthrin	tba	$1.14*10^{-3}$ Beta-cyfluthrin	1.14 Beta-cyfluthrin	55	0.064 Beta-cyfluthrin	64 Beta-cyfluthrin
imidacloprid	230	0.5047	504	256	1.691	1,691
clothianidin	88	0.0079	8	96	1.197	1,197
chloran traniliprole	0.01	0.0133	13	0.03	0.1795	179

2019 Chronic 11 day adult monarch bioassay, 2 replicate experiments in Sept 2019
Acute bioassays cannot be done as the number of treatments and the number of adults used per treatment is 180 adults/chemical x 2 for 2 replicate experiment per chemical. For 4 chemicals we would need a total of 360 x 4 chemicals=1440 adults

2019 Sept data	**PLB Larval LC50**	**dose**	**mortality**	**No eggs/female**	**No eggs hatching**
bifenthrin (30)	55 ppm	7 ppm 2nd rep 0.1 ppm Sept 10	Yes day1-11	Yes day 4,8	Yes day 7, 12

2019 Sept data	PLB Larval LC50	dose	mortality	No eggs/female	No eggs hatching
Imidacloprid (30)	256 ppm	50 ppb 2nd rep same Sept 10	Yes Day 6-8	No diff	No diff
Clothianidin (30)	96 ppm	10 ppm 2nd rep same Sept 10	Yes day1-11	No diff	No diff
Chloran traniliprole (30)	0.03 ppm	1 ppb 2nd rep same Sept 10	no	Yes day 8	No diff

Adults were force fed 30% sugar syrup on day 1 only and data were collected from day 1-11

2019 Chronic 30 day adult monarch bioassay; 2 replicate experiments in Sept 2019

clothianidin

	Larval weight	Pupal weight	Days to pupation	# pupae	Days to emerg	# males	# females	Days to death	Mean no eggs
0 ppb									
10 ppb	No diff	No diff	No diff	No diff	No diff	No diff	No diff	No diff	No diff
20 ppb	No diff	No diff	No diff	No diff	No diff	No diff	No diff	No diff	No diff
40 ppb	less	less	No diff	less	No diff	No diff	No diff	No diff	No diff

Acute LD50 Painted lady butterfly, *Vanessa cardui*, Third instar bioassay

Insecticide	n[a]	LC_{50}[b]	Krishnan LC_{50}	95% FL[c]	LC_{90}[b]	95% FL[c]	Slope ±SE[d]	X^{2e}	P^f	Bioassay date
clothianidin	180	88	8	(62 , 121)	539	(329 , 1,286)	0.7 ±0.1	0.5	0.9	6/12/2019
imidacloprid	180	230	50	(165 , 302)	967	(676 , 1,727)	0.9 ±0.1	3.8	0.3	6/7/2019
chlorantraniliprole	180	0.01	13	(0.008 , 0.02)	0.1	(0.06 , 0.3)	0.6 ±0.1	5.2	0.2	7/3/2019
bifenthrin	pending		1.14 betac							Oct 2019

Acute LD50 Painted lady butterfly, Vanessa cardui, Third instar bioassay										
Insecticide	**n[a]**	**LC_{50}[b]**	**Krishnan LC_{50}**	**95% FL[c]**	**LC_{90}[b]**	**95% FL[c]**	**Slope ±SE[d]**	**X^{2e}**	**P^f**	**Bioassay date**
clothianidin	180	96.2	1,200	(50 , 144)	801	(487 , 2,050)	0.6 ±0.1	2.8	0.6	Rep 1: 6/3/19 Rep 2 & 3: 6/28/17
imidacloprid	180	256	1,691	(190 , 348)	1309	(823 , 2,864)	0.8 ±0.1	3	0.4	6/29/2019

bifenthrin	180	55	64 betac	(38 , 84)	432	(239 , 1,122)	0.6 ±0.1	0.5	0.9	7/3/2019
chlorantraniliprole	180	0.03	180	(0.02 , 0.04)	0.2	(0.1 , 0.5)	0.7 ±0.1	0.5	0.9	6/27/2019

Acute LD50 Monarch butterfly, Danaus plexippus, Third instar bioassay

Insecticide	n[a]	LC_{50}[b]	95% FL[c]	LC_{90}[b]	95% FL[c]	Slope ±SE[d]	X^2[e]	*P*[f]	Bioassay date
clothianidin	262	3.7	(2 , 6)	89	(46 , 263)	0.4 ±0.05	8.1	0.2	Rep 1: 8/20/19 Rep 2 & 3: 8/22/19
imidacloprid	Pending								Oct 2019
chlorantraniliprole	300	0.2	(0.1 , 0.3)	2.8	(1.6 , 7)	0.5 ±0.07	2	0.8	Rep 1:8/15/19 Rep 2: 8/16/19 Rep 3: 8/21/19
bifenthrin	Pending								Oct 2019

Activity 2 Status as of February 15, 2020

Most insecticide experiments were completed. We determined the LD50 for Painted Lady and Monarch butterflly larvae, which has not been researched previously. Monarch butterfly larvae had an LD50 of 3.7 ppm clothianidin, while painted lady butterflies could tolerate 96 ppm clothianidin. LD50 studies showed that Monarch and Painted lady butterflies tolerated higher doses of bifenthrin, imidacloprid, and clothianidin than bumble bees. We are finishing LD50 studies for Painted Lady and Monarch butterflies adults. Monarch adults were not able to fly at 5 ppm and had reduced flight at 1ppm clothianidin.

However, bumblebees at 100 times lower dose of 20 ppb clothianidin had reduction in nest weight and brood production. These data show that bumblebees are more sensitive to the neonicotinoid clothianidin compared to two species of butterfly.

The new bee friendly insecticide chlorotraniliprole did not kill bumblebees at 4 ppm. However, Monarch butterfly larvae had an LD50 of 0.2 ppm clothianidin, while painted lady butterflies could tolerate 0.03 ppm clothianidin. This new and highly popular bee friendly insecticide is not butterfly friendly. This is important research as this insecticide is used in urban landscapes and it is not known that it is very toxic to butterfly larvae.

Monarch butterflies were t4sted at field relevant doses of 4 insecticides and there was no effect on egg laying, percent egg hatch, or 6 behaviors.

A new graphic for the LCCMR grant was attached at the end of the work plan.

For Activity 2, we wrote 3 new outreach bulletins and developed 1 new website. The bulletin"2020 toxicity of pesticides to pollinators" is a valuable tool to help IPM mangers identify and use bee friendly insecticides. Our lab has provided 4 workshops/year and 28 talks/year to professionals and consumers on issues related to the grant's research.
2-1. Guide to integrated pest management (IPM), 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider
2-2. Think IPM for pollinator conservation, 12x16 color poster, by Dr. Vera Krischik, Laurie Schneider
2-3. 2020 Toxicity of pesticides to pollinators, 4 pages by Dr. Vera Krischik

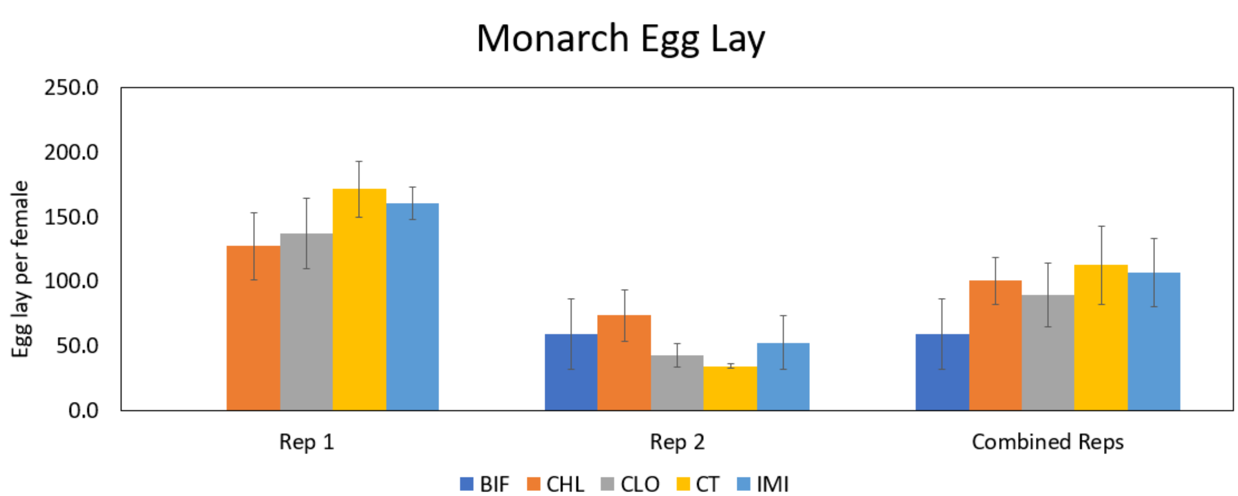
Our lab has provided 4 workshops/year and 28 talks/year to professionals and consumers on issues related to the grant's research.

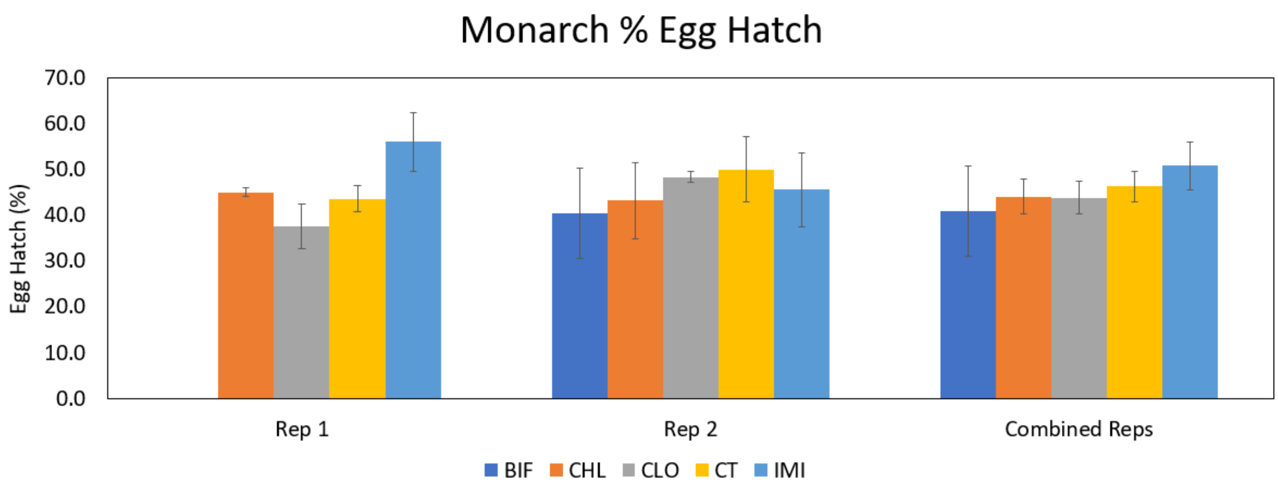
Acute LD50 Monarch butterfly, Danaus plexippus, Third instar bioassay

Insecticide	n[a]	LC_{10} [b]	95% FL[c]	LC_{50} [b]	95% FL[c]	LC_{90} [b]	95% FL[c]	Slope ±SE[d]	X^2 [e]	*P*[f]	*Concentrations used in bioassays (ppm)*	Bioassay date
clothianidin	262	0.15	(0.03 , 0.4)	3.7	(2 , 6)	89	(46 , 263)	0.4 ±0.05	8.1	0.2	0, 1, 5, 10, 50, 100, 250, 500, 750	Rep 1: 8/20/19 Rep 2 & 3: 8/22/19
imidacloprid	99	0.19	(0.04, 0.4)	1.09	(0.7, 1.8)	6.24	(3.2, 34.2)	0.7 ±0.18	1.2	0.3	0, 0.3, 1, 3	Rep 1: 2/10/20 Rep 2: 2/10/20 Rep 3: 2/11/20
bifenthrin	191	0.18	(0.08, 0.3)	1.51	(1.0, 2.5)	12.49	(6.4, 35.9)	0.6 ±0.08	4.2	0.4	0, 0.03, 0.1, 0.3, 1, 3, 30	Rep 1: 1/22/20 Rep 2: 1/23/20 Rep 3: 2/10/20
Chlorantran-iliprole	300	0.02	(0.005 , 0.03)	0.2	(0.1 , 0.3)	2.8	(1.6 , 7)	0.5 ±0.07	2	0.8	0, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100	Rep 1:8/15/19 Rep 2: 8/16/19 Rep 3: 8/21/19

[a]total number of insects used, [b]Lethal concentration expressed in parts per million (ppm), [c]fiducial limits, [d]probit slope ± standard error, [e]Chi-square value, [f]*P* value

Egg laying and % egg hatch for adult monarch butterflies after feeding on milkweed treated with insecticide. 20-30 butterflies were used per insecticide (n = 3). Insecticides used: control = 0 ppb; bifenthrin (pyrethroid) = 100 ppb; clothianidin (neonicotinoid) = 10 ppm; imidacloprid (neonicotinoid) = 50 ppb; chlorantraniliprole = 1 ppb. Bifenthrin was not used in Replicate 1.





Behavior parameters for adult monarch butterflies after feeding on milkweed treated with insecticide. 20-30 butterflies were used per insecticide (n = 3). Replicate 1 was conducted August 5th-14th, 2019, and Replicate 2 September 5th-19th, 2019. Insecticides used: control = 0 ppb; bifenthrin (pyrethroid) = 100 ppb; clothianidin (neonicotinoid) = 10 ppm; imidacloprid (neonicotinoid) = 50 ppb; chlorantraniliprole = 1 ppb. Bifenthrin was not used in Replicate 1.

Parameter name	Definition	Rep	Univariate Repeated Measures[1]	ANOVA Day 1[2]	ANOVA Day 2[3]	ANOVA Combined Days[4]
Flailing bout Fbout	movement between rest	1	Time: $F(4,36)=2.62$ $p = 0.051$ Trt: $F(3,9)=0.98$ $p = 0.44$ Trt*Time: $F(12,36)=1.10$ $p = 0.39$	$F(3, 9) = 1$ $p = 0.435$	$F(3,9) = 2.98$ $p = 0.089$	$F(3,70) = 1.50$ $p = 0.22$
		2	Time: $F(4,40)=6.66$ $p = 0.0003$ Trt: $F(4,10)=7.08$ $p = 0.006$ Trt*Time: $F(16,40)=1.64$ $p = 0.10$ Sig for CLO	$F(4,10) = 0.87$ $p = 0.51$	$F(4,10) = 0.09$ $p = 0.98$	$F(4,70) = 5.10$ $p = 0.0012$ Sig for CLO
		Combined 1 and 2	Time: $F(4,92)=3.95$ $p = 0.005$ Trt: $F(4,23)=4.23$ $p = 0.01$ Trt*Time: $F(16,92)=0.87$ $p = 0.61$ Not sig for any.	$F(4,23) = 0.72$ $p = 0.59$	$F(4,23) = 0.42$ $p = 0.79$	$F(4,144) = 5.20$ $p = 0.0006$ Sig for CLO
Proboscis extension Pext	butterfly held upside down by	1,2	Time: $F(4,36)=3.43$ $p = 0.02$ Trt: $F(3,9)=1.84$ $p = 0.21$	$F(3, 9) = 9.56$ $p = 0.0037$ Sig for IMI	$F(3, 9) = 0.92$ $p = 0.47$	$F(3,70) = 2.10$ $p = 0.11$

Parameter name	Definition	Rep	Univariate Repeated Measures[1]	ANOVA Day 1[2]	ANOVA Day 2[3]	ANOVA Combined Days[4]
	wings and proboscis extension		Trt*Time: F(12,36)=2.63 p = 0.01			
		2	Time: F(4,40)=3.13 p = 0.025 Trt: F(4,10)=2.72 p = 0.09 Trt*Time: F(16,40)=1.65 p = 0.10	F(4,10) = 0.70 p = 0.61	F(4,10) = 0.98 p = 0.46	F(4,70) = 4.84 p = 0.0017 Sig for CHL
		Combined 1 and 2	Time: F(4,92)=4.08 p = 0.004 Trt: F(4,23)=2.41 p = 0.078 Trt*Time: F(16,92)=2.44 p = 0.004	F(4,23) = 3.15 p = 0.46*	F(4, 23) = 1.14 p = 0.36	F(4,144) = 3.50 p = 0.0093 Sig for CHL
Legs used Luse	maximum number of legs moving at once	1	Time: F(4,36)=3.04 p = 0.029 Trt: F(3,9)=1.38 p = 0.31 Trt*Time: F(12,36)=1.19 p = 0.33	F(3,9) = 7.75 p = 0.007 Sig for CLO	F(3,9) = 2.04 p = 0.18	F(3,70) = 1.61 p = 0.19
Abdomen jerks Ajerk	movement of the abdomen past the hind wing edge	1	Time: F(4,36)=3.51 p = 0.016 Trt: F(3,9)=0.47 p = 0.71 Trt*Time: F(12,36)=1.47 p = 0.18	F(3,9) = 2.92 p = 0.09	F(3,9) = 0.68 p = 0.58	F(3,70) = 0.39 p = 0.76
Melon test Mtest	binary test: sat on melon for 10 secs, was proboscis extended	2	Time: F(4,40)=12.47 p = <0.0001 Trt: F(4,10)=1.72 p = 0.22 Trt*Time: F(16,40)=1.91 p = 0.049	F(4,10) = 0.32 p = 0.86	F(4,10) = 4.98 p = 0.018 Not sig for any trt.	F(4,70) = 2.23 p = 0.07
Drop test Dtest	Binary test: when dropped, before reaching ground do wings open	2	Time: F(4,40)=6.71 p = 0.003*** Trt: F(4,10)=2.18 p = 0.14 Trt*Time: F(16,40)=2.47 p = 0.011	X^2 (4) = 0 ** p = 1.0	X^2 (4) = 5.75 ** p = 0.22	X^2 (4) = 15.11 ** p = 0.005 Sig for IMI and CLO

[1] Univariate Repeated Measures ANOVA: ANOVA examining the effect of treatment (Trt), time, and the treatment x time interaction (Trt*Time) on given parameter. Used because measurements were taken on the same individuals every day, so observations were not independent. Analysis includes observations from Days 1-5 for all experiments but excludes Day 6 due to missing data. **This analysis is most appropriate to describe data over several days.**

[2] ANOVA Day 1: ANOVA comparing treatment effects on Day 1 of measuring factor for Replicate 1 (R1), Replicate 2 (R2), and the Combination or R1 and R2.

[3] ANOVA Day 2: ANOVA comparing treatment effects on Day 2 of measuring factor for Replicate 1 (R1), Replicate 2 (R2), and the Combination or R1 and R2.

[4] ANOVA Combined: **This analysis should not be used to describe data, as data seriously violate assumptions of independence necessary for ANOVA.** This test was carried out on observations combined from all days (1-6).

*ANOVA p value was $p = 0.03$. However, failed to meet assumption of equal variances (Levene's Test $p < 0.05$), so value reported is Welch's.

** Failed to meet assumptions for normality and equal variances, so nonparametric Kruskal-Wallis test was used.

***May have failed assumptions for normality. Might consider analyzing nonparametrically (e.g. Friedman's).

Status as of August 15, 2020:
Activity 2 Final Report Summary:
All objectives on IPM and cultural methods for conserving beneficial insects were completed. Research investigated the best insecticides to conserve beneficial insects that can be used in green space. The new bee friendly insecticide chlorotraniliprole was highly toxic to butterflies and should not be used near butterfly habitat. Chlorotraniliprole did not kill bumblebees at 4 ppm, however Monarch butterfly larvae were killed at 0.2 ppm, while Painted lady butterfly larvae were killed at 0.03 ppm and adults were killed at 0.05 ppm chlorotraniliprole. This new and highly popular bee friendly insecticide is not butterfly friendly.

In contrast, the neonicotinoid insecticide chlothianidin that was commonly used as a seed treatment and foliar applied insecticide in agriculture, is highly toxic to bees, but not butterflies. Monarch butterfly larvae were killed at 4 ppm clothianidin, while Painted lady butterflies larvae were killed at 96 ppm clothianidin, and adults were killed at 13 ppm clothianidin. At 20 ppb clothianidin bumblebees colonies had reduced nest weight and brood production. Bumblebees are more sensitive to the neonicotinoid clothianidin (40 ppb lethal dose, 20 ppb sublethal dose) compared to two species of butterfly (4, 96 ppm lethal dose).

Summary of the research:

The following tables use the LC50 research and relate it to the levels of insecticides sprayed in the field to determine if butterflies and bumblebees will be killed with current insecticide use rates.

Table 1 is a summary of the rates used.

Table 2,3,4 demonstrates that label rates of insecticides will kill monarch and painted lady butterfly larvae.

Table 5 demonstrates that label-rates of insecticide will kill adult honey bees and bumble bees.

Table 6,7, 8 demonstrates that field rates of insecticides will kill monarch and painted lady butterfly larvae.

Table 9 demonstrates that field rates of insecticide will kill adult honey bees and bumble bees.

Table 1. Pesticide label application rates. All values refer to quantity of active ingredient (AI).

Pesticide (AI)	Foliar/soil	AI content			Maximum label application rate per year			Life Stage	Pest insect label rate for ornamentals/flowerbeds (per application)			
		% AI	lb/gal	g/fl oz	lb/acre/yr	g/acre/yr	g/sq ft/yr		fl oz/ 100 gal	fl oz/1000 sq ft	g/1000 sq ft	mg/sq ft
Acelepryn (chlorantraniliprole)	Foliar	18.4	1.67	5.918	0.5	226.795	0.00521	adult	NA	0.37	2.182	2.182
								grub	NA	0.37	2.182	2.182
Arena 50 WDG (clothianidin)	Foliar	50	NA	NA	0.4	181.4	0.00416	adult	NA	NA	4.150	4.150
								grub	NA	NA	4.150	4.150
Flagship 25 WG (thiamethoxam)	granular (ornamentals)	NA	NA	NA	0.266	120.655	0.00277	adult	NA	NA	2.770	2.770
								grub	NA	NA	2.770	2.770
Meridian 25 WG (thiamethoxam)	granular (turf)	NA	NA	NA	0.266	120.655	0.00277	adult	NA	NA	3.016	3.016
								grub	NA	NA	2.770	2.770
Merit 2F (imidacloprid)	Foliar	21.4	2.0	7.087	0.4	181.4	0.00416	adult	1.5	0.03	0.213	0.213
								grub	NA	0.6	4.252	4.252
Talstar Professional (bifenthrin)	Foliar	7.9	0.67	2.35	0.5	226.795	0.00521	adult	NA	0.5	1.181	1.181
								grub	NA	1.0	2.359	2.359

Table 2. Toxicity of label-rate insecticide applications to larval *Danaus plexippus* (mon) and *Vanessa cardui* (plb) based on 96h acute dietary exposure bioassay. Butterfly LC50 values from Krischik (unpublished). Calculations use estimate of 1 mL solution/leaf. All values refer to insecticide active ingredient (AI).

Pesticide	larval LC50 (ug/L)			larval LC50 (mg/mL)			larval LC50 (mg/sq ft)			High label rate (mg/sq ft)	Does label rate kill butterflies?		
	mon5	plb5	plb3	mon5	plb5	plb3	mon	plb	plb3		mon5	plb5	plb 3
Acelepryn (chlorantraniliprole)	200	30	10	0.0002	0.00003	0.00001	0.012	0.0018	0.00062	2.182	yes	yes	yes
Arena 50 WDG (clothianidin)	3700	96200	88000	0.0037	0.0962	0.088	0.228	5.93	5.42	4.150	yes	no	no
Merit 2F (imidacloprid)	1090	256000	230000	0.0011	0.256	0.23	0.067	15.77	14.17	4.252	yes	no	no
Talstar (bifenthrin)	1510	9000	200	0.0015	0.009	0.0002	0.093	0.55	0.012	2.359	yes	yes	yes

Table 3. Toxicity of label-rate insecticide applications to *Danaus plexippus* 5th instar (mon5) larvae and *Vanessa cardui* 5th instar (plb5) and 3rd instar (plb3) larvae based on 96h acute dietary exposure bioassay. Butterfly LC10 values from Krischik (unpublished). Calculations use estimate of 1 mL solution/leaf. All values refer to insecticide active ingredient (AI).

Pesticide	larval LC10 (ug/L)			larval LC10 (mg/mL)			larval LC10 (mg/sq ft)			High label rate (mg/sq ft)	Does label rate kill butterflies?		
	mon5	plb5	plb3	mon5	plb5	plb3	mon5	plb5	plb3		mon5	plb5	plb3
Acelepryn (chlorantraniliprole)	20	4	1	0.00002	0.000004	0.000001	0.0012	0.00025	0.000062	2.182	yes	yes	yes
Arena 50 WDG (clothianidin)	150	28900	14300	0.00015	0.0289	0.014	0.0092	1.78	0.88	4.150	yes	yes	yes
Merit 2F (imidacloprid)	190	43300	54600	0.00019	0.0433	0.055	0.012	2.67	3.36	4.252	yes	yes	yes
Talstar (bifenthrin)	180	7100	20	0.00018	0.0071	0.00002	0.011	0.44	0.0012	2.359	yes	yes	yes

Table 4.Toxicity of label-rate insecticide applications to *Danaus plexippus* 5th instar (mon5) larvae and *Vanessa cardui* 5th instar (plb5) and 3rd instar (plb3) larvae based on 96h acute dietary exposure bioassay. Butterfly LC90 values from Krischik (unpublished). Calculations use estimate of 1 mL solution/leaf. All values refer to insecticide active ingredient (AI).

Pesticide	larval LC90 (ug/L)			larval LC90 (mg/mL)			larval LC90 (mg/sq ft)			High label rate (mg/sq ft)	Does label rate kill butterflies?		
	mon5	plb5	plb3	mon5	plb5	plb3	mon5	plb5	plb3		mon5	plb5	plb3
Acelepryn (chlorantraniliprole)	2800	200	100	0.0028	0.0002	0.0001	0.17	0.012	0.0062	2.182	yes	yes	yes
Arena 50 WDG (clothianidin)	89000	801000	539000	0.089	0.801	0.539	5.48	49.35	33.21	4.150	no	no	no
Merit 2F (imidacloprid)	6240	1309000	967000	0.00624	1.309	0.967	0.38	80.65	59.58	4.252	yes	no	no

Talstar (bifenthrin)	12490	70000	1600	0.0125	0.07	0.0016	0.77	4.31	0.099	2.359	yes	yes	yes

Table 5. **Toxicity of label-rate insecticide applications to adult honey bees (*Apis mellifera*) and bumble bees (*Bombus spp*.).** Calculations use estimate of 1 mL solution/leaf. LC50 are from EPA and other published sources. All values refer to insecticide active ingredient (AI).

Pesticide	LC50 (ug/L) (ppb)		LC50 (mg/mL)		LC50 (mg/sq ft)		High label rate (mg/sq ft)	Does label rate kill bees?	
	honey bee	bumble bee	honey bee	bumble bee	honey bee	bumble bee		honey bee	bumble bee
Acelepryn (chlorantraniliprole)	7060[h+]	13000[c]	0.0071	0.013	0.435	0.801	2.182	yes	yes
Arena 50 WDG (clothianidin)	40[g]	2[d]	0.00004	0.000002	0.002	0.0001	4.150	yes	yes
Merit 2F (imidacloprid)	40[i]	59[e]	0.00004	0.000059	0.002	0.004	4.252	yes	yes
Talstar (bifenthrin)	1000[i]	360[f]	0.001	0.00036	0.062	0.022	2.359	yes	yes

+ value based on topical application. No available data for oral LC50.

Table 6. Toxicity of insecticide field residues to *Danaus plexippus* 5th instar (mon5) larvae, and *Vanessa cardui* 5th instar (plb5) and 3rd instar (plb3) larvae, honey bees (Apis mellifera), and bumble bees (Bombus spp.). Butterfly LC50s based on 96h acute dietary exposure bioassay conducted by Krischik (unpublished). Calculations use estimate of 1 mL solution/leaf. All values refer to insecticide active ingredient (AI). Created June 2020 (ML).

Pesticide	larval LC50 (ug/L)			larval LC50 (ng/g milkweed)			Honey bee LC_{50} (ug/L) (ppb)	Bumble bee LC_{50} (ug/L) (ppb)	Maximum field residue (ng/g foliage)	Does field residue kill butterflies?			Does field residue kill bees?	
	mon5	plb5	plb3	mon5	plb5	plb3				mon5	plb5	plb3	Honey bee	Bumble bee
Acelepryn (chlorantraniliprole)	200	30	10	730	109	36.5	7060[h+]	13000[c]	66.0[a]	no	no	yes	no	no
Arena 50 WDG (clothianidin)	3700	96200	88000	13504	351095	321168	368[i] 40[g]	2[d]	352.3[a]	no	no	no	yes	yes
Merit 2F (imidacloprid)	1090	256000	230000	3978	934307	839416	40[i]	59[e]	36.5[b]	no	no	no	yes	no

Talstar (bifenthrin)	1510	9000	200	5511	32847	730	1000[i]	360[f]	53.1[b]	no	no	no	no	no

Table 7. Toxicity of insecticide field residues to *Danaus plexippus* 5th instar (mon5) larvae and *Vanessa cardui* 5th instar (plb5) and 3rd instar (plb3) larvae based on 96h acute dietary exposure bioassay. Butterfly LC10 values from Krischik (unpublished). Calculations use estimate of 1 mL solution/leaf. All values refer to insecticide active ingredient (AI).

Pesticide	larval LC10 (ug/L)			larval LC10 (ng/g milkweed)			Maximum field residue (ng/g foliage)	Does field residue kill butterflies?		
	mon5	plb5	plb3	mon5	plb5	plb3		mon5	plb5	plb3
Acelepryn (chlorantraniliprole)	20	4	1	73.0	14.6	3.6	66.0[a]	no	yes	yes
Arena 50 WDG (clothianidin)	150	28900	14300	547	105475	52190	352.3[a]	no	no	no
Merit 2F (imidacloprid)	190	43300	54600	693	158029	199270	36.5[b]	no	no	no
Talstar (bifenthrin)	180	7100	20	657	25912	73.0	53.1[b]	no	no	no

Table 8. **Toxicity of insecticide field residues to *Danaus plexippus* 5th instar (mon5) larvae and *Vanessa cardui* 5th instar (plb5) and 3rd instar (plb3) larvae based on 96h acute dietary exposure bioassay.** Butterfly LC90 values from Krischik (unpublished). Calculations use estimate of 1 mL solution/leaf. All values refer to insecticide active ingredient (AI).

Pesticide	larval LC90 (ug/L)			larval LC90 (ng/g milkweed)			Maximum field residue (ng/g foliage)	Does field residue kill butterflies?		
	mon5	plb5	plb3	mon5	plb5	plb3		mon5	plb5	plb3
Acelepryn (chlorantraniliprole)	2800	200	100	10219	730	365	66.0[a]	no	no	no
Arena 50 WDG (clothianidin)	89000	801000	539000	324818	2923358	1967153	352.3[a]	no	no	no
Merit 2F (imidacloprid)	6240	1309000	967000	22774	4777372	3529197	36.5[b]	no	no	no

Talstar (bifenthrin)	12490	70000	1600	45584	255475	5839	53.1[b]	no	no	no

Table 9. Krischik (unpublished) monarch 5^{th} instar (mon5) and painted lady 5^{th} instar (plb5) and 3^{rd} instar (plb3) butterfly LC_{50}; Krishnan et al. 2020 5^{th} instar monarch butterfly LC_{50}; honey bee oral LC_{50}; bumble bee oral LC_{50}.

Pesticide	Krischik larval LC_{50} (ug/g milkweed) (ppm)			Krishnan et al. 2020 Monarch 5^{th} LC_{50} (ug/g) (ppm)	Honey bee LC_{50} (mg/L) (ppm)	Bumble bee LC_{50} (mg/L) (ppm)	Are honey bees more sensitive than butterflies?	Are bumble bees more sensitive than butterflies?
	mon5	plb5	plb3					
Acelepryn (chlorantraniliprole)	0.730	0.109	0.036	0.97	7.06[a+]	13[c]	no	no
Arena 50 WDG (clothianidin)	13.504	351.095	321.168	0.80 (2^{nd} and 3^{rd} instars were 4.2 and 7.8, respectively)	0.368[b] 0.04[g]	0.002[d]	yes	yes
Merit 2F (imidacloprid)	3.978	934.307	839.416	9.4	0.04[b]	0.059[e]	yes	yes
Talstar (bifenthrin)	5.511	32.847	0.730	NA	1.0[b]	0.36[f]		
beta-cyfluthrin	NA	NA	NA	0.62	0.50[b]	0.12[g]		
chlorpyrifos	NA	NA	NA	10	2.5[b]	0.23[g]		
thiamethoxam	NA	NA	NA	33	0.0265[b]	0.12[e]	yes	yes

References

Besarda, L., V. Mommaerts, J. Vandeven, X. Cuvelier, G. Sterk, and G. Smagghe. 2010. Compatibility of traditional and novel acaricides with bumblebees (Bombus terrestris): a first laboratory assessment of toxicity and sublethal effects. *Pest Management Science* 66:786-793. doi10.1002/ps.1943

Halsch, C.A., A. Code, S.M. Hoyle, J.A. Fordyce, N. Baert, and M.L. Forister. 2020. Pesticide contamination of milkweeds across the agricultural, urban, and open spaces of low-elevation northern California. *Frontiers in Ecology and Evolution* 8:162. doi:10.3389/fevo.2020.00162

Krishnan, N., Y. Zhang, K.G. Bidne, R.L. Hellmich, J.R. Coats, and S.P. Bradbury. 2020. Assessing field-scale risks of foliar insecticide applications to monarch butterfly (*Danaus plexippus*) larvae. *Environmental Toxicology and Chemistry* 00(00):1-19. doi:10.1002/etc.4672

Minnesota Department of Agriculture. 2020. Pesticides & bee toxicity. Web.

Mommaerts, V., S. Reynders, J. Boulet, L. Besard, G. Sterk, and G. Smagghe. 2009. Risk assessment for side effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology* 19:207-215. doi:10.1007/s10646-009-0406-2

Pettis, J.S., E.M. Lichtenberg, M. Andree, J. Stitzinger, R. Rose, and D. vanEngelsdorp. 2013. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLOS One* 8(7): e70182. doi:10.1371/journal.pone.0070182

Sanchez-Bayo, F. and K. Goka. 2014. Pesticide residues and bees – a risk assessment. *PLOS One* 9(4): e94482. doi:10.1371/journal.pone.0094482

Smagghe, G., J. Deknopper, I. Meeus, and V. Mommaerts. 2013. Dietary chlorantraniliprole suppresses reproduction in worker bumblebees. *Pest Management Science* 69:787-791. doi:10.1002/ps.3504

Wade, A., C.H. Lin, C. Kurkul, E.R. Regan, and R.M. Johnson. 2019. Combined toxicity of insecticides and fungicides applied to California almond orchards to honey bee larvae and adults. *Insects* 10, 20. doi:10.3390/insects10010020

Hopwood, J., A. Code, M. Vaughn, D. Biddinger, M. Shepherd, S. Hoffman Black, E. Lee-Mader, and C. Mazzacano. 2016. *How neonicotinoids can kill bees: the science behind the role these insecticides play in harming bees*. 2nd Ed. 76pp. Portland, OR. Xerxes Society for Invertebrate Conservation.

The 3 tables below contain all of the data from acute LC50 experiments and chronic experiments for monarch and painted lady butterflies and bumblebees.

2017-2020 Butterfly insecticide bioassays acute and chronic					
Acute LC50 studies					
experiment	**insecticide**	**lethal dose (ppm)**			**dose**
		LC10	**LC50**	**LC90**	
acute plb 3rd instar	clothianidin	14.3	88	539	ppm= 0, 10, 25, 50, 100, 250, 500, 1000
acute plb 3rd instar	imidacloprid	54.6	230	967	ppm= 0, 100, 200, 500, 1000, 2000

acute plb 3rd instar	bifenthrin	0.02	0.2	1.6	ppm= 0, 0.1, 0.3, 1, 3, 10, 30, 100, 300
acute plb 3rd instar	chlorantraniliprole	0.001	0.01	0.1	ppm= 0, 0.001, 0.005, 0.01, 0.05, 0.1
acute plb 5th instar	clothianidin	28.9	96.2	801	ppm= 0, 50, 100, 250, 500, 1000, 2000
acute plb 5th instar	imidacloprid	43.3	256	1309	ppm= 0, 50, 100, 250, 500, 1000, 2000
acute plb 5th instar	bifenthrin	7.1	9	70	ppm= 0, 5, 10, 50, 100, 500, 2000
acute plb 5th instar	chlorantraniliprole	0.004	0.03	0.2	ppm= 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5
acute monarch 5th instar	clothianidin	0.15	3.7	89	ppm= 0, 1, 5, 10, 50, 100, 250, 500, 750
acute monarch 5th instar	imidacloprid	0.19	1.09	6.24	ppm=0, 0.3, 1, 3
acute monarch 5th instar	bifenthrin	0.18	1.51	12.29	ppm=0, 0.03, 0.1, 0.3, 1, 3, 30
acute monarch 5th instar	chlorantraniliprole	0.02	0.2	2.8	ppm= 0, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100
plb adult	clothianidin	5.98	13.15	28.94	ppm=0, 1, 5, 10, 50
plb adult	clothianidin	flight reduced at 10 ppm and 50 ppm			
plb adult	imidacloprid	8	48	284	ppm=0,5,10,25,50
plb adult	imidacloprid	no effect on flight			
plb adult	bifenthrin	3.4	9	24	ppm=0,5,10,25,50
plb adult	bifenthrin	flight reduced at 10, 25, and 50 ppm			
plb adult	chlorantraniliprole	0.03	0.6	5	ppm=0.025,0.05,0.25,0.5
plb adult	chlorantraniliprole	flight reduced at 0.5, 0.25, and 0.05 ppm			
monarch adult	clothianidin	NA	NA	NA	TBA June 2021
	imidacloprid	NA	NA	NA	TBA June 2021
	bifenthrin	NA	NA	NA	TBA June 2021
	chlorantraniliprole	NA	NA	NA	TBA June 2021

EXP: Butterfly chronic adult and chronic larvae to adult							
	chemical	**Larval wt**	**survival**	**No pupae**	**Days pupae**	**ecl**	**dose**
chronic adult: fed treated sugar syrup only once and behavior measured exp1 at day 2-8 and exp2 at day 8-14.							
monarch adult rep 1	imidacloprid clothianidin bifenthrin chlorantraniliprole		NS	NS		NS	imi= 50 ppb clo= 10,000 ppb chl=1 ppb

monarch adult rep 2	imidacloprid clothianidin bifenthrin chlorantraniliprole		NS	NS		NS	imi= 50 ppb clo= 10,000 ppb chl=1 ppb bif= 100 ppb
chronic larvae to adult: fed treated leaves daily							
plb chronic larvae to adult rep 1,2	clothianidin	NS	P=0.018 0,40	P=0.025 0,40	NS	NS	0, 10, 40 ppb
monarch chronic larvae to adult rep 1 ,2	clothianidin	NS	NS	P=0.002 0,40	P=0.002 0,40	P=0.04 0,40	0, 10, 20, 40ppb

EXP: Bumblebees chronic larvae to adult					
	chemical	**colony wt**	**brood**	**consump**	**movement**
Bumblebees 2 reps	clothianidin	S, yes	S, yes	S, yes	S, yes
Bumblebees 2 reps	chlorantraniliprole	NS	NS	NS	NS

A summary of some of the research is presented in a poster given to the online meeting of the Society of Toxicology and Environmental Sciences in April 13 2020.

UNIVERSITY OF MINNESOTA

Residues in landscape plants and effects on bumblebees and two species of butterflies

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Introduction

Neonicotinoid insecticides are commonly used in landscapes and agriculture in the US, but levels of residue in ornamental plants are rarely measured. Neonicotinoid research showed lethal and sublethal effects on bee behavior and colony health (Scholer & Krischik 2014, Baron et al. 2017, Arce et al. 2017) and on butterfly survival, development, and behavior (Pecenka & Lundgren 2015, James 2019, Peterson et al. 2019, Krishnan et al. 2020).

Objective 1: Materials & Methods

Objective 1: Comparing neonicotinoid residues in ornamentals and agriculture. Plants were treated with label rates of imidacloprid, harvested at 5-10 wks, and residue was quantified by HPLC GC at the USDA lab in Gastonia, NC

Objective 1: Results

Objective 1. Imidacloprid is common in ornamentals and residues are significantly higher compared to clothianidin residues near ag fields. The LD_{50} is around 4 ng/bee for both and was shown to have similar colony affects on bumblebees (Scholer and Krischik 2014).

Table 1. Neonicotinoid residues in urban and ag plants.

Species/ label application	Flowers imid ppb	Leaves imid ppb	Many cloth ppb	Ref
Neonicotinoid residues in agricultural fields (4mg/sqft)				
Asclepias syriaca			1.14 0.71	1,2
Brassica napus pollen		0.09 0.10		3
Brassica napus Wildflowers pollen	1.4 0.16			4
Bee Urban Bee Rural pollen	20		5 35	5
Taraxacum pollen	2.9		6.3	6,7
Neonicotinoid residue in ornamentals (pot 300 mg/sqft)				
Tilia cordata 25 cm DBH trunk inj	1,340 yr1 45 yr 2	36,283 yr1 680 yr 2		8
Tilia cordata 25 cm DBH soil dr	34 yr 1 38 yr 2	290 yr 1 680 yr 2		8
Tilia cordata 70 cm DBH soil dr	30 yr1 88 yr2	554 yr1 737 yr2		8
Cornus racemosa 4 cm DBH soil dr	762	21,062		8
Rosa soil dr	812	na		8
Asclepias incarnata soil dr	86	132		8
Agastache soil dr	94	561		8
Calibrachoa hybrid Pot dr, 1L pot	333	25, 933		8
Ruellia humilis Pot dr, 1L pot	502	2,086		8
Asclepias incarnata pot dr, 6L pot	1568			8
Agastache Pot dr 6 L pot	1973			8

1. Pecenka and Lundgren 2015, 2. Olaya-Arenas and Kaplan 2017, 3. Blacquiere et al. 2012, 4. Botias et al. 2015, 5. David et al. 2016, 6. Krupke et al. 2012, 7. Krupke et al. 2017, 8. Krischik unpublished

Objective 2: Materials & Methods

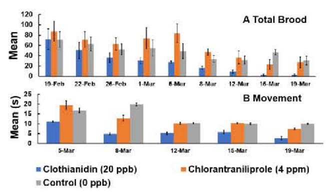
Objective 2: Flight cage studies on lethal and sublethal doses on bumblebees.
Bumblebees, *Bombus impatiens* (Koppert Biological Systems, Howell, MI), colonies were fed untreated Koppert Bee Happy syrup (35%) for 3 weeks after which clothianidin (20 ppb) or chlorantraniliprole (4 ppm) were dissolved in the syrup and fed ad libitum for 5 weeks (10 colonies/trt). Colonies were measured weekly for weight (g), syrup consumption (ml), and movement (sec). Photos were taken bi-weekly to measure brood numbers, brood cell age (1-3), worker numbers, and disease. Movement is the time for a worker to cross the brood.

Objective 2: Results

Objective 2: *Bombus impatiens* fed 20 ppb of clothianidin (LC_{50} 100 ppb, Scholer and Krischik 2014) in syrup had reduced brood production and movement. At 4 ppm chlorantraniliprole (LC_{50} 7 ppm, Smagghe et al. 2015) caused no effects

Figure 1. Clothianidin (20 ppb) had lower colony weight and brood.

Figure 2. (A) Brood numbers (mean ± SE) and (B) movement (mean ± SE) , both were lower for clothianidin (20 ppb).



Objective 3: Materials & Methods

Objective 3: Lab studies on lethal and sublethal doses on butterflies
For LC_{50} bioassays larvae were fed swamp milkweed, *Asclepias incarnata (Danaus plexippus)* or common mallow, *Malva sylvestris (Vanessa cardui)* dipped in insecticides. Adults in 3 m cages were fed Bee Happy syrup at eclosion. On day 1 adults (30/trt) were force fed syrup containing bifenthrin, (0.1 ppm), clothianidin (10 ppm), imidacloprid (0.05 ppm), or chlorantraniliprole (0.001 ppm). Butterflies were dropped and the ability to open wings was measured on 3 days (day 1, 8, 10).

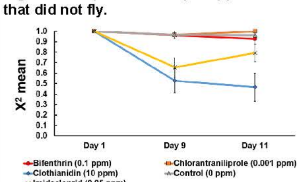
Objective 3: Results

Objective 3: For clothianidin, larval *D. plexippus* had an LC_{50} of 4 ppm and *V. cardui* 96 ppm and for chlorantraniliprole 0.20 ppm and 0.03 ppm. Sublethal effects of clothianidin (10 ppm) reduced flight (wing opening), but did not lower fecundity

Table 2. LC_{50} ppm values for *D. plexippus* and *V. cardui*

Insecticide	n	LC_{10}	LC_{50}	LC_{90}	Slope ±SE	X^2	*P*
LC_{50} fifth instar painted lady larvae, *Vanessa cardui*							
clothianidin	180	28.9	96.2	801	0.6 ± 0.1	2.8	0.6
imidacloprid	180	43.3	256	1309	0.8 ± 0.1	3	0.4
bifenthrin	180	7.1	9	70	0.6 ± 0.1	0.5	0.9
chlorantraniliprole	100	0.004	0.03	0.2	0.7 ± 0.1	0.06	0.9
LC_{50} fifth instar monarch larvae, *Danaus plexippus*							
clothianidin	262	0.15	3.7	89	0.4 ± 0.05	8.1	0.2
imidacloprid	99	0.19	1.1	6.24	0.7 ± 0.18	1.2	0.3
bifenthrin	191	0.18	1.5	12.49	0.6 ± 0.08	4.2	0.4
chlorantraniliprole	300	0.02	0.2	2.8	0.5 ± 0.07	2.0	0.8
LC_{50} adult painted lady adults, *Vanessa cardui*							
clothianidin	100	6.0	13.2	28.9	1.6 ± 0.4	1.5	0.5

Figure 3. Proportion of *D. plexippus* adults that did not fly.



References

Arce AN, TI David, EL Randall, et al. 2017. Journal Applied Ecology 54:1199-1208.
Baron, GL VAA Jansen, MJF Brown et al. 2017. Nature Ecology & Evol 1:1308-1316.
Baron GL, NE Raine & MJF Brown. 2017. Proceeds Royal Society B 284:20170123.
Blacquiere T, G Smagghe, CAM van Gestel et al. 2010. Ecotoxicology 21:973-992.
David A, C Botías C, EM Hil, D Goulson. 2016. Environ Int 88:169–178
James DG. 2019. Insects 10(9):276
Krischik, VK unpublished.
Krishnan N, Y Zhang, G Bidne et al. 2020. Environ. Toxicol. & Chem. 39(4):923-941.
Krupke CH, GJ Hunt, BD Eitzer et al. 2012. PLoS One 7(1):e29268.
Krupke CH, JD Holland, EY Long et al. 2017.JApplied Ecology 54(5):1449-1458
Olaya-Arenas P & I Kaplan. 2019. Front. Ecol. Evol. 7(223).
Pecenka JR & JG Lundgren. 2015. Sci. Nat. 102:19.
Peterson EM, KR Shaw & PN Sith et al. 2019. EnvirToxicol.&Chem.38(12):2629-36.
Scholer J & V Krischik. 2014. PLoS ONE 9(3): e91573.
Smagghe G, J Deknopper. I. Meeus et al. 2013. Pest Manag. Sci. 69(7): 787-791.

Discussion & Conclusion

Two butterfly species had higher LC_{50} for the neonicotinoids clothianidin (4, 96 ppm) and imidacloprid (1, 256 ppm) than bumblebees (100 ppb imidacloprid, clothianidin). LC_{50} for butterflies was lower for chlorantraniliprole (30, 200 ppb) than bumblebees (7 ppm). Clothianidin (10 ppm) reduced flight in monarch butterflies. The 20 year decline of endangered Rusty patched bumblebee, *B. affinis,* may be correlated to ubiquitous neonicotinoid residues and bumblebee sensitivity (20 ppb).

An undergraduate student performed a meta-analysis on the effects of neonicotinoid insecticides on birds. Data showed that neonicotinoid insecticide residues were found in livers and other organs of field collected dead birds.

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Do neonicotinyl insecticides affect birds as they do bees?

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Background

Neonicotinoids are a family of insecticides commonly used for pest treatment in agricultural and horticultural applications. They are applied through soil drenches, seed coatings, foliar sprays, and tree trunk injections. Systemic in nature, the insecticide will move from the application site through the plant to the leaves, pollen, nectar, seeds, and fruits following application.

In bees, neonicotinoids have been shown to be lethal in high doses. Sub-lethal doses can cause deleterious effects on foraging, navigation, and colony health. With growing concern of their usage, introductory research has begun on the effects of neonicotinyl insecticides on bird populations.

This study may serve as a foundation for further research into non-target effects of these insecticides.

Objectives

- Objective 1: Determine residue levels in leaves and seeds of green ash trees, (*Fraxinus pennsylvanica)* following an Imidacloprid (neonicotinoid) soil drench.
- Objective 2: Determine lethal and sub-lethal effects of neonicotinyl insecticides on birds.

Methods

- We collected samples of leaves and seeds from ash trees treated with Imidacloprid insecticides.
- We performed meta-analysis of published research to find the LD50 (lethal dose) and the negative effects of neonicotinoids on birds.
- Using the results of our meta-analysis, we calculated how many treated ash seeds would need to be eaten for lethal and sub-lethal effects to occur.

Results

Imidacloprid and other neonicotinyl insecticides have been found in the bodies of wild birds. These studies show that there are lethal and sub-lethal effects of neonicotinyl insecticides, observed in both field and lab experiments.

We found that ash trees, following an Imidacloprid soil-drench, had a mean residue of 35 ppb in the seeds.

We have determined how many treated ash seeds must be ingested to see deleterious effects on birds, using known LD50 levels and our residue analysis for the amount of imidacloprid in an ash seed.

Table 1: Lethal and Sub-lethal effects on birds

Bird Species	*LD50 (mg/kg)	Lethal and Sub-Lethal Effects	References
Field Surveys			
Many species	unknown	Correlation between bird population declines and neonicotinoids in water	Hallmann 2014
Many Species	unknown	Nervous disorders present in 29.6% of 103 deaths with neonicotinoid residues detected. Primarily pigeons/partridges	Millot 2016
American Robin	unknown	Poisoning after ingesting grubs from a treated lawn	Gibbons 2014
Rufous Hummingbird Anna's Hummingbird	unknown	Neonicotinoids (thiamethoxam, clothianidin, and imidacloprid) were measured in cloacal fluid with a concentration of 3.63 ppb	Bishop 2018
Eurasian Eagle-Owl	unknown	Imidacloprid residue in blood after exposure was measured at a concentration of 3.28 ppb	Taliansky-Chamudis 2017
White-crowned Sparrow	unknown	Imidacloprid residue was found in the blood of 78% of wild sparrows tested	Hao 2018
European Honey Buzzard	unknown	Neonicotinoid residue was present in 80% of the blood samples taken from both adult and juvenile buzzards	Byholm 2018
Greater Prairie-Chicken	unknown	Neonicotinoids were found in 67% of prairie-chicken livers tested (imidacloprid: 8.3 ppb, clothianidin 4.2 ppb, thiamethoxam 1.1 ppb)	Roy 2019
Sharp-tailed Grouse	unknown	Neonicotinoids were present in 89% of grouse livers tested. (Max levels measured: imidacloprid: 84.5 ppb, clothianidin: 3.58 ppb, thiamethoxam: 1.18 ppb)	Roy 2019
Wild Turkey	unknown	43% of turkeys tested were positive for one or more neonicotinoid present in liver samples	MacDonald 2018
Experimental Studies			
Brown-headed Cowbird	unknown	Impaired coordination, retching, reduced consumption of imidacloprid-treated seeds	Millot 2016 Avery 1993
Red-winged Blackbird	unknown	Impaired coordination, retching, reduced consumption of imidacloprid-treated seeds	Millot 2016 Avery 1993
Red Munia	31	Thyroid disruption interfering with seasonal reproductive stages gonad development	Pandey 2017
Canary	35	Incapacitation at 10mg/kg	Mineau 2013
House Sparrow	41	Ingestion of 1.5 beet seeds can result in death, reduced coordination, inability to fly	Gibbons 2014 Mineau 2013 Millot 2016
White-crowned Sparrow	41	4 imidacloprid-treated canola seeds, or 0.2 treated corn seeds can result in death, respiratory distress, reduced body mass	Eng 2017 Goulson 2013
Red-legged Partridge	31-53	The highest dose (0.14-0.7mg/g) of wheat seeds treated with imidacloprid killed 100% of partridges in 21 days Low dose resulted in reduced fecundity	Gibbons 2014 Lopez-Antia 2014 Lopez-Antia 2016
Grey Partridge	13.9	5 maize seeds, six beet seeds or 32 oilseed rape seeds will reach LD50. Sub-lethal effects include failed eggs	Gibbons 2014 Millot 2016 Goulson 2013
Japanese Quail	31	Imidacloprid: Severe signs at 6mg/kg Clothianidin: clinical signs at 25 mg/kg; Incapacitation at 100 mg/kg	Mineau 2013 MacDonald 2018
Mallard	283	Severe clinical signs at 25mg/kg; mortality 8 days post dose	Mineau 2013 Millot 2016
Northern Bobwhite	152	Slight clinical signs at 25 mg/kg; incapacitation 50-100 mg/kg	Mineau 2013 Ertl 2018 MacDonald 2018
Rock Pigeon	25	50% mortality with the ingestion of <4 treated wheat seeds Severe clinical signs at 12.5 mg/k	Millot 2016, Mineau 2013, MacDonald 2018, Berney 1999
Chicken	104	Chick embryo mortality due to malformed crest and neural cells; mortality in 5-24 hours	Wang 2016 Kammon 2010 Liu 2016

*LD50 amounts are the lethal dose of ingested imidacloprid

Table 2: Imidacloprid residue in leaves and seeds of Ash Trees treated annually via soil drench

Ash tree (number)	Imidacloprid Residue	Recovery
July leaves (6), no residue (2)	94ppb	100%
July seeds (2) no residue (2)	36 ppb	119%
July leaves control (3)	0 ppb	100%
July seeds control (3)	0 ppb	132%
August leaves (6) no residue (2)	66 ppb	95%
August seeds (6) no residue (2)	34 ppb	105%
August leaves control (3)	0 ppb	91%
August seeds control (3)	0 ppb	107%

Table 3: Number of ash seeds (5g) with a residue of 35 ppb imidacloprid that must be eaten to reach the LD50

Bird Species and Weight	Ash Seeds Ingested for Lethal/Sub-lethal Effects
Rock Pigeon 900 g	Lethal: 24,545 seeds Sub-lethal: 4,909 seeds
Mallard 1,500g	Lethal: 32,706,818 seeds
Japanese Quail 100 g	Lethal: 14,090 seeds
Northern Bobwhite 150 g	Lethal: 10,227 seeds
House Sparrow 30 g	Lethal: 5,590 seeds
Canary 20 g	Lethal: 27,272 seeds
White-crowned Sparrow 26 g	Lethal: 5,590 seeds Sub-lethal: 1,363 seeds

Conclusion

The amount of imidacloprid residue in an ash seed after treatment is 35 ppb, not enough to kill birds. Seeds of treated crops, such as corn, canola, sunflower, and beets carry a greater risk. These have around 1-2 mg applied per seed compared to those of ash trees which have 35 ppb, a much smaller amount.

A meta-analysis of papers showed that neonicotinoids are found in free-ranging birds, resulting in detrimental or fatal effects. The residue we've found in ash seeds is not enough to constitute a risk to normal behaviors or physiological functions.

Further research is needed to determine lethal and sub-lethal amounts of neonicotinoids in birds including clothianidin, thiamethoxam, etc. Research is also needed to measure neonicotinoid residues in treated vegetation and seeds.

An updated graphic with the results for the research and outreach programs

UNIVERSITY OF MINNESOTA

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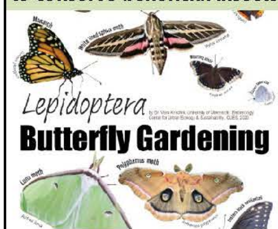
LCCMR, August 2017 - June 2020 Conservation Biocontrol

Activity 1A: Conservation biocontrol in restorations. Research and outreach educational programs will be performed to conserve beneficial insects by researching different cultural tactics in restorations.



New IPM outreach bulletins on Best IPM Practices for Back yard Landscape, Best IPM Practices for Open Spaces, Guide to IPM, Pollinator Lawn IPM, Butterfly Gardening booklet, Butterfly & Plant ID booklet, Conserving Rusty Patched Bumble Bee, Conservation Guide , poster Think IPM

Research on best habitat for nesting and overwintering beneficial insects called beetle banks were installed at 3 sites in Washington county. At a citizen science field day, 36 banks at 3 sites had a mean of 131 insects in a sample of 10% of each beetle bank compared to control plots with 1 insect.

Research on best habitat for native stem nesting bees called reed huts were installed at 3 sites in Washington county. At a citizen science field day, 36 reed bee huts were inspected at 3 sites which contained 236 occupied reeds or 95% of the huts occupied by nesting bees.

Research on best larval host plants and adult nectar plants for Minnesota butterflies are listed in bulletins.

Activity 1B: Conservation biocontrol in restorations. Wild flowers in restorations will be investigated for pesticide residue levels that may affect beneficial insect conservation.

Outreach poster, Think IPM; **Outreach talks were** 4 workshops/year and 28 talks/year to professionals and consumers.
Outreach website at https://ncipmhort.dl.umn.edu

Research on LD50 of butterflies and residue of insecticide in the field permit correlation of field doses of insecticides on survival and behavior of butterflies and bumble bees.

Research on pesticide residue on flowers near potato fields showed that 100% of 36 samples tested contained at least 2 and up to 15 different pesticides.

Research on pesticide residue on flowers near corn fields showed that of 40% of 32 samples tested contained only 1 pesticide and it was atrazine.

Activity 2: Beneficial insect friendly pesticides. Research will investigate what pesticides conserve beneficial insects.

Outreach IPM bulletins describe IPM practices based on our research that will conserve bees, butterflies, and other beneficial insects that kill pests.

Research on Acelepryn, a bee-friendly insecticide used for killing Japanese beetles showed that bumble bees are able to tolerate 4 ppm sub-lethal dose. In contrast, Monarch and Painted lady butterfly larvae are killed at 0.030 ppm dose, around 133 times less than bumble bees. bifenthrin

Research showed that Monarch and Painted lady butterflies tolerated higher doses of imidacloprid and clothianidin, and bifenthrin than bumble bees.

V. DISSEMINATION:

Description:

Develop user workshops and friendly websites, containing webinars, videos, and bulletins on ways to conserve beneficial insects in restorations/landscapes and in plant propagation.

Status as of February 15, 2018: From February to August 15 2018, 14 talks to commodity groups, pesticide applicators, pollinator groups, and Master Gardeners are provided.

A website has been created that identifies the 24 families of bees that pollinator plants, as well as videos on important nectar plants and videos on bee life histories. USDA NCIPM webinars website+pollinator+plant videos: http://ncipmhort.cfans.umn.edu/

Since August 2017, 10 talks to commodity groups, pesticide applicators, pollinator groups, and Master Gardeners have been given.

Status as of August 15, 2018:

1. St. Croix Bluffs Regional Parks is a model for other park systems to integrate beetle bumps and hanging nest in their habitat restoration and wild areas. Counts clearly demonstrate beneficial insects used the beetle bumps for overwintering and nesting thus increasing populations of good bugs and predatory insects to naturally control unwanted insects and increase biodiversity. The beetle bump project was visually engaging for 10,000's of park visitors who were curious and learned about them. Washington County Parks integrated the beetle bumps and bee huts into their public educational and naturalist programs. In March 2019, a fact sheet and talk will be presented at the *Best Practices for Pollinators Summit* in Minneapolis, Minnesota for 400 state, county, local and private industry land managers.
2. In October 2019, Washington County Parks, Wild Ones, Washington Conservation District and Pollinator Friendly Alliance partnered to present a citizen science project at the site of St. Croix Bluffs Regional Park with the beetle bumps including citizen scientists working alongside entomologists to identify and count insects in the beetle bumps.
3. A Best Management Practices (BMP) website called "Pollinator conservation biocontrol LCCMR" for small and municipal restorations and has been created: http://ncipmhort.cfans.umn.edu/.
4. A blog on our research can be found at http://ncipmhort.cfans.umn.edu/krichiks-bee-butterfly-research-news
5. We have developed and maintained other websites about proper insecticide use to conserve bees for professional IPM managers in greenhouse and landscape.
6. UM/MNLA/MDA Pesticide Certification Training turf and ornamentals.
7. http://pesticidecert.cfans.umn.edu/
8. UM CFANS CUES website cues.cfans.umn.edu/

Status as of February 15, 2019:

Status as of August 15 2019:

1. From August 15, 2018 to Feb 15, 2019, 11 talks to commodity groups, pesticide applicators, pollinator groups, and Master Gardeners were provided.
2. A workshop was given on Feb 26, 2019 to 44 greenhouse operators on how to grow greenhouse plants free of systemic insecticides that harm bees and other beneficial insects.

Status as of February 15, 2020:
Activity 1: Conservation biocontrol in restorations.
1-1. Pollinator lawn IPM, 2 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Pollinator Friendly Alliance
1-2. Conservation guide: pollinators, plants, pesticides, 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Emily Tenczar
1-3. Best practices for pollinators: conserving biodiversity in open spaces, 2 page color bulletin, by Dr. Vera Krischik
1-4. Conserving the endangered rusty patched bumble bee, 2 page color bulletin, by Dr. Vera Krischik, Xerces Society
Activity 2: Beneficial insect friendly pesticides that do not kill bees
2-1. Guide to integrated pest management (IPM), 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider
2-2. Think IPM for pollinator conservation, 12x16 color poster, by Dr. Vera Krischik, Laurie Schneider
2-3. 2020 Toxicity of pesticides to pollinators, 4 pages
A new graphic for the LCCMR grant was attached at the end of the work plan.

Our lab has provided 4 workshops/year and 28 talks/year to professionals and consumers on issues related to the grant's research.

Status as of August 15, 2020:
Final Report Summary:
All objectives on IPM and cultural methods for conserving beneficial insects were completed. The bee friendly insecticide chlorantraniliprole was highly toxic to butterflies and cannot be used near butterfly habitat. Pesticide residue was highest on wildflowers near potatoes and demonstrates the need for buffer strips.
Outreach/research products are found at https://ncipmhort.cfans.umn.edu/

The grant produced 8 new outreach bulletins, 1 new poster, and new research results which are presented at a new website These outreach bulletins are attached as a pdf file to the work plan. The bulletins, poster, and research summaries are available at the website to download and were handed out at outreach events. Our lab has provided 4 workshops/year and 28 talks/year to professionals and consumers on issues related to the grant's research. After 2020 we will continue to use these bulletins at outreach events to educate consumers on IPM programs to protect bees, butterflies, and beneficial insects, such as the parasitoids of the emerald ash borer.

The bulletins and posters are attached in a pdf file to the work plan.
Activity 1: Conservation biocontrol in restorations, new outreach bulletins
1-1. 2020 Pollinator lawn IPM, 2 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Pollinator Friendly Alliance
1-2. 2020 Conservation guide: pollinators, plants, pesticides, 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider, Emily Tenczar
1-3. 2020 Best practices for pollinators: conserving biodiversity in open spaces, 2 page color bulletin, by Dr. Vera Krischik
1-4. 2020 Best practices for pollinators: conserving biodiversity in backyard landscapes, 2 page color bulletin, by Dr. Vera Krischik
1-5. 2020 Butterfly gardening, 19 page color bulletin, Dr. Vera Krischik
1-6. 2020 Conserving the endangered rusty patched bumble bee, 2 page color bulletin, by Dr. Vera Krischik, Xerces Society
1-7. 2020 Research summary: Beetle bumps
1-8. 2020 Research summary: Pesticide residue wildflowers

Activity 2: Beneficial insect friendly pesticides that do not kill bees, new outreach bulletins

2-1. 2020 Guide to integrated pest management (IPM), 8 page color bulletin, by Dr. Vera Krischik, Laurie Schneider
2-2. 2020 Think IPM for pollinator conservation, 12x16 color poster, by Dr. Vera Krischik, Laurie Schneider
2-3. 2020 Toxicity of pesticides to pollinators, 4 pages by Dr. Vera Krischik
2-4. 2020 Protecting bees from systemic insecticides, 2 pages by Dr. Vera Krischik
2-6. 2020 acute chronic butterfly summary
2-5. 2020 SETAC research poster, bumblebee research
2-6. 2020 acute chronic butterfly summary
2-7. 2019 UROP research poster on neonicotinoids and birds
2-8. 2020 LCCMR graphic with results updated June

VI. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview:

***This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report.**

Budget Category	$ Amount	Overview Explanation
Personnel: Students: Grad student, $21/hr, 16.9% health insurance, $16,240 tuition; $7,500 summer salary= $42, 500/yr x 3yr=$128,000; 1.5 FTE; Students: UG summer technicians $10.00/hr x40 hrs x 20wk=$8,00 x 2 yr=$16,000, 0.2 FTE; Non-students: Lab scientist: 26pp x 80hrs x$19.00/hr x 1.26 fringe=$49,800/yr x 3yr=$150,000, 3.2 FTE	$289,000	Grad student, summer UG technician, research technician, and web master
Professional Technical Contracts: Residue analysis of imidacloprid performed at USDA AMS Lab in Gastonia, NC, EPA approved lab, GLP (180 x $352=$63,600; +$225 x 24=$5,400= total=$70,000); UM Soil testing lab 180 samples x $25=$5,000	$75,000	Residue analysis must be done at the EPA approved USDA AMS, Gastonia, NC lab to be valid
Equipment/Tools/Supplies: Research supplies Bumblebee colonies 30@$120 each =$4,000; bee food, $1,000; *Osmia* bees, lacewings, lady beetles, monarch butterflies, parasitoids for experiments and insecticide tests, $12,000; greenhouse space for research $140/mo x 36mo=$6,000; flowering plants, (plugs, pots, soil, fertilizers) $1,000; insecticides, $1,000; UM field charges, $1,000	$25,000	Equipment and supplies to maintain beneficial insects for bioassays with biorational and conventional insecticides; insecticides and supplies for growing plants for bioassays ; greenhouse space for maintaining insect colonies and performing bioassays; field supplies to investigate different cultural management tactics; field supplies to collect samples
Capital Expenditures over $5,000:	$0	
Printing: Outreach bulletins for distribution at meetings, University contract printing at Kinko $0.11BW/pg x6 pg=$0.66 x 500=$330 x 4bulletins=$1,300 + other handouts=$2,000; peer-review article publication costs journal $2,000	$4,000	Cost for duplicating management bulletins for use at meetings and talks; software for website

Budget Category	$ Amount	Overview Explanation
Travel: Instate travel (mileage) to research sites. Three tentative field sites for restoration research, Brainerd, Chaska, Stillwater, rental car, one year: 2 wk/mo x 5 mo=10 x $260/wk= $2,600 x $01.7mi/UM fee=$442+$2600=$3,042/yr x 2yr=$6,100/field; Instate travel to outreach activities: 2 Workshops at arboretum and 10 talk= 20 days x $45/day=$900	$7,000	Instate travel to research sites using UM car or personal car and reimbursed mileage.
TOTAL ENRTF BUDGET:	**$400,000.00**	

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, 0.2FTE for undergraduate technicians, and 3.0 FTE for a technician, = total of 4.7 FTE

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: USDA AMS NC residue lab to quantify imidacloprid, 0.5 FTE, = total 0.5 FTE

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
35% UM PI cost share ($29,400/yr x 3 yrs (in kind)	$88,200	$0	
50% UM overhead (in kind)	$200,000	$0	
UM AES and extension funds	$3,500		
State			
	$0	$	
TOTAL OTHER FUNDS:	**$291,700**	**$0**	

VII. PROJECT STRATEGY:

A. Project Partners:

Partners receiving ENRTF funding - None

Partners NOT receiving ENRTF funding

Listserve team: A listserve and website will be generated in the first months of the grant to connect with interested parties on the outreach and applied research.

1. Minnesota Honey Producers (Dan Whitney, Pres, statewide);

2. & 3. MN Beekeepers (Steve Ellis , St Cloud and Jeff Anderson, Eagle Bend, MN);

4. Colorado State Beekeepers (President Beth Conrey, Denver, CO);

5. Minnesota Pollution Control Agency (Sarah Rudolf, St. Paul, MN);

6. Minneapolis Park and Recreation Board (Ralph Siefert, Minneapolis, MN);

7. MDA (Raj Mann, Geir Friisoe, St Paul, MN);

8. MNLA (Cassie Larson, Roseville, MN);

9. UM Landscape Arboretum (Sandy Tanck);

10. UM Master Gardeners (Tim Kenny, Chaska, MN);
11. UM Master Naturalists (Brit Forsberg ; Amy Rager, Morrris, MN);
12. MN DNR (Sarah Pennington, Brainerd, MN);
13. Wild Ones Native Plant (Stillwater Chapter);
14. UM Landcare (Les Potts);
15. Xerces Society (Eric Mader adjunct extension educator, UMN);
16. Pesticide Action Network NA, PANNA (Lex Horan, Minneapolis, MN);
17. MN Zoo (Erik Runquist, Apple Valley, MN);
18. Pollinator Friendly Org (Laurie Schneider, Stillwater, MN);
19. UM Raptor Center (Julia Ponder, St. Paul, MN);
20. Erin Rupp (Pollinate Minnesota, Lobbyist St Paul);
21. UM Hort (Mary Meyer, Chaska, MN); and any other interested groups or people.
22. *Don MacSwain, Natural Resources Coordinator of Washington County Parks, St Croix Bluffs Regional Park;*
23. Jennifer Vieth, Executive Director of Carpenter Nature Center.
24. MN DOT.

Through conversations with different MN NGO's on the "MN pollinator google group" the listserve will expand its membership (Erin Rupp (Pollinate Minnesota, St Paul), Laurie Schneider (Pollinator Friendly Alliance, Stillwater), Margot Monson (Pollinator Friendly Alliance, St Anthony Park), Julia Kay (Wildones, Stillwater, MN), Julia Vanatta (Pollinator Revival, Minneapolis, MN), Patricia Hauser (Humming for bees, Minnetonka, MN), and Julia Kay). I already have worked with Laurie Schneider, Stillwater, "Pollinator Friendly Alliance" on picking out study sites; Sarah Foltz Jordon of the Xerces Society, Don MacSwain Natural Resources Coordinator of Washington County Parks, St Croix Bluffs Regional Park; and Jennifer Vieth, Executive Director of Carpenter Nature Center. In Brainer, Sarah Pennington of the MN DNR has contacted me to work on some restorations in her area. I have spoken to Brit Frosberg of the UM Extension MN Naturalist Program and Sandy Tanck of the MN Arboretum on working with us on the research and outreach program. The outreach program will be posted on the three UM websites for which I am the webmaster:

- UMinnesota Extension website http://www.extension.umn.edu/garden/plant-nursery-health/;

- the CFANS college website http://cues.cfans.umn.edu; and

- the NCIPM webinars website+pollinator+plant videos website http://ncipmhort.cfans.umn.edu/. I will create a blog that will be updated each week with links to relevant information about beneficial insect conservation in restorations. The first year I will set up the website and the email listserve on the research.

- Every year in May I will organize a workshop.

- Each year I will produce at least one bulletin directed at different end users such as greenhouse/nursery growers, small restorations in county parks, and consumers on proper pesticide use and different restoration techniques.

- Our lab each year will provide 6 talks each year to small groups and at least 6 talks to UM associated groups.

B. Project Impact and Long-term Strategy:

The project's goals are to educate landscape managers (parks; state, county, city; municipal buildings, restoration managers, etc.), and consumers on ways to conserve beneficial insects (bees, butterflies, predators, and parasitoids) thru conservation biocontrol, IPM , and proper insecticide use by providing workshops, websites, webinars, and bulletins based on the applied research.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
LCCMR 221G Mitigating Pollinator Decline	2010-2013	$297,000
LCCMR 6e Understanding Systemic Insecticides as Protection Strategy for Bees	2014-2017	$326,000
Non-state		
USDA SARE grant, Effects of neonicotioids on bees	2010-2012	$175,000
2015 MNLA, MN Nursery Association Grant, New Bee Labeling Laws: Determination of Residue in Flowers and Leaves from imidacloprid, dinotefuran, and pymetrozine use in greenhouse pots	2015	$10,000
2015 USDA, NC IPM, grant develop webinar and website on pollinators, Mitigating Pollinator Decline webinar, website, Arboretum citizen science project	2015	$20,000
TOTAL OTHER FUNDS:		**$828,000**

VIII. REPORTING REQUIREMENTS:

- The project is for three years, will begin on 7/1/2017 and end on 6/30/2020.
- Periodic project status update reports will be submitted August 15 and February 15 of each year.
- A final report and associated products will be submitted between June 30 and August 15, 2020.

IX. VISUAL COMPONENT or MAP(S):

Please see attached updated graphic summary
28. 2020 Krischik 153F updated graphic.

X. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:
n/a

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2017 LCCMR Work Plan Final Report

2017 LCCMR Project Title: ***Promoting Conservation Biocontrol of Beneficial Insects*** Feb. 23, 2020

PI: Dr. Vera Krischik, Department of Entomology, University of Minnesota, krisc001@umn.edu
http://www.entomology.umn.edu/faculty-staff/vera-krischik; http://cues.cfans.umn.edu/;
https://ncipmhort.dl.umn.edu/

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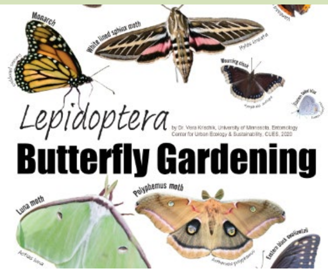

New IPM outreach bulletins on Best IPM Practices for Back yard Landscape, Best IPM Practices for Open Spaces, Guide to IPM, Pollinator Lawn IPM, Butterfly Gardening booklet, Butterfly & Plant ID booklet, Conserving Rusty Patched Bumble Bee, Conservation Guide , poster Think IPM

Research on best habitat for nesting and overwintering beneficial insects called beetle banks were installed at 3 sites in Washington county. At a citizen science field day, 36 banks at 3 sites had a mean of 131 insects in a sample of 10% of each beetle bank compared to control plots with 1 insect.

Research on best habitat for native stem nesting bees called reed huts were installed at 3 sites in Washington county. At a citizen science field day, 36 reed bee huts were inspected at 3 sites which contained 236 occupied reeds or 95% of the huts occupied by nesting bees.

Research on best larval host plants and adult nectar plants for Minnesota butterflies are listed in bulletins.

Activity 1B: ***Conservation biocontrol in restorations.*** **Wild flowers in restorations will be investigated for pesticide residue levels that may affect beneficial insect conservation.**

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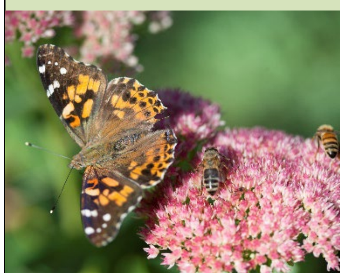
Outreach poster, Think IPM;
Outreach talks were 4 workshops/year and 28 talks/year to professionals and consumers.
Outreach website at https://ncipmhort.dl.umn.edu

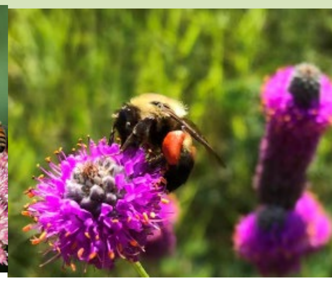
Research on LD50 of butterflies and residue of insecticide in the field permit correlation of field doses of insecticides on survival and behavior of butterflies and bumble bees.

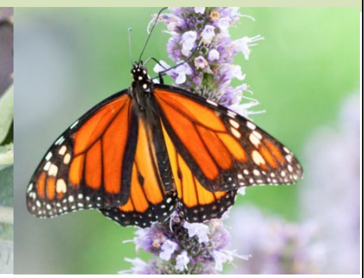
Research on pesticide residue on flowers near potato fields showed that 100% of 36 samples tested contained at least 2 and up to 15 different pesticides.

Research on pesticide residue on flowers near corn fields showed that of 40% of 32 samples tested contained only 1 pesticide and it was atrazine.

Activity 2: *Beneficial insect friendly pesticides.* Research will investigate what pesticides conserve beneficial insects.







Outreach IPM bulletins describe IPM practices based on our research that will conserve bees, butterflies, and other beneficial insects that kill pests.

Research on Acelepryn, a bee-friendly insecticide used for killing Japanese beetles showed that bumble bees are able to tolerate 4 ppm sub-lethal dose. In contrast, Monarch and Painted lady butterfly larvae are killed at 0.030 ppm dose, around 133 times less than bumble bees.

LD50 toxicity, behavior, and flight research showed that Monarch and Painted lady butterflies tolerated higher doses of bifenthrin, imidacloprid, and clothianidin than bumble bees.

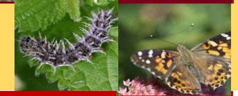
Environment and Natural Resources Trust Fund
M.L. 2017 Project Budget
Project Title: 153-F, Promoting conservation biocontrol of
Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 08b
Project Manager: Vera Krischik
Organization: University of Minnesota
M.L. 2017 ENRTF Appropriation: $ 400,000
Project Length and Completion Date: 3 Years, July 1 2017 until June **August 15 2020**
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET
BUDGET ITEM
Personnel (Wages and Benefits): Overall:
Students: Grad student, $21/hr, 16.9% health insurance, $16,240 tutiion; $7,500 summer salary= $42, 500/yr x 3yrs=$128,000; 1.5 FTE
Students: UG summer technicians $10.00/hr x40 hrs x 20wks=$8,000 x 2yrs=$16,000, 0.2 FTE
Non-students: Lab scientist: 26pp x 80hrs x$19.00/hr x 1.26 fringe=$49,800/yr x 3yrs=$150,000, 3.2 FTE
Professional Technical Contracts: Residue analysis of imidacloprid performed at USDA AMS Lab in Gastonia, NC, EPA approved lab, shipping samples overnight express on dry ice (180 x $352=$63,600; +$225 x 24=$5,400= total=$70,000); UM Soil testing lab 180 samples x $25=$5,000
Equipment/Tools/Supplies: Research supplies Bumblebee colonies 30@$120 each =$4,000; bee food, $1,000; *Osmia* bees, lacewings, lady beetles, monarch butterflies, parasitoids for experiments and insecticide tests, $12,000; greenhouse space for research $140/mo x 36mos=$6,000; flowering plants, (plugs, pots, soil, fertilizers)
Printing: Outreach bulletins for distribution at meetings, University contract printing at Kinko $0.11BW/pg x6 pg=$0.66 x 500=$330 x 4bulletins=$1,300 + other handouts=$2,000; peer-review article publication costs journal $2,000
Travel: Instate travel (mileage) to research sites. Three tentative field sites for restoration research, Brainerd, Chaska, Stillwater, rental car, one year: 2 wks/mo x 5 mos=10 x $260/wk= $2,600 x $01.7mi/UM fee=$442+$2600=$3,042/yr x 2yrs=$6,100/field;

UNIVERSITY OF MINNESOTA

Residues in landscape plants and effects on bumblebees and two species of butterflies

Vera Krischik, Dept Entomol, Univ. Minnesota, krisc001@umn.edu;

Lab: R. Gutierrez-Moreno, T. Balaxashvili, M. Pounds, M. Lagus, N. Partington, L. Schneider



Introduction

Neonicotinoid insecticides are commonly used in landscapes and agriculture in the US, but levels of residue in ornamental plants are rarely measured. Neonicotinoid research showed lethal and sublethal effects on bee behavior and colony health (Scholer & Krischik 2014, Baron et al. 2017, Arce et al. 2017) and on butterfly survival, development, and behavior (Pecenka & Lundgren 2015, James 2019, Peterson et al. 2019, Krishnan et al. 2020).

Objective 1: Materials & Methods

Objective 1: Comparing neonicotinoid residues in ornamentals and agriculture. Plants were treated with label rates of imidacloprid, harvested at 5-10 wks, and residue was quantified by HPLC GC at the USDA lab in Gastonia, NC

Objective 1: Results

Objective 1. Imidacloprid is common in ornamentals and residues are significantly higher compared to clothianidin residues near ag fields**.** The LD_{50} is around 4 ng/bee for both and was shown to have similar colony affects on bumblebees (Scholer and Krischik 2014).

Table 1. Neonicotinoid residues in urban and ag plants.

Species/ label application	Flowers imid ppb	Leaves imid ppb	Many cloth ppb	Ref
Neonicotinoid residues in agricultural fields (4mg/sqft)				
Asclepias syriaca			1.14 0.71	1,2
Brassica napus pollen		0.09 0.10		3
Brassica napus Wildflowers pollen	1.4 0.16			4
Bee Urban Bee Rural pollen	20		5 35	5
Taraxacum pollen	2.9		6.3	6,7
Neonicotinoid residue in ornamentals (pot 300 mg/sqft)				
Tilia cordara 25 cm DBH trunk inj	1,340 yr1 45 yr 2	36,283 yr1 680 yr 2		8
Tilia cordara 25 cm DBH soil dr	34 yr 1 38 yr 2	290 yr 1 680 yr 2		8
Tilia cordara 70 cm DBH soil dr	30 yr1 88 yr2	554 yr1 737 yr2		8
Cornus racemosa 4 cm DBH soil dr	762	21,062		8
Rosa soil dr	812	na		8
Asclepias incarnata soil dr	86	132		8
Agastache soil dr	94	561		8
Calibrachoa hybrid Pot dr, 1L pot	333	25, 933		8
Ruellia humilis Pot dr, 1L pot	502	2,086		8
Asclepias incarnata pot dr, 6L pot	1568			8
Agastache Pot dr 6 L pot	1973			8

1. Pecenka and Lundgren 2015, 2.Olaya-Arenas and Kaplan 2017, 3. Blacquiere et al. 2012, 4. Botias et al. 2015, 5. David et al.2016, 6. Krupke et al. 2012, 7. Krupke et al. 2017, 8. Krischik unpublished

Objective 2: Materials & Methods

Objective 2: Flight cage studies on lethal and sublethal doses on bumblebees. Bumblebees, *Bombus impatiens* (Koppert Biological Systems, Howell, MI), colonies were fed untreated Koppert Bee Happy syrup (35%) for 3 weeks after which clothianidin (20 ppb) or chlorantraniliprole (4 ppm) were dissolved in the syrup and fed ad libitum for 5 weeks (10 colonies/trt). Colonies were measured weekly for weight (g), syrup consumption (ml), and movement (sec). Photos were taken bi-weekly to measure brood numbers, brood cell age (1-3), worker numbers, and disease. Movement is the time for a worker to cross the brood.

Objective 2: Results

Objective 2: *Bombus impatien*s fed 20 ppb of clothianidin (LC_{50} 100 ppb, Scholer and Krischik 2014) in syrup had reduced brood production and movement. At 4 ppm chlorantraniliprole (LC_{50} 7 ppm, Smagghe et al. 2015) caused no effects

Figure 1. Clothianidin (20 ppb) had lower colony weight and brood.

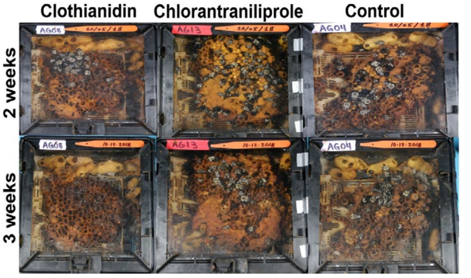
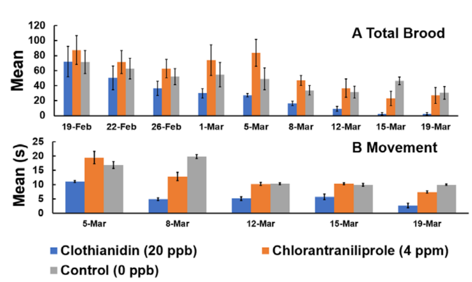


Figure 2. (A) Brood numbers (mean ± SE) and (B) movement (mean ± SE) , both were lower for clothianidin (20 ppb).



Objective 3: Materials & Methods

Objective 3: Lab studies on lethal and sublethal doses on butterflies

For LC_{50} bioassays larvae were fed swamp milkweed, *Asclepias incarnata (Danaus plexippus)* or common mallow, *Malva sylvestris (Vanessa cardui)* dipped in insecticides. Adults in 3 m cages were fed Bee Happy syrup at eclosion. On day 1 adults (30/trt) were force fed syrup containing bifenthrin, (0.1 ppm), clothianidin (10 ppm), imidacloprid (0.05 ppm), or chlorantraniliprole (0.001 ppm). Butterflies were dropped and the ability to open wings was measured on 3 days (day 1, 8, 10).

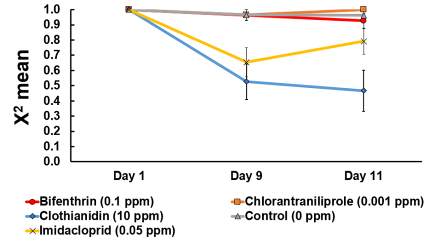
Objective 3: Results

Objective 3: For clothianidin, larval *D. plexippus* had an LC_{50} of 4 ppm and *V. cardui* 96 ppm and for chlorantraniliprole 0.20 ppm and 0.03 ppm. Sublethal effects of clothianidin (10 ppm) reduced flight (wing opening), but did not lower fecundity

Table 2. LC_{50} **ppm values for** ***D. plexippus*** **and** ***V. cardui***

Insecticide	n	LC_{10}	LC_{50}	LC_{90}	Slope ±SE	X^2	*P*
LC_{50} fifth instar painted lady larvae, *Vanessa cardui*							
clothianidin	180	28.9	96.2	801	0.6 ± 0.1	2.8	0.6
imidacloprid	180	43.3	256	1309	0.8 ± 0.1	3	0.4
bifenthrin	180	7.1	9	70	0.6 ± 0.1	0.5	0.9
chlorantraniliprole	180	0.004	0.03	0.2	0.7 ± 0.1	0.06	0.9
LC_{50} fifth instar monarch larvae, *Danaus plexippus*							
clothianidin	262	0.15	3.7	89	0.4 ± 0.05	8.1	0.2
imidacloprid	99	0.19	1.1	6.24	0.7 ± 0.18	1.2	0.3
bifenthrin	191	0.18	1.5	12.49	0.6 ± 0.08	4.2	0.4
chlorantraniliprole	300	0.02	0.2	2.8	0.5 ± 0.07	2.0	0.8
LC_{50} adult painted lady adults, *Vanessa cardui*							
clothianidin	100	6.0	13.2	28.9	1.6 ± 0.4	1.5	0.5

Figure 3. Proportion of ***D. plexippus*** **adults that did not fly.**



References

Arce AN, TI David, EL Randall, et al. 2017. Journal Applied Ecology 54:1199-1208.
Baron, GL VAA Jansen, MJF Brown et al. 2017. Nature Ecology & Evol 1:1308-1316.
Baron GL, NE Raine & MJF Brown. 2017. Proceeds Royal Society B 284:20170123.
Blacquiere T, G Smagghe, CAM van Gestel et al. 2010. Ecotoxicology 21:973-992.
David A, C Botías C, EM Hil, D Goulson. 2016. Environ Int 88:169–178
James DG. 2019. Insects 10(9):276
Krischik, VK unpublished.
Krishnan N, Y Zhang, G Bidne et al. 2020. Environ. Toxicol. & Chem. 39(4):923-941.
Krupke CH, GJ Hunt, BD Eitzer et al. 2012. PLoS One 7(1):e29268.
Krupke CH, JD Holland, EY Long et al. 2017.JApplied Ecology 54(5):1449-1458
Olaya-Arenas P & I Kaplan. 2019. Front. Ecol. Evol. 7(223).
Pecenka JR & JG Lundgren. 2015. Sci. Nat. 102:19.
Peterson EM, KR Shaw & PN Sith et al. 2019. EnvirToxicol.&Chem.38(12):2629-36.
Scholer J & V Krischik. 2014. PLoS ONE 9(3): e91573.
Smagghe G, J Deknopper. I. Meeus et al. 2013. Pest Manag. Sci. 69(7): 787-791.

Discussion & Conclusion

Two butterfly species had higher LC_{50} for the neonicotinoids clothianidin (4, 96 ppm) and imidacloprid (1, 256 ppm) than bumblebees (100 ppb imidacloprid, clothianidin). LC_{50} for butterflies was lower for chlorantraniliprole (30, 200 ppb) than bumblebees (7 ppm). Clothianidin (10 ppm) reduced flight in monarch butterflies. The 20 year decline of endangered Rusty patched bumblebee, *B. affinis,* may be correlated to ubiquitous neonicotinoid residues and bumblebee sensitivity (20 ppb).

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Driven to Discover®

Do neonicotinyl insecticides affect birds as they do bees?

Michaela Sanford, Vera Krischik, Angela Begosh
Department of Entomology, University of Minnesota

Background

Neonicotinoids are a family of insecticides commonly used for pest treatment in agricultural and horticultural applications. They are applied through soil drenches, seed coatings, foliar sprays, and tree trunk injections. Systemic in nature, the insecticide will move from the application site through the plant to the leaves, pollen, nectar, seeds, and fruits following application.

In bees, neonicotinoids have been shown to be lethal in high doses. Sub-lethal doses can cause deleterious effects on foraging, navigation, and colony health. With growing concern of their usage, introductory research has begun on the effects of neonicotinyl insecticides on bird populations.

This study may serve as a foundation for further research into non-target effects of these insecticides.

Objectives

- Objective 1: Determine residue levels in leaves and seeds of green ash trees, (*Fraxinus pennsylvanica)* following an Imidacloprid (neonicotinoid) soil drench.
- Objective 2: Determine lethal and sub-lethal effects of neonicotinyl insecticides on birds.

Methods

- We collected samples of leaves and seeds from ash trees treated with Imidacloprid insecticides.
- We performed meta-analysis of published research to find the LD50 (lethal dose) and the negative effects of neonicotinoids on birds.
- Using the results of our meta-analysis, we calculated how many treated ash seeds would need to be eaten for lethal and sub-lethal effects to occur.

Results

Imidacloprid and other neonicotinyl insecticides have been found in the bodies of wild birds. These studies show that there are lethal and sub-lethal effects of neonicotinyl insecticides, observed in both field and lab experiments.

We found that ash trees, following an Imidacloprid soil-drench, had a mean residue of 35 ppb in the seeds.

We have determined how many treated ash seeds must be ingested to see deleterious effects on birds, using known LD50 levels and our residue analysis for the amount of imidacloprid in an ash seed.

Table 1: Lethal and Sub-lethal effects on birds

bulletin 27

Bird Species	*LD50 (mg/kg)	Lethal and Sub-Lethal Effects	References
Field Surveys			
Many species	unknown	Correlation between bird population declines and neonicotinoids in water	Hallmann 2014
Many Species	unknown	Nervous disorders present in 29.6% of 103 deaths with neonicotinoid residues detected. Primarily pigeons/partridges	Millot 2016
American Robin	unknown	Poisoning after ingesting grubs from a treated lawn	Gibbons 2014
Rufous Hummingbird Anna's Hummingbird	unknown	Neonicotinoids (thiamethoxam, clothianidin, and imidacloprid) were measured in cloacal fluid with a concentration of 3.63 ppb	Bishop 2018
Eurasian Eagle-Owl	unknown	Imidacloprid residue in blood after exposure was measured at a concentration of 3.28 ppb	Taliansky-Chamudis 2017
White-crowned Sparrow	unknown	Imidacloprid residue was found in the blood of 78% of wild sparrows tested	Hao 2018
European Honey Buzzard	unknown	Neonicotinoid residue was present in 80% of the blood samples taken from both adult and juvenile buzzards	Byholm 2018
Greater Prairie-Chicken	unknown	Neonicotinoids were found in 67% of prairie-chicken livers tested (imidacloprid: 8.3 ppb, clothianidin 4.2 ppb, thiamethoxam 1.1 ppb)	Roy 2019
Sharp-tailed Grouse	unknown	Neonicotinoids were present in 89% of grouse livers tested. (Max levels measured: imidacloprid: 84.5 ppb, clothianidin: 3.58 ppb, thiamethoxam: 1.18 ppb)	Roy 2019
Wild Turkey	unknown	43% of turkeys tested were positive for one or more neonicotinoid present in liver samples	MacDonald 2018
Experimental Studies			
Brown-headed Cowbird	unknown	Impaired coordination, retching, reduced consumption of imidacloprid-treated seeds	Millot 2016 Avery 1993
Red-winged Blackbird	unknown	Impaired coordination, retching, reduced consumption of imidacloprid-treated seeds	Millot 2016 Avery 1993
Red Munia	31	Thyroid disruption interfering with seasonal reproductive stages gonad development	Pandey 2017
Canary	35	Incapacitation at 10mg/kg	Mineau 2013
House Sparrow	41	Ingestion of 1.5 beet seeds can result in death, reduced coordination, inability to fly	Gibbons 2014 Mineau 2013 Millot 2016
White-crowned Sparrow	41	4 imidacloprid-treated canola seeds, or 0.2 treated corn seeds can result in death, respiratory distress, reduced body mass	Eng 2017 Goulson 2013
Red-legged Partridge	31-53	The highest dose (0.14-0.7mg/g) of wheat seeds treated with imidacloprid killed 100% of partridges in 21 days Low dose resulted in reduced fecundity	Gibbons 2014 Lopez-Antia 2014 Lopez-Antia 2016
Grey Partridge	13.9	5 maize seeds, six beet seeds or 32 oilseed rape seeds will reach LD50. Sub-lethal effects include failed eggs	Gibbons 2014 Millot 2016 Goulson 2013
Japanese Quail	31	Imidacloprid: Severe signs at 6mg/kg Clothianidin: clinical signs at 25 mg/kg; Incapacitation at 100 mg/kg	Mineau 2013 MacDonald 2018
Mallard	283	Severe clinical signs at 25mg/kg; mortality 8 days post dose	Mineau 2013 Millot 2016
Northern Bobwhite	152	Slight clinical signs at 25 mg/kg; incapacitation 50-100 mg/kg	Mineau 2013 Ertl 2018 MacDonald 2018
Rock Pigeon	25	50% mortality with the ingestion of <4 treated wheat seeds Severe clinical signs at 12.5 mg/k	Millot 2016, Mineau 2013, MacDonald 2018, Berney 1999
Chicken	104	Chick embryo mortality due to malformed crest and neural cells; mortality in 5-24 hours	Wang 2016 Kammon 2010 Liu 2016

*LD50 amounts are the lethal dose of ingested imidacloprid

Table 2: Imidacloprid residue in leaves and seeds of Ash Trees treated annually via soil drench

Ash tree (number)	Imidacloprid Residue	Recovery
July leaves (6), no residue (2)	94ppb	100%
July seeds (2) no residue (2)	36 ppb	119%
July leaves control (3)	0 ppb	100%
July seeds control (3)	0 ppb	132%
August leaves (6) no residue (2)	66 ppb	95%
August seeds (6) no residue (2)	34 ppb	105%
August leaves control (3)	0 ppb	91%
August seeds control (3)	0 ppb	107%

Table 3: Number of ash seeds (5g) with a residue of 35 ppb imidacloprid that must be eaten to reach the LD50

Bird Species and Weight	Ash Seeds Ingested for Lethal/Sub-lethal Effects
Rock Pigeon 900 g	Lethal: 24,545 seeds Sub-lethal: 4,909 seeds
Mallard 1,500g	Lethal: 32,706,818 seeds
Japanese Quail 100 g	Lethal: 14,090 seeds
Northern Bobwhite 150 g	Lethal: 10,227 seeds
House Sparrow 30 g	Lethal: 5,590 seeds
Canary 20 g	Lethal: 27,272 seeds
White-crowned Sparrow 26 g	Lethal: 5,590 seeds Sub-lethal: 1,363 seeds

Conclusion

The amount of imidacloprid residue in an ash seed after treatment is 35 ppb, not enough to kill birds. Seeds of treated crops, such as corn, canola, sunflower, and beets carry a greater risk. These have around 1-2 mg applied per seed compared to those of ash trees which have 35 ppb, a much smaller amount.

A meta-analysis of papers showed that neonicotinoids are found in free-ranging birds, resulting in detrimental or fatal effects. The residue we've found in ash seeds is not enough to constitute a risk to normal behaviors or physiological functions.

Further research is needed to determine lethal and sub-lethal amounts of neonicotinoids in birds including clothianidin, thiamethoxam, etc. Research is also needed to measure neonicotinoid residues in treated vegetation and seeds.