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

**Date of Report:** Dec 29, 2009
**Date of Next Progress Report:** Jan 30, 2011
**Date of Work Program Approval:**
**Project Completion Date:** June 30, 2013 or June 30, 2010

## I. PROJECT TITLE: 221G Mitigating Pollinator Decline


**Project Manager**: Vera Krischik
**Affiliation:** University of Minnesota
**Mailing Address:** 1980 Folwell Ave 3219
**City / State / Zip:** St. Paul, MN 55108
**Telephone Number:** 612.625.7044
**E-mail Address:** krisc001@umn.edu
**FAX Number:** 612.625.5299
**Web Site Address:** www.entomology.umn.edu/cues


**Location: University of Minnesota, Department of Entomology, St. Paul Campus**

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

**Legal Citation: ML 2010, Chap.[____], Sec.[____], Subd._____.**

**Appropriation Language:**

## II. PROJECT SUMMARY AND RESULTS: Jan 2010


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

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

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

**III. PROGRESS SUMMARY AS OF** Jan 2011**:**

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

**RESULT 1: In the field, apply systemic neonicoinyl insecticides (imidacloprid and clothianidin) to the soil. Collect flowers to determine the amount of insecticide translocated to nectar and pollen. Determine the amount of insecticides in flowers through residue analysis with a HPLC-Mass Spec. Determine the effects of these amounts in nectar and pollen on survival and behavior of pollinators by the use of controlled bioassays.**

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

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

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

**Field research: Growing dandelion, rose, and linden trees and treating soil with insecticides.**
For all research, we will always perform 2-3 experiments (replicated experiments). We will use 6-10 plants per treatment. These numbers increase the amount of plants used in the experiments, but are necessary for appropriate statistical analysis.

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

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

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

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

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

**Greenhouse research: Bioassays on effects of amounts in nectar and pollen on insect survival and behavior**
Insects need to have natural light to forage on flowers. Research bioassays on pollinators will be accomplished in the greenhouse.

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

Behavioral observation of beneficial insects (passive pollinators). Beneficial insects, green lacewing (*Chrysoperla carnea*, 1 species of wasp (*Anagyrus psuedococci*), and 3 species of

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

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

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

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

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

Effects of imidacloprid on native bumble bee health. In this study, we will use microcolonies of bumble bees following previously established methods to measure lethal and sublethal effects of insecticides on bumble bees (Regali and Rasmont 1995; Tasei et al, 2000; Babendreier et al, 2008). Microcolonies of *B. impatiens* will be established by placing three newly emerged bumble bee workers in wooden boxes. Within a few days, a hierarchy will be established and one dominant worker in each microcolony will develop her ovaries and lay eggs. The eggs of these uninseminated false queens will develop into haploid male progeny. The two other workers will care for the male brood of the false queen, allowing us to quantify brood care. All male offspring reared from the worker's colonies will be removed at the day of emergence and stored at –20C.

Bees will be provided with a feeder containing sucrose solution spiked with concentrations of imidacloprid (Bayer Chemical Co, Analytical Grade), 0, 20, 40, 400 ppb in year 1, and concentrations derived from field experiment in year 2 and 3. They also will be provided a Petri dish containing pollen dough, prepared by mixing ground floral pollen with sucrose solution (50%) at a ratio of 1:0.4 (pollen: sucrose solution). To calculate food consumption, the pollen dough will be changed every other day and weighed at the beginning and the end of each time interval. Feeders will be replaced three times a week and weighed at the beginning and the end of each time interval. The bumble bees will be allowed to feed *ad libitum* for 80 days.

Survival of adult worker bees will be checked daily and dead individuals will be removed and stored at –20C. Survivorship will be analyzed using Cox proportional hazard model. The whole experiment will be terminated after 80 days and all surviving bees stored at –20C. Male offspring and the three workers per colony will be dried at 80C for 4 h and weighed on a microbalance (Mettler Toledo MX5, d = 1 g; ± 2g) (Mettler-Toledo GmbH, Greifensee, Switzerland). In summary, from the microcolonies, we will obtain measures of bumble bee survivorship after the different imidacloprid treatments, mean weight of surviving bumble bees, number of offspring produced, and consumption of sucrose and pollen. The experiment will be repeated three times, using new hives for each replicate.

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

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

Behavioral effects on honey bee learning: We will use the odor conditioning PER assay, used with bumble bees, to study the effects of imidacloprid on learning in honey bees. We will age-mark newly emerged bees by painting a spot of Testor's enamel paint on the thorax and collect them when they are 7-12 days old (pre-foraging age), and another set when they are 20-25 days old (foraging age). We will collect bees from 3 colonies at each treatment level and the control colonies (20 bees at each age from each of 12 colonies). We will conduct PER learning trials, as described in above, to compare any sub-lethal effects of the imidacloprid treatments on the learning and memory of adult bees.

**Deliverable 1-1.** We will publish research papers (at least 3) in peer reviewed journals on the amount of imidacloprid and clothianidin translocated to nectar and pollen and effects on pollinators. We will present these data at research meetings and to our electronic "Outreach Committee". We will work with state agencies and landscape-related associations that use insecticides to make them aware of the potential damage to pollinators. We will discuss the research with MN Pollution Control Agency, MDA, and DNR. We will discuss the data at the National level with the EPA.

**Deliverable 1-2**. We will mitigate pollinator loss by developing a landscape pest management bulletin and insecticide list using pollinator-friendly insecticides and EPA approved low risk insecticides. Copies of the bulletin and insecticide use will be handed out at consumer and professional events over the 3 year grant period. We will also develop pollinator friendly insecticide recommendations and place them on the front page of the very popular CUES website (www.entomology.umn.edu/cues). We will work with state agencies and landscape-related associations that use insecticides to make them aware of the potential damage to pollinators. We will share the bulletin with our electronic "Outreach Committee". The PI will give talks around the state of MN and provide handouts at various landscape-related events, such as MDA Pesticide certification, MNLA (MN Nursery and Landscape Association) certification, MSA (MN Society of Arboriculture) certification, MN DNR lakeside restoration programs, and Master Gardeners Annual Meetings and others.

**Deliverable 1-3.** We will develop a section of the CUES website (www.entomology.umn.edu/cues) called "Mitigating Pollinator Decline" where we will discuss the research and publication to conserve pollinators.

**Deliverable 1-4.** We alter each landscape pest profile in the UMN Agricultural Station Publication "IPM of Midwest landscapes, 316 pp" by Vera Krischik and John Davidson. We will change the insecticide recommendation for each of 200 pests to include pollinator-friendly insecticides featuring EPA registered reduced risk insecticides (http://www.entomology.umn.edu/cues/Web/196Sawflies.pdf). We will produce 50 copies of the

update manual and provide them to state agencies and landscape-related associations that use insecticides to make them aware of the potential damage to pollinators. The IPM Manual will also be available online.

**Summary Budget Information for Result 1:**

**ENRTF Budget:** $146,984 labor
$110,478 supplies
$4,000 printing
$0 travel

**Amount Spent: $ 0**
**Balance: $ 261,462**

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

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

**RESULT 2: We will determine through research the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators.**
**Description:**
**Research result 2.** Most plant recommendations are derived from published lists that were not based on research. When you study these recommended plants in the field, many are not visited by bees or beneficial insects. It is very difficult to find a list of native plants for restorations that support pollinators.

We will study established plantings at the MN Landscape Arboretum (Chaska, MN) and St. Paul Campus to determine the best plants for seasonal phenology of food for pollinators in MN. Twenty-four stations (divided into 3 plots) will be chosen based on proximity to specific plant species in order to obtain replicated samples. These stations will be used for data collection on behavioral observations of beneficial insects visiting flowers and sticky trap collection. Teams of 2 will observe flowers at each of the 24 flagged station, 8 times each month. Observations will last for ten-2 minute intervals between 1000 and 1500 h and the number of insects visiting the flowers and taxa of insect will be recorded. Standard yellow sticky traps (Gempler's No bait, length x width: 20.3 x 30.5 cm) will be placed at the flagged stations for a 48 h period 4 times a month. We will visit another restoration and perform behavioral observation, such as Kelly Farm (Elk River, MN). From our observations, we will add bee plants to the present restoration on Gortner, north of the greenhouses. We have a completed manuscript that will be submitted soon, where we compared visits of beneficial insects to native and bedding plants. We will use the same procedures worked out in that study.

**Deliverable 2-1.** We will publish research papers (at least 1) in peer reviewed journals on the preferred native plants for pollinators. We will present these data at research meetings and to our electronic "Outreach Committee". We will work with state agencies and landscape-related associations to make them aware of the best pollinator plants for landscapes, restorations and rain gardens. We will discuss the research with Xerces Society (National Insect Conservation Society, Portland, OR, www.xerces.org), MDA ,and DNR.

**Deliverable 2-2.** The Xerces Society has developed pollinator lists for the Pacific Northwest. We will develop these lists for the Midwest and produce a bulletin to explain how to restore pollinator habitat. We will have a table on the best pollinator plants determined through research. We will make a poster to give to parks, state agencies, and consumer and landscape associations to make them aware of how to conserve pollinators.

**Deliverable2- 3.** We will mitigate pollinator loss by developing a section of the CUES website on "Mitigating pollinator loss through best pollinator plants" as we did for the collaborative plant restoration project with the Washington-Ramsey Watershed District and DNR http://www.entomology.umn.edu/cues/gervais/gv_links.htm).

**Deliverable 2-4.** We will add plants to the native plant restoration at the UMinnesota St. Paul Campus, on the corner of Gortner, north of the Horticulture Greenhouses. We will make a permanent display with the poster and add a mailbox to house bulletins of the best pollinator plants.

**Summary Budget Information for Result 2:** **ENRTF Budget:** $24,000 labor
$3,000 supplies
$3,000 printing
$3,000 travel

**Amount Spent: $ 0**
**Balance: $ 33,000**

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

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

**RESULT 3:** Use an email listserve to an "Outreach Committee" to disseminate research results and deliverables around Minnesota. Deliver 2 workshops on pollinator conservation using the research and demonstration project developed on the St. Paul Campus. Also, the PI will travel to established meetings around greater Minnesota to deliver talks and disseminate grant products.

**Description:**
**Result 3 and Deliverable 3:** In order for associations and state agencies to change management practices, it is often best to discuss the progress of the research and its deliverables throughout the grant period. We will convene an electronic email "Outreach Committee" that includes members from diverse MN landscape related groups, such as MN Pollution Control Agency, MN DNR, MN DA, MNDOT, MN Honey bee Producers, MN Hobby Bee Keepers, Native Plant Society, Xerces Society, MNLA (MN Nursery and Landscape Association), and others. We will create an electronic listserve and send them progress reports and updates every 6 mo on the research and deliverables. We will alert them as the components of the research are posted on the website.

The PI will use travel funds to provide 8 talks in established meetings around the state. The PI is often requested to deliver talks, but there is no budget, so the talks must be declined. The funds will permit the PI to present the research results and deliverables to a wide range of professionals and consumers.

We will deliver 2 workshops in Spring 2013 on the results of the grant. We will charge a small registration fee to cover advertising, room, and food. We will spend 5 hours with presentations and a visit to the demonstration project on the St. Paul campus. Travel funds are requested for Mr. Eric Mader, Xerces Insect Conservation Society (Portland, OR) to attend and provide talks at the meeting since he is active in developing legislation and literature on pollinator conservation.

**Summary Budget Information for Result 3:**

**ENRTF Budget:**
$0 labor
$0 supplies
$0 printing
$2,538 travel

**Amount Spent:** **$ 0**
**Balance:** **$ 2,538**

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

| Deliverable | Completion Date | Budget |
|---|---|---|
| **1. Listserve to share information with "Outreach Committee"** | June 2013 | $0 |
| **2. PI to deliver at least 8 talks around Minnesota** | June 2013 | $1,038 travel |
| **3. Workshop delivered 2 times, travel funds for invited speaker, Eric Mader, Xerces Society, Portland, OR** | June 2013 | $1,500 travel |

**Result Completion Date:** June 2013
**Result Status as of report 1:** Jan 2011
**Result Status as of report 2:** June 2011
**Result Status as of report 3:** Jan 2012
**Result Status as of report 4:** June 2012

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

**V. TOTAL ENRTF PROJECT BUDGET:**

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

**TOTAL ENRTF PROJECT BUDGET: $297,000**

**VI. PROJECT STRATEGY:**

**A. Project Partners:**

**ENRTF Budget**: $0 labor
$0 supplies
$0 printing
$1,500 travel

**Amount Spent: $ 0**
**Balance: $1,500 (see Result 3)**

no salary, in-kind: Krischik 30%/yr X 3 yr = $89,022
no salary, no in-kind, Dr. Marla Spivak, Department of Entomology, University of Minnesota
no salary, no in-kind, yes travel funds (see Result 3) Mr. Eric Mader, Xerces Society for Pollinator Conservation, Portland, OR and UM Adjunct Extension Research Educator, $1,500 travel funds to participate in MN workshops

Electronic listserve "Outreach Committee**"**
1. MN Nature Conservancy, TBA
2. National Honey Bee Advisory Board, Clint Walker, co-chair and Darren Cox, co-chair
3. MN Honey Bee producers, Darel Rufer, President
4. Old Mill Honey Co, Steve Ellis
5. California-Minnesota Honey Farms, Jeff Anderson
6. MN Hobby Beekeepers Association, Dan Malmgren
7. MDA, invasive species group,TBA
8. DNR, native shoreland restoration and upland restoration groups, TBA
9. MN DOT, Todd Carroll, LLA, ASLA, http://dotapp7.dot.state.mn.us/plant/
10. MNLA, MN Landscape Association, Bob Fitch
11. MNTIF, MN Turf and Grounds Foundation, Kathy Aro
12. MNGCSA, MN Golf course Superintendents Association, TBA
13. Sustainability Project Coordinator, City of Minneapolis, June Mathiowetz
14. Native Plants Society, TBA
15. Organic Growers Association, TBA

**B. Project Impact and Long-term Strategy:**
Use research to justify the development of pest management programs for landscapes that use insecticides that do not harm pollinators. Develop outreach materials that promote pollinator-friendly plants for use in land management, such as roadsides, restorations, conservation plantings, and urban areas. Deliver these recommendations to state agencies, landscape

industry, commodity groups, and homeowners. Share the results of the research and deliverables with the email listserve "Outreach Committee". The PI will travel around the state of MN to provide talks and discussions on pollinator conservation. We will provide 2 workshops to consumers and professionals using the restoration on the St. Paul Campus.

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

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

**D. Spending HIstory:**

Research and outreach products related to imidaclopird and native plants:
7 published papers on imidacloprid use, UM-DNR extension bulletin on plants for restorations, UM-DNR poster on plants for restorations, and CUES website (www.entomology.umn.edu/cues)

**Research funds related to imidacloprid and native plants**

2009 USDA SARE, ""In field grown canola, translocation to flowers from seed and soil treatments ", $175,000
2008 Bayer Chemical Company, "Effects of application methods of imidacloprid", $28,000
2007 Bayer Chemical Company, "Effects of application methods of imidacloprid", $14,000
2006 MNLA "Effects of imidacloprid on green lacewing, $4,000
2006 Bayer Chemical Company, "Effects of application methods of imidacloprid", $21,000
2006 International Paper, " Cottonwood leaf beetle and imidacloprid in poplars" $18,000
2005 Bayer Chemical Company, "Effects of application methods of imidacloprid", $19,500
2004 Bayer Chemical Company, "Effects of imidacloprid on growth enhancement of NM6 cottonwoods and management of cottonwood leaf beetle on wood and leaves", $25,000
2003 International Paper, "Use of imidacloprid in cottonwood saplings", $10,000
2002 AURI PRO, “Management of cottonwood leaf beetle in Minnesota”, $40,000
2001 AURI PRO, “BC and pesticide use in interiorscapes”, $40,000
1997 Minnesota Met Council, "Improving water quality through sustainable management”, $43,000
1995 University of Minnesota Extension Service Collegiate Grant, "CUES: Center for sustainability in urban ecosystems", $98,000

**Total grant funds:$534,500**

## VII. DISSEMINATION:

Research results will be disseminated through 3 research papers, website, electronic email listserve of "Outreach Committee", talks, workshops, 2 bulletins, and poster.

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

## IX. RESEARCH PROJECTS:

Peer review document submitted separately as LCCMR 221GPeerReview.doc

**Timetable of research and deliverables:**

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

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

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

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

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

**2010 Trust Fund Budget**

| BUDGET ITEM | Result 1 Residue analysis, bioassays | Amount Spent July 2010 | Balance July 2010 | Result 2 Best bee plants | Amount Spent July 2010 | Balance July 2010 | Result 3 Workshop | Amount Spent July 2010 | Balance July 2010 | TOTAL BUDGET | TOTAL BALANCE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **PERSONNEL:**<br>**Graduate Student, research**<br>$35,000/yr, fringe is calculated at 17.14%. Increase of 3.25% is included for year 2 and 3=$104,984 | $104,984 | 0 | $104,984 | 0 | 0 | $0 | 0 | 0 | 0 | 104,984 | 104,984 |
| **PERSONNEL:**<br>**Undergraduate Student, research residue and bioassay , best bee plants**<br>$14,000/yr, fringe is calculated at 9% =$42,000 | 22,00 | 0 | 22,000 | 20,000 | 0 | 20,000 | 0 | 0 | 0 | 42,000 | 42,000 |
| **PERSONNEL:**<br>**Undergraduate Student, research best bee plants, development of website, outreach bulletins (update IPM manual, bee-friendly insecticide bulletin and table, and best bee plant bulletin and table)**<br>$8,000/yr, fringe is calculated at 9% =$24,000 | 0 | 0 | 0 | 24,000 | 0 | 24,000 | 0 | 0 | 0 | 24,000 | 24,000 |
| **Research supplies: Purchase bumble bees, honey bees, and beneficial insects from insectaries, rearing supplies, cages, plants, bioassay containers, analytical grade imidacloprid/clothianidin**<br>Bumble bee colonies w/queen (Koppert),<br>$120 x 48 colonies =$4,800<br>Bumblebees boxes 30 x $100=$3,000<br>Honey bee packages (70 pkgs x $65/pkg) =$4,500<br>beekeeping equipment (boxes, covers, wooden frames) =$1,500<br>Beneficial insects from Rincon-Vitova Insectaries<br>lady beetles 500 for $10 = 5 cages<br>5 trts X 2 reps X 8 reps/tr =80/5 X $10=$160 X 3 species lady beetles= $500 | 22,478 | 0 | 22,478 | 0 | 0 | 0 | 0 | 0 | 0 | 22,479 | 22,478 |
| green lacewings 240 for $52 = 2 cages<br>5 trts X 2 reps X 8 reps/trts=80/2 x $52=$2,100<br>Anagyrus 250 for $50 = 5cages<br>5 trts X 2 reps X 8 reps/trts=80/5= 16 X 50= $800<br>Bioquip cages $200 x 26cages= $5,200<br>Misc: netting replacement, diet, petri dishes, cotton, plastic film, wipes, postage, analytical grade imidacloprid and clothianidin, etc=$1,078 | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Research field space for demonstration project on best bee plants:**<br>Purchasing young native plants, soil amendments, mulch, planting tools, irrigation, permanent display =$2,700<br>Land rental<br>$100 per plot (60 ft x30 ft) X 3yr = $300 | 0 | 0 | 0 | 3,000 | 0 | 3,000 | 0 | 0 | 0 | 3,000 | 3,000 | |
| **Research field space for growing dandelion, rose, linden for rsidue analysis and bioassays:**<br>**Field space:**<br>Dandelion and linden field space:<br>$100 per plot (60 ft x30 ft), 1 plot dandelion and 2 plots linden=$300/yr X 3 years= $900<br>purchasing linden whips and planting = $1,000<br>**Rose field space:**<br>more expense since irrigation is needed.<br>Each block is 960 sq ft. = $600/mo X 5 mo =$3,000 X 2 yr=$6,000<br>Insecticides for treating lindens, dandelion, rose = $100 | 8,000 | 0 | 8,000 | 0 | 0 | 0 | 0 | 0 | 0 | 8,000 | 8,000 | |
| **Research greenhouse space for bioassays:**<br>8 treatments (= 3 passive (ladybeetle, lacewing, wasp) + 3 bee + 2 plant rearing) x 100 sg ft x $0.64/ sg ft x 12 mo=$6,600 /yr X 3 yr =$20,000 | 20,000 | 0 | 20,000 | 0 | 0 | 0 | 0 | 0 | 0 | $20,000 | 20,000 | |
| **Residue analysis:**<br>Measure amount of imidacloprid/clothianidin in pollen and nectar of dandelion, rose, linden with HPLC-mass spec.<br>ALS Laboratory Group, Environmental Division<br>Residue analysis $300/sample for preparing samples and HPLC-MS analysis<br>imidacloprid, olefin, hydroxy and analysis for pollen and nectar<br>imidacloprid X 3 plant species (dandelion, rose, linden) X4 plants/species X 2 plant parts (pollen, nectar) X 2 rep exper X 3 trts =146 samples X $300=$43,000<br>clothianidin on rose X 5 plants/species X 2 plant parts (pollen, nectar) X 2 rep exper X 3 trts =60 samples X $300=$17,000<br>Total = $60,000 | 60,000 | 0 | 60,000 | 0 | 0 | 0 | 0 | 0 | 0 | $60,000 | 60,000 | |
| **Travel to sites for research on best bee plants :** In Minnesota mileage @$0.55 X 800 mi =$400 mileage + $600 food (40 days X $15/day)= $1,000 yr X 3yr= $3,000 | $0 | $0 | $0 | $3,000 | **0** | $3,000 | $0 | $0 | $0 | $3,000 | 3,000 | |
| **Travel to outreach, workshops:** In Minnesota mileage @$0.55 x 200 mi=$400 mileage + $120 food (8 days X $15/day)= $520 x 2yr=$1,037 | $0 | $0 | $0 | $0 | **0** | $0 | $1,038 | $0 | $1,038 | $1,038 | $1,038 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Printing best bee plants bulletin, table, and poster:**<br>1. Bulletin with best bee plant table: available at demonstration site , for outreach, and for workshops $0.09/pg x 10 pg + cost paper = $1.00 @ 1000 copies=$1,000<br>2. Best bee plant poster 500 copies=$2,000 | $0 | $0 | $0 | $3,000 | **0** | $3,000 | $0 | $0 | $0 | $3,000 | $3,000 | |
| **Printing updated IPM Manual:**<br>350 pages x $0.10/pg=$35 = colored pages/cover=$40 x 50 copies=$2,000 | $2,000 | $0 | $2,000 | $0 | **0** | $0 | $0 | $0 | $0 | $2,000 | $2,000 | |
| **Printing reduced risk insecticides bulletin and table:**<br>Bulletin and table on reduced risk insecticides: $0.09/pg x 10 pg = $1.00 @ 1000 copies=$1,000 /yr<br>Green Expo = 300 copies/yr<br>Pesticide Certification Workshops =200copies/yr<br>Master Gardener Training = 200 copies/yr<br>State agencies, landscapers = 100 copies/yr<br>Workshop = 200 copies/yr | 2,000 | $0 | 2,000 | $0 | **0** | 0 | $0 | $0 | 0 | 2,000 | 2,000 | |
| **Workshop invited speaker: Airfare, hotel, per diem, no honorarium:**<br>Eric Mader, UM Adjunct and Xerces Society for Invertebrate Conservation, Pollinator Outreach Coordinator, Portland, OR<br>2 day visit: $400 airfare + $220 hotel + $100 food + misc = $750 x 2 workshops=$1500 | $0 | $0 | $0 | $0 | 0 | $0 | 1,500 | 0 | 1,500 | $1,500 | 1,500 | |
| **COLUMN TOTAL** | **$219,462** | **$0** | **$219,462** | **$53,000** | **$0** | **$53,000** | **$2,538** | **$0** | **$2,538** | **$297,000** | 297,000 | |