

Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

Project Manager Name: **Vera Krischik**

Project Manager Email address: **krisc001@umn.edu**

Project Title: **Mitigating Pollinator Decline**

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1. Abstract

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 extremely high rates 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.

2. Background

Native plants used in restoration for wildlife and food plants from apples to zucchini require pollinators. Bees and other pollinators offer valuable ecosystem services in both natural and managed agriculture ecosystems, so it is essential to protect them. Pollinators are experiencing serious decline due to insecticide use, lack of nutritionally rich native plants for pollen and nectar, and lack of habitat. Continued loss of pollinators will have an impact on the natural resources and the economy of Minnesota.

This issue has been addressed by the Xerces Society, National Research Council Report, the Congressional Research Report, testimony by the National Academy of Sciences to the US Congress, and the media in newspapers and television programs. For the first time a group at Pennsylvania State University are investigating Colony Collapse Disorder (CCD) and what weakens bees, such as the interaction of *Varroa* mites, insecticides, fungus, and virus in the hive. However, systemic neonicotinyl insecticides used on landscape plants and crops are considered as a major factor in pollinator decline. After the 1998 ban in France of the systemic seed treatment Gaucho (active ingredient, imidacloprid), French researchers found that imidacloprid is translocated from coated seeds at planting thru the growing plant to nectar and pollen in flowers. In May 2008 a large number of bees died in Germany and the government banned the use of 5 neonicotinyl insecticides, including imidacloprid and clothianidin.

However, in the US use of these 5 neonicotinyl insecticides is very common in greenhouse, landscape, and crops. Native plants grown in greenhouses and transplanted outside may contain high levels of imidacloprid which may kill pollinators. Research in France on the seed treatment Gaucho used in corn, sunflower, and canola demonstrated that imidacloprid was translocated to nectar and pollen. Our

research demonstrated that nectar and pollen from greenhouse plants treated with soil applications of imidacloprid contained significantly higher amounts of imidacloprid and its metabolites, than from a Gaucho-seed treatment. The label of Gaucho states that 0.375 mg AI for corn and 0.11 mg AI of for canola of imidacloprid should be applied. The greenhouse rate used on perennial landscape plants states that 300 mg AI/ 3gallon pot with 1 plant can be used. This is an 800 times higher rate for corn and 2700 times higher rate for 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 more than the seed treatment Gaucho where most of the research has been done.

Our research on greenhouse rates of imidacloprid showed that the amount of imidacloprid found in nectar of 2 flowering plants was 20 ppb to 41 ppb from a single soil application compared to 1.9 ppb imidacloprid in sunflower nectar and 0.6 to 0.8 ppb in canola nectar from a seed treatment. For buckwheat and milkweed landscape plants, a label rate of soil applied imidacloprid (Marathon 1%G) was translocated to buckwheat nectar at 16 ppb (Krischik et al. 2007) and milkweed at 41 ppb/flower (Krischik et al. 2010). These concentration of caused high mortality of beneficial insects, such as lady beetles, lacewings, and a small parasitic wasp (Smith and Krischik 1990, Rogers et al. 2007, Krischik et al. 2007, Krischik et. al 2009 submitted).

There are multiple ways that plants in urban landscapes can contain imidacloprid -contaminated nectar, since it is commonly applied in the landscape for many pests (Krischik and Davidson 2004) and many greenhouse plants are treated with prior to sale and transplanting. Imidacloprid may persist in nectar for a long time, since soil applications were effective against foliar pests for 1 to 2 years in containers (Szczepaniec and Raupp 2007, Gupta and Krischik 2007, Tenczar and Krischik 2007) and landscape trees (Cowles et al. 2006, Frank et al. 2007, Tenczar and Krischik, 2007). Injections of concentrated volumes of imidacloprid (Imicide, Pointer) applied to trees trunks and roots were effective for 12 months for ash (McCullough et al. 2003) and linden (Johnson and Williamson 2007). Tree injections of imidacloprid at flowering are cause for concern, since linden flowers are a good source of nectar and pollen for bees, butterflies, and other beneficial insects.

However, the effect of these higher levels of imidacloprid used on landscape plants on bees has not been researched. Please see Table 1 for information on how much imidacloprid and clothianidin can alter behavior or kill bees.

3. Hypothesis

Research result 1. Investigate whether systemic insecticides, imidacloprid and clothianidin, are translocated to nectar and pollen in sufficient amounts to kill or alter behavior of pollinators.

Deliverable 1. We will mitigate pollinator loss by developing a section on the CUES website on "Pollinator compatible insecticides for landscapes." We will amend the 2004 "IPM of Midwest Landscapes" that is online at the CUES website to contain a section on pollinator compatible insecticides (<http://www.entomology.umn.edu/cues/ipmbook.htm>). We will mitigate pollinator loss by developing landscape pest management protocols using pollinator-friendly insecticides.

Research result 2. We will determine the best plants to be used in Minnesota landscapes to provide season-long nectar and pollen for pollinators. 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.

Deliverable 2. We will mitigate pollinator loss by developing a section on the CUES website on "Mitigating pollinator loss through proper plant selection" 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). We will mitigate pollinator loss by developing recommendations for restorations and landscapes with seasonal phenology of pollinator plants.

Research 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.

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. Eric has been given status as Adjunct Extension and Research Educator, University of Minnesota (no salary).

Table 1. Levels of imidacloprid applied to commodities and levels detected in nectar/pollen		
<i>Commodity</i>	<i>(AI) treatment rate</i>	<i>residue in nectar (research paper)</i>
Seed treatment Gaucho* *most research on this treatment level	0.11 mgAI /plant	0.6-0.8 ppb canola nectar (Scott-Dupree and Spivak 2001) 1.9 ppb sunflower nectar (Schmuck et al. 2001) 6 ppb found in bee pollen loads in France (Chauzat et al. 2006)
field crops Formula: Admire Pro	4 mg AI/sg ft	not researched
Greenhouse/nursery pots before planting outdoors; can reapply Formula: Marathon	300 mg AI /3galpot	20 to 54 ppb (Krischik et al. 2007, 2009)
3 ft plant in landscape, rose	630 mg AI	not researched
?DBH apple tree-method? ?DBH eucalyptus-method? 16 in DBH tree-soil drench 24inDBH tree- soil drench	unknown unknown 50 g AI 76 g AI	4000 ppb unpublished, Bayer 550 ppb, unpublishedTim Payne, UCRiverside not researched not researched
Levels of imidacloprid that alter behavior or kill pollinators or beneficial insects (passive pollinators)		
<i>Insect species</i>	<i>Level imidacloprid</i>	<i>Research paper</i>
Greenhouse research Kills beneficial insects: ladybeetle predator (4 sp), green lacewing predator, parasitic wasp	15 ppb	(Krischik et al. 2007, 2009)
Kills honeybees in one sip NOEC	158-185 ppb <5ppb	(Bayer report 2007) (PAN-Europe 2009 letter)

acute oral	40 ng/bee	(Suchail et al 2001)
acute contact	40-102 ng/bee	(Nauen 2001)
Level altering honeybee behavior	6-100 ppb	24 ppb disrupts learning & olfactory conditioning (Decourtye et al. 2004) 6 ppb disruption of feeding (Colin et al. 2004) 100 ppb decrease in foraging (Kirchener 1999)
Level altering bumblebee behavior <i>B. impatiens</i> <i>B. terrestris</i> <i>B. impatiens</i>	10-30 ppb	30 ppb slower foraging rate (Morandin and Winston 2003) 10 ppb reduced brood survival (Tasei et al. 2000) clothianidin and thiamethoxam are deadly at extremely low dosages (Biobest 2008)
Kills honeybees acute oral	43 ng/bee clothianidin	US EPA Clothianidin Factsheet Clothianidin (Arena) is a major metabolite of thiamethoxam which is also labeled for landscape (Flagship- flowers, Meridian-turf)

4. Methodologies

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. Levels of insecticide residues 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.

We will investigate whether systemic insecticides are translocated to nectar and pollen in sufficient amounts to kill or alter behavior of pollinators. We will study the amount of systemic neonicotinyl imidacloprid that is translocated to nectar and pollen from a soil application of imidacloprid (Merit) on rose, dandelion, and small linden trees. We will use residue analysis to determine the parent compound

and metabolites in nectar and pollen. For clothianidin, we will only determine amounts of parent compound and metabolites in pollen of rose from a soil application (Arena), due to costs and time. We will not bioassay with insects, since the LD50 are very close for imidacloprid and clothianidin. We will use a residue analysis lab since it is cost effective and we have used this lab for past research (ALS Laboratory Group, www.alsenviro.com, Environmental Division, Edmonton, Canada). We will determine the effects of these levels of imidacloprid on behavior and mortality of beneficial insects, honey bees and bumble bees.

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.

We will plant dandelion, rose, and linden trees in field plots at the University of MN, St Paul Campus. For dandelion and rose, the imidacloprid treatments will include: 1) control, 2) 1X landscape rate soil application, and 3) 2X landscape soil applications since labels permit reapplication. For linden trees, the treatments will include: 1) control, 2) 1X soil drench, 3) 1X trunk injection, and 4) 2X trunk injection. We will replicate the study 3 times. We will use 21.4% A. I., Merit 2F, Bayer Chemical Co. For clothianidin (Arena) we will use the treatments as outlined above on rose.

We will collect open flowers at mid-day, at the height of nectar production, from approximately 30 different plants, for a mean of 65 flowers per 10-ml scintillation vial and will place the collected flowers in a cooler filled with ice. The vials will be stored in an ultralow freezer at -79°C.

Lab research: Residue analysis

Residue analysis. We will determine the concentration of , olefin, and hydroxy translocated to nectar and pollen both in flowers and collected by bees by 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, although that analysis is not outlined here.

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 °C, 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.

Statistical Analysis. For all research, we will first use Levene's test to determine homogeneity of variance. If variances are unequal, a Welch test will be used (JMP, SAS 2005). If variances are equal, data will be analyzed with one way ANOVA for treatment, replicate and replicate by treatment interactions using

PROC GLM (SAS 2004). Means will be compared with Tukey's HSD test. We will use PROC MIXED for any data that needs repeated measures statistics (SAS 2004).

Greenhouse research: Determine the effects of imidacloprid on the behavior and survivorship of pollinators.

Behavioral observation of beneficial insects. 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 x 30 cm x 30 cm) (BioQuip, Rancho Dominguez, 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 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 to be conducted over 2 years, 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 unseminated 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.

Field bioassays combined with lab bioassays:

Effects of imidacloprid on managed honey bee health. We will start this study in year 2 and 3, after we have worked out the methods with bumble bees. 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.

If Api-Guard (an essential oil made from thymol) is used to control *Varroa* mites in any of the experimental honey bee colonies, we will make sure to have an untreated control group to quantify potential effects this treatment might have on brood production. M. Spivak has used ApiGuard for many years and has not observed effects on the queen's egg laying or brood production during the times of year when it is applied (when ambient temperatures range from 60-85F). We understand that sometimes at higher temperatures, or when colonies are weak in the spring, there can be effects on brood.

Measures of 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 5th (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.

We will definitely monitor Varroa and Nosema levels in all experimental colonies, and we can determine virus presence/ absence using PCR. To determine viral load would necessitate using real-time PCR which is quite expensive and not budgeted in this proposal. We may be able to send samples to a different lab for viral load analysis, budget permitting. However, we agree that it is very important to monitor other pathogens and parasitic mites as they could definitely have an effect on colony health.

As for the quantity and viability of drone sperm: This is research being conducted by D. Tarpay at North Carolina State University, but we could possibly collaborate with him on this aspect.

Behavioral effects on 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.

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.

RESULT 3: Provide research results and offer ways to mitigate pollinator decline to the consumer and professional community in the state of Minnesota.

Description research result 3.

Use an email listserve to an "Outreach Committee" to disseminate research results and information. 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

5. Results and Deliverables

Deliverable research result 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 table 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 January Green Expo, 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.

Deliverable research result 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.

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. Eric has been given status as Adjunct Extension and Research Educator, University of Minnesota (no salary).

6. Timetable

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
Demonstration plot					x	x			x	x		
Result 3 and deliverable. 1. Listserve, 2. PI to deliver at least 8 talks around Greater 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 travel to deliver talks		x	x	x		x	x	x		x	x	x
Workshops (2)												x

7. Budget

See budget provided in pdf format

8. Credentials

Project Manager Qualifications and Organization Description

Dr. Vera Krischik, Assoc. Professor Ecology of Urban Landscapes, Department of Entomology, University of Minnesota, St. Paul Campus

The PI is a tenured Faculty in the Entomology Department of the College of Food, Agricultural and Natural Resource Sciences at the University of Minnesota. One of the goals of the College is to develop viable food and agricultural systems, while maintaining healthy natural resources. Bees and other pollinators provide ecosystem services in both natural and managed agriculture ecosystems, so it is essentially to protect them. Determining ways to prevent pollinator loss and promote functioning sustainable ecosystems are a goal of my research and outreach programs. The PI has over 30 years of research expertise and publications in this area. Equipment and facilities are available for this research.

Vera obtained her PhD from the University of Maryland in 1984, held at Post Doc at the University of Maryland, was a researcher at the New York Botanical Garden (NSF sponsored Visiting Professor for Women, 1991-1993), and was an IPM coordinator at USDA, Washington DC from 1988-1994. Since 1995, she is a professor in the Department of Entomology at the St. Paul, University of Minnesota. She teaches 2 courses: ENT 5009, Pesticide Use and Misuse and ENT 4015, Ornamental and Turf IPM. She has 6 published papers on the non target effects of imidacloprid on beneficial insects and 2 published papers and 2 in manuscript on the proper use of imidacloprid for landscape plants. She has two books: one published in 1991 by John Wiley entitled "Microbial Mediation of Plant Insect Interactions" and another published in 2004 by the MN Agricultural Experiment Station on "IPM of Midwest Landscapes", 316 pp. She has partnered with MDA, DNR, MNLA, MNTGF, and watershed districts for her outreach and research programs and publications. She has developed a plant restoration bulletin and poster in cooperation with the DNR and Ramsey Watershed District. She teaches at least 5 large workshops each year on proper pesticides use in cooperation with MDA and MNLA. She has trained 6 graduate students and 1 post doc. She is director of CUES: Center for sustainable urban ecosystems that promote natural resource management, online at www.entomology.umn.edu/cues. Dr. Krischik was contacted by MN National Public Radio on June 23, 2009 for an interview on the non-target effects of imidacloprid on birds and bees.

Dr. Vera Krischik's experience with imidacloprid and management of landscape pests

online at www.entomology.umn.edu/cues/krischiklab/krischik.htm

1. Tenczar, E. G., and V. A. Krischik. 2007. Comparison of standard (granular and drench) and novel (tablet, stick soak, and root dip) treatments for cottonwood leaf beetle (Coleoptera: Chrysomelidae) management on hybrid poplar. *J. Econ. Entomol.* 100: 1611-1621.
2. Krischik, V. A., A. Landmark, and G. Heimpel. 2007. Soil-applied is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) *Environ. Entomol.* 36(5): 1238-1245.
3. Rogers, M. A., V. A. Krischik, and L. A. Martin. 2007. Effect of soil application of on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), used for biological control in greenhouse. *Biological Control* 42(2): 172-177.
4. Gupta, G., and V. A. Krischik. 2007. Professional and consumer insecticides for the management of adult Japanese beetle on hybrid tea rose. *J. Econ. Entomol.* 100(3): 830-837.
5. Tenczar, E. G., and V. A. Krischik. 2006. Management of cottonwood leaf beetle (Coleoptera: Chrysomelidae) with a novel transplant soak and biorational insecticides to conserve coccinellid beetles. *J. Econ. Entomol.* 99(1): 102-108.
6. Smith, S. F. and V. A. Krischik. 2000. Effects of biorational insecticides and imidacloprid on four coccinellid species (Coleoptera: Coccinellidae). *J. Econ. Entomol.* 93(3): 732-736.
7. Smith, S. F. and V. A. Krischik. 1999. Effects of systemic imidacloprid on *Coleomegilla maculata*. (Coleoptera: Coccinellidae). *Environ. Entomol.* 28(6): 1180-1195.

Research completed and manuscript finished

1. Tenczar, E. G., and V. A. Krischik. 2010. Effects of irrigation in hybrid polar fields on efficacy of different formulations of imidacloprid.
2. Krischik, V. A., Gupta, G, and Vasei, A. 2010. Efficacy and duration of imidacloprid in roses.
3. Krischik, V. A., Gupta, G, Rogers, M, and Vasei, A. 2010. Soil-applied imidacloprid is translocated to nectar and reduces survival of 4 species of coccinellid beetles, but not 2 species of butterflies (submitted, accepted, and in revision)

Books

1. Krischik, V and J. Davidson. 2004. IPM of Midwest landscapes, UMAg Exp Stat., 316pp.
2. Barbosa, P., V. Krischik, and C. Jones. 1991. Microbial mediation of plant insect interactions. John Wiley and Sons, 530pp.

Collaborators

Dr. Marla Spivak, Professor and Extension Entomologist Apiculture, Department of Entomology, University of Minnesota

Marla Spivak obtained her PhD from the University of Kansas in 1989, and was a post-doctoral fellow at the Center for Insect Science at the University of Arizona from 1989-1992. Her position at the University of Minnesota began in 1992, and her responsibilities include research, teaching and extension for the 5 state area (MN, WI, IA, SD, ND). Other research projects include understanding the neural mechanisms mediating honey bee hygienic behavior (funded by the NSF since 1993) and studying the social behavior of the bumble bee, *Bombus impatiens*. She teaches courses on Social Insects, Bee Management, and Scientific Communication and Ethics. Other extension projects include publishing a manual on how to rear bumble bees for use in pollination, using solitary bees for apple pollination, and the pollination requirements for cranberries in WI.

Mr. Eric Mader: Xerces Society, National Pollinator Outreach Coordinator, Portland, OR

Eric works to raise awareness of native pollinator conservation techniques among growers and government agencies. His previous work includes commercial beekeeping and crop consulting for the Agrecol Corporation where he provided weekly insect and disease scouting on hundreds of native plant crops. He is a graduate of the University Of Minnesota Department Of Horticulture's Masters program, and has recently written a book on how to manage non-Apis bee species for the USDA Sustainable Agriculture Network and the Natural Resource, Agriculture, and Engineering Service at Cornell University. He is an adjunct extension educator at the University of Minnesota. He held a Native Pollinator Conservation workshop on June 9, from 9-4, at the University of Minnesota, St. Paul. He has worked with various groups on developing pollinator conservation recommendations.

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

9. Dissemination and Use

This project will address and mitigate pollinator decline with research and outreach programs.

Research result 1. Residue analysis and bioassays will be developed into research papers and a bulletin. Research and bulletin will be posted on the CUES website. The data will be discussed at the 2 workshops and delivered at outreach activities around the state.

Deliverable. We will mitigate pollinator loss by developing landscape pest management protocols using pollinator-friendly insecticides. We alter each landscape pest profile in the online "IPM of Midwest landscapes, 316 pp" by Vera Krischik and John Davidson, to have new entries on pollinator compatible insecticides featuring EPA registered reduced risk insecticides (<http://www.entomology.umn.edu/cues/Web/196Sawflies.pdf>). We will also develop pollinator friendly insecticide recommendations and place them on the front page of the very popular CUES website (<http://www.entomology.umn.edu/cues>). and develop a bulletin.

Research result 2. Data on bee friendly plants will be developed into research papers and a bulletin. Research and bulletin will be posted on the CUES website. . The data will be discussed at the 2 workshops and delivered at outreach activities around the state. We will work with state agencies and commodity groups to restore habitat with pollinator-friendly plants.

Deliverable. We will mitigate pollinator loss by developing a section of the CUES website on "Mitigating pollinator loss through proper plant selection" 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). We will develop a bulletin, poster, and plant list.

Result 3 and Deliverable. 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.

Appendix 1: Letters of support

Letters of support:

- 1. Xerces Society, Portland, OR, Eric Mader, National Pollination Outreach Coordinator
- 2. National Honey Bee Advisory Board, Clint Walker, Rogers, TX, co-chair and Darren Cox, Logan, UT, co-chair
- 3. MN Honey Bee producers, Waverly, MN, Darel Rufer, President

- 4. MN Hobby Beekeepers Association, St. croix, MN, Dan Malmgren
- 5. California-Minnesota Honey Farms, Eagle Bend, MN, Jeff Anderson
- 6. Old Mill Honey Co, Barrett, MN, Steve Ellis



THE XERCES SOCIETY
FOR INVERTEBRATE CONSERVATION
 4828 Southeast Hawthorne Boulevard Portland, Oregon 97215, USA
 Telephone 503-232-6639 Fax 503-234-6794 www.xerces.org

September 15, 2009

To Whom It May Concern,

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On behalf of the Xerces Society for Invertebrate Conservation I would like to express my strong support for Dr. Krischik's LCCMR proposal titled "Mitigating Pollinator Decline in Minnesota."

While the non-native European honey bee (*Apis mellifera*) has been the focus of highly publicized reports following its dramatic nationwide decline over the past several years, our native bees, including Minnesota's approximately 500 endemic bee species are suffering equally alarming losses. In many cases these bees are significantly more effective pollinators on a bee-for-bee basis than the non-native honey bee, especially for native food crops such as blueberry, cranberry, and squash, and for the native wildflowers which form the foundation of healthy natural ecosystems.

In fact nearly 70% of all native plants require insect pollination for reproduction, and according to our best available data, the fruits and seeds produced through insect pollination comprise nearly 25% of the direct food supply for terrestrial birds and mammal—including species as diverse as songbirds and black bears.

As an example of the pollinator declines taking place in Minnesota, three of the state's 12 endemic, and formerly common bumble bee species, the yellow banded bumble bee (*Bombus terricola*), the rusty patched bumble bee (*B. affinis*), and the cuckoo bumble bee (*B. anthus*), have not been observed in almost a decade. Their likely extirpation represents a loss of approximately one-fourth of the state's bumble bee biodiversity, and an alarming threat to the profoundly important ecological service of pollination.

The threats facing pollinators are many. However two widely recognized reasons for the loss of pollinator diversity and abundance are the loss of habitat, and the use of pesticides. Dr. Krischik's proposal directly addresses those threats and proposes well reasoned and much needed solutions for mitigating further losses. This proposal is grounded in the best available science and engages precisely the constituent audiences most capable of affecting positive change. I urge to fully support this important work.

Sincerely,

Eric Mader
National Pollination Outreach Coordinator



September 21, 2009

Dear LCCMR committee members,

The National Honey Bee Advisory Board represents the vast majority of the beekeeping industry of the United States on issues relating to pesticides. We work directly with EPA, USDA, and university researchers around the country in an effort to improve pollinator protection.

Systemic pesticides such as imidacloprid are a major concern to our group. Unlike conventional pesticides, which are applied topically, these new compounds actually are taken up by the plant tissue and moved through out the plant including the pollen and nectar. Chemical levels of great concern have been found in floral nectar and pollen, but more research is desperately needed to quantify the hazard to pollinators.

Dr. Vera Krischik is a leading researcher in this field. She is well-qualified to conduct this proposed project to mitigate pollinator decline in Minnesota. In fact, we believe that the research she is requesting you to fund will play a profoundly important role in addressing what has become a national as well as worldwide problem.

The great value of pollinators to a healthy planet is often not fully appreciated. A recent BBC Radio 4 broadcast, *Earthwatch* described bees as "Irreplaceable—the world's most invaluable species," noting these facts:

- * 250,000 species of flowering plants depend on bees for pollination
- * Bees pollinate 1/3 of the 80 major world crops
- * Their economic value was estimated in 2005 at \$130 billion for pollination services
- * 25% terrestrial species would go extinct without them

You have a unique opportunity to help solve a major issue of worldwide importance by approving this important grant proposal. The beekeepers of Minnesota and the nation need your assistance; please help us.

Sincerely,

Clint Walker III, Co-Chair
Darren Cox, Co-Chair

1007-2007 MINNESOTA HONEY PRODUCERS ASS'N., INC. POLLINATION

1907-2007
MINNESOTA HONEY PRODUCERS ASS'N., INC.

OFFICERS:
 DAREL RUFER
 President
 3499 75th St SW
 Waverly, MN 55389
 763-688-4038

JEFF WALL
 Vice President
 PO Box 371
 Bettelheim, MN 56315
 218-844-5294

VIKki HULL
 Secretary
 PO Box 371
 Bettelheim, MN 56315
 218-844-5296

CONNIE LESATY
 Treasurer
 2612 36th St
 St Joseph, MN 56374
 509-252-9943

DIRECTORS:
 STEVE ROSE
 4152 215 St E
 Mankato, MN 56001
 561-643-6718

DAN WHITNEY
 PO Box 41
 Orono, MN 56571
 751-388-1177

BEAN WETZELAND
 1871 Luma Dr
 Bettelheim, MN 56313
 763-421-8644

DIRECTOR-AT-LARGE:
 FRED HOLTE
 4308 Miller Ave
 Mankato, MN 56002
 763-689-1569

EDITORS:
 MARK & TANYA SUNBERG
 125 W. Vermont Ave
 Fargo, ND 58103
 701-796-2868

RESEARCH EDUCATION PROMOTION

September 25, 2009

To Whom It May Concern:
 (Which is all of us!)

The MN Honey Producer Association (MHPA) is in strong support of your research efforts to determine what is killing bees and pollinators in general. Honey bees and the pollination services that they provide are under threat from a combination of factors called Colony Collapse Disorder (CCD). One contributing factor to bee decline may be the presence of insecticides in flower nectar in the foraging environment. We need immediate research to help determine some of the causes of bee decline and reduction in pollination.

We have provided annual financial contributions for research at the University of Minnesota on honey bees for over 25 years. We feel this particular issue of insecticides is one of the most important ones we have right now. We will cooperate with the University of Minnesota by providing access to our members so they can conduct surveys and collect information on CCD. We would like the state to provide research funds so data can be collected to determine if systemic insecticides are accumulating in nectar and pollen and contributing to the loss of our honey bee colonies.

It not only affects honey bees, but all other native pollinators and more plants of a wider time frame of pollen and nectar production whether native planting and some native planting. It is extremely important! This research could be a lifeline to our pollinators that are our "angels of agriculture".

Sincerely,
 Darel J. Rufer, President
 Minnesota Honey Producers, Assoc.

Minnesota Hobby Beekeepers Association
 14787 Morgan Av N
 Marine on the St. Croix, MN 55047
 "Better Beekeeping Through Education"

September 30, 2009

Legislative-Citizen Commission on Minnesota Resources
Room 65, State Office Building
100 Rev. Dr. Martin Luther King Jr. Blvd.
St. Paul, Minnesota 55155

Dear LCCMR,

The Minnesota Hobby Beekeepers Association strongly supports and encourages you to support the proposed research by Drs Krischik and Spivak on determining the effects of neonicotinyl insecticides on bees and on providing more floral resources to support our pollinators. Systemic insecticides accumulate in nectar and pollen, and bees are very susceptible to the neonicotinyl class of insecticides. This is a very important emerging issue for natural resources and agriculture in MN since bees pollinate 75% of most native plants including most of the domestic crops and home gardens. This research is a very important step in understanding why honey bees are experiencing Colony Collapse Disorder, and why our native bee pollinators are in decline. If these insecticides are contributing to the demise of bees, we need to be very proactive about protecting bees from these compounds. The best way to be proactive is to promote plantings of bee pollinated flowers in our urban and agricultural landscapes.

The University of Minnesota Entomology Department has been world-renowned reputation for its research, education and contributions to insect science and in particular, to beekeeping and bee health. Drs. Marla Spivak and Vera Krischik, and Scientist Gary Reuter (Spivak's technician) share their research giving presentations to state, national and world entomology and beekeeping organizations. They have very effective Extension and Outreach programs to help get research findings out to beekeepers and the public.

The Minnesota Hobby Beekeepers Association is proud to be an affiliate of the University of Minnesota. We are comprised of beekeepers as a hobby and small business. We have over 300 members in Minnesota and Wisconsin. Our mission is better beekeeping through education. We have been privileged to have Doctor Spivak and Scientist Gary Reuter as our advisors, and look forward gleaming more knowledge from Dr. Krischik also in the future.

If you have any questions, please feel free to contact me.

Thank you for your consideration,

Dan Malmgren
Dan Malmgren, President
(651)-503-9239



Jeff & Christian Anderson
721 Wells Street
Eagle Bend, MN 56446
(218) 738-6712

October 1, 2009
Page 2

September 23, 2009

Dear LCCMR committee members:
Please fund... ["Mitigating Pollinator Decline in Minnesota - G_Creative Ideas"](#)

You should be aware that pollinators are in decline in Minnesota. Pollinators are critical for a healthy environment, pollinating plants and keeping them viable for wildlife's food and shelter. *Without healthy pollinators much of what LCCMR is trying to accomplish for Minnesota environment won't be successful.*

12/31/08 BBC Radio 4 broadcast. [Earthwatch](#) described bees as "irreplaceable - the world's most invaluable species" noting these facts...

- 250,000 species of flowering plants depend on bees for pollination
- They pollinate 1/3 of the 80 major world crops
- Their economic value was estimated in 2005 at £130 billion for pollination services
- 23% terrestrial species would go extinct without them

Insecticides kill insects, many pollinators are insects.

Managed honeybee colonies are an excellent gauge of the health of native pollinators. *EPA uses managed honeybees as the barometer for all insect pollinators.* As a commercial beekeeper I can attest to the fact that *use of insecticides on blooming plants kills honeybees.* One can extrapolate similar losses to native pollinators.

The method of applying insecticides is changing. Until relatively recently pesticide applications were generally topical. Today many are applied as soil drenches and seed treatments designed to treat the plant systemically. Many of the systemics are nicotine based. Nicotines, as they are referred to, work systemically in plants making them, their pollen, and nectar toxic. There is a very fine line between the dose which kills pests and one that kills beneficial insects. A number of countries are taking a hard look at systemic nicotinic's. Here are several articles of interest... excerpts from

Germany 2008:

<http://www.greensunflower.org/en/forum/general-discussion/real-cause-cd-inimidacloprid-its-family>

"(NaturalNews) German government researchers have concluded that a bestselling Bayer pesticide is responsible for the recent massive die-off of honeybees across the country's Baden-Württemberg region..."

"Researchers found buildup of the pesticide clothianidin in the tissues of 99 percent of dead bees"...

"nearly 97 percent of honeybee deaths had been caused directly by contact with the insecticide"...

"the government has banned an entire family of pesticides, fueling accusations that pesticides may be responsible for the current worldwide epidemic of honeybee die-offs."

England 2009

<http://www.buzzallfor.org.uk/Resources/Default.aspx?NewsContentId=20§id=2&report=1.pdf>

"...unexplained collapse in Britain's bee population is being exacerbated by a group of widely used systemic pesticides called neonicotinoids already banned in much of the European Union"...

"... This is the most comprehensive review of the scientific evidence yet and it has revealed the disturbing amount damage these pesticides can cause to bees..."

US 2009

<http://pollinatorthis.org/beeblog/2009/05/24/us-honey-bee-advisory-board-asks-epa-to-ban-imidacloprid/>

"...American beekeepers, who claim the product is a cause of colony collapse disorder, which has cost many commercial U.S. beekeepers at least a third of their bees since 2006, and threatens the reliability of the world's food supply..."

"...Bayer scientists found imidacloprid in the nectar and pollen of flowering trees and shrubs at concentrations high enough to kill a honeybee in minutes. The disclosure recently set in motion product reviews by the California Department of Pesticide Regulation and the EPA..."

"...2007 studies, Bayer applied standard doses of imidacloprid to test trees, including apple, lime and dogwood. Its scientists found imidacloprid in nectar at concentrations of up to 4,000 parts per billion. (Honeybees can withstand a dose of up to 185 ppb...)"

"National Honeybee Advisory Board, which represents the two biggest beekeeper associations in the U.S., recently asked the U.S. Environmental Protection Agency to ban the product. "We believe imidacloprid kills bees"

EPA currently only looks at the acute toxicity of pesticides. They do not consider that bio-accumulating systemic insecticides are doing damage to pollinators when used according label directions. EPA can only take action when they have scientific facts in front of them. Obviously chemical manufacturers only supply EPA with required information, so even if "they" know of problems, they are not "required" to submit that information. This is why private research is crucial. EPA will not act unless and until scientific evidence is supplied showing a need for change.

Professor Vera Krischik, University of Minnesota, has submitted a research proposal, ["Mitigating Pollinator Decline in Minnesota - G_Creative Ideas"](#). I strongly recommend funding of this research proposal. It is imperative to learn if nicotinic's are "safe enough" for use in our Minnesota environment.

I anticipate hearing that this vital research is to be funded. Thanks for your consideration.

Sincerely,

Jeff Anderson
California-Minnesota Honey Farms
721 Wells Street
Eagle Bend, MN 56446
(218) 738-6712

2

OLD MILL HONEY CO.
20501 Co Rd 5
Barrett, MN 56311
September 15, 2009

To: MN LCCMR Committee:

My name is Steve Ellis. I have been a commercial beekeeper for 28 years. My business, Old Mill Honey, operates 2,300 hives of bees in California and Minnesota. Commercial beekeepers like myself pollinate crops from almonds to apples to cherries as well as hundreds of other blooming crops. Unfortunately, the honeybee industry and, in fact all pollinators, are suffering an unprecedented and catastrophic decline.

For the last four years a previously unknown ailment has been decimating the U.S. beekeeping industry. This affliction kills off entire colonies of bees in a matter of only a few days; 60-80% of entire operations can disappear within a few short weeks. This phenomenon is so new in fact that a name had to be invented for it: CCD or Colony Collapse Disorder. Much media attention has been given to the subject. From people on the street to flight attendants on commercial airlines, people see my beekeeping cap and ask, "What's happening to the bees?"

This heightened public awareness has carried with it the "silver lining" of underscoring the vital importance of honeybees in our food production systems. Without honeybees and their pollination of food crops, we will loose fully one-third of our diet. Simply put, we will all be eating gruel, because cereal grains are one of the few foods that can be produced without insect pollination.

Put this way most people then ask me, "What do you think is causing this problem and what can I do to help?" I have been active in the beekeeping industry, attending regularly our state and national beekeeping association meetings, and I try hard to stay as informed on industry concerns as I can. My strong belief is that this dramatic decline of pollinators which we have experienced is directly related to the recent heavy use of neonicotinoid systemic insecticide.

Unfortunately the way things work in the United States today most research done on newly registered pesticide compounds is commissioned and paid for by the chemical industry. The beekeeping industry does not have sufficient funds to commission independent researchers to determine safety for pollinators. A project funded by the LCCMR would have a critically important feature: *independence.*

Here in Minnesota we are very fortunate to have at the University of Minnesota researchers with experience testing both honeybees and this new chemical class of neonicotinoids. Professors Vera Krischik and Marla Spivak would bring the

necessary expertise to be able to make a significant contribution to this critically timely issue. I personally know both of these researchers and I can tell you that the state's money will be very well invested in their competent hands.

This proposal will definitely help solve the mystery of what is killing off our essential pollinators. Minnesota is in a unique position to assist in this essential work. Dr. Krischik is one of the leading research scientists, and her emphasis in Neonicotinoid systemic insecticides places her in a position to really make a difference. As a beekeeper, I can tell you this grant is desperately needed.

Sincerely,

Steve Ellis

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