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: **Landscape management of EAB: Nontarget consequences**

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1. Abstract

Management of Emerald Ash Borer (EAB**),** *Agrilus plannipennis* (Coleoptera: Buprestidae) in landscapes employs two methods, removal of dead or dying trees and annual treatment of trees with insecticides. The report recommends a soil drench of imidacloprid for trees smaller than 12" DBH (diameter at breast high), but the ability for a tree to uptake imidacloprid depends on soil moisture. Minnesota receives less rainfall than the states where research was preformed for the recommendations. The chemical producer recommends insecticide treatments only in spring or fall, but consumers and professionals will apply insecticides in summer when they may not be absorbed by the tree and may result in runoff. Flowering plants growing under treated trees may uptake imidacloprid that is translocated to pollen and nectar and may cause mortality in beneficial insects. Imidacloprid is listed by the EPA as having potential for mobility in soils. Emamectin benzoate is highly unsoluable in water and dose not move in soils.

The purpose of this research is:

1. We will investigate the amount of insecticide present thru the year in trunk and leaves for different times of seasonal treatments (May, early August, and September). We will determine the efficacy and duration of imidacloprid treatmentss in MN environment where low levels of rainfall in midsummer may alter uptake of treatements. Research would relate these amounts to the known toxicity of imidacloprid and emamectin benzoate to emerald ash borer found in published studies. If it is demonstrated that uptake of imidacloprid from soils in midsummer reduces efficacy, then we can further stress the importance of spring and fall treatments in managment bulletins. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration.

2. We will determine the amount of insecticides used as a soil surface treatment that is translocated into the pollen of flowering plants growing under trees. We will investigate mortality and behavior of beneficial insects feeding on the pollen. This research will help us understand any potential nontarget effects of imidacloprid.

3. We will investigate the amount of insecticides that move from the point of application to soils under the tree. This research will help us understand any potential nontarget effects of imidacloprid.

4. We will relate the amounts of insecticide in trunks to the published toxicity of these insecticides to birds. This research will help us understand any potential nontarget effects of imidacloprid.

2. Background

Recommendations for chemical management of ash trees

Management of Emerald Ash Borer (EAB**),** *Agrilus plannipennis* (Coleoptera: Buprestidae) in landscapes employs two methods, removal of dead or dying trees and annual treatment of landscape trees with insecticides. Efficacy of insecticides in controlling larval EAB was performed during the last 8 years in EAB infested states (McCollough et al. 2005, extension.entm.purdue.edu/EAB/ , Smitley et al. 2010a,b). The report recommends a soil drench of imidacloprid as one of the best control options for trees smaller than 12" DBH (diameter at breast high), but the ability for a tree to uptake imidacloprid depends on soil moisture. MN receives rainfall on average of 26 in/yr which is 32% less rainfall than Ohio (38 in/yr) where most of the research was performed and less rainfall than the EAB infested states, such as Indiana, 39 in/yr, Illinois, 33 in/yr, and Michigan, 32 in/yr. In MN high pressure soil injections or trunk injections may be more effectively taken up by the tree and may be the better option.

The chemical producer recommends insecticide application only in spring or fall, but consumers and professionals will apply insecticides in summer when they may not be absorbed by the tree and may result in runoff. In addition, the report indicated that trunk treatments of a recently registered insecticide, emamectin benzoate (Tree-Age) offers the best efficacy. However, limited research funded by industry was done on this insecticide, which was previously registered for use in farmed salmon, fruit trees, and cole crops.

Soils high in humus bind imidacloprid, but clay or sandy soils may permit imidacloprid to leach away from the site of application (Smitley 2010 a,b). Imidacloprid is listed by the EPA as having potential for mobility in soils. It moves more in sandy soil and binds to humus in soils (Peterson 2007, Arora et al. 2009, Ping et al. 2010, Cowles 2010, Dilling et al. 2010). Imidacloprid was detected at concentrations of 0.2 to 7 ppb in 12 monitoring wells and 16 down gradient private homeowner wells. Imidacloprid was detected at 0.24 ppb in two Suffolk County, NY community water supply wells (85 feet and 90 feet deep). Additionally, imidacloprid was detected at a golf course monitoring well (0.43 ppb) and at monitoring wells near trees (0.2 to 5.1 ppb) that have been treated with imidacloprid by trunk injection for the Asian Longhorned Beetle (ALB). Imidacloprid in New York State is a Restricted-Use Product and is banned on LI from all consumer products as of October 2004.
EXTONET http://pmep.cce.cornell.edu/profiles/insect-mite/fenitrothion-methylpara/imidacloprid/imidac_reg_1004.html (see Table 1). Dr. Phil Lewis (EAB lab, USDA APHIS, MA) if these results were contradicted in subsequent analysis. He referred me to National Program Director Christine Markham, USDA APHIS (Washington DC, Telephone: (919) 855-7328, E-mail: Christine.Markham@aphis.usda.gov) whom I emailed, but have no reply. However, imidacloprid is still classified as a restricted use pesticide with no use by consumers on LI, NY.

In 2009, California initiated a review of imidacloprid's potential for soil mobility and to harm nontarget beneficials feeding on pollen and nectar. Imidacloprid is under review for preregistration by the EPA until 2014. The Natural Resources Defense Council sued the EPA in 2008 about the nontarget effects of imidacloprid on beneficials because EPA would not release industry research on the effects of imidacloprid on honeybees. In 2008 the German organization "Coalition against Bayer Dangers" in cooperation with German beekeepers sued Bayer after a proposed large bee poisoning by clothianidin in May 2008. Clothianidin has replaced imidacloprid in many products since imidacloprid went off patent in 2008. Clothianidin and imidacloprid are both neonicotinyl insecticides and have very similar LD50 for honeybees.

Why EAB is not controlled by natural forces such as predators and host plant chemistry
Minnesota ash trees are under threat from an invasive beetle accidentally introduced from Asia that attacks only ash trees. Larvae tunnel into the wood and feed on the inner bark, ultimately killing the tree. Signs of EAB infestation are: wood pecker activity and holes, waterspouts, crown dieback, D-shaped exit holes in the bark (1/8 inches in width), and feeding galleries under the bark.

Emerald ash borer has already killed more than 40 million ash trees nationwide and is found in 13 US states, including Minnesota, Wisconsin, Missouri, Illinois, Indiana, Michigan, Kentucky, Ohio, Virginia, West Virginia, Pennsylvania, New York, Maryland, and 2 Canadian provinces, Ontario and Quebec. Although the borer is able to fly 2 – 5 miles, it is moved long distances in infested logs, firewood and nursery stock. The first location of infestation was Detroit, Michigan in 2002 it is thought to have resided there for 10 or more years before it was finally discovered. DNA testing has revealed that 3 distinct populations of EAB occur in the US. EAB was first detected in the St. Anthony neighborhood in St. Paul near the University of Minnesota in May of 2009.

EAB in Michigan forests killed all ash regardless of age of stand or tree age. Even one year old ash is killed. Ash seeds only survive a year in the forest floor. Since young ash is killed, then the potential for ash to disappear from forests is possible. All native ash trees and their cultivars are susceptible to EAB. This includes Green Ash (*Fraxinus pennsyvanica*), White Ash (*F. americana*), Black Ash (*F. nigra*) and Blue Ash (*F. quadrangulata*). Manchurian ash (*F. mandshurica*) has an evolutionary history with the beetle in Asia and thus has developed pest (Rebek et al. 2008, Herms. The borer is devastating because native ash does not have chemicals that convey resistance, such as specific phenolic chemicals in their bark, as Asian ash (Eyles et al. 2007, Rebek et al. 2008, Cipollini et al 2010).

Numbers of EAB in Asia are very low because of resistant host plants, climatic conditions and natural enemies. USDA scientists are currently evaluating three parasitoids (small wasps) from China for biological control of EAB in the U.S. *Spathius agrilis* was found parasitizing up to 90 percent of EAB larvae in ash trees in China. *Tetrastichus planipennisi* is another parasitoid of EAB from China where it attacks and kills up to 50 percent of EAB larvae. *Oobius agrili* kills up to 60 percent of EAB eggs laid during the summer (www.aphis.usda.gov/plant_health/plant_pest_info/emerald_ash_b/downloads/eab-biocontrol.pdf)

Economic impact of EAB

The total losses for Ohio communities, including ash landscape losses, tree removal and replacements, are estimated to range between $1.8 and $7.6 billion. The potential total costs in Ohio are estimated to be between $157,000 and $665,000 per 1000 residents (Sydney et al. 2007). The USDA Forest Service used a computer model to predict EAB costs. The simulations predict a growing EAB infestation that is likely to include most of the 25 states. Responses to the infestation include treatment, removal, and replacement of more than 17 million ash trees at an estimated cost of $10.7 billion. (nrs.fs.fed.us/disturbance/invasive_species/eab/effects_impacts/cost_of_infestation/)

Number of ash trees in St. Paul and Minneapolis

Trees are important resources for cities as they reduce heat, absorb pollution, absorb runoff, create shade, and promote a sense of community and well-being. Trees are estimated to be worth over $290 million to Minnesota communities each year (MN DNR, www.dnr.state.mn.us/volunteer/mayjun09/ash_trees.html). There are about 937 million ash trees in Minnesota (www.ci.minneapolis.mn.us/news/20090518AshBorer.asp) which is the second highest number for a state. Minnesota ash trees typically supply between 30,000 and 40,000 cords of wood each year, mainly for pulp and paper, but also for firewood and specialty products such as cabinets, furniture, veneer, and basket-making.

In Minnesota, ash was used as replacement for elms because they grow fast and are drought and salt tolerant. The Minneapolis urban forest includes more than 200,000 ash trees on boulevards and on private property. That is 21 percent of all Minneapolis trees (Ralph Sievert, Director of Forestry, Minneapolis Park and Recreation Board (MPRB)). Minneapolis has 38,000 boulevard ash trees, which will cost an estimated $27 million to remove and replant (Jim Hermann, MPRB). Saint Paul has more than 150,000 trees on street right-of-ways and boulevards and 300,000 more trees in Saint Paul open spaces, such as parks, golf courses, and natural areas for a total of approximately 450,000 in Saint Paul. It is estimated that about 25 percent of all public trees are a variety of ash species.

Minneapolis and St. Paul recommendations for protecting ash trees

Both Twin Cities maintain an EAB awareness websites. Homeowner recommendations for treating tress are available (www.stpaul.gov/DocumentView.aspx?DID=12693). In St. Paul, there is a policy of an annual permit to chemically treat any public ash tree by hiring a City of Saint Paul licensed tree service that is bonded and insured in Minnesota. Application is limited to state approved trunk injections only. In Minneapolis, the MPRB is giving permits for trunk injection treatment of public property trees. However, a new resolution in July 2010 in Minneapolis urges residents to consider other options, such as replacing ash.

Insecticides used for managing EAB (see Table 1)

Management of EAB in landscapes employs two methods, removal of dead or dying trees and annual treatment of landscape trees with insecticides. EAB insecticide recommendations were posted in May of 2009 on the web in a multistate (Michigan, Ohio, Indiana, and Wisconsin), nonpeer reviewed report http://www.emeraldashborer.info/files/Multistate_EAB_In secticide_Fact_Sheet_22May09.pdf and summarized in a question-based website http://www.entm.purdue.edu/EAB/faq.shtml
Homeowner recommendations for treating tress are available (www.stpaul.gov/DocumentView.aspx?DID=12693).

Management bulletins suggest homeowners purchase Xytect and Bayer Advanced Tree & Shrub at local garden centers or hire an ISA Certified Arborist. Smaller trees (<less than 12 in DBH (diameter at breast height) can be treated with a soil application such as Bayer Advanced Tree and Shrub or a soil injection of imidacloprid by a certified Arborist. Larger trees (>12 in DBH) should be treated using a trunk injection method. However, insecticides need to be applied each year for all products, except trunk injections of emamectin benzoate which should last 2-3 years (McCullough et al. 2005, Smitley et al. 2010). Research results on the duration and efficacy in Michigan and Ohio can be found on numerous websites, such as USDA FS (nrs.fs.fed.us/disturbance/invasive_species/eab/control_management/systemic_insecticides, proceedings of USDA FS EAB workshops (2004-2007) (nrs.fs.fed.us/disturbance/invasive_species/eab/pubs/), and EAB multistate site (www.emeraldashborer.info/Research.cfm). Some of these abstracts are published in peer-reviewed papers.

Treatments of insecticides

Minnesota's homeowner recommendations for treating tress are available (www.stpaul.gov/DocumentView.aspx?DID=12693).

1. Passive soil surface drench: Apply liquid or granular imidacloprid to soil surface under tree with amount based on diameter of tree at breast height (DBH) or circumference for trees < 12 in DBH. Water around tree after treatment. This is the method that is available to homeowners.

2. Soil injection: Use a soil injector or deep root fertilizer probe connected to a storage tank with pressurized pump to apply pressurized imidacloprid under the soil surface for trees < 12 in DBH. Prices of soil injectors range from $300 to $17000. This is the method that is performed by professional landscapers, but cheaper probes can be purchased by homeowners.

3. Tree injection: Inject imidacloprid or emamectin benzoate into tree trunk of < 12 in DBH. Different companies produce trunk injectors, some work better than others. This is the method that is performed by professional arborists.

4. Trunk bark spray/drench: Apply insecticide (Onyx, bifenthrin or Safari, dinotefuran) to bark of tree and kill larval borers and eggs. This is the method that is performed by professional arborists. It is the least used method.

Annual costs of applying insecticides in Milwaukee and Cedarburg, Wisconsin

Milwaukee, Wisconsin started in May 2009 to treat 32,000 ash trees located on city property over two years at a cost of $475 a liter for 1300 liters or $40/tree (www.jsonline.com/news/wisconsin/42749267.html). The cost of trunk injections of emamectin benzoate is $1.2 million. By the city's estimate, it would cost $27 million to cut down and replace the city's street trees. Milwaukee has an estimated 500,000 ash trees on public and private property.

Cedarburg, Wisconsin (population 11,000) is using a similar proactive approach to Milwaukee (www.ci.cedarburg.wi.us/Forestry%20Web%20Page/emerald_ash_borer_information.htm). Cedarburg is using a different insecticide, imidacloprid. The price of replacing dead ash in Cedarburg has been estimated at $1.3 million. The suburb hired a contractor to apply insecticide to 654 trees last year and the entire public inventory of 1,600 ash trees that are 12 in DBH or larger. Repeated annual treatments are expected for at least several years. Estimated annual treatments are $70,000.

Amount AI/acre of imidacloprid for landscapes compared to agricultural fields

This high use of insecticides in landscapes for EAB is unprecedented. In agriculture, the limits of active ingredient are listed on the insecticide label. For instance, imidacloprid used for potatoes in the common formulation Admire Pro (Bayer CropScience, Kansas City, MO), places a limit of 0.3 lb/yr or 136g/yr for a 1 acre potato field which is around 4 mg AI/sqft.

In Minnesota, two imidacloprid labels have different limits; Merit 2F is labeled at 1.4 g A.I./inch (1X rate, Merit 2F) and Xytect 2F is labeled at 2.8 g A.I./inch (2X rate, Xytect 2F). Xytect 2F and Merit 2F are the same product manufactured by Bayer and provided to Rainbow Tree for distribution. Xytect 2F label of imidacloprid is available to consumers and arborists and is locally distributed (RainbowTrees, Rainbow Scientific, Treescaredirect, Minnetonka, MN) at retail outlets such as Bachmans. Although Bayer petitioned the EPA to alter its label to the higher amount, that request is pending. The Bayer Advanced

Tree and Shrub product is also available to consumers and is a different formula (Bruce Monke of Bayer (Bayer Crop Sciences, Kansas City, MO, (816) 506-3197).

Current Xytect 2F label (Rainbow Tree Care, Rainbow Scientific, Treecaredirect, Minnetonka, MN) rate: 12 ml, 0.4 lb/acre (On November 18 2010 we checked all calculations with Bruce Monke of Bayer (Bayer Crop Sciences, Kansas City, MO).
10 x 2.8=28 g/AI/10 in DBH tree; 6.5 trees-10 in DBH/acre
16 x 2.8=45 g/AI/16 in DBH tree; 4 trees-16 in DBH/acre
24 x 2.8=67g/AI/240 in DBH tree; 3 trees-24 in DBH/acre
Current Merit 2F label (Bayer, Kansas City, MO) rate: 6 ml, 0.4 lb/acre
10 x 1.4=14 g/AI/10 in DBH tree; 13 trees-10 in DBH/acre
16 x 1.4=23 g/AI/16 in DBH tree; 8 trees-16 in DBH/acre
24 x 1.4=34 g/AI/240 in DBH tree; 6 trees-24 in DBH/acre

For EAB, a surface soil application of imidacloprid is limited to 0.4 lb/yr or 181g A.I. yr. For Xytect, a 24 in DBH tree requires 67g A.I. (3 trees-24 in DBH/acre is the yearly limit) and a 16 in DBH tree requires 45g AI (4 trees-16 in DBH/acre is the yearly limit**). If we calculate the sq ft directly under a 24 in ash to be 400 sq ft, then the amount of imidacloprid applied is 168 mg/sq ft compared to 4mg/sq ft in agriculture.** In addition, imidacloprid is also the most widely used insecticide for all homeowner formulations and is the common insecticide used on lawns. The amounts used for lawn, flower, shrub, and other tree care needs to be added to the limit on AI per year. However, there is no regulatory control of the amount of AI used. Consequently, yearly treatments of insecticide for management of EAB can make the total of amount of insecticide used in landscape very high. No research has addressed the movement of imidacloprid treatments into flowering plants growing near treated trees. The consequences of these higher amounts applied to soils and then translocated to nectar and pollen on beneficial insects and pollinators is not researched and is unknown.

Landscape plant management: How are systemic insecticides different than contact insecticides in terms of translocating to pollen and nectar and affects on pollinators and beneficial insects
Systemic insecticides are applied to the soil, sprayed on foliage, or injected into the trunk and are translocated through the plant into leaves to kill target insects. These insecticides are translocated to nectar and pollen, which can alter the behavior and survivorship of nectar-feeding beneficial insects, such as predators, parasitoids, and pollinators such as bumblebees and honey bees. One of the first organophosphate insecticides found to be translocated to nectar was Schradan which was developed in WWII as a nerve gas and was discontinued in 1964. It was found at concentration of 5500 ppb in nectar of white mustard (*Sinapis alba*) flowers 3-12 d post-spraying of unopened buds (Jones and Thomas 1953). Honey bees collected the contaminated nectar and stored it as honey in the colony with no breakdown of Schradan for at least two and a half months (Anderson and Atkins 1968), but it did not cause honey bee mortality.

Another organophosphate, dimethoate (dimethoate EC, 0.1% AI no company given) applied as a spray, caused 40% mortality to honey bees when they consumed floral nectar from California bluebell, *Phacelia campanularia* Gray, borage, *Borago officinalis* L. and Argentine rape, *Brassica napus* L . Nectar from flowers that opened post-spray was toxic to honey bees for 4 d (Jaycox 1964). In another study, a foliar spray of dimethoate on containerized alfalfa, *Medicago sativa* L., (Cygon 2E, 23.4% AI, American Cyanaimid Company, Wayne, NJ) resulted in 16,000 ppb in florets that were uncovered and 5,000 ppb in covered florets. After 2 weeks, 1,000 ppb dimethoate was found in both covered and uncovered florets. Syrup spiked with 1,000 ppb dimethoate killed 8% of honey bees when fed for 7 days. However, 10,000 ppb killed 82% of honey bees in 1 day (Barker et al. 1980).

Rather than diminishing in use due to their potential effects on pollinators and beneficial insects, the use of systemic neonicotinyl insecticides have increased since other systemic neonicotinyl insecticides were registered: acetamiprid, clothianidin, dinotefuran, thiacloprid, and thiamethoxam. These insecticides were registered for forestry, poplar biomass production, crops, trees, turf, greenhouse, nursery, and urban landscapes. Imidacloprid is one of the most commonly used neonicotinyl insecticides. There are many formulations of imidacloprid which vary in the concentration of active ingredient and rate that is applied, which may affect its efficacy and duration. Formulations include Allectus and Merit, which are registered for turfgrass, Merit and Confidor for landscape, Marathon for greenhouse and nursery, Pointer and Imicide for tree injections, Admire and Provado for crops, and Gaucho for seed treatments. Since Gaucho was banned as a seed application in France, French research focused on the toxicity of this seed application to honey bees. However, the crop and urban landscape labels of imidacloprid in the U.S. use higher concentrations of active ingredients which merit attention. **No research has investigated the effects of imidacloprid applied to the soil of landscape trees and consequent runoff to nearby flowering plants, such as hosta, rose and petunia on mortality and behavior of beneficial insects and bumblebee pollinators.**

Research on effects of low levels of imidacloprid in seed treatment on bees (see Table 2)
Honey bees, *Apis mellifera*, are the primary pollinating insect in North America. The value of the increased agricultural yield and quality achieved through pollination by honey bees alone was $9.3 billion in 1989, and rose to $14.6 billion in 2000 (Morse and Calderone 2000). About 2 million colonies are rented by growers each year to service over 50 different crops. Among food crops dependent on pollination are almonds, apples, blueberries, cranberries, cherries, asparagus, broccoli, carrots, cauliflower, celery, cucumbers, onions, pumpkins, squash, sunflowers, and soybeans. 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.

Considerable research in France was done to determine if imidacloprid was translocated to nectar and pollen, and whether it altered behavior and reduced survivorship of honey bees and bumblebees. Research on Gaucho the seed treatment used in maize, sunflower, and canola demonstrated that imidacloprid was translocated to nectar and pollen, and although some studies present conflicting results, there is evidence for increased mortality and altered behavior of *Bombus* sp. and *A. mellifera,* as explained below.

For honey bees, it was found that imidacloprid is more toxic when orally ingested than by contact exposure (Suchail et al. 2000). Bayer researchers demonstrated that there was no effect on *A. mellifera* at <20 ppb (Schmuck 1999, Schmuck et al. 2001), while at concentrations >20 ppb behavior was changed as measured by a reduction in recruitment to food sources (Schmuck 1999). Data demonstrate that imidacloprid in syrup can alter behavior and kill bees. After ingesting imidacloprid for 8 d, *A. mellifera* mortality was 50% at concentrations between 0.1 and 10 ppb (Suchail et al. 2001). In another study, imidacloprid presented to *A. mellifera* at 0.5 ppb and 5 ppb in syrup for 13 days caused changes in subtle behavioral changes, such as higher frequency of pollen carrying and larger number of capped brood cells, which was reversed when contaminated syrup was no longer provided. The authors give explanations of how imidacloprid may affect behavior (Faucon et al. 2005). Imidacloprid reduced the orientation abilities of *A. mellifera* at 25 ppb (Lambin et al. 2001). Foraging bees reduced their visits to syrup feeders that had concentrations of imidacloprid at 6 ppb (Colin et al. 2004) and 50 ppb (Kirchner 1999). Reduction in recruitment was postulated as a result of decrease in effectiveness of dances at the hive to recruit bees (Kirchner 1999). Oral toxicity was identified at a LD50 of 50 ppb (Suchail et al. 2000). In another study, oral toxicity to *A. mellifera* was 370 ppb at 72 h. The olefin metabolite was more toxic (290 ppb) and the hydroxy metabolite less toxic (2060 ppb) compared to imidacloprid (Suchail et al. 2001). Chronic feeding tests revealed that imidacloprid at 48–96 ppb were lethal to caged worker bees (Decourtye et al. 2003).

Bumblebees, *B. impatiens* Cresson and *B. occidentalis* Greene exposed to 7 ppb imidacloprid showed no change in foraging rate, while bees exposed to 30 ppb had slower foraging rates and longer handling time (Morandin and Winston 2003). At 10 ppb imidacloprid in syrup *B. terrestris* L had 10% reduced survival, less brood production, and lower larval ejection by workers (Tasei et al. 2000). *Bombus impatiens* was not affected by a soil application of imidacloprid that was irrigated, although residue analysis was not done to confirm its uptake (Gels et al. 2002). Research at Biobest (2008), an international biological supplier, reported that two neonicotinyls acetamiprid and thiacloprid applied orally in syrup was toxic to bumblebees at high dose, but imidacloprid and thiamethoxam are deadly even at extremely low dosages

Residue analysis from samples collected in France from 2000 to 2003 demonstrated that imidacloprid was found in leaves, pollen, and nectar after Gaucho seed application (Bonmatin et al. 2005a,b). In maize pollen, Gaucho application resulted in 0.1 to 18 ppb (mean of 2 ppb) imidacloprid (Bonmatin et al.

2005a,b). In sunflower pollen, Gaucho application resulted in 3 ppb (Bonmatin et al. 2005a,b) and 13 ppb imidacloprid at 1.3X label rate (Laurent and Rathahao 2003). In canola pollen, Gaucho application resulted in 4.4 to 7.6 ppb imidacloprid (Scott-Dupree and Spivak 2001). Other research demonstrated that sunflower and maize pollen contained 3.3 ppb imidacloprid (Schmuck et al. 2001). Gaucho application resulted in 1.9 ppb imidacloprid in sunflower nectar (Schmuck et al. 2001) and 0.6 to 0.8 ppb in canola nectar (Scott-Dupree and Spivak 2001). A review paper concluded that honey bees were exposed to lethal and sublethal doses in fields that regularly used imidacloprid (Rortais et al. 2005). However, Bayer researchers reviewed some of the literature and plotted their results in figures, and concluded that field exposure was negligible (Maus et al. 2003). It must be reiterated that current treatments of imidacloprid are not limited to seed treatments. Foliar and soil treatments of this compound are delivered are higher rates but residue analysis and effects of bees of these common treatments has not been investigated.

Research on effects of imidacloprid on beneficial insects (see Table 2)
V. Krischik's research demonstrated that more residue ends up in nectar when applied to soil of flowering landscape plants. The label of Gaucho Grande seed application (48.7% AI, Bayer CropScience, NC), for corn and canola states that 0.375 mg AI per seed should be used. The label of Marathon 1%G (1% AI) used on perennial landscape plants states that 300 mg AI per 3-gallon or 15-cm-diameter pot should be used, which is an 800 times higher rate for one plant. 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. In contrast, canola seed treatments are diluted by the biomass of the plants as they grow and then flower 70 days after application. As a basis for comparison, Gaucho seed application resulted in 1.9 ppb imidacloprid in sunflower nectar (Schmuck et al. 2001) and 0.6 to 0.8 ppb in canola nectar (Scott-Dupree and Spivak 2001). But for buckwheat and milkweed landscape plants, a label rate of soil applied imidacloprid (Marathon 1%G) was translocated to buckwheat nectar at 18 ppb (Krischik et al. 2007) and milkweed at 41 ppb/flower (Krischik et al. 2008). These concentration of imidacloprid 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 2010 submitted). Imidacloprid foliar sprays in cotton, decreased longevity by 25% and host finding by 77% in the wasp parasitoid, *Microplitis croceipes* (Cresson) when it fed on extra floral nectaries (Stapel et al., 2000). However, the effects on bees of these higher concentrations have not been studied.

Ways in which treating landscape plants can impact pollinators and beneficial insects
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 imidacloprid prior to sale and transplanting. Imidacloprid may persist in nectar for a long time, since soil treatments 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 at flowering are cause for concern, since linden flowers are a good source of nectar and pollen for bees, butterflies, and other beneficial insects.

Nontarget effects on soil organisms
After soil drench treatments of imidacloprid to lawns, population size for 10 beneficial insects were reduced by 60% (Peck 2009). Imidacloprid applied to soil caused adverse effects on litter-dwelling earthworms and the LD50 was 25 ppm (Kreutzweiser et al. 2008, 2009). Fahem et al. 2010 determined the LD50 for earthworms was 0.11 ppm. Growth and feeding rates of isopods were reduced after imidacloprid was applied to the soil (Drobne et al. 2008). In hemlock, soil drench treatments significantly reduced abundance and species richness for the detritivore and phytophaga guilds. Of the 293 species documented to be associated with eastern hemlocks, 33 species were found to be directly effected (Dilling et al. 2009). Soil treatments of imidacloprid for hemlock wooly adelgid caused reduction in abundance and richness of Collembola springtail (Reynolds 2008).

Nontarget effects on birds
It is documented that woodpeckers increase their activity on EAB infested ash. Levels of woodpecker predation on EAB were variable, ranging from zero to 26.3 woodpecker attacks per m2 for green ash

(n=15 sites) and from 2.3 to 37.1 attacks per m2 for white ash (n=7sites). Woodpecker predation level was positively associated with the EAB density in a tree. (Lindell et al. 2006). In some trees, woodpeckers removed up to 95% of EAB larvae (Cappaert et al. 2005b). Numerous woodpeckers and sapsuckers eat sap from weeping holes in trunks (www.birdwatchersdigest.com/site/backyard_birds/bird_feeding/saptappers.aspx)

In Minnesota, there is public concern that EAB treatments may negatively affect birds. Concerns about imidacloprid and birds did not originate with Krischik; the interest in Minnesota was generated from other individuals. On June 23, 2009 Stephanie Hemphill, of Minnesota Public Radio interviewed Krischik and produced an online report entitled "Ash borer pesticide has birders concerned". In that report and interview Krischik states birds should have no harmful effect from eating EAB containing imidacloprid (minnesota.terprod.publicradio.org/display/web/2009/06/23/ash_borer_pesticide_questions/). On August 11, 2009 in the Minneapolis MN Star Tribune an article by Jim Williams entitled "Of borers and birds" reported an interview with Dr. Mike Raupp of the University of Maryland who came to the same conclusion as Krischik (www.startribune.com/lifestyle/homegarden/52963292.html).

On March 4 2010 the online Twin Cities Naturalist an article that raised the question of whether insecticide treatments for EAB could harm bees and birds (www.twincitiesnaturalist.com/2010/03/fighting-emerald-ash-borer-could-harm.html)
The article references the City of St. Paul's EAB Response Management Plan (www.highlanddistrictcouncil.org/uploads/Emerald%20Ash%20Borer/EAB%20Response%20Management%20Plan%206-15.pdf) that states on page 18...Imidacloprid is toxic to birds and wildlife and mildly toxic to fish. Imidacloprid use has been linked to eggshell thinning in birds [3], reduced egg production and reduced hatching success at exposures of 234 ppm in food. [4] It is highly toxic to certain species including the house sparrow [5], pigeon, canary, and Japanese quail [6] and on page 19... The reproductive health of birds is also affected with reduced egg production, and egg thinning. It affects a multitude of beneficial insects, as well as earthworms.

On May 29, 2010 in a radio interview and online report entitled "Urban pesticide under scrutiny report" Dan Gunderson of Minnesota Public Radio interviewed Krischik on the nontarget effects of imidacloprid on bees minnesota.publicradio.org/display/web/2010/05/28/pesticides/. During two field days in summer 2009 in Minnesota arborists discussed the dripping of imidacloprid from woodpecker holes in ash trees after they were treated with trunk injections. Since there is interest in Minnesota on the possible effects of imidacloprid on birds it is included in this grant proposal. Although no research on birds would be performed, we can relate levels of insecticides in trunk to LD50 of birds. Data and discussion could reduce the concerns of Minnesotans.

Mota-Sanchez et al 2009 that reported the amount of imidacloprid in ash wood. Trees were injected on June 15, 2004 with 6 ml (660 mg A.I.) of Imicide (10% imidacloprid A.I., J.J. Mauget, Arcadia, CA) 14C-imidacloprid, at 15 cm above ground level with two injection ports on opposite sides of the tree using Systemic Tree Injection Tubes (STITs). The research reported that at 2 m above ground by 20 d wood contained 3 ng/g which is far below the LD50 for birds; Japanese quail (100 g) has an LD50 of 31 mg/kg or 3.1 mg. However, injection of 6 ml in 2 injection sites (330 mg A.I.) could possibly produce concentrated exudates the day of injection that could affect birds.The caution here is possible, but not probable**.** It is important for concerned Minnesotans to understand the science behind injections, just as has been discussed here. In the proposed research, we would discuss the possible interaction of imidacloprid and birds. References such as Mota-Sanchez et al and our data would be discussed to alleviate concerns. So it may be possible, but not probable for a bird the day of injection to be killed by drinking imidacloprid from weeping bark holes. Again we will look for such phenomena and if it does not occur we will report it to alleviate concerns. Dialog and discussion is what we hope to generate to reduce concerns; we have no predetermined purpose to show injections are harmful to birds.

Relation of this proposal to research funded in Krischik's lab
We have the facilities and expertise to perform the research outlined in this grant proposal. We have evaluated the efficacy and duration of various Bayer products in rose and hybrid poplar for 6 years. In July of 2010 we received an LCCMR to investigate higher amounts of imidacloprid used in urban landscapes on the mortality of honeybees and bumblebees. The project does not include ash trees, trunk

injections, or measuring the seasonal amount of insecticide in wood and leaves. In 3 months of research, our preliminary results show that landscape rates of imidacloprid alter learning in bumblebees, and increase mortality and reduce pollen foraging in honeybees. In Feb of 2010 we received a USDA SARE grant to look at the residue of imidacloprid in canola and effects on pollinators. The flowers are frozen awaiting residue analysis.

Consequently our lab has experience and knowledge in applying imidacloprid, measuring residues, and bioassays with beneficial insects and bees. We will perform the ELISA residue analysis in our lab after training with Dr.Phil Lewis, Program Leader, Insecticide and Applied Technologies Section, USDA APHIS, Buzzards Bay, MA. Also, we will send samples for cross checking by the more expensive HPLC GC method to residue labs, either Dr. Roger Simmonds, USDA ARS, Gastonia, NC or Dr. Susan Nelson, ALS Laboratories, Edmonton, Al, Canada. We will hire by contract S&S trees to apply the insecticides as they are certified MN pesticide applicators and ISA certified arborists. We have contacted collaborators to identify ash trees near the St. Paul Campus.

3. Hypothesis (Objectives)
The purpose of this research is:

1. We will investigate the amount of insecticide present thru the year in trunk and leaves for different times of seasonal treatments (May, early August, and September). We will determine the efficacy and duration of imidacloprid treatmentss in MN environment where low levels of rainfall in midsummer may alter uptake of treatements. Research would relate these amounts to the known toxicity of imidacloprid and emamectin benzoate to emerald ash borer found in published studies. If it is demonstrated that uptake of imidacloprid from soils in midsummer reduces efficacy, then we can further stress the importance of spring and fall treatments in managment bulletins. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration.

2. We will determine the amount of insecticides used as a soil surface treatment that is translocated into the pollen of flowering plants growing under trees. We will investigate mortality and behavior of beneficial insects feeding on the pollen. This research will help us understand any potential nontarget effects of imidacloprid.

3. We will investigate the amount of insecticides that move from the point of application to soils under the tree. This research will help us understand any potential nontarget effects of imidacloprid.

4. We will relate the amounts of insecticide in trunks to the published toxicity of these insecticides to birds. This research will help us understand any potential nontarget effects of imidacloprid.

4. Methodology

Objective 1. Investigate the amount of insecticide present thru the year in trunk and leaves for different times of seasonal application (May, early August, and September). Research would relate these amounts to the known toxicity of imidacloprid and emamectin benzoate to emerald ash borer larvae found in published studies. We will determine the efficacy and duration of imidacloprid treatments in the MN environment where low levels of rainfall in midsummer may alter uptake of the soil surface and soil injection treatments. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration

1.1. Insecticide application

EAB insecticide recommendations were posted in May of 2009 on the web in a multistate (Michigan, Ohio, Indiana, and Wisconsin), nonpeer reviewed report http://www.emeraldashborer.info/files/Multistate_EAB_In secticide_Fact_Sheet_22May09.pdf and summarized in a question-based website http://www.entm.purdue.edu/EAB/faq.shtml
Homeowner recommendations for treating tress are available in the MN Dept of Ag bulletin (www.stpaul.gov/DocumentView.aspx?DID=12693). We are not recommending any treatment that was not suggested in the MN Dept of Ag bulletin. We only treat with emamectin benzoate in Spring as recommended. We treat with trunk injections of imidacloprid in Spring and Fall as recommended.

However, the Xytect 2F label does not state that passive drench and soil injection treatments should not be performed in mid Summer. In addition, mid Summer treatments are being performed by homeowners and some arborists in MN. This proposal will determine whether mid Summer soil and trunk injections are as effective in creating residue levels that have the same efficacy and duration as Spring and Fall treatments. We know of no other published research that addressed this issue.

The grant funds are available in late July 2011 for 3 years (July 2011 to June 2014). We will perform no new treatments in 2013-2014, but will sample treated trees. In the first Summer, 2011, we will inventory trees and perform fall treatments of passive soil applied imidacloprid, soil injection of imidacloprid, and trunk injection of imidacloprid. In Spring 2012 we will perform treatments of emamectin benzoate and the 3 imidacloprid treatments. In August 2012 we will perform the 3 imidacloprid treatments. S & S trees (Contact Gail Nozal, certified ISA arborist and MDA pesticide applicator, South St. Paul, MN) will use the standard treatments that they offer to customers. We will use trees greater than 8 inch DBH as they can use the higher rate on the Xytect 2F label for soil drench and soil injection. We will use trunk injection with M3 infuser of imidacloprid (Xytect Infusible) (Rainbow Treecare Scientific, Treecaredirect, Minnetonka, MN) at 5 ml/DBH, 1 injection site/2inch DBH. We will use the Tree IV injection system forTREE-age (emamectin benzoate) medium rate (55 ml/ 10-12 inch DBH).

Landscape trees will be used on the UM St. Paul campus, MPRB areas, and St. Paul, Roseville, Falcon Heights areas. In July 2011 tree locations will be arranged. Each tree chosen for this study was marked with a permanent aluminum tag and its coordinates recorded by a global positioning system to help locate the tree at later dates.

In Minnesota, two imidacloprid labels have different limits; Merit 2F is labeled at 1.4 g A.I./inch (1X rate, 6 ml, Merit 2F) and Xytect 2F is labeled at 2.8 g A.I.inch (2X rate, 12 ml, Xytect 2F). Xytect 2F and Merit 2F are the same product manufactured by Bayer and Xytect 2F is sold to Rainbow Tree for distribution. Xytect 2F label of imidaclopird is available to consumers and arborists and is locally distributed (RainbowTrees, Rainbow Scientific, Treescaredirect, Minnetonka, MN) at retail outlets such as Bachmans. Although Bayer petitioned the EPA to alter its label to the higher amount, that request is pending. We will not use the Bayer Advanced Tree and Shrub product that is also available to consumers, which is a different formula (Bruce Monke of Bayer (Bayer Crop Sciences, Kansas City, MO, (816) 506-3197).

On November 18 2010 the we checked all calculations with Bruce Monke of Bayer (Bayer Crop Sciences, Kansas City, MO, (816) 506-3197).

Current Xytect 2F label (Rainbow Tree Care, Rainbow Scientific, Treecaredirect, Minnetonka, MN) rate: 12 ml, 0.4 lb/acre

10 x 2.8=28 g/AI/10 in DBH tree; 6.5 trees-10 in DBH/acre
16 x 2.8=45 g/AI/16 in DBH tree; 4 trees-16 in DBH/acre
24 x 2.8=67g/AI/240 in DBH tree; 3 trees-24 in DBH/acre

Current Merit 2F label (Bayer, Kansas City, MO) rate: 6 ml, 0.4 lb/acre

10 x 1.4=14 g/AI/10 in DBH tree; 13 trees-10 in DBH/acre
16 x 1.4=23 g/AI/16 in DBH tree; 8 trees-16 in DBH/acre
24 x 1.4=34 g/AI/240 in DBH tree; 6 trees-24 in DBH/acre

Treatments and methods of applying insecticides:

Rates to be used:

1. Soil drench of liquid around the base of the tree with Xytect 2F (Rainbow Treecare, Rainbow Scientific, Treecaredirect, Minnetonka, MN); 0.4 fl oz or 12 ml/inch DBH; dispersed in the area beneath the tree; Basal system: Make application 6 to 12 inch from base of tree,
n=6 spring 2012 + n=6 August 2012+ n=6 Fall 2011=18 trees
2. Soil injection with Xytect 2F (Rainbow Treecare, Rainbow Scientific, Treecaredirect, Minnetonka, MN) and fertilizer probe (American Arborist FMC soil injector, West Chester, PA); 0.4 fl oz or 12 ml/inch DBH; dispersed in the area beneath the tree; Basal system: Make application 6 to 12 inch from base of tree,
n=6 spring 2012 + n=6 August 2012+ n=6 Fall 2011=18 trees
3. Trunk injection with M3 infuser of imidacloprid (Xytect Infusible) (Rainbow Treecare, Rainbow Scientific, Treecaredirect, Minnetonka, MN); rate 5 ml/DBH, 1 injection site/2inch DBH

n=12 spring (6 trees for weeping sap) 2012 + n=6 August 2012+ n=6 Fall 2011=24 trees
4. Trunk injection with Tree IV of emamectin benzoate(TREEage) (ArborJet, Woburn, MA); medium rate 55 ml, 10 to 12 inch DBH, Inject at base of tree within 12 inches of soil with every 6 inches circumference.
n=8 spring 2012 = 8 trees
5. Untreated control
n=12 spring 2012 + n=6 August 2012+ n=6 fall 2011=24 trees
Total trees = 92

1.2 Sampling leaves, trunk cores, and exudates
The grant funds are available in late July 2011 for 3 years (July 2011 to 2014). We will perform no new treatments in 2013-2014, but will sample treated trees for 2 years. In the first Summer, 2011, we will inventory trees and perform fall treatments of passive soil applied imidacloprid, soil injection of imidacloprid, and trunk injection of imidacloprid. In Spring 2012 we will perform treatments of emamectin benzoate and the 3 imidacloprid treatments. In August 2012 we will perform the 3 imidacloprid treatments.

We will use a sampling method similar to Mota-Snchez et al 2010. Samples will be take from ash trees at 0, 4, 8, 12 weeks after treatment for year 1 and then the same dates in year 2 starting in Spring 2012. We will sample in summer 2012, 2013, and 2014. At each sampling date from each tree (6 trees per treatment) we will collect 12 samples of 20 g wet weight leaves for ELISA (9 samples) and 3 samples frozen for HPLC GC analysis. Samples of leaves will be taken from the lower, mid and upper canopy of each tree with a pole pruner or the use of a S &S bucket tree. Samples of the trunk will be collected using a 0.5 cm diameter corkborer to remove a 0.4 cm thick disk of bark and wood at 1 m and 2 m above ground line at a 90 degree angle to the injection point. Mota-Snchez et al 2010 indicates that such trunk core samples were comprised mainly of bark and phloem, but also contained a small amount (generally <10%) of xylem material. After trunk injection treatment of imidacloprid in Spring 2012 we will drill 6 holes/ tree at 1.3 m above the injection site and determine if sap leeks out of those holes for the next 3 days and store the exudate in 20 ml scintillation vials (Fisher Scientific)(n=6 trees imidacloprid trunk injection and n=6 controls) for HPLC analysis. Samples for HPLC will be frozen.

1.3 Statistics
We will use the PROC GLM (SAS, JMP) for most statistical analysis, unless otherwise stated. We will first use Levene's test to determine homogeneity of variance. If variances are unequal, a Welch test will be used (JMP, SAS). If variances are equal, data will be analyzed with one way for treatment, replicate and replicate by treatment interactions. Means will be compared with Tukey's HSD test. We will use PROC MIXED for any data that needs repeated measures statistics (SAS).

1.4 Relating residue to EAB toxicity
We will relate levels of imidacloprid found in leaves and trunk to published LD50 values for adult beetles. In bioassays LD50 for the adults of EAB was 7.1 ng/beetle (around 71 ng/g= 71 ppb if an adult weighs 0.1g), which confirms that EAB is very susceptible to imidacloprid in comparison to other insect species (Cregg et al. 2005, www.invasive.org/eab/chemicalcontrol.cfm#8). Levels of 4-40 ppm imidacloprid caused mortality in EAB adults (Cappaert et al. 2007). In a more recent study, EAB adult mortality ranged from 70 to 100% at 64 ug/g (ppm) (Mota-Ssanchez et al. 2009).

Emamectin benzoate is highly toxic to EAB larvae and adults as verified by the high mortality in published studies (Smitley et al. 2010). However, we could only find an LD50 of 0.6 ppb for another order of insect on fruit trees, the obliquebanded leaf roller (Proclam label)
(www.nysaes.cornell.edu/ent/faculty/jentsch/pdf/historical-perspectives-on-apple-production.pdf
We spoke with Dr. Phil Lewis, Program Leader, Insecticide and Applied Technologies Section, USDA APHIS, MA if he knew of any published studies on the LD50 of emamectin benzoate and he did not.

1.5. Cleanup of samples for leaves and trunk wood (Lewis and McCollough 2004, Cowles et al. 2006, Eisenbeck et al. 2009, Arora 2009
A 20 g sample of leaves will blended with 50 mL acetonitrile in a blender and filtered through Whatman filter paper No.1. The acetonitrile extract will be evaporated to near dryness (5 mL) using rotary vacuum evaporator and diluted with 50 mL of saturated sodium chloride solution and partitioned thrice into hexane

(3 9 50 mL). Discard the hexane layers and again partition the lower aqueous phase with hexane: ethyl acetate (98:2, v/v). Again discard the organic layers. Lower aqueous phase is again partitioned thrice into dichloromethane using 50 mL each time. The pooled dichloromethane extracts are passed through anhydrous sodium sulfate, treated with 500 mg activated charcoal powder for 2 h. Filter the clear extract through Whatman filter paper No. 1 along with rinsing of dichloromethane and evaporated to near dryness using rotary vacuum evaporator. The sample will be frozen until analysis. The assay values for tissues are converted to ppb of their fresh weight equivalent.

Trunk samples will be taken with a sample (1 g) of pulverized tissue added to 10.00 ml of histological grade acetone in a 60-ml environmental sample vial and shaken horizontally overnight (2 cycles/s). After allowing particulate matter to settle (1 h), a 1.000-ml aliquot will be converted to an aqueous suspension by allowing the acetone to evaporate and vortexing the residue in 1.000 ml of distilled water. The sample will be frozen until analysis. The assay values for tissues are converted to ppb of their fresh weight equivalent.

1.6. Cleanup for flower pollen (according to Krischik et al. 2007, 2010)

Since pollen collection is easier than nectar collection, we will collect pollen form plants growing under treated ash trees. We discussed flower collection in Section 2.1. For residue analysis, each sample of 1.0 g of pollen (Mexican Milkweed (*Asclepias curassavica*, 100 flowers in 1 g), buckwheat *(Fagopyrum esculentum,* 424 flowers in 1 g) and Rosa (30 flowers in 1 g)) will be used. Each 1 g sample 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 sample will be frozen until analysis. The assay values will be converted to ppb flower.

1.7. Cleanup for soils (Cleanup for soils Arora 2009, Cowley 2010)

A representative 50 g soil will be mixed with 50 mLmixture of acetonitrile:water (7:3, v/v) in 250 mL conical flask and shaken for 3 h on an electrical shaker at 150 rpm. Soil suspension will be filtered through Whatman filter paper No.1, washed twice with 50 mL acetonitrile and water mixture (7:3 v/v) and finally with 30 mL acetonitrile only. Filtrate will be concentrated to about 50 mL on rotary vacuum evaporator and partitioned thrice into dichloromethane using 50 mL each time. Dichloromethane fractions will be collected and dried over anhydrous sodium sulfate. The extract will finally be concentrated to near dryness under rotary vacuum evaporator and the residues will be dissolved in 5 mL acetonitrile (HPLC grade). The sample will be frozen until analysis. The assay values will be converted to ppb for 1g soil.

1.8. Imidacloprid and emamectin benzoate, ELISA method (Lewis and McCollough 2004, Cowles et al. 2006, Eisenbeck et al. 2009)

Krischik and grant supported researchers were invited by Dr. Lewis to the USDA EAB lab to learn the ELIZA method for imidacloprid and emamectin benzoate.

We spoke to Dr. Richard Cowles (Conn. Ag. Exp. Station, New Haven, CT) and Dr. Phil Lewis about the ELISA procedures. Dr. Cowles altered the procedures in his published paper, most notably not performing a duplicate analysis for each sample. Dr. Phil Lewis has used the method in his published papers and has offered to let our lab visit him and receive training on the ELISA methods for imidacloprid and emamectin benzoate. The ELISA method for imidacloprid is a premade test kit with instructions available for purchase from EnviroLogix (EP-006 Imidacloprid Quantiplate Kits, EnviroLogix, Portland, ME) (www.xygen.com/pesticides/ImidaclopridSeed.htm). The EnviroLogix Imidacloprid Plate Kit is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, Imidacloprid pesticide residues in the sample extract compete with enzyme (horseradish peroxidase) labeled Imidacloprid for a limited number of antibody binding sites on the inside surface of the test wells. After a simple wash step, the outcome of the competition is visualized with a color development step. As with all competitive immunoassays, sample concentration is inversely proportional to color development (darker color = lower concentration). In my discussion with Dr. Lewis he indicated that we should purchase a EMax microplate reader and software (www.selectscience.net/products/emax-microplate-reader/?prodID=11247) .

The emamectin benzoate test kit is available from Horiba (Horiba, Ltd., 2 Miyanohigashi, Kisshoin, Minami-ku, Kyoto 601-8510) www.horiba.com/us/en/analysis-based-on/immunochemistry/immunoassay/details/emamectin-1657/.

1.9 Imidacloprid and emamectin benzoate, HPLC GC method

Residue amounts are crossed checked by analyzing some samples with HPLC at the USD-ARS lab in Gastonia, NC, lab supervisor Dr. Roger Simonds (Laurent and Rathahao 2003).

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 spiking standards are prepared in 20% acetonitrile/0.1% acetic acid. Samples are fortified with imidacloprid, hydroxy, and olefin at 0.05 and 0.10 ppm. Retention time will be 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 is 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 is 95%, 74%, and 96% respectively at 0.05, 0.10, and 15 ppm.

For emaemctin benzoate HPLC equipped with a fluorescence detector will be used in the laboratory of Dr. Roger Simonds, USDA ARS (Takei et al. 2003a,b). The column needed is a reversed phase, and 418nm emission. I do not have personal experience with this method.

Objective 2. Determine through residue analysis the amount of insecticides used as a soil surface treatment into the pollen of flowering plants growing under trees. Determine the consequences on beneficial insects feeding on the pollen. This research will help us understand any potential nontarget effects of imidacloprid.

2.1. Uptake of imidacloprid from passive soil drench treatment under ash trees into flowers

We will treat a different set of trees for the uptake of imidacloprid by flowers. In order to care for the flowers, we need to establish these gardens under trees in the UMinnesota Campus that are near a hose for irrigation. A soil drench of liquid around the base of the tree with Xytect 2F (Rainbow Treecare, Rainbow Scientific, Treecaredirect, Minnetonka, MN); 0.4 fl oz or 12 ml/inch DBH; dispersed in the area beneath the tree; Basal system: Make application 6 to 12 inch from base of tree. In Spring under each tree we will plant fully grown plants: 6 shrub roses (Carefree Beauty), 12 Mexican milkweeds (*Ascelpius curasavica*), and 24 buckwheat *(Fagopyrum esculentum),* which amounts to 42 plants under each tree (n = 10 soil drench and n = 10 controls). All three of these plants are indeterminate and produce new flowers all the time until frost. We performed HPLC GC residue analysis on pollen for imidacloprid passive soil treatments on Mexican Milkweed (*Asclepias curassavica*, 100 flowers in 1 g), buckwheat *(Fagopyrum esculentum,* 424 flowers in 1 g). Currently, Rosa Mr. Lincoln (30 flowers in 1 g) is at the residue lab (ALS, Edmonton, Canada). For HPLC GC residue analysis a single sample needs to be 1g. *A* spray of 5 gallons of water M, W, and F will simulate irrigation. This is similar to current practices as flowers need additional watering. Every 3 weeks for 3 months flowers will be collected for analysis of imidacloprid in pollen only. Flowers from the same plant will be used in bioassays. We used these protocols in published studies (krischik .

1. Soil drench of liquid insecticide around the base of the tree with Xytect 2F (Rainbow Treecare, Rainbow Scientific, Treecaredirect, Minnetonka, MN); 0.4 fl oz or 12 ml/inch DBH; dispersed in the area beneath the tree; Basal system: Make application 6 to 12 inch from base of tree (n = 10 soil drench and n = 10 controls).
2. Plants will be ordered from North Creek Nuurseries (Landenburg, PA) as plugs and 3 gallon roses from Linders Greenhouse (St. Paul, MN) grown in the GH prior to planting. Under each of the 20 trees, 6 shrub roses (Carefree Beauty), 12 Mexican milkweeds (*Ascelpius curasavica*), and 24 buckwheat *(Fagopyrum esculentum),* which amounts to 42 plants under each tree.

3. Flowers will be cut off from the plant once a week and placed in plastic bags labeled as to tree and plant individual on ice. They will be returned to the lab. Within 2 hrs the anthers will be cut from the flowers and placed in a 20 ml scintillation vial (Fisher Scientific) in the ultralow freezer. We need 1 g for HPL GC analysis. We will collect enough flowers for 5-1g samples of each plant for under each tree for each month (May to September). Thd number of flowers/g based on our previous research is: Mexican Milkweed (*Asclepias curassavica*, 100 flowers in 1 g), buckwheat *(Fagopyrum esculentum,* 424 flowers in 1 g). Currently, Rosa Mr. Lincoln (30 flowers in 1 g)

2.2 Mortality and behavior of beneficial insects (Smith and Krischik 1990, Rogers et al. 2007, Krischik et al. 2007, Krischik et. al 2010 submitted).

Preconditioning insects. Beneficial insects, green lacewing (*Chrysoperla carnea*, 1 species of *parasitoid* 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 field-collected flowers, commercial artificial diet for lacewings and lady beetles (Rincon-Vitova), and 50% honey-water (Aquatube, Syndicate Sales, Kokomo, IN).

Lady beetle adult survival bioassays on rose and milkweed. Three replicate experiments will be performed with 3 treatments, each with 10 bioassay containers with 10 ladybeetles for 2 flower species (rose and milkweed). Treatments will be: C (20 containers in each of 3 experiments) and 2X (Xytect label rate) (20 containers in each of 3 experiments). Bioassay containers are10 cm × 2 cm (diameter) Aquapic® (Syndicate Sales, Inc., Kokomo, Indiana) water tubes. In the plastic cap of each water tube a 0.5 ml centrifuge tube with a hinged plastic cap and pointed end was inserted. A hole was made in the pointed end to accommodate a milkweed flower stalk or a rose flower stalk. Water was placed inside the centrifuge tube to keep the flower hydrated. Flowers were changed every other day to insure pollen availability. All bioassay chambers were kept in laboratory incubators and maintained under a photoperiod of 12:12 (L: D) h, 25 °C, and 70 to 75% RH (Krischik et al. 2010 submitted). Data analysis by PROC GLM (SAS, JMP).

Lacewing and parasitoid wasp adult survival bioassays on rose and milkweed. Mesh cages used to precondition insects will be used as bioassay containers. Three replicate experiments will be performed. Treatments will be C and 2X (Xytect label rate) for 2 flower species (rose and milkweed). Each treatment/ plant species will have 10 bioassay containers with 50 green lacewings or parasitoid *Anagyrus psuedococci. Anagyrus psuedococci* and lacewings are available from Rincon Vitova, both can live for 30 days, and feed on nectar and pollen. In each box, 10-20 ml scintillation vials will be filled with flowers daily. Each day survial and trembling will be recorded. Data analysis by PROC GLM (SAS, JMP)

Cold anthrone test. A cold anthrone test was performed to determine the presence of fructose sugars from nectar within the guts of *C. carnea* and ladybeetles after feeding on flowers for 24 h (Van Handel, 1967). The anthrone reagent was prepared by adding 380 ml of sulfuric acid to150 ml deionized water and 0.75 g of anthrone was stirred over an ice bath (Van Handel 1965). After 1 h, a dark green color indicated a positive reaction, confirming the presence of fructose in nectar, indicating that the insects were feeding. Thie test confirms that the insects were feeding. Data analysis by Chi-square (X^2) test (SAS JMP).

2.3 Mortality and behavior of individual bees We will obtain commercially purchased bumblebee 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 will provide these colonies with sugar syrup at the doses found in the flowers and study affects on mortality, behavior, and colony parameters (number of workers, drones, etc).

We will follow published protocols to study the effects on the behavior and survivorship of bumblebees (Regali and Rasmont 1995, Tasei et al. 2000, Babendreier et al. 2008). Thirty large (forager) bumblebee 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 at levels found in residue analysis and 2 other levels: at X ppb (found in residue analysis), a second colony with 40 ppb (concentration found in milkweed nectar in our previous studies), and a third colony at 200 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 bumblebee at the feeder. The experiment will last for 5 days. The experiment will be repeated three times, using new hives for each replicate. Data analysis by PROC GLM (SAS, JMP).

2.4. Effects of imidacloprid on bumblebee 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 bumblebees, 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 bumblebees 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). Data analysis by PROC GLM (SAS, JMP) and PERS learning curves (Simone et al. 2010).

2.5. Effects of imidacloprid on bumblebee health. In this study to be conducted over 2 years, we will use micocolonies of bumblebees following previously established methods to measure lethal and sublethal effects of insecticides on bumblebees (Regali and Rasmont 1995; Tasei et al, 2000; Babendreier et al, 2008). Microcolonies of *B. impatiens* will be established by placing three newly emerged bumblebee 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), used in 2.4 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 bumblebees 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 bumblebee survivorship after the different imidacloprid treatments, mean weight of surviving bumblebees, number of offspring produced, and consumption of sucrose and pollen. The experiment will be repeated three times, using new hives for each replicate. Analysis is by PROC GLM and PERS learning curves (Simone et al. 2010).

Objective 3. We will investigate the amount of insecticides that move from the point of application to soils under the tree. This research will help us understand any potential nontarget effects of imidacloprid.

3.1 Movement of insecticides in soil away from the site of placement: Field collection from soil under trees

The grant funds are available in late July 2011 for 3 years (July 2011 to 2014). We will perform no new treatments in 2013-2014, but will sample treated trees for 2 years. In the first Summer, 2011, we will inventory trees and perform fall treatments of passive soil applied imidacloprid, soil injection of imidacloprid, and trunk injection of imidacloprid. In Spring 2012 we will perform treatments of emamectin benzoate and the 3 imidacloprid treatments. In August 2012 we will perform the 3 imidacloprid treatments.

We will sample soil under treated trees when we collect leaves and trunk cores Samples will be take from under ash trees at 0, 4, 8, 12 weeks after treatment for year 1 and the same time periods in year 2. At each sampling date from each tree (6 trees per treatment) we will collect 6-50g samples next to the trunk and 6-50g samples 3 ft for ELISA (4 samples) and HPLC GC (2 samples) analysis. Data analysis by PROC GLM (SAS, JMP)

Objective 4. We will relate the amounts of insecticide in trunks to the published toxicity of these insecticides to birds. This research will help us understand any potential nontarget effects of imidacloprid.

4. 1 Statistical correlation of LD50 of imidacloprid and enamcetin benzoate residue levels in trunk cores on published LD50 of birds

Mota-Sanchez et al 2009 that reported the amount of imidacloprid in ash wood. Trees were injected on June 15, 2004 with 6 ml (660 mg A.I.) of Imicide (10% imidacloprid A.I., J.J. Mauget, Arcadia, CA) 14C-imidacloprid, at 15 cm above ground level with two injection ports on opposite sides of the tree using Systemic Tree Injection Tubes (STITs). The research reported that at 2 m above ground by 20 d wood contained 3 ng/g which is far below the LD50 for birds; Japanese quail (100 g) has an LD50 of 31 mg/kg or 3.1 mg. However, injection of 6 ml in 2 injection sites (330 mg A.I.) could possibly produce concentrated exudates the day of injection that could affect birds. The caution here is possible, but not probable**.** It is important for concerned Minnesotans to understand the science behind injections, just as has been discussed here. In the proposed research, we would discuss the possible interaction of imidacloprid and birds. References such as Mota-Sanchez et al and our data would be discussed to alleviate concerns. So it may be possible, but not probable for a bird the day of injection to be killed by drinking imidacloprid from weeping bark holes. Again we will look for such phenomena and if it does not occur we will report it to alleviate concerns. Dialog and discussion is what we hope to generate to reduce concerns; we have no predetermined purpose to show injections are harmful to birds. Data analysis by PROC REG (SAS, JMP)

The EPA has published information on the acute LD50 of imidacloprid and emamectin to a few species of birds (bobwhite quail, Japanese quail, and in some cases starlings) based on mg insecticide to kg of body weight. We will make a regression of body weight of birds used in toxicology tests and native woodpeckers (red-headed, downy, hairy, red-bellied woodpeckers, common flicker, and yellow-bellied sapsucker), and hummingbirds (ruby-throated) found in the bird literature. Then, we will relate the LD50 based on body weight to the weight of native species. This will provide a rough estimate if the ranges found in trunk could potentially affect birds eating sap from woodpecker holes.

Table 1. Potential for neonicotinyl insecticides (imidacloprid, thiamethoxam, dinotefuran, clothianidin) and emamcetin benzoate to move to flowering plants and water

Insecticide	Water solubility Koc=soil binding	Soil half life (aerobic)	LD50 birds	LD50 fish	LD50 crustaceans	LD50 bees
imidacloprid 1994 Bayer "restricted use" in LI, New York State, as of January 1, 2005	Sol=514ppm Koc= 132-310 Potential to leach	27-299 days	Bobwhite quail 152 mg/kg Japanese quail 31 mg/kg Toxic to birds.	fish 211ppb	Daphnia 85 ppm Very toxic	bees 80ppb highly toxic
The low Koc of 132 to 310, combined with a high water solubility of 514 ppm, suggests a potential to leach to ground water http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/imid.pdf The three other neonicotinoids included in this reevaluation of imidacloprid, clothianidin, dinotefuran, and thiamethoxam, are in the same chemical family (nitroguanidines) as imidacloprid have soil mobility characteristics and half-lives that are very similar to imidacloprid. A University of California found imidacloprid residues in eucalyptus nectar at levels of up to 550 parts per billion (ppb) and the lethal concentration of imidacloprid needed to kill 50 percent of a test population (LC50) of honey bees is 185 ppb. http://www.cdpr.ca.gov/docs/registration/canot/2009/ca2009-02.pdf I imidacloprid has been detected at concentrations (0.2 to 7 ppb) in 12 monitoring wells and 16 down gradient private homeowner wells. Imidacloprid has also been recently detected at 0.24 ppb in two Suffolk County community water supply wells (85 feet and 90 feet deep). Additionally, imidacloprid has now been detected at a golf course monitoring well (0.43 ppb) and at monitoring wells near trees (0.2 to 5.1 ppb) that have been treated with imidacloprid by trunk injection for the Asian Longhorned Beetle (ALB). Imidacloprid in New York State as Restricted-Use Products 10/04 EXTONET http://pmep.cce.cornell.edu/profiles/insect-mite/fenitrothion-methylpara/imidacloprid/imidac_reg_1004.html						
thiamethoxam 2003 Syngenta	Sol=327mg/l Koc=64 Leaching highly mobile	229 days	Bobwhite quail 1552ppm	fish 100ppm	Daphnia 100ppm	bees 120ppb highly toxic
Thiamethxam converted to dinotefurn by plants and animals. Mobile, high potential for leaching EXTONET http://pmep.cce.cornell.edu/profiles/insect-mite/propetamphos-zetacyperm/thiamethoxam/thiameth_sln_0602.html						
dinotefuran 2004 Bayer	Sol=259mg/l Koc=23-33 Leaching highly mobile	214 days	Bobwhite quail 5000mg/kg Japanese quail 2000mg/kg	fish 100ppm	Dapnia 1000ppm	bees 43ppb highly toxic
Dinotefuran may also potentially be present in drinking water, given its high water solubility, high mobility in soils, and potential persistence in the environment. Therefore, exposures and risks from food and drinking water need to be assessed, as well as from residential uses. EXTONET http://pmep.cce.cornell.edu/profiles/insect-mite/ddt- EPA Pesticide Factsheet 2004 http://www.epa.gov/opprd001/factsheets/dunotefuran.pdf						
clothianidn 2003	Sol=259mg/l Koc=160 Leaching highly mobile	495 days	Bobwhite quail 2000mg/kg	fish 117ppm	Daphnia 1000ppm	bees 43ppb highly toxic
BOH's primary concerns are that clothianidin is likely to reach surface waters contributing to contaminant loading and potentially impacting non-target aquatic species, and that nontarget pollinators attracted to pollen or nectar in treated areas will be exposed to potentially toxic residues in						

those resources. EXTONET http://pmep.cce.cornell.edu/profiles/insect-mite/cadusafos-cyromazine/clothianidin/clothianidin_den_0707.pdf						
emamectin benzoate MN State 2009 EPA2010 Syngenta, TreeAge	Sol=101mg/l 0.024 g/l (pH 7, 25°C). Koc=283,000 Low mobility so remain in trees for long duration.	427 days	Bobwhite quail 264 mg/kg highly toxic	fish 174ppb highly toxic	Daphnia 1ppb highly toxic	bees 35ppb highly toxic
EPA 24C as restricted use pesticide due to hazards to humans. A group of chemically related macrlacytic lactones (Abamectin" originally a 80:20 mixture of avermectins) produced by fermenting *Streptomyces avermitilis*.						

Table 2. Potential nontarget effects on pollinators and beneficial insects		
Commodity	***imidacloprid(A.I.) treatment rate***	***residue in nectar or pollen (research paper)***
DBH apple tree-method DBH eucalyptus-soil drench **16 in DBH tree- passive soil drench** **roughly 4 -16 in DBH trees trees/acre (1 acre=5 St. Paul lots of 0.2 acre) for the 0.4lb/acre limit** **24in DBH tree- passive soil drench** **roughly 3 -24 in DBH trees trees/acre (1 acre=5 St. Paul lots of 0.2 acre) for the 0.4lb/acre limit**	label rate label rate **Xytect 2X rate 16inDB =45 gA.I.** **Xytect 2X rate 24inDBH= 67gA.I.**	4000 ppb unpublished, CA Dept 550 ppb, Payne 2010 **not researched, this proposal** **not researched, this proposal**
3 ft plant in landscape, rose	300-600 mg	Krischik lab in progress 2010
Greenhouse/nursery pots before planting outdoors; can reapply	300 mg /3galpot	20 to 54 ppb (Krischik et al. 2007, 2009)
field crops most formulas Admire Pro,etc	4 mg/sg ft	range 30-101 ppb in pollen and 4-14 ppb in nectar cucurbits (not published yet) (UMaryland, 2010)
Seed treatment Gaucho* *99% of research on this application	0.11 mg / canola plant 0.600 mg / corn or sunflower 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)
Table 2B. Levels that alter behavior or kill pollinator or beneficial insect (passive pollinators)		
Insect species	***imidacloprid level***	***level residue affecting insects (research paper)***
Kills beneficial insects: 4 species ladybeetle predator, lacewing predator, parasitic wasp Reduces honeybee foraging and pollen storage Reduces bumblebee learning	15 ppb 20-200 ppb 1ng/bee(10 ppb)	(Krischik et al. 2007, 2009) Krischik lab in progress 2010 Krischik lab in progress 2010
Kills honeybees one sip NOEC (no effect concentration)	158-185 ppb <5ppb	(CA imid review, 2009; Bayer report 2007) (PAN-Europe letter 2009)

actute oral acute contact	40 ng/bee=400ppb 40-102 ng/bee	(Suchail et al 2001) (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 *B. impatiens* *B. terrestris* *B. impatiens*	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)

5. Results and Deliverables, Total budget: $340,000

Result 1.We will investigate the amount of insecticide present thru the year in trunk and leaves for different times of seasonal treatments (May, early August, and September). We will determine the efficacy and duration of imidacloprid treatmentss in MN environment where low levels of rainfall in midsummer may alter uptake of treatements. Research would relate these amounts to the known toxicity of imidacloprid and emamectin benzoate to emerald ash borer found in published studies. If it is demonstrated that uptake of imidacloprid from soils in midsummer reduces efficacy, then we can further stress the importance of spring and fall treatments in managment bulletins. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration.
Budget: $200,000

Deliverable 1.	Completion Date
1. Determine concentration of imidacloprid and emamectin benzoate in leaves and trunk at 3 seasonal times (May, July, and September).	2014
2. Determine the duration and efficacy of treatments by comparing residue levels to published LD50 for EAB,	2014

Result 2. We will determine through residue analysis the amount of insecticides useds as a soil surface treatment that is translocated into the pollen of flowering plants growing under trees. We will investigate mortality and behavior of beneficial insects feeding on the pollen. This research will help us understand any potential nontarget effects of imidacloprid. Budget: $70,000

Deliverable 2.	Completion Date
1. Determine concentration of imidacloprid in leaves and pollen of flowers growing under treated trees.	2014
2. Determine the effects of these concentrations on behavior and mortality through bioassays with beneficial insects (lady beetles, green lacewing, and parasitic wasp, bumble bee).	2014

Result 3. We will investigate the amount of insecticides that move from the point of application to soils under the tree. This research will help us understand any potential nontarget effects of imidacloprid.
Budget: $70,000

Deliverable 3.	Completion Date
1. Determine movement of imidacloprid in soil at 3 distances from treated ash.	2014

Result 4. We will relate the amounts of insecticide in trunks to the published toxicity of these insecticides to birds. This research will help us understand any potential nontarget effects of imidacloprid. Budget: $0

Deliverable 4.	Completion Date
1. This research will help us understand any potential nontarget effects of imidacloprid and submit recommendations.	2014

6.Timetable

Research result 1. We will investigate the amount of insecticide present thru the year in trunk and leaves for different times of seasonal treatments (May, early August, and September). We will determine the efficacy and duration of imidacloprid treatmentss in MN environment where low levels of rainfall in midsummer may alter uptake of treatements. Research would relate these amounts to the known toxicity of imidacloprid and emamectin benzoate to emerald ash borer found in published studies.

Deliverable 1. If it is demonstrated that uptake of imidacloprid from soils in midsummer reduces efficacy, then we can further stress the importance of spring and fall treatments in managment bulletins. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration.

	July 2011 -June 2012				**July 2012-June 2013**				**July 2013-June 2014**			
	Su	Fall	Win	Sp	Su	Fall	Win	Sp	Su	Fall	Win	Sp
Establish plants, treat with	x	x		x	x							
Collect leaves and trunk	x	x		x	x	x		x	x	x		x
Perform residue analysis		x	x	x	x	x	x	x		x	x	x
Research paper								x		x	x	x
Website				x	x	x	x	x		x	x	x

Research result 2. We will determine through residue analysis the amount of insecticides useds as a soil surface treatment that is translocated into the pollen of flowering plants growing under trees. We will investigate mortality and behavior of beneficial insects feeding on the pollen.

Deliverable 2. This research will help us understand any potential nontarget effects of imidacloprid.

	Su	Fall	Win	Sp	Su	Fall	Win	Sp	Su	Fall	Win	Sp
Establish plants, treat with				x	x	x		x	x	x		
Collect leaves and flowers				x	x	x		x	x	x		
Perform residue analysis				x	x	x		x	x	x		
Research paper											x	x
Website					x	x	x	x	x	x	x	x

Research result 3. We will investigate the amount of insecticides that move from the point of application to soils under the tree. This research will help us understand any potential nontarget effects of imidacloprid.

Deliverable 3. This research will help us understand any potential nontarget effects of imidacloprid and submit recommendations.

	Su	Fall	Win	Sp	Su	Fall	Win	Sp	Su	Fall	Win	Sp
Establish plants, treat with				x	x	x		x	x	x		
Collect soil				x	x	x		x	x	x		
Perform residue analysis				x	x	x		x	x	x		
Research paper											x	x
Website					x	x	x	x	x	x	x	x

Research result 4. We will relate the amounts of insecticide in trunks to the published toxicity of these insecticides to birds.

Deliverable 4: This research will help us understand any potential nontarget effects of imidacloprid.

	Su	Fall	Win	Sp	Su	Fall	Win	Sp	Su	Fall	Win	Sp
Website					x	x	x	x	x	x	x	x

7. Budget

Project Budget: 124E-Landscape management of EAB: Nontarget consequences
Vera Krischik UM Entomology

IV. TOTAL PROJECT REQUEST BUDGET

BUDGET ITEM	TOT AMT
Result 1: Research effects of soil moisture on translocation of imidacloprid from roots to xylem/phloem in cambium to leaves of ash. Determine if MN low moisture soils cause imidacloprid not to be translocated, but instead runoff into soil. Use 2 levels of soil moisture (none, 1/2"wk irrigated) for 3 methods of application (passive drench, high pressure drench, injection). Measure insecticide residue in cambium, leaves, plants under trees, and soil at 3 distances from tree. Research on trees on UM Campus landcare, MPRB land, and others.	
Personnel: Graduate Student $19.39/hr + fringe (16.86% health insurance and $12,012 tuition)	$111,789
Personnel: Graduate Student $19.39/hr + fringe (16.86% health insurance and $12,012 tuition)	$111,789
Contracts: S&S trees to apply insecticides to 100, 10-14inch trees; Bayer Chem co will donate all imidacloprid; will request ArborJet to donate emamectin benzoate	$13,800
Subtotal personnel:	$237,378
Research supplies: Bioassays in UMN Quarantine Facility on St. Paul Campus, rearing cages, bioassay containers, beneficial insects from insectaries, equipment for applying insecticides, insecticides. pilot studies: smaller DBH ash trees to be planted on St. Paul campus for flowering plants under trees and runoff; landscape trees preferred; landscape ash identified and located by S&S trees, MPRB, St Paul landcare and others.	$24,422
Residue analysis: Measure amount of imidacloprid in leaves, cambium, leave/pollen/nectar of plants under trees, and soil adjacent to tree with ELISA quick test (Envirologix, Portland, ME, $400/40samples) and HPLC-mass spec (Roger Simonds,USDA AMS, Gastonia, NC, $160/sample)	$70,100
Travel: to study sites, collaboraotrs, presentations; Mileage $0.50/mi for 1400 miles/year=$700/yr	$2,100
Publication: Cost for duplicating management recommendations, factsheets, handouts for use at meetings and talks. Publication costs for research papers.	$6,000
Subtotal supplies:	$102,622
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND $ REQUEST	**$340,000**
Acquisition (Including Easements):	NA
Restoration:	NA
Other:	NA

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT
Other Non-State $ Being Applied to Project During Project Period:	
Other State $ Being Applied to Project During Project Period:	NA
In-kind Services During Project Period:	NA
Remaining $ from Current ENRTF Appropriation (if applicable):	NA
Funding history: USDA SARE grant 2010 $175,000: Bayer Chem Co 2004-2008 $90,000	$265,000

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8. Credentials - brief background of the principal investigators and cooperators
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. 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 papers on the movement of imidacloprid in trees and shrubs. She has 3 books: one published in 1991 by John Wiley entitled "Microbial Mediation of Plant Insect Interactions"; another published in 2004 by the MN Agricultural Experiment Station on "IPM of Midwest Landscapes", 316 pp.; and another published in 1995 and 1992. by Oklahoma State University "Stored Product Management" 204 pp. Vera 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 online at
www.entomology.umn.edu/cues/krischiklab/krischik.htm

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3. Rogers, M. A., V. A. Krischik, and L. A. Martin. 2007. Effect of soil application of imidacloprid 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 imidaclopirid 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.
Outreach: National Public Radio June 23, 2009 Interview on imidacloprid use in ash trees
Courses: ENT 4015 Ornamental and turf entomology; ENT 5009 Pesticides
Books: 1. Krischik, VA and J. Davidson. 2004. *IPM of Midwest Landscapes*, MN Ag Exp Stat, 316pp; 2. Barbosa, P, V. Krischik, and CG Jones (eds.).1991.*Microbial mediation plant-herbivore interactions*, JWileySons, 530 pp, 3. Krischik, V. A., G. Cuperus, and D. Galliart (eds.). 1995 and 1992. *Stored Product Management.* Oklahoma State University 204 pp. 1st and 2nd editions.

Project Team/Partners
The research will be performed in the lab of Dr. Vera Krischik (Landscape Plant Pest Management), Department of Entomology at the University of Minnesota, St. Paul Campus.
The research will be reported to the new forest entomologist, Dr. Brian Aukema. The research will be reported to the MDA EAB Task force, Mark Abrahmson. Two PhD students will work on the research. S & S Tree Service will apply the insecticides. Collaborators and interested parties will be sent email reports every 3mo.
1. Nila Hines, Pesticide Registration Review Coordinator, Minnesota Department of Agriculture, collaboration approved
2. June Mathiowetz, Sustainability Project Coordinator, City of Minneapolis, collaboration approved
3. Lois Eberhart, City of Minneapolis Surface Water & Sewers Administrator, Department of Public Works, collaboration approved
4. Robert Blair, Bird Population Biology, Dept Fisheries and Wildlife, UMinnesota, collaboration approved
5 Mark Stennes, certified arborist, S & S Tree Service, collaboration approved
6. Gail Nozal, certified arborist, S & S Tree Service, collaboration approved
7. Bob Fitch, Executive Director, MNLA, MN Landscape Association, collaboration approved
8. Ralph Siefert, MPRB, Minneapolis Park and Recreation Board, collaboration approved
9. Les Potts, Supervisor, Landcare, UMinnesota, collaboration approved
10. Eric Mader, Xerces Society and adjunct extension educator, UMinnesota
Interested parties
Rachael Coyle, Parks and Rec, St. Paul Forestry
Judy L. Crane, Ph.D., Research Scientist, Environmental Analysis and Outcomes Division,
Minnesota Pollution Control Agency Steve Hennes, MPCA ecological risk assessor, collaboration approved Environmental Analysis and Outcomes Division, Minnesota Pollution Control Agency (Comment: Unfortunately, Steve or I can not be listed as formal collaborators on your LCCMR project. The MPCA is going to be shrinking due to our budget problems so management are trying to prioritize the work we'll be able to

do. On an informal basis, you can still contact us with questions that fall within the purview of the MPCA. We would also be interested in hearing about any seminars you and your grad students present at the UMN on this project.)

9. Dissemination and Use

The research will be posted on the CUES website (www.entomology.umn.edu/cues) and updated every 6 mo. We will develop peer reviewed publications and present outreach talks. We will discuss the research protocols and results with collaborators every 3 months. We will present our finding to the Emerald ash Borer Task Force and results can be used to update their EAB management bulletin. We will present our results to our collaborators to use in their work on pesticide registration and recovery, and urban bird population management. They are: Nila Hines, Pesticide Registration Review Coordinator, Minnesota Department of Agriculture; June Mathiowetz, Sustainability Project Coordinator, City of Minneapolis; Lois Eberhart, City of Minneapolis Surface Water & Sewers Administrator, Department of Public Works; and Robert Blair, Bird Population Biology, Dept Fisheries and Wildlife, UMinnesota.

MINNESOTA DEPARTMENT OF AGRICULTURE

June 21, 2010

Dr. Vera Krischik
219 Hodson Hall, 1980 Folwell Ave.
University of Minnesota
St. Paul, MN 55108

Dear Dr. Vera Krischik:

Thank you for forwarding to the Minnesota Department of Agriculture (MDA) your 2011-2012 LCCMR proposal: *Landscape management of EAB: Nontarget consequences*.

Although we only recently became aware of this proposal, and have had limited opportunity to assess it, the MDA shares your concern about the potential impacts to water quality and non-target organisms from the increased use of insecticides to control emerald ash borer (EAB). In fact, just this spring the MDA began reconnaissance sampling of water resources for imidacloprid in urban settings where EAB insecticides may be used, and more recently completed the "*Emerald Ash Borer: Homeowner Guide to Insecticide, Selection, Use, and Environmental Protection*."

Additionally, the MDA intends to conduct a special pesticide registration review of the chemistries and products used to manage EAB, and to develop Minnesota-specific recommendations on the use of insecticides to minimize impacts to water quality and non-target organisms. The MDA administers and enforces state and federal pesticide laws and regulations, under the Pesticide Control Law, Chap. 18B. The special registration review is scheduled for completion in 2010-2011.

Minnesota-specific pesticide registration reviews are a collaborative process with researchers from the Minnesota Pollution Control Agency (MPCA), Minnesota Department of Health (MDH), Department of Natural Resources (DNR) and the University of Minnesota. When conducting registration reviews, the MDA tracks emerging science through a variety of mechanisms, primarily by accessing available EPA science documents, consulting with EPA scientists through state-federal regulatory channels and by consulting peer-reviewed scientific literature.

Your research project will be of interest to the MDA and certainly others engaged in the EAB special registration review process.

The MDA looks forward to learning more about your research.

Sincerely,

Nila I. Hines
Pesticide Registration Review Coordinator
Pesticide and Fertilizer Management Division (PFMD)
Minnesota Department of Agriculture
651-201-6208

CC: Greg Buzicky, MDA PFMD Director

Minneapolis
City of Lakes

Office of the City Coordinator

Steven Bosacker
City Coordinator

350 South 5th Street – Room 301M
Minneapolis MN 55415-1393

Office 612 673-3992
Fax 612 673-3250
TTY 612 673-2157

steven.bosacker@ci.minneapolis.mn.us

June 16, 2010

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

Dear LCCMR Members:

I am writing to express strong support for the LCCMR grant proposal "Landscape Management of Emerald Ash Borer: Nontarget Consequences." Dr. Krischik's research proposal is important to all Minnesota communities and residents as they face the question of whether or not to commit to multi-year insecticide treatments to save ash trees.

The emerald ash borer was found in Minneapolis in February 2010. The City has more than 200,000 ash trees or 20 percent of its tree canopy at stake as the emerald ash borer moves through the area. While Minneapolis residents are interested in taking the best possible steps to minimize the beetles' damage, they are also very concerned about the overall ecosystem and the lack of available research on the impacts of the particular insecticides being used, especially on water, soil, bees and people.

The City of Milwaukee is spending $1.6 million in 2009-2010 to treat its 33,000 ash trees. Before local governments in Minnesota begin investing large sums of public dollars in synthetic treatments, it needs research gaps filled to protect the public interest and avoid potential liabilities. Without it, Minnesota cities and residents lack sufficient information in weighing their options.

The results of Dr. Krischik's research will provide us with the science and information that will further the development of management recommendations in addressing emerald ash borer. It will also allow us to communicate best practices to our residents who want to do the right thing in preserving our ash trees while also protecting the ecosystem and health.

I hope the LCCMR will look closely at Dr. Krischik's proposal and consider what it will potentially mean for all towns and cities across Minnesota as they face the emerald ash borer's arrival.

Sincerely,

June Mathiowetz

June Mathiowetz
Sustainability Project Coordinator
City of Minneapolis

call 311 Minneapolis
City Information and Services

Minnesota Nursery &
Landscape Association

651-633-4987 • Fax 651-633-4986
1813 Lexington Ave N • Roseville, MN 55113

www.MNLA.biz
GardenMinnesota.com

June 21, 2010

Dear LCCMR Committee and grant program:

The Minnesota Nursery & Landscape Association supports Dr. Vera Krischik's cooperative proposal between the MDA and University of Minnesota on reducing the spread of emerald ash borer (EAB). Our members – including growers, arborists and landscape professionals – are concerned about the economic and ecological consequences of losing trees and other landscape plants to pests such as EAB. Since EAB was detected in Minnesota just last year, the risk to Minnesota is immediate and these funds will help to minimize environmental harm from the pest and the pesticides used for treatment. Our industry and our association stand ready to cooperate and support all efforts to prevent or minimize the impact of EAB.

Sincerely,

Bob Fitch
Executive Director, Minnesota Nursery & Landscape Association

Date: Thu, 16 Sep 2010 08:13:27 -0500
From: "Gail Nozal" <gail@sstree.com>
To: <krisc001@umn.edu>
Cc: "Mark Stennes" <mark@sstree.com>

Vera- Mark Stennes sent an email on regarding a study you will be doing next year. We are interested in working with you on this project. Mark is out at meetings today and I understand that you need to get this information soon, so I thought I would get started on it for you.
As far as the estimated costs here is what we are looking at (approximately without having the exact size of trees). **These prices are based on using Quali Pro brand for the imidacloprid on the soil drench and soil inject, Xytect (single dose rate injectable) for the Pointer application and Tree-Age medium rate using the Tree IV.** If we need to using name brand product let me know, same thing with the Pointer system.

Treatment Cost (8 trees per treatment, using the list below) $ 4640 (I assumed that it was only the soil drench and soil inject for the August application). *Also I used an average 10" tree to estimate the costs.*
Times of treatments
1. May (spring) = 32 trees
2. August (summer) = 16 trees
3. Sept (fall) = 32 trees
Total 80 trees, maybe impossible

Please feel free to give me a call to review the pricing. Just want to make sure we are on the same page with number of trees and the treatments mentioned. I will be attending the MnSTAC meeting today so I will be out of the office in the middle of the day.
Gail
Cell: 651-442-7153
gail@ sstree.com
Gail Nozal
Director-Plant Health Care & Urban Forestry
ISA Certified Arborist #MN-276A
651.451.8907
www.sstree.com

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