

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**

Project number: **124E**

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 landscape trees with insecticides. 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. Minnesota receives less rainfall than the states where research was performed for the recommendations. 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. Flowering plants growing under treated trees may uptake imidacloprid which then can be translocated to pollen and nectar and may cause mortality in beneficial insects and pollinators.

Imidacloprid is listed by the EPA as having potential for mobility in soils. 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. Emamectin benzoate is highly unsoluble in water and does not move in soils.

The purpose of this research is:

1. We will investigate the amount of insecticide present thru the year in trunk sap and leaves for different times of seasonal application (May, early August, and September). We will determine the efficacy and duration of imidacloprid applications in the MN environment where low levels of rainfall in midsummer may alter uptake of the soil surface and soil injection applications. Research would relate these amounts to the known toxicity of imidacloprid and emamectin benzoate to emerald ash borer larvae and adults found in published studies. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration.
2. We will investigate the amount of insecticides that move from the point of application to flowering plants growing under trees and also to adjacent soil. Research would investigate the amount of the insecticide in the pollen and nectar of flowering plants and consequences on beneficial insects feeding on the nectar and pollen. This research will help us understand any potential nontarget effects of imidacloprid and submit recommendations.
3. We will relate the amounts of insecticide in trunks and liquid pooled in woodpecker holes to

the published toxicity of these insecticides to birds. This research will help us understand any potential nontarget effects of imidacloprid and submit recommendations.

5. We will provide insecticide application methods for MN.

2. Background

Recommendations for chemical management of ash trees

Management of Emerald Ash Borer (EAB), *Agilus 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 applications 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).

Emamectin benzoate is highly unsoluble in water and remains in the tree for long periods and has much lower potential to move offsite. There are concerns that high levels of both insecticides can pool into woodpecker holes when birds forage on ash for emerald ash borer larvae and these concentrations in sap may be above the LD50 (lethal dose to 50% of the population) for birds, since so much active ingredient (AI) of product is injected into the tree and the LD50 for birds is very low for both chemicals (Table 1, MN Public Radio interview of Krischik, June 23, 2009).

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 reregistration 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 pennsylvanica*), 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 resistance. The borer is devastating because native ash does not have phenolic chemicals in their bark to protect them as do Asian ash.

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 agrili* was found parasitizing up to 90 percent of EAB larvae in ash trees in China. *Tetrastichus planipennis* 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: High use of insecticides in cities to protect ash trees

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 (Sydnor 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 trees 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_Insecticide_Fact_Sheet_22May09.pdf and summarized in a question-based website <http://www.entm.purdue.edu/EAB/faq.shtml>

Homeowner recommendations for treating trees are available (www.stpaul.gov/DocumentView.aspx?DID=12693).

Management bulletins suggest homeowners purchase 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.

Methods of applying insecticides

- 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) for trees < 12 in dbh. Water around tree after application. 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 landscapers. 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 per acre of imidacloprid used in 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.

For EAB, a surface soil application of imidacloprid is limited to 0.4 lb/yr or 181g AI (active ingredient)/yr. A 24 in dbh tree requires 76g AI (2.5 trees is the yearly limit per acre) and a 16 in dbh tree requires 50g AI (3.5 tree is the yearly limit per acre). **If we calculate the sq ft directly under a 24 in ash to be 150 sq ft, then the amount of imidacloprid applied is 500 mg/sq ft compared to 4mg/sq ft in agriculture.** In St. Paul the average lot size is 0.20 acre lot, so in 5 city lots only 2.5 (24 in dbh) -3.5 (16 in dbh) trees can be treated per year.

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 applications of insecticide for management of EAB can make the total of amount of insecticide used in landscape very high.

Also, no research has addressed the movement of imidacloprid applications into flowering plants or distant soil under treated trees. It is known that soil treatments and trunk injections of imidacloprid move into nectar and pollen. The consequences of these higher amounts in nectar and pollen on beneficial insects that kill pest insects and pollinators are not researched and 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 Cyanamid 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 has increased since four 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 applications. 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 applications of imidacloprid are not limited to seed treatments. Foliar and soil applications of this compound are delivered at higher rates but residue analysis and effects of bees of these common applications 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 applications 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, Rogers and Krischik, 2008

submitted, Krischik et. al 2008 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 applications were effective against foliar pests for 1 to 2 years in containers (Szczeplaniec 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 applications 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 applications 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

There are concerns that high levels of both insecticides imidacloprid and emamectin benzoate can pool into woodpecker holes when birds forage on ash for emerald ash borer larvae and these concentrations in sap may be above the LD50 for birds, since so much active ingredient (AI) of product is injected into the tree (Table 1, MN Public Radio interview of Krischik, June 23, 2009). No research has addressed this issue.

However 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=7 sites). 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).

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. We will be able to document the duration of the standard imidacloprid applications in

ash trees growing under MN conditions. This research will help arborists plan their management tactics. Also, we will study the effect of soil surface application on imidacloprid runoff to nearby soils and flowering plants, imidacloprid accumulation in nectar and pollen, and effects on nontarget beneficial insects such as predatory beetles, lacewings, and bumblebees.

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 sap 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 I have experience and knowledge in applying imidacloprid, measuring residues, and bioassays with beneficial insects and bumblebees. I have contacted researchers that perform residue analysis for hire, S&S trees to apply the insecticides, and collaborators to find ash trees to use in the research.

3. Hypothesis (Objectives)

The purpose of this research is:

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). We will determine the efficacy and duration of imidacloprid applications in the MN environment where low levels of rainfall in midsummer may alter uptake of the soil surface and soil injection applications. Research would relate these amounts to the known toxicity of imidacloprid and emamectin benzoate to emerald ash borer larvae found in published studies. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration.
2. Investigate the amount of insecticides to move from the point of application to flowering plants growing under trees and also to adjacent soil. Research would investigate the amount of the insecticide in the pollen and nectar of flowering plants and consequences on beneficial insects feeding on the nectar and pollen. This research will help us understand any potential nontarget effects of imidacloprid.
3. Investigate the amount of insecticides to move thru the soil.
4. Relate the amounts of insecticide in trunks and liquid pooled in woodpecker holes to the published toxicity of these insecticides to birds. This research will help us understand any potential nontarget effects of imidacloprid.
5. Provide insecticide application methods for MN.

4. Methodology

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 applications in the MN environment where low levels of rainfall

in midsummer may alter uptake of the soil surface and soil injection applications. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration

1.1. Insecticide application

Insecticide application will be made in collaboration and contract with Mark Stennes and Gail Nozal of S & S Tree Service (South St. Paul, MN) to apply the treatments. They have the expertise, equipment, and are MN certified arborists and MN certified pesticide applicators. They also have bucket trucks for sampling leaves during the season. Ash trees of around 14 in dbh will be used for injection and around 12 in dbh for soil surface and soil injection. Landscape trees will be used on the UM St. Paul campus, MPRB areas, and St. Paul, Roseville, Falcon Heights areas. In Spring 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.

Application methods of insecticide compared are:

1. Soil drench of liquid around the base of the tree with Bayer Advanced Garden Tree and Shrub Insect Control (Bayer Advanced LLC, Birmingham, AL);
n=6 spring + n=6 August+ n=6 Fall=18 trees
 2. Soil injection with fertilizer probe (John Deere soil injector) dispersed in the area beneath the tree canopy of Merit 75 WP insecticide (Bayer, Kansas City, MO);
n=6 spring + n=6 August+ n=6 Fall=18 trees
 3. Trunk injection with M3 infuser of imidacloprid (Xytect single dose) (Rainbow Tree, TreeCareDirect, Minnetonka, MN);
n=6 spring + n=6 August+ n=6 Fall=18 trees
 4. trunk injection with Tree I.V. of emamectin benzoate(TREEAge) (ArborJet,Woburn, MA);
n=6 Spring + n=6 + n=6 Fall=12 trees
 5. Untreated control
n=6 spring + n=6 August+ n=6 Fall=18 trees
- Total trees = 84

1.2 Relating residue to EAB toxicity

We will relate levels of imidacloprid found in leaves and trunk sap to beetle mortality. Research studies have determined the LD50 for imidacloprid on EAB. In bioassays, a high percentage of knockdown in EAB adults was observed 24 hours after. Three days after exposing the adults to the foliage, mortality increased. The percent of knockdown plus dead beetles was 71 percent at 20 days after treatment and 77 percent at 45 days after treatment. Other sublethal effects, including reduced feeding, and slowed movement were also observed. These effects may severely affect the fitness of surviving beetles. The LD50 for the adults of EAB was 7.1 ng/beetle, 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). Emamectin benzoate is highly toxic to EAB larvae and adults. I could only find an LD50 of 0.6 ppb for obliquebanded leaf roller (Proclam) (www.nysaes.cornell.edu/ent/faculty/jentsch/pdf/historical-perspectives-on-apple-production.pdf)

However, if EAB larva and adults are available, we can use the UM MDA Quarantine Lab, St. Paul Campus to determine the LD50.

1.3 Standards and statistics for residue analysis

The standards (imidacloprid, olefin, and hydroxy will be purchased from Bayer CropSciences (Research Triangle Park, NC), Fischer Scientific (Pittsburg, PA), and ArborJet (Woburn, MA). For residue statistical analysis, we will first use Levene's test to determine homogeneity of variance. If variances are unequal, a Welch test will be used (JMP, SAS 2005). If variances are equal, data will be analyzed with one way ANOVA for treatment, replicate and replicate by treatment interactions using PROC GLM (SAS 2004). Means will be compared with Tukey's HSD test. We will use PROC MIXED for any data that needs repeated measures statistics (SAS 2004).

1.4. Cleanup for leaves and trunk sap (Lewis and McCollough 2004, Cowles et al. 2006, Eisenbeck et al. 2009, Arora 2009)

A 20 g sample of leaves will be 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.

1.5. Cleanup for flowers, nectar and pollen (according to Krischik et al. 2007, 2010)

For residue analysis, each sample of 1.0 g of pollen or nectar (approximately 200 flowers combined from at least 3 vials) will be placed in 15 ml of water in a 50 ml culture tube, followed by an ultrasonic bath for 2 min, then placed on a wrist shaker for 2 hr, filtered, partitioned with dichloromethane, filtered, and evaporated to dryness. The residue will be dissolved in 20% acetonitrile/0.1% acetic acid and brought to 1 ml, frozen, and then extracted with acetonitrile and concentrated with a rotovaporator. The sample will be frozen until analysis. The assay values will be converted to ppb flower.

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

A representative 50 g soil will be mixed with 50 mL mixture 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.

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

Samples will be analyzed using ELISA (enzyme linked immunosorption assay) kits (EnviroLogix EP-006 Imidacloprid Quantiplate Kits, EnviroLogix, Portland, ME) using the methods described by Cowles et al. (2006). Sample dilution ranges will be from 1:50 to 1:50,000 to establish a reading for that sample within the standard curve. Concentrations of the original samples are calculated from the standard curve and the sample dilution. Resulting data are log transformed and analyzed by repeated measures for analysis of variance, with treatment used as a between-subject factor and depth and day used as within-subject factors (SAS).

The sample and conjugate are premixed in a 96-well plastic tray; and an eightchannel pipette issued to transfer samples and reagents to the ELISA plate. Three sets of imidacloprid standards (0, 0.2, 1, and 5 ppb) are placed at the diagonal corners and the center of each 96-well plate. The plate is shielded from temperature gradients during incubation by placing it in an insulated box. To read the ELISA plates 96-a well plate reader will be used. Standard curves will be graphed using SigmaPlot software (SAS) to provide a linear regression with log of the concentration versus the optical density measurements from the standards. The regression parameters of slope and intercept will then be used to calculate the concentration of unknowns. Measurements of color intensity generated with digital images of ELISA plates will require log transformation on both axes (imidacloprid concentration versus color intensity) to generate a linear standard curve from which concentrations of unknowns will be calculated.

1.8 Imidacloprid, HPLC method (Roger Simonds, USDA ARS, Gastonia, NC, (Krischik et al. 2007 and Krischik et al. 2009 submitted; and others, such as Laurent and Rathahao 2003)

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

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 μ m particle size, 40 $^{\circ}$ C, mobile phase A 0.1% acetic acid in water and mobile phase B 0.1% acetic acid in acetonitrile, flow rate 0.5 ml/min, and injection volume 15 μ l. 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.

1.9 Residue analysis: Uptake of insecticides in trunk sap and leaves for emamectin benzoate, HPLC method: (Roger Simonds, USDA ARS, Gastonia, NC) (Takei et al. 2003a,b)

High-performance liquid chromatography equipped with a fluorescence detector will be used in the laboratory of Dr. Roger Simonds, USDA ARS. The column needed is a reversed phase, and 418nm emission. I do not have personal experience with this method.

Objective 2. Investigate the amount of insecticides to move from the point of application to flowering plants growing under trees. Research would investigate the amount of the insecticide in the pollen and nectar of flowering plants and consequences on beneficial insects feeding on the nectar and pollen. This research will help us understand any potential nontarget effects of imidacloprid.

2.1. Uptake of insecticides by plants growing under ash trees: Flowers and soil under trees (soil surface, soil injected, and trunk injected imidacloprid)

Trees receiving Spring and August insecticide applications (surface drench, soil injection, and trunk injection) will have flowering plants placed in the soil under the trees prior to insecticide applications. We will use 6 shrub roses (Carefree Beauty), 12 Mexican milkweeds (*Asclepius curasavica*), and 12 Texas sage (Native plant, *Salvia coccina*). A spray of 40 gallons of water once a week will simulate irrigation. After 3 weeks flowers will be collected for analysis of imidacloprid in nectar and pollen. Also, soil samples at 4 distance from application at 6 in under the surface (0ft, 2ft, 5 ft and 10 ft) will be taken for imidacloprid analysis.

If the residues found in flower nectar and pollen are similar to levels in bioassays that we already performed, we will not continue with new bioassays. We will relate the data to these previous studies.

2.2 Mortality and behavior of beneficial insects Beneficial insects, green lacewing (*Chrysoperla carnea*, 1 species of wasp (*Anagyrus psuedococci*), and 3 species of lady beetles (*Harmonia axyridis*, *Hippodaemia convergens*, *Coleomegilla maculata*) will be ordered from Roncon Vitova Insectaries (Ventura, CA) or field-collected. Procedures developed by Krischik et al (2007, 2009) will be followed. Mesh cages (30 cm × 30 cm × 30 cm) (BioQuip, Rancho Dominguez, CA) will be daily supplied with cut flowers and water. When insects are received and prior to the study they will be conditioned with commercial artificial diet for lacewings and lady beetles (Rincon-Vitova) and 20% honey-water for all species (Aquatube, Syndicate Sales, Kokomo, IN). For 2 weeks, mortality and trembling will be observed 2X daily. Flowers from field studies will be used. At least 10 cages for each treatment will be used and the experiment will be replicated 3 times.

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), 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. Repeated measures ANOVA will be used to analyze differences in number and duration of bee visits to the feeders across the treatments. In year 2 and 3, these behavioral observations may be repeated using concentrations derived from field studies.

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 sublethal 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).

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 -20°C .

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 -20°C . Survivorship will be analyzed using Cox proportional hazard model. The whole experiment will be terminated after 80 days and all surviving bees stored at -20°C . Male offspring and the three workers per colony will be dried at 80°C for 4 h and weighed on a microbalance (Mettler Toledo MX5, $d = 1 \text{ g}; \pm 2\text{g}$) (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.

3. Movement of insecticides in soil away from the site of placement:

3.1 Leaching investigation according to Cowles 2009

Three treatments will be analyzed for leaching, trunk injection, surface application, and soil injection for treatments in Spring and in early August. Trees used will be UM campus trees or trees planted in a plantation on the St. Paul campus. We will use 3 concentrations of label rate imidacloprid (1X, 2X, and 3X) for pouring into the columns.

Soil (540 g dry weight) will be packed into columns constructed from PVC pipe (52 mm I.D.), to fill a volume of 490 ml, or a soil depth of 23 cm. Each column will be constructed from 2 pieces, each 15 cm long. When assembled, these two pieces will be held together with duct tape wrapped around the exterior of the column. Aluminum window screen will be glued to the bottom of the column, and a piece of Scotch-Brite¹ scouring pad (3M Co., St. Paul, MN) will be cut to fit within the column on top of the screen, to hold the soil within the column. Ports will be drilled into the side of each column, positioned at 2, 17, and 22 cm from the bottom of the column. A 6.4 mm diameter tube, will be predrilled with several 2 mm diameter holes, and inserted across the greatest diameter of the column for each port. When the column is filled with soil, these ports allow removal of soil solution 5, 10, and 25 cm below the depth at which imidacloprid will be loaded. There will be 16 columns constructed, enough to randomize three replicates of 5 treatments, plus one soil blank. Prior to loading soil columns with imidacloprid, they will be saturated with water to confirm that soil solution can be extracted, and then allowed to drain for 1 d.

Syringes (60 ml) will be used for extracting soil solution; one syringe will be kept for each port. The tip of the syringe will form an airtight connection with the 6.4 mm diameter tubing used to construct the extraction ports. The columns will be left with air gaps at their lower ends so that

they can drip into a Petri dish. If it is too difficult to draw a water sample from the 20 cm depth port, then the sample will be taken from the leachate captured in the dish. On each work day (Monday through Friday) 54 ml of water will be added at the top of each column at 8:30 a.m. (simulating 2.5 cm of precipitation). The water is allowed to percolate through the soil until 1:00 p.m., at which time soil solution samples will be taken, stored in 1.5 ml microcentrifuge tubes, and frozen at -20°C . Samples will be collected on days 1–3, 7–10, 14–17, 20, 21, 24, 27, 30, and 34 d after loading the soil columns with imidacloprid.

3.2. Field collection from soil under trees

The methods described in the leaching study above will be used, but imidacloprid will not be added to the soil columns. On each work day (Monday through Friday) 54 ml of water will be added at the top of each column at 10:00 a.m. (simulating 2.5 cm of precipitation).

Three treatments will be analyzed for trunk injection, surface application, and soil injection for treatments in Spring and in early August. A total of 18 trees (n=6 per treatment) for the 2 seasonal times (n=36 trees) will be used. Trees used will be UM campus trees.

4. Relate the amounts of insecticide in trunks and liquid pooled in woodpecker holes to the published toxicity of these insecticides to birds. This research will help us understand any potential nontarget effects of imidacloprid

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 sap could potentially affect birds eating sap from woodpecker holes.

5. Development of MN based insecticide recommendations to protect water quality and nontarget species

We will share our data with Minnesota state agencies, our collaborators, and interested parties. We will gain insight whether Minnesota rainfall in midsummer allow movement of insecticide from soil drench and soil injection into trees. We will identify whether imidacloprid moves from point of application away from the tree. We will gain some insight into whether sap pooled in woodpecker holes cause toxicity to sap-feeding birds. If needed we will use the data to update MN recommendations for managing EAB. Please see point 8 for our collaborators and interested parties.

Table 1. Potential for neonicotinyl insecticides (imidacloprid, thiamethoxam, dinotefuran, clothianidin) and emamectin 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
<p>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</p> <p>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</p> <p>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</p> <p>Concern that chemicals from tree injection collect in woodpecker holes made during foraging for EAB larvae and birds feed on exudate.</p>						
thiamethoxam 2003 Syngenta	Sol=327mg/l Koc=64 Leaching highly mobile	229 days	Bobwhite quail 1552ppm	fish 100ppm	Daphnia 100ppm	bees 120ppb highly toxic
<p>Thiamethoxam converted to dinotefuran 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</p>						
dinotefuran 2004 Bayer	Sol=259mg/l Koc=23-33 Leaching highly mobile	214 days	Bobwhite quail 5000mg/kg Japanese quail 2000mg/kg	fish 100ppm	Daphnia 1000ppm	bees 43ppb highly toxic
<p>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</p>						
clothianidin 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	WS=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 macrolactone lactones (Abamectin" originally a 80:20 mixture of avermectins) produced by fermenting *Streptomyces avermitilis*. **Concern that chemicals from tree injection collect in woodpecker holes made during foraging for EAB larvae and birds feed on exudate.**

Table 2. Potential nontarget effects on pollinators and beneficial insects		
Commodity	imidacloprid(AI) 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 7 trees in 7 lots use up the 0.4lb/acre limit 24inDBH tree- soil passive drench roughly 5 trees in 5 lots use up the 0.4lb/acre limi	label rate label rate 50 g AI 76 g AI	4000 ppb unpublished, CA Dept 550 ppb, Payne 2010 not researched, this proposal not researched, this propo
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) acute oral acute contact	158-185 ppb <5ppb 40 ng/bee=400ppb 40-102 ng/bee	(CA imid review, 2009; Bayer report 2007) (PAN-Europe letter 2009) (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 <i>B. impatiens</i> <i>B. terrestris</i> <i>B. impatiens</i>	10-30 ppb .	30 ppb slower foraging rate (Morandin and Winston 2003) 10 ppb reduced brood survival (Tasei et al. 2000) clothianidin, and thiamethoxam are deadly at extremely low dosages (Biobest 2008)

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 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 and adults found in published studies. We will determine the efficacy and duration of imidacloprid applications in the MN environment where low levels of rainfall in midsummer may alter uptake of the soil surface and soil injection applications. 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 sap at 3 seasonal times (May, July, and September).	2014
2. Determine the effects of these concentrations on behavior and mortality of EAB larvae and adults.	2014

Result 2. Investigate the amount of insecticides to move from the point of application under the tree to flowering plants growing under trees and also to adjacent soil. Research would investigate the amount of the insecticide in the leaves, pollen and nectar of flowering plants and consequences on beneficial insects feeding on the nectar and 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 flowers of plants growing under 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. Investigate the amount of insecticides to move from thru the soil. **Budget: \$70,000**

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

Result 4. Relate the amounts in insecticide in trunk and liquid pooled in woodpecker holes 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. Develop MN based EAB management recommendations that protect water quality and nontarget species from insecticides used for EAB insecticides offsite.	2014

Result 5. Provide insecticide application methods for MN. **Budget: \$0**

Deliverable 5.	Completion Date
1. Develop MN based EAB management recommendations that protect water quality and nontarget species from insecticides used for EAB. We will determine the effects of low and high soil moisture on the movement of insecticides offsite.	2014

6. Timetable

	July 2011 -June 30 2012				July 2012-June 30 2013				July 2013-June 2014			
	Su	Fall	Win	Sp	Su	Fall	Win	Sp	Su	Fall	Win	Sp
Research result 1. 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 applications in the MN environment where low levels of rainfall in midsummer may alter uptake of the soil surface and soil injection applications. This research will help arborists and homeowners plan their management tactics for the best efficacy and duration.												
Deliverable 1. Understand the effects of application type and seasonal time on amount of insecticide translocated to leaves and trunk sap												
Establish plants, treat with insecticides	x	x		x	x							
Collect leaves and trunk sap	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. Investigate the amount of insecticides to move from the point of application to flowering plants growing under trees and also to adjacent soil. Research would investigate the amount of the insecticide in the pollen and nectar of flowering plants and consequences on beneficial insects feeding on the nectar and pollen. This research will help us understand any potential nontarget effects of imidacloprid												
Deliverable 2. Understand the effects of application type and seasonal time on amount of insecticide translocated to leaves and flowers growing under trees.												
Establish plants, treat with insecticides				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. Investigate the amount of insecticides to move from thru the soil.												
Deliverable 3. Understand the effects of application type and seasonal time on amount of insecticide translocated to leaves and flowers on movement in soil.												
Establish plants, treat with insecticides				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. Relate the amounts in insecticide in trunk and liquid pooled in woodpecker holes to the published toxicity of these insecticides to birds.												
Deliverable 4: This research will help us understand any potential nontarget effects of imidacloprid												
Bulletin on website and data in residue paper				x	x	x		x	x	x	x	x
Research result 5. Provide insecticide application methods for MN.												
Deliverable 5: Bulletin on website												
Bulletin on website and data in residue paper				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 (Erviroligix, Portland, ME, \$400/40samples) and HPLC-mass spec (Roger Simonds, USDA AMS, Gastonia, NC, \$160/sample)	\$70,100
Travel: to study sites, collaboratoers, 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

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. Dr. Krischik was contacted by MN National Public Radio on June 23, 2009 for an interview on the non-target effects of imidacloprid on birds and bees.

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

1. [Tenczar, E. G., and V. A. Krischik. 2007. Comparison of standard \(granular and drench\) and novel \(tablet, stick soak, and root dip\) imidacloprid treatments for cottonwood leaf beetle \(Coleoptera: Chrysomelidae\) management on hybrid poplar. J. Econ. Entomol. 100: 1611-1621.](#)
 2. [Krischik, V. A., A. Landmark, and G. Heimpel. 2007. Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding Anagyrus pseudococci \(Girault\) \(Hymenoptera: Encyrtidae\) Environ. Entomol. 36\(5\): 1238-1245.](#)
 3. [Rogers, M. A., V. A. Krischik, and L. A. Martin. 2007. Effect of soil application of 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 imidacloprid on four coccinellid species \(Coleoptera: Coccinellidae\). J. Econ. Entomol. 93\(3\): 732-736.](#)
 7. [Smith, S. F. and V. A. Krischik. 1999. Effects of systemic imidacloprid on Coleomegilla maculata. \(Coleoptera: Coccinellidae\). Environ. Entomol. 28\(6\): 1180-1195.](#)
- 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. Galliard (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. 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.)

The research will be reported to the EAB Task force, Mark Abrahamson and the new forest entomologist in the Department of Entomology at UMinnesota, Dr. Brian Aukema

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 on a monthly basis. 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: Nile 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.



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.

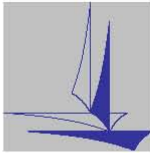
The MDA looks forward to learning more about your research.

Sincerely,

A handwritten signature in black ink, appearing to read "Nila Hines", written over a light blue horizontal line.

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
Sustainability Project Coordinator
City of Minneapolis



www.ci.minneapolis.mn.us
Affirmative Action Employer



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,

A handwritten signature in black ink that reads 'Bob Fitch'.

Bob Fitch

Executive Director, Minnesota Nursery & Landscape Association

Date: Thu, 16 Sep 2010 08:13:27 -0500
From: "Gail Nozal" <gail@sstree.com>
To: <kris001@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



Literature Citations

- Anderson, L.D. and E.L. Atkins, Jr. 1968. Pesticide usage in relation to beekeeping. Annual Review of Entomology 13: 213-238
- Apiservices. 2005. Virtual Beekeeping.
http://www.beekeeping.com/articles/us/aventis_regent.htm
- Arora, P.K. Jyot, Singh, Singh, Battu, Singh and Aulakh. 2009. Persistence of imidacloprid on grape leaves, grape berries and soil. Soil Bull Environ Contam Toxicol. 82:239–242
- Babendreier, D. B. Reichhart, J. Romeis and F. Bigler. 2008. Impact of insecticidal proteins expressed in transgenic plants on bumblebee microcolonies. Entomologia Experimentalis et Applicata (126): 148–157
- Barker R.J., Y. Lehner and M.R. Kunzmann. 1980. Pesticides and honey bees: nectar and pollen contamination in alfalfa treated with dimethoate. Arch. Environ. Contam Toxicol 9: 125-133
- Berenbaum, M. 2007. Testimony to Congress on pollinator decline.
 (http://www7.nationalacademies.org/ocga/testimony/Colony_Collapse_Disorder_and_Pollinator_Decline.asp)
- Biobest. 2008. and D-Manager Guido Sterk of Biobest concerning neonicotinoids:
<http://207.5.17.151/biobest/en/bulletin/r&dManagerguidosterk.htm>
- Bitterman, M.E., R. Menzel, and A. Rietz. 1983. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*) J. Comp Psychol. 97: 107-119.
- Bonizzoni, L., X. A. Martinez, M. Hederer, P. Bross, J. M. Bohet, F. Hamdan, C. Dauw, M. R. Henckes, G. Jesse, F. Veillerette, H. Mardulyn, M. Fichers, S. Parente, Y. Védrenne, F. Berger, F. Panella, H. Clément, and J. Y. Saliez. 2006. Pesticides and bees protection: the case of imidacloprid, fipronil, thiamethoxam and clothianidin. Open letter.
[http://www.foeeurope.org/activities/chemicals/LettreMonsieurKyprianou\(en\).pdf](http://www.foeeurope.org/activities/chemicals/LettreMonsieurKyprianou(en).pdf)
- Bonmatin, J. M., I. Moineau, R. Charvet, M. E. Colin, C. Fleche and E. R. Bengsch. 2005b. Behaviour of imidacloprid in fields. Toxicity for Honey Bees. In: Environmental chemistry green chemistry and pollutants in ecosystems, pp:483-494, edits, (Eric. Lichtfouse, Jan. Schwarzbauer, Didier Robert), Springer Berlin Heidelberg, 780 pages
- Bonmatin, J. M., P. A. Marchand, R. Charvet, I. Moineau, E. R. Bengsch and M. E. Colin. 2005a. Quantification of imidacloprid uptake in maize crops. J. Agric. Food Chem. 53: 5336-5341
- Cappaert, D., D.G. McCullough and T. Poland. 2005. The upside of the emerald ash borer catastrophe: a feast for woodpeckers, p. 69–70. In: V. Mastro and R. Reardon (comps.) (eds.). Emerald Ash Borer Research and Technology Development Meeting, Romulus, Michigan, 5–6 Oct. 2004. U.S. Dept. Agric. For. Serv. Pub.
- Colin, M. E., J. M. Bonmatin, I. Moineau, C. Gaimon, S. Brun and J. Vermandère. 2004. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Arch. Environ. Contam. Tox. 47: 387-395
- Commings, J. 2008. Emergency pesticide ban for saving the honeybee. 2008. Institute of Science in Society. (<http://www.i-sis.org.uk/honeybeePesticideBan.php>)
- Cowles, R. S. 2009. Optimizing dosage and preventing leaching of imidacloprid for management of hemlock woolly adelgid in forests. Forest Ecology and Management 257: 1026–1033

- Cowles, R. S., M. E. Montgomery and C.A.S.-J. Cheah. 2006. Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. *J. Econ. Entomol.* 99(4): 1258-1267
- Cowles, R. S., M. E. Montgomery and C.A.S.J. Cheah. 2006. Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. *J. Econ. Entomol.* 99: 1258–1267
- Cowles, R. S. 2009. Optimizing dosage and preventing leaching of imidacloprid for management of hemlock woolly adelgid in forests. *Forest Ecology and Management* 257 1026–1033
- Cox-Foster, D. 2007. Prepared testimony of Diana Cox-Foster professor Department of Entomology the Pennsylvania State University before the U.S. House of Representatives Committee on Agriculture Subcommittee on Horticulture and Organic Agriculture on colony collapse disorder in honey bee colonies in the United States
- Cox-Foster, D. L., S. Conlan, E. C. Holmes, G. Palacios, J. D. Evans, N. A. Moran, P.-L. Quan, T. Briese, M. Hornig, D. M. Geiser, V. Martinson, D. van Engelsdorp, A. L. Kalkstein, A. Drysdale, J. Hui, J. Zhai, L. Cui, S. K. Hutchison, J. F. Simons, M. Egholm, J. S. Pettis, W. I. Lipkin. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318 (5848): 283–287
- Decourtye A., E. Lacassie and M. Pham-Delègue. 2003. Learning performance of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to season. *Pest Manag. Sci.* 59: 69-278
- Delaplane, K.S. and D. F. Mayer, 2000. *Crop pollination by bees.* CAB Publishing, CAP International: Wallingford.
- Dilling, C., P. Lambdin, J. Grant and R. Rhea. 2009. Community response of insects associated with eastern hemlock to imidacloprid and horticultural oil treatments. *Environ. Entomol.* 38: (1) 53-66
- Dilling, C., P. Lambdin, J. Grant and R. Rhea. 2010. Spatial and temporal distribution of imidacloprid in eastern hemlock in the southern Appalachians. *J. Econ. Entomol.* 103(2): 368-373
- Drobne, D. Blazic, M. Gestel, C. A. M. van Leser, V. Zidar, P. Jemec and A. Trebse, P. 2008. Toxicity of imidacloprid to the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea) *Chemosphere.* 71: (7) 1326-1334
- Eisenbeck, B. M., D. E. Mullins, S.M. Salom and L. T. Kok. 2009. Evaluation of ELISA for imidacloprid detection in eastern hemlock (*Tsuga canadensis*) wood and needle tissues. *Pest Manag. Sci.* 65: 122–128
- Evans, E., I. Burns and M. Spivak. 2007. *Befriending bumble bees: a practical guide to raising local bumble bees.* University of Minnesota Extension. Pub # 08484. 65pp
- Evans, J.D. 2006. Beepath: An ordered quantitative-PCR array for exploring honey bee immunity and disease. *J Invertebr Pathol* 93: 135-139
- Fahem, Muhammad and M. F. Khan. 2010. Toxicity of imidacloprid (nicotinoid) against earthworm, *pheretima posthuma* with reference to its effects on protein. *Journal of Basic and Applied Sciences* Vol. 6, No. 1, 55-62
- Faucon, J. P., C. Aurières, P. Drajnudel, L. Mathieu, M. Ribière, A. C. Martel, S. Zeggane, M. P. Chauzat and M. F. Aubert. 2005. Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Manag. Sci.* 61:111-125
- Frank, S., R. Ahern and M. J. Raupp. 2007. Does imidacloprid reduce defoliation by Japanese beetles on linden for more than one growing season? *Arboric. Urban For.* 33: 392–396

- Gels, J.A., D.W.Held and D.A.Potter. 2002. Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae), foraging on flowering white clover in turf. J. Econ. Entomol. 95(4): 722-728
- Grosman, D.M., S. R. Clarke and W.W. Upton. 2009. Efficacy of two systemic insecticides injected into loblolly pine for protection against southern pine bark beetles (Coleoptera: Curculionidae). J. Econ. Entomol. 102(3): 1062-1069
- Gupta, G. and V. Krischik. 2006. Professional and consumer insecticides for the management of adult Japanese beetle. J. Econ. Entomol. 100: 830-839
<http://www.isis.org.uk/honeybeePesticideBan.php>
- Jaycox, E.R. 1964. Effect on honey bees of nectar from systemic insecticide-treated plants. J. of Econ. Entomol. 57:1
- Johnson, T.A., and R.C. Williamson. 2007. Potential management strategies for the linden borer (Coleoptera: Cerambycidae) in urban landscapes and nurseries. J. Econ. Entomol. 100: 1328-1333
- Jones, G.D.G. and W.D.E. Thomas. 1953. Contamination of nectar with the systemic insecticide 'Schradan'. Nature 4345: 263
- Kirchner, W. H. 1999. Mad-bee-disease? Sublethal effects of imidacloprid ("Gaucho") on the behavior of honey-bees. Apidologie 30: 422
- Koppert Biological Systems. 2005. <http://www.koppert.nl/e0110.html>
- Kreutzweiser, D. P., K. Good, D. Chartrand, T. Scarr, S. B. Holmes and D. G. Thompson. 2008. Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects. Pest Manag. Sci 64:112–118
- Kreutzweiser, D. P., D. G. Thompson, and T.A. Scarr. 2009. Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. Ecotoxicology and Environmental Safety 72 1053–1057
- Krischik, V, A Landmark, G Heimpel. 2007. [Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding Anagyrus pseudococci \(Girault\) \(Hymenoptera: Encyrtidae\)](#) J. Environ. Entomol. 36(5): 1238-1245
- Krischik, V, A, G. Gupta, M. Rogers and A. Varshney. 2010. Effects of soil applied imidacloprid on 4 species of lady beetles and 2 species of butterflies. Environ Entomol. submitted and accepted
- Krischik, V. and J. Davidson. 2004. IPM of Midwest landscapes. MN Agr. Exp Station, 316 pp
- Laloi, D., J. C. Sandoz, A. L. Picard-Nizou, A. Marchesi, A. Pouvreau, J. N. Tasei, G. Poppy and M. H. Pham-Delegue. 1999. Olfactory conditioning of the proboscis extension in bumble bees. Entomol. Exp. Appl. 90: 123-129
- Lambin, M., C. Armengaud, S. Raymond and M. Gauthier. 2001. Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. Arch. Insect Biochem 48: 129-134
- Laurent F.M. and E. Rathahao. 2003. Distribution of (¹⁴C) imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. J. Agric and Food Chem 51:8005-8010
- Lewis, P. and McCollough, D. 2004. Developing a fast, inexpensive method to extract and analyze imidacloprid (merit) residue in plant tissue, www.emeraldashborer.info/files/meritresidue.pdf
- Lindell, C.A., D.G. McCullough, D. Cappaert, M.L. Apostolou, and M.B. Roth. 2007. Factors influencing woodpecker predation on emerald ash borer Am. Midl. Nat. 159:434–44

- Masterman R, R. Ross, K. Mesce and M. Spivak. 2001. Olfactory and behavioral response thresholds to odors of diseased brood differ between hygienic and non-hygienic honey bees (*Apis mellifera* L.) J. Comp Physiol. A 187: 441-452
- Maus, C., G. Cure and R. Schmuck. 2003. Safety of imidacloprid seed dressings to honey bees: A comprehensive overview and compilation of the current state of knowledge, Bulletin of Insectology 56(1): 51-57
- McCullough, D. G., D. R. Smitley and T. M. Poland. 2003. Evaluation of insecticides to control emerald ash borer adults and larvae. Michigan State University, Lansing. <http://www.emeraldashborer.info/files/bulletin.pdf>
- McCullough, D.G., D.A. Cappaert, T.M. Poland, P. Lewis and J. Molongoski. 2006. Evaluation of neo-nicotinoid insecticides applied as non-invasive trunk sprays. emerald ash borer and Asian longhorned beetle research and development review meeting.
- Morandin, L. A. and M. L. Winston. 2003. Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. Environ. Entomol. 32: 555–563
- Morse, R.A. and N.W. Calderone. 2000. [The value of honey bees as pollinators of US crops in 2000. Cornell University, Itaca, NY, 31 pp.](http://www.masterbeekeeper.org/pdf/pollination.pdf)
- Peck, D. T. 2009. Comparative impacts of white grub (Coleoptera: Scarabaeidae) control products on the abundance of non-target soil-active arthropods in turfgrass. Pedobiologia 52: 287-299
- Peterson, C. J. 2007. Imidacloprid mobility and longevity in soil columns at a termiticidal application rate. Pest Manag Sci 63:1124-1132
- Arora, P.K., G. Jyot, B. Singh, R.S. Battu, B. Singh, P.S. Aulakh. 2009. Persistence of imidacloprid on grape leaves, grape berries and soil. Bull Environ Contam Toxicol 82:239-242
- Ping, L. C., Zhang, Y. Zhu, M. Wu, F. Dai, X. Hu, H. Zhao and Z. Li. 2010. Imidacloprid adsorption by soils treated with humic substances under different pH and temperature conditions. African Journal of Biotechnology Vol. 9(13), pp. 1935-1940
- Reynolds, W. M. 2008. Imidacloprid insecticide treatments for hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), affect a non-target soil arthropod community surrounding eastern hemlock, *Tsuga canadensis* (L.) Carriere. MS thesis, University of Tennessee, Knoxville, TN.
- Rogers, M. A., V. A. Krischik and L.A. Martin. 2007. Effects of soil application of imidacloprid on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), used for biological control in greenhouses. Biological Control. 42: 172-177
- Rortais, A., G. Arnold, M. P. Halm and F. Touffet-Briens. 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie. 36:71-83
- SAS Institute. 2004. SAS for Windows, version 9.1. SAS Institute, Cary, NC
- Schmuck R., 1999. No causal relationship between Gaucho seed dressing in sunflowers and the French bee syndrome. Pflanzenschutz Nachrichten Bayer 52: 257-299
- Schmuck, R., R. Schöning, A. Stork and O. Schramel. 2001. Risk posed to honeybees (*Apis mellifera* L., Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Manag Sci. 57: 225-238
- Scott-Dupree, C. D. and M. Spivak. 2001. The impact of Gaucho and TI-435 seed-treated canola on honey bees, *Apis mellifera* L., Université de Guelf, Ontario Canada Université du Minnesota, USA, Web available report 110405, April 11, 2001. <http://www.honeycouncil.ca/users/getdownload.asp?DownloadID=83>

- Smith, S. F. and V. A. Krischik. 1999. Effects of systemic imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environ. Entomol.* 28: 1189-1195
- Smitley, D., J.A. Decola and D.J. Cox, 2010. Multiple-year protection of ash trees from emerald ash borer with a single trunk injection of emamectin benzoate, and single-year protection with an imidacloprid basal drench. *Arboriculture & Urban Forestry.* 36(5): 206–211
- Smitley, D.R., E. J. Rebek, R. N. Royalty, T. W. Davis, and K. F. Newhouse. 2010. Protection of individual ash trees from emerald ash borer (Coleoptera: Buprestidae) with basal soil applications of imidacloprid. *J. Econ. Entomol.* 103(1): 119-126
- Stapel, J.O., A.M. Cortesero and W.J. Lewis. 2000. Disruptive sublethal effects of insecticides on biological control: altered foraging ability and life span of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. *Biol. Control* 17, 243–249
- Suchail, S., D. Guez and L. Belzunces. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ. Toxicol. Chem.* 20: 2482-2486.
- Suchail, S., D. Guez and L. Belzunces. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ. Toxicol. Chem.* 20: 2482-2486
- Suchail, S., D. L. Guez and L. P. Belzunces. 2000. Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. *Environ. Toxicol. Chem.* 19: 1901-1905.
- Suchail, S., D. L. Guez and L. P. Belzunces. 2000. Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. *Environ. Toxicol. Chem.* 19: 1901-1905
- Sydnor, T.D., M. Bumgardner and A. Todd. 2007. The potential economic impacts of emerald ash borer (*agrilus planipennis*) on Ohio, U.S., communities. *Arboriculture & Urban Forestry* 2007. 33(1):48-54
- Szczepaniec A. and M.J. Raupp. 2007. Residual toxicity of imidacloprid to hawthorn lace bug, *Corythuca cydoniae*, feeding on cotoneasters in landscapes and containers. *J. Environ. Hort.* 25:4346
- Takai, K., T. Suzuki and K. Kawazu. 2003a. Development and preventative effect against pine wilt disease of a novel liquid formulation of emamectin benzoate. *Pest Manag Sci.* 59: 365-370.
- Takai, K., T. Suzuki and K. Kawazu. 2003b. Distribution and persistence of emamectin benzoate at efficacious concentrations in pine tissues after injection of a liquid formulation. *Pest Manag Sci.* 60: 42-48.
- Tasei, J.N., G. Ripault and E. Rivault. 2000. Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. [Entomologia Experimentalis et Applicata](#) *J. Econ. Entomol.* 126(2): 148–157.
- Tasei, J.N., G. Ripault and E. Rivault. 2000. Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. [Entomologia Experimentalis et Applicata](#) *J. Econ. Entomol.* 126(2): 148–157
- Tasei, J.N., G. Ripault and E. Rivault. 2001. Hazards of imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower. *J. Econ. Entomol.* 94(3): 623–627
- Tasei, J.N., G. Ripault and E. Rivault. 2001. Hazards of imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower. *J. Econ. Entomol.* 94(3): 623–627
- Tenczar, E. and V. Krischik. 2006. Management of cottonwood leaf beetle, *Chysomela scripta* (Coleoptera: Chrysomelidae) with a novel transplant soak and biorational insecticides to conserve coccinellid beetles. *J. Econ. Entomol.* 99(1):102-108

- Tenczar, E. and V. Krischik. 2006. Management of cottonwood leaf beetle, *Chysomela scripta* (Coleoptera: Chrysomelidae) with a novel transplant soak and biorational insecticides to conserve coccinellid beetles. J. Econ. Entomol. 99(1):102-108
- Tenczar, E. and V. Krischik. 2007. Comparison of standard (granular and drench) and novel (tablet, stick soak, root dip) imidacloprid treatments for cottonwood leaf beetle management on hybrid poplar J. Econ. Entomol. 100: 1611-1621
- Tenczar, E. and V. Krischik. 2007. Comparison of standard (granular and drench) and novel (tablet, stick soak, root dip) imidacloprid treatments for cottonwood leaf beetle management on hybrid poplar J. Econ. Entomol. 100: 1611-1621
- Tomizawa, M. and J.E. Casida. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45:247-68
- Tomizawa, M. and J.E. Casida. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45:247-68
- Winter, K., L. Adams, R. W. Thorp, D. W. Inouye, L. Day, J. Ascher and S. Buchmann, 2006. Importation of non-native bumble bees into North America: potential consequences of using *Bombus terrestris* and other non-native bumble bees for greenhouse crop pollination in Canada, Mexico, and the United States. Pages 33. A White Paper of the North American Pollinator Protection Campaign (NAPCC).
http://www.pollinator.org/Resources/BEEIMPORTATION_AUG2006.pdf
- Winter, K., L. Adams, R. W. Thorp, D. W. Inouye, L. Day, J. Ascher and S. Buchmann. 2006. Importation of non-native bumble bees into North America: potential consequences of using *Bombus terrestris* and other non-native bumble bees for greenhouse crop pollination in Canada, Mexico, and the United States. Pages 33. A White Paper of the North American Pollinator Protection Campaign (NAPCC).
http://www.pollinator.org/Resources/BEEIMPORTATION_AUG2006.pdf
- Xerces Society. 2006. Pollinator conservation program for healthy ecosystems and bountiful harvests (http://www.xerces.org/Pollinator_Insect_Conservation/index.htm)