

2012 Ohio Report: NCERA-224

Dan Herms

Department of Entomology, The Ohio State University
The Ohio Agricultural Research and Development Center

Wooster, OH 44691

herms.2@osu.edu

Tree health, carbon sequestration, and sustainability of urban forests

Investigators: Alejandro Chiriboga (Ph.D. candidate) and Daniel A. Herms

Summary: Global climate change concerns have increased the need to create multiple mitigation scenarios to reduce carbon dioxide (CO₂) emissions. Strategic management of urban forests at different times and levels (e.g. nursery and landscape) can contribute to reduce atmospheric CO₂ rates over time. Our assessments of the structure and functions of urban street trees in the City of Wooster, Ohio, have suggested that the economic value of environmental services can increase substantially throughout time with strategic management. Nursery production practices such as fertilization and irrigation can play a crucial role on the establishment and the future of trees in the landscape. In urban ecosystems, tree establishment can become more challenging because of the wide variety of stressful environmental conditions. Thus, the goal of this long-term multidisciplinary research is to investigate how nursery production practices affect resource allocation patterns, insect herbivory, and carbon sequestration of urban trees. Results of this study will contribute to identify management strategies that can alter the rates of carbon sequestration of urban trees over time and can also enhance the economic value other environmental services. In addition, this research will increase our understanding of physiological responses of trees in urban environments.

An Integrated, Comprehensive Program to Develop Emerald Ash Borer Resistant Ash Trees

Investigators: D.A. Herms and O. Mittapalli (Dept. of Entomology); Don Cipollini (Dept. of Biological Sciences, Wright State University); Jennifer Koch, Kathleen Knight and Therese Poland (USDA Forest Service), Pierluigi Bonello (Dept. of Plant Pathology).

Summary: This 3-year project will identify EAB-resistant germplasm and associated mechanisms of resistance, as well as the underlying genes and genetic markers. This information will be used in an accelerated program to develop EAB-resistant ash trees for use in reforestation of natural and urban forests. Emerald ash borer (EAB, *Agrilus planipennis*) has killed millions of ash trees (*Fraxinus* spp.) in managed and natural landscapes of North America since its accidental introduction from Asia. All eastern North American ashes are susceptible, including green (*F. pennsylvanica*), white (*F. americana*), black (*F. nigra*), blue ash (*F. quadrangulata*), and pumpkin ash (*F. profunda*). Ash mortality now exceeds 99% in southeast Michigan (Herms et al. 2009), and as EAB continues to spread, it threatens the existence of ash in North America on a continental scale, with devastating economic and environmental impacts. The development of EAB resistant ash trees will be critical for long-term reforestation and preservation of ash in natural and urban forests. The objectives of the project are to

(1) identify, breed and screen ash germplasm for EAB resistance and silvicultural traits, including characterize EAB-resistance status of Asian species; identify, propagate, and breed surviving North American “lingering ash”; generate genetic crosses and hybrids between Asian and North American genotypes; develop bioassays to screen ash germplasm in early ontogenetic stages to confirm resistance to EAB; and evaluate horticultural and silvicultural properties of

resistant genotypes; **(2)** Identify mechanisms of EAB resistance to facilitate breeding and screening, including manipulate susceptibility of resistant Asian species in the field, and correlate larval performance with physical and chemical traits affecting host quality; identify induced and constitutive biochemical mechanisms of resistance using comparative metabolomics and proteomics; extract and purify putative resistance compounds, and confirm their efficacy in bioassays using artificial diet; and **(3)** Functional genomics of ash and EAB to accelerate development of resistant ash trees, including identify and sequence EAB resistance genes of ash; identify and sequence detoxification and other EAB genes responsible for successful colonization of ash; functional characterization of the genes obtained from objective 1 and 2; develop a gene knock-out (RNA interference) strategy for EAB to identify metabolic traits (e.g. detoxification enzymes) that can be counteracted by resistance traits; and identify genetic markers to trace resistance traits through breeding programs.

Induced responses of ash to emerald ash borer neonate larvae: effects of girdling and fertility

Investigators: Vanessa Muilenburg, David Showalter, Sourav Chakraborty, Pierluigi Bonello, Daniel Herms

Summary: Previous work identified potential constitutive metabolic and transcriptomic resistance mechanisms of ash to emerald ash borer (EAB). However, the role of induced defenses to EAB is unknown. Girdling has been identified as an effective tool for studying tree resistance to wood-boring insects. After a tree is girdled, current photosynthate accumulates above the girdle, fueling defensive responses, but tissues below the girdle become depleted of photosynthate because the flow is cut-off to tissues below the girdle. This results in phloem tissues that are resistant above the girdle and susceptible below the girdle. Therefore, green, white and Manchurian ash were girdled on May 5th, 2011 in a common garden in Novi, MI. Natural induced responses of ash were elicited by inoculating girdled and un-girdled trees with EAB eggs above and below the girdle on July 7th, 2011. Phloem around larval galleries resulting from EAB egg inoculation was excised to study the localized, induced resistance mechanisms. Phloem without galleries was also sampled to analyze constitutive resistance mechanisms. Phloem samples will be analyzed to compare phenolic and proteomic profiles among/between (1) species, (2) above and below the girdle, and (3) un-girdled and girdled trees in order to characterize putative metabolic and gene-level resistance mechanisms. Additionally, both girdled and un-girdled trees were harvested and dissected to reveal EAB larval galleries in order to relate colonization levels with potential resistance mechanisms. The effect of fertility on ash resistance was also analyzed by inoculating green, white, and Manchurian ash with EAB eggs in 2012. As previously described, phenolic profiles of phloem and larval galleries were examined to relate patterns of colonization with potential resistance mechanisms.

The use of methyl jasmonate for insecticide-free control of the emerald ash borer

Investigators: Vanessa Muilenburg, David Showalter, Sourav Chakraborty, Pierluigi Bonello, Daniel Herms

Summary: Methyl jasmonate (MeJA) is a natural phytohormone, ubiquitous in plants, that is associated with plant resistance mechanisms to pests and pathogens. Research by Whitehill (2011) indicates that application of MeJA to susceptible North American ash seedlings (2.5-3.5 cm DBH) induces endogenous defenses that results in resistance to EAB. Building off this work, we will evaluate the efficacy of MeJA application to mature white and green ash trees via tree injection and determine its potential for replacement of synthetic insecticides for control of EAB. Previous work indicated that 1 μ mol/g FW of MeJA in phloem tissue confers protection in

ash against EAB colonization. Therefore, we are evaluating five rates of application: 0X, 1/2X, 1X, 2X, and 4X the concentration needed to achieve 1umol/g FW of MeJA in phloem tissue. This experiment was conducted at Wright Patterson Airforce Base in Dayton, OH in 2011 and 2012. EAB adult emergence and ash condition were analyzed in 2011 and 2012. A final evaluation of EAB adult emergence and effectiveness of treatments will be conducted in spring 2013.

Effects of water and nutrient availability and exogenous methyl jasmonate application on ash phloem chemistry and resistance to the emerald ash borer

Investigators: David Showalter, Robert Hansen, Daniel Herms, Pierluigi Bonello

Summary: Emerald ash borer (EAB) has the potential to devastate North American ash populations in urban and forest settings, resulting in enormous ecological and economic damage until ash is functionally eliminated or management strategies improve. It is hypothesized that by virtue of a shared evolutionary history, Asian ash species possess biochemically-mediated resistance to the emerald ash borer (1). Phloem chemistry of numerous ash cultivars has been previously characterized under constitutive and induced conditions. Additionally, the relationship between phenolic chemistry and EAB resistance has been explored in ambient water and fertility conditions (2-4). This study takes a water- and nutrient-stress treatment approach to modulate the resistance phenotypes of ash species previously identified as resistant and susceptible and associate those phenotypes with changes in physiology and phloem chemistry. In May 2012, 128 ash trees of 1" average diameter were potted and arranged in 8 blocks. Half of the trees were constitutively EAB-resistant Manchurian ash cv. 'Mancana' and half were EAB-susceptible white ash cv. 'Autumn Purple'. Two levels of fertilization and of irrigation were provided by a computer-controlled system at rates of 30 or 150 ppm N, with N/P₂O₅/K₂O, at a ratio of 3:1:2 from calcium nitrate, monoammonium phosphate, and potassium nitrate. Each pot received a delivery of nutrient solution whenever the potting medium moisture tension dried to -5 or -10 kPa, as measured by soil tensiometers. Growth and physiology were monitored as trunk diameter, net CO₂ assimilation, stomatal conductance, specific leaf area, and leaf C:N ratio. Fertilization and irrigation treatment as well as physiological measurements will continue in 2013 with the application of differential phytohormone pretreatments, challenges with EAB eggs, and collection of larva and ash phloem samples. Relationships between nutrient and water availability, larval performance, ash phloem chemistry, and phytohormone treatment will be compared between ash species.

Changes in regeneration dynamics of ash (*Fraxinus* spp.) since invasion by emerald ash borer (*Agrilus planipennis* Fairmaire) in the Central Hardwood forests in the United States

Investigators: W.S. Klooster, D.A. Herms, K.S. Knight (USDA Forest Service), C.P. Herms (Dept. of Horticulture and Crop Science), D.G. McCullough (Dept. of Entomology and Forestry, Michigan State University), A.M. Smith, K.J.K. Gandhi, and J. Cardina (Dept. of Horticulture and Crop Science).

Summary: Emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) has already killed tens of millions of native ash (*Fraxinus* spp.) trees and threatens ash throughout the eastern United States and Canada. Unless ash is able to regenerate, the entire genus could become functionally extirpated from North American forests. We quantified ash mortality and regeneration potential over time in mixed hardwood forests containing *F. Americana* L. (white ash), *F. pennsylvanica* Marsh. (green ash), and *F. nigra* Marsh. (black ash) near the epicenter of EAB invasion in southeastern Michigan, and across Ohio. Ash were the most important canopy tree species in hydric stands (based on importance value), and second most important species in mesic and xeric

stands. In Michigan, mortality of ash with dbh ≥ 2.5 cm increased rapidly from 40% in 2005 when the study began to 99.7% in 2009, before declining slightly to 97% in 2010. As ash mortality exceeded 99%, the seed bank was rapidly depleted, and density of new ash seedlings declined to zero. Established ash seedlings <1.37 m tall were the most abundant demographic class in the understory. Patterns of ash seedling demography in relation to ash decline were similar in Ohio. Given the large extent of EAB-induced ash mortality, cessation of reproduction, and lack of a persistent seed bank, the fate of ash in eastern North American forests will depend on the long-term survival of the orphaned cohort of established ash seedlings and saplings and its dynamic equilibrium with EAB.

Direct and indirect impacts of emerald ash borer on forest bird communities

Investigators: L.C. Long, K.S. Knight (USDA Forest Service), D.A. Herms

Summary: Emerald ash borer (*Agrilus planipennis*, EAB), an invasive wood-boring beetle, has killed millions of ash trees since its accidental introduction from Asia. Ash can be common in the fragmented forests of Midwestern states, and comprises 10% of trees in Ohio's forests. The rapid generation of canopy gaps, dead standing trees, and regeneration in subsequent forest strata that result from EAB-induced ash mortality may create new niches for some organisms and narrow or close existing ones. As a result EAB-induced ash decline may induce a cascade of direct and indirect ecological effects on the structure and function of forest communities. We are documenting the effects of ash mortality on communities of breeding birds in 32 fragmented forest sites, representing a gradient of ash decline, across western Ohio. Specifically, our aim is to determine if high densities of EAB adults emerging from and ovipositing on infested trees provides a resource pulse in late spring and early summer that can be exploited by bark foraging birds. Utilizing point transect foraging observations, we showed that as EAB-induced ash decline progressed, bark-foraging birds increased the proportion of time spent foraging on severely declining ash relative to other tree genera. Additionally, we quantified the relationship between EAB-induced ash mortality and alterations in bird community composition related to changes in habitat. We found that bird diversity increases during the late stages of ash decline.

Dendrochronological study of non-ash trees in emerald ash borer-impacted forest stands: effects of gap formation on release of associated vegetation

Investigators: W.S. Klooster (Post-doctoral scientist) and D.A. Herms

Summary: This study will examine radial growth patterns of non-ash canopy, subcanopy, and understory trees in response to forest disturbance caused by emerald ash borer-induced ash mortality. The specific objectives include: 1) quantifying any radial growth responses in non-ash tree species within EAB-impacted stands; 2) comparing among species to determine whether certain species responded more to ash mortality than other species (given a response); and 3) describing the nature of any response. I hypothesize that the radial growth of non-ash trees will be affected by their proximity to dead ash trees as well as by the size and density of the dead ash; specifically, I expect non-ash trees will exhibit increased growth rates beginning when the nearby ash tree(s) declined, and a marked increase the season after the ash died.

Methods will involve collecting cores from all non-ash trees ≥ 12.5 cm dbh within a subset of previously established plots within the Huron River Watershed in southeastern Michigan, where ash mortality exceeds 95%. All trees will be identified to species and will be mapped in relation to the former locations of the ash trees within the plots using ArcGIS software. The tree core data will be used to calculate radial growth rates for the different species to determine if there was an increase in growth following ash mortality. Spatial analyses will be performed to determine how any growth responses varied by distance to ash trees. Data will also be compared among stand

hydrological class, time since ash mortality, and the pre-EAB size, density, and importance value of ash in the stand.

Effects of emerald ash borer-induced ash mortality and gap formation on establishment and growth of invasive plants

Investigators: W.S. Klooster, D.A. Herms, K.S. Knight (USDA Forest Service), C.P. Herms (Dept. of Horticulture and Crop Science), A.M. Smith, and J. Cardina (Dept. of Horticulture and Crop Science).

Summary: Emerald ash borer (EAB; *Agrilus planipennis*) is causing widespread ash (*Fraxinus* species) mortality forests of southeastern trees, resulting in widespread, simultaneous formation of canopy gaps throughout the forest, which may facilitate establishment and spread of shadeintolerant

invasive plants. Hemispherical photographs, used to calculate percent gap fraction, were taken at a height of 1.5 m to determine how loss of ash was affecting radiant energy resources available to the understory vegetation. Variation among gap fraction values indicated high heterogeneity in light reaching the forest understory. Percent gap fraction was negatively correlated with percent plant cover within the 2 – 5 m and > 5 m layers, suggesting that while gaps may have formed in the canopy layer, light was intercepted before reaching the forest understory. Plant abundance and composition within the forest understory did not change dramatically during the course of the study, nor was there a direct connection between species abundance or diversity and gap fraction. Plant communities were slightly differentiated by the soil hydrological conditions, and less diverse communities occurred in mesic and xeric sites than in hydric sites. Growth of invasive and native species in the understory was not influenced by percent gap fraction; invasive species typically outgrew native species regardless of light level and soil hydrological condition. In a comparison of emergence and survival of *Lonicera maackii* and *Rosa multiflora* under various native and invasive leaf litters we found no difference in establishment success for either invasive species under the different leaf litters over two or three growing seasons. However, leaf litter from *Fraxinus* and many of the invasive species contained similarly high concentrations of Ca and P, suggesting some degree of functional overlap, which may play an important ecological role if *Fraxinus* species are eliminated from North American forests following EAB-induced mortality and if invasive species increase in abundance. In EAB-infested forests, plant community responses to ash mortality will be based on the hierarchical structure of what plant species are able to grow in the climate, soil conditions, and topography within the impacted sites, as well as the stochastic nature of which species are poised to colonize gaps following canopy tree mortality. As EAB infestation continues to spread throughout North American forests, fewer vegetation patches will include ash as an important component, potentially altering the overall mosaic of habitat and ecosystem function across the landscape. The results of these studies did not conclusively answer the question of how EAB will impact spread of invasive plants, perhaps because the time course of the study was too short. However, these data provide an excellent baseline for future research on long-term impacts of EAB on mixed hardwood forests.

Indirect effects of ash mortality caused by emerald ash borer on the forest floor invertebrate community

Investigators: Kayla I. Perry and Daniel A. Herms

Summary: Emerald ash borer (EAB), *Agrilus planipennis*, an invasive wood-boring beetle from Asia, has killed millions of ash trees (*Fraxinus spp.*) in North America since its introduction. Ash mortality caused by EAB has resulted in widespread simultaneous formation of canopy gaps

and increased ash coarse woody debris (CWD). Because soil invertebrates are sensitive to environmental change, CWD can stabilize environmental conditions on the forest floor (e.g. temperature, moisture) and buffer changes to the forest floor habitat caused by increased light. An influx of CWD also has the potential to increase habitat heterogeneity on the forest floor, and thus increase available resources. Soil invertebrates are important in forest ecosystems because they mediate the process of decomposition and nutrient cycling, thereby linking above and below ground food webs. The effects of light gaps and CWD on the forest floor invertebrate community were investigated in southeastern Michigan forests as well as in forest stands at the NASA Plum Brook Station in northern Ohio. Abundance and diversity of soil invertebrate were assessed with pitfall traps, and leaf litter decomposition was quantified to assess their activity. Soil temperature and moisture were measured adjacent to pitfall traps, and canopy gap size was assessed with a densiometer.

In Michigan, the soil invertebrate community associated with existing canopy gaps and ash CWD was assessed to observe natural patterns that may exist between these two environmental variables. Observed canopy gaps ranged between 4-24% openness with an average of 10%. Soil temperature and moisture was found to increase with increasing canopy openness, but there was no effect for CWD. Preliminary results for 2011 suggest there was no effect of gap size or CWD on soil invertebrate richness or diversity.

At NASA Plum Brook, a full factorial experiment with two treatments: presence/absence of light gaps and CWD was designed to understand how these two environmental variables interact to influence invertebrate community composition. No effect was found for CWD. The forest floor environment and invertebrate community responded to the presence of light gaps (20-26% canopy openness). Soil temperature increased in gaps, and soil moisture followed a similar trend. There was an interacting effect of light gap and CWD on invertebrate diversity. In terms of the light treatment alone, invertebrate diversity and species richness decreased in the presence of light gaps. Additionally, the abundance of ground beetles, scarab beetles, globular springtails, and harvestmen decreased in light gaps. Leaf litter decomposition was not influenced by light gaps or CWD.

Cascading ecological impacts of emerald ash borer: Tritrophic interactions between prickly ash, giant swallowtail butterfly larvae, and larval predators

Investigators: K.B. Rice and D.A. Herms

Summary: Extensive ash tree mortality caused by emerald ash borer (EAB, *Agilus planipennis*) has generated widespread canopy gaps, resulting in increased light penetration to the understory. Foliage of the native shrub prickly ash (*Zanthoxylum americanum*) contains furanocoumarins, which are secondary metabolites that deter most herbivores, especially as they become more toxic when photoactivated by UV light. Furthermore, furanocoumarin biosynthesis is energy intensive, and their concentration increases when photosynthesis is enhanced by increased light availability. Female plants typically invest more resources in defense, while males allocate more towards growth. Therefore, male and female prickly ash located in canopy gaps may differ in their furanocoumarin concentrations, growth rates, and reproductive effort.

Giant swallowtail butterfly larvae (*Papilio cresphontes*) are specialist herbivores on prickly ash capable of detoxifying furanocoumarins. Energetic costs of furanocoumarin detoxification can slow larval development, and thus increase exposure to natural enemies. In a series of field and lab experiments, we examined the effects of EAB-induced canopy gaps on resource allocation of prickly ash, and growth and survival of *P. cresphontes*.

Prickly ash located in canopy gaps had, increased levels of furanocoumarins, lower specific leaf

area, increased growth, and increased thorn densities compared to shaded plants. Male prickly ash grew faster than females, but females produced more flowers and fruits. We hypothesize that the slower growth of females results from higher allocation to reproduction and defense. In laboratory bioassays, *P. cressphontes* larvae fed foliage from plants in gaps had lower growth rates than larvae feeding on shaded plants. There was no difference in survival of larvae placed on plants in gaps compared to understory plants, with mortality over 48 hours close to 70% in both habitats. We conclude that larval survival may be lower in gaps because decreased growth rates may increase the amount of time larvae are exposed to natural enemies.

Characterizing adult emergence patterns of emerald ash borer across a latitudinal gradient

Investigators: Samuel A. Discua, Robin A.J. Taylor, and Daniel A. Herms

Summary: Timely forecasting the start and end of emerald ash borer (EAB) adult emergence is required to inform regulatory decisions and control practices. The cumulative number of growing degree-days (GDD) required for first EAB emergence in southeastern Michigan has been determined. However, full emergence phenology period, and especially the end of emergence, has not been adequately characterized, nor has EAB phenology been characterized in other regions. The objectives of this study are (1) to characterize adult emergence patterns of EAB across latitudinal gradient; and (2) to use adult emergence and temperature data to generate degree-day models for predicting key phenological events, including first, 10%, 50%, 75%, and 99% adult emergence. In 2011 and 2012, we monitored EAB emergence at five locations across a north to south latitudinal gradient extending from Midland, MI at the north extreme progressing south to Toledo, Wooster, Delaware, and Cincinnati, OH. Infested ash trees felled at each location were cut into bolts ca 2m in length. Bolts were held in shade under ambient conditions, and EAB adult emergence was monitored by marking all preexisting exit holes, and then counting new emergence holes weekly from first adult emergence until three consecutive weeks of no new emergence. As expected, emergence commenced earliest in Cincinnati, OH and latest in Midland, MI. Adult emergence period was larger in Cincinnati, OH (12 weeks) than Midland, MI (7 weeks). These results provide evidence of the effect of latitude and different climatic conditions on EAB adult phenology. Adult emergence and average daily temperature data will be used to generate a GDD models for predicting EAB adult emergence across much of its current north-south distribution.

Multiyear evaluation of efficacy of soil and trunk applied systemic insecticides for ash canopy conservation

Investigator: Daniel A. Herms

Summary: In 2006, studies were initiated in Toledo, Ohio to evaluate the effectiveness of systemic insecticides for protecting large caliper ash street trees from emerald ash borer in the face of extremely high pest pressure. Specific objectives were to: (1) evaluate efficacy of different rates of imidacloprid (Merit and Xytect) soil drenches applied annually in spring or fall, and (2) to determine the number of years of control provided by different rates of a single emamectin benzoate (Tree-äge) trunk-injection.

Objective 1, Efficacy of imidacloprid soil drenches applied in spring or fall: The following imidacloprid soil drench treatments were applied from 2006-2011 to a green ash (*Fraxinus pennsylvanica*) street tree planting ranging in size from 11-21 in DBH (mean = 15 in): (1) untreated control: (2) Merit 2F, 1.42 g ai / inch DBH applied in May, (3) Merit 2F, 1.42 g ai / inch DBH, applied in October, (4) Xytect WSP, 1.42 g ai / inch DBH, applied October, (5) Xytect WSP, 2.84 g ai / inch DBH, applied in May, (6) Xytect WSP, 2.84 g ai / inch DBH, applied in October. No trees showed visible symptoms of canopy decline in 2006 when

treatments began. Untreated trees began to decline in 2008 and by 2010, all were dead. Trees treated in spring or fall with the high rate of Xytect, or in spring with Merit, maintained healthy canopies through 2012 with percent canopy thinning less than 10% in all cases. Trees treated in the fall with the low rate (1.42 g ai/inch dbh) exhibited significantly greater canopy decline (averaging 60-80%), and were ultimately declared hazards and removed by the city foresters. These results suggest that when applied annual applications of imidacloprid soil drenches can effectively protect trees in the 14-20 inch DBH size class even under intense pest pressure, and at lower rates that spring applications are more effective than fall applications.

Objective 2: Duration of efficacy of emamectin benzoate (Tree-äge) trunkinjections:

The following rates of emamectin benzoate (Tree-äge) were applied as trunk injections using the Arborjet Viper Tree IV on 14 June 2006 to a green ash street tree planting ranging in size from 13-24 in DBH (mean = 21 in): 0.1, 0.2, 0.4, and 0.8 g ai/in DBH. Pest pressure was intense at the study site and canopy decline of untreated trees averaged 53% and 96% in 2008 and 2009 respectively, and all untreated trees had died by 2010. All rates provided excellent control through two years (less than 10% canopy decline in 2008). However, in the third year, canopy decline increased significantly in all treatments, indicating that control was beginning to relax. In 2009, canopy decline averaged 25%, 55%, 60%, and 13% in the 0.1, 0.2, 0.4, and 0.8 g ai/in DBH treatments, respectfully. These results indicate that trees in the 20-25 inch DBH size class can be protected effectively for two years by a single application of emamectin benzoate. However, under intense pest pressure, treatment efficacy began to weaken in the third year, and trees began to decline.

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Diseases in Nurseries and Landscapes
(Multistate Research Coordinating Committee and Information Exchange Group)
2011 – 201 Texas Report/Landscape Tree Pathology

Prepared by

D.N. Appel, Department of Plant Pathology and Microbiology, Texas A&M University, College
Station, TX 77843

Introduction

The major diseases and pathogens of landscape trees in Texas can be grouped into three categories. The first consists of native pathogens such as cankers and root rots. The damage caused by these pathogens can be periodically devastating, depending mostly on climatic trends such as drought. For example, *Hypoxylon atropunctatum* (more recently *Biscogniauxia atropunctatum*), the fungus causing Hypoxylon canker, has increased dramatically following the historic drought of 2011. Another category of diseases contains the highly virulent oak wilt pathogen *Ceratocystis fagacearum*. Oak wilt continues to be a highly recognized lethal presence to valuable oaks in woodlands and urban landscapes throughout a huge region in central Texas. Another, similar disease on the increase in Texas in recent years is the well-known Dutch elm disease, caused by *Ophiostoma ulmi*. The third category is exotic pathogens originating elsewhere but imminently threatening to invade susceptible trees and nursery crops in Texas landscapes.

The current research priorities are on oak wilt and surveys for some potentially devastating exotic pathogens. The following report briefly describes some of these efforts and summarizes where future needs will lie.

Oak Wilt: *Ceratocystis fagacearum*

Oak wilt annually kills trees in epidemic proportions, largely in the central and upper coastal plain regions of Texas. The impact is felt in both urban and rural landscapes throughout nearly 70 counties. *Ceratocystis fagacearum* is a vascular parasite with a long-distance insect vector, the nitidulid beetle. The pathogen may also be spread by movement of contaminated firewood, and spreads locally from tree to tree through connected root systems among healthy and diseased trees. The biology of the pathogen and disease cycle of oak wilt are well understood. A comprehensive control program is successful in reducing losses from oak wilt and is promoted through dozens of workshops held annually by the Texas Forest Service and Texas AgriLife Extension Service. However, in spite of the benefits derived from oak wilt control, there are still aspects of the program in need of improvement.

Oak wilt recommendations for control include a variety of tools designed to prevent infection of healthy trees as well as save trees destined to become infected. The success of these measures depends largely on early detection. Oak wilt is currently diagnosed through a combination of symptoms, signs, and laboratory isolation of the pathogen. The clinical diagnosis can take weeks, and is subject to false negative results. Therefore, we have initiated an effort to develop a quantitative real time polymerase chain reaction (QRT PCR) protocol for oak wilt diagnostics. ITS (internal transcribed spacer) regions of *Ceratocystis* were selected and compared with databases of fungal DNA sequences using BLAST (Basic Local Alignment Search Tool). Based on these selections, primers and fluorescent labeled probes specific for *C. fagacearum* were designed using Primer Express[®] software (Applied Biosystems, Foster City, CA 94404). These primer/probe sets were tested using an Applied Biosystems 7300 real-time PCR System with DNA extracted from mycelium and spore concentrations of the fungus. Following confirmation of the results from fungal cultures, the primer/probe set was used for testing field samples from diseased and healthy oaks.

Field testing consisted of removing tissues from trees with a drill. After removing a small patch of bark, the 5/16" drill bit was inserted into the vascular system and removed to extract filings. These wood filings were captured in small plastic bags and returned to the laboratory for extraction and analysis by QRT PCR.

The results of QRT PCR analyses were successful in detecting *C. fagacearum* in diseased trees. In Table 1, the results of testing 11 trees growing in an active oak wilt center are depicted. The Ct values for samples from live oaks exhibiting typical foliar symptoms of oak wilt were relatively low (< 33), in the range providing a fair degree of confidence to detect the fungus in the vascular systems of the symptomatic trees. Results were different in another set of trees where no oak wilt was suspected or uncertain (Table 2). The live

Tree No.	Tree Description	#PCR +	Ave. Ct Value	Isolation results	Diagnosis
Hen-1	Veinal necrosis, scorch	1/4	29.85	+	Positive ow
Hen-2	dead	0	33.47	n/a	n/a
Hen-6	thinning	2/4	29	n/a	Positive ow
Hen - 9	Bronzing, necrosis (red oak)	1/4	38.38	n/a	questionable
Hen - 10a	Veinal necrosis, scorch	3/4	24.9	+	Positive ow
Hen - 10b	Thinning, half leaf scorch	4/4	17.6	n/a	Positive ow
Hen- 11	Veinal necrosis, scorch	3/4	28.53	n/a	Positive ow
Hen - 12	Veinal necrosis, scorch	3/4	29.36	n/a	Positive ow
Hen - 13	Veinal necrosis, scorch	3/4	24.70	n/a	Positive ow
Hen - 17	Vein banding, scorch	3/4	33.09	+	Positive ow
Hen - 18	Vein banding, scorch	2/4	28.37	n/a	Positive ow

Table 1. QRT PCR results on trees with oak wilt symptoms.

oaks numbered 1 - 6 from Leakey were all presumed to be damaged by the severe drought of 2011 and were not considered to be at high risk of infection by *C. fagacearum*. Even so, some of the trees yielded fairly low Ct values (no.s 3 and 5), and one was in the range that might be construed as being a potential oak wilt infection. No values were obtained from the cedar elm. Again, the live oak in the LBJ Wildflower center (no. 9), with clear oak wilt symptoms, gave a low Ct value, strongly suggesting an oak wilt diagnosis. Another anomaly included the high, weak reaction from the presumably resistant post oak (no. 12).

These and other results obtained from the field testing for a QRT PCR method to diagnose

Tree No./ Location	Tree Description	Ct Values			Diagnosis
1. Leakey, TX	L.O./no symptoms	0	0	0	No O.W./drought
2. Leakey, TX	L.O./no symptoms	0	0	0	No O.W./drought
3. Leakey, TX	L.O./severe thinning	30.63	30.44	32.42	Questionable/ drought
4. Leakey, TX	L.O./severe thinning	37.40	36.31	0	No O.W./ drought
5. Leakey, TX	L.O./no symptoms	34.68	33.51	31.60	No O.W./drought
6. Leakey, TX	L.O./no symptoms	35.37	35.21	0	No O.W./drought
7. LBJ Ctr.	Cedar elm	0	0	0	No O.W./drought
8. LBJ Ctr.	L.O./vein banding	19.61	20.59	19.85	Positive O.W.
9. LBJ Ctr.	L.O./discolored	29.29	35.79	0	Questionable
10. LBJ Ctr.	L.O./no symptoms	0	0	0	No O.W.
11. LBJ Ctr.	L.O./no symptoms	34.41	37	0	No O.W.
12. LBJ Ctr.	Post oak	39.01	35.90	0	No O.W.
13. clinic	L.O./suspect O.W. – clinic neg.	0	0	0	No O.W.
14. clinic	L.O./suspect O.W. – clinic neg.	0	0	0	No O.W.

Table 2. QRT PCR results on trees with unknown problems or species other than susceptible oaks.

oak wilt are very encouraging and indicate that the method will eventually be adopted for routine use in a clinical setting such as the Texas Plant Disease Diagnostic Clinic at Texas A&M University in College Station. However, there are clearly some issues that will require further research before we reach a sufficient level of confidence to incorporate QRT PCR into our routine protocols. The apparent false positives, for example, must be eliminated. An upper threshold for the Ct values must be established to minimize the number of "inconclusive" results. As with all plant diagnostic routines, the interpretation by the diagnostician must also be accounted for prior to using the protocol for routine clientele submissions.

Disease Surveys for Exotic Pathogens

Citrus Greening (Huanglongbing) is the most serious citrus disease in the world. Having caused a 40% reduction of Florida citrus production statewide, the same pathogen (*Candidatus Liberabacter asiaticus*) has just recently been found in two groves in the Rio Grande Valley, commercial citrus producing area of south Texas. Prior to this discovery, *Diophorina citri*, the

psyllid vector of the greening pathogen, arrived in Texas sometime around 2004 and has now become widespread through the lower half of the state. Since then, psyllid monitoring has been an important activity to determine the eventual impact of the disease. We have been involved with the survey and collection of psyllids throughout the upper gulf coast of Texas for the past 2 years. Abundant populations have been found in "dooryard" trees and small, backyard groves. The psyllids occur year around with increases occurring during periods of flush in trees. Collected psyllids are returned to the Texas Plant Disease Diagnostic Lab for processing to determine the presence of the pathogen, but no contaminated psyllids have been found on the upper Gulf Coast.

Another survey currently underway is that for the Sudden Oak Death pathogen, *Phytophthora ramorum*. Since 2004, *P. ramorum* has been found on plants in 11 nurseries and one residence, all of which were the result of importation of nursery stock from California. However, periodic surveys have found no further introductions or persistence of the pathogen in Texas since 2009. In the past year, several previously contaminated nurseries were surveyed by streamside sampling outside of nurseries and soil sampling within nurseries. The streamside surveys are conducted by floating baits for weeks in water sources downstream from the nursery. Soil sampling consists of returning the soil to the lab, saturating the soil with water, and baiting for the pathogen with the same bait used in the streamside surveys, rhododendron leaves.

Impending Disease Issues in Texas Landscapes

The drought and subsequent predisposition of trees to contributing factors was the major tree health issue in Texas in 2011. Hypoxylon canker, bacterial leaf scorch caused by *Xylella fastidiosa*, native elm wilt caused by *Dothierella ulmi*, and root rot caused by *Ganoderma aplanatum* are some of the diseases that may increase in incidence and severity following the drought. In addition, there are numerous exotic pathogens yet to be addressed, such as *Geosmithia morbida* (thousand cankers disease), *Xanthomonas axonopodis* (citrus canker), and *Raffaelea lauricola* (laurel wilt). There are also a number of existing problems that may require attention in the near future such as Dutch elm disease, Giant Asian Dodder, and Texas lethal palm decline. There will be much needed work on the diagnosis and control of these diseases, as well as increased emphasis on public education regarding the impact they may have on Texas landscapes.

Michigan Report for NCERA 224

David Smitley
November 30, 2012

Causes of Variation in Results of Research Tests on Protecting Ash Trees from Emerald Ash Borer With Systemic Insecticides.

Introduction. Since 2002 when emerald ash borer was first discovered to be a new invasive pest in North America several new insecticide treatments have been researched and found to be effective, including basal drenches of imidacloprid and dinotefuran, and trunk injections of imidacloprid, dinotefuran and emamectin benzoate. Although trunk injection of emamectin benzoate provides a consistently high level of protection under almost any condition, the level of protection given by other treatments vary considerably from one research test to another, and sometimes even within a test. More detailed analysis of treatment rates, size of test trees and infestation pressure elucidated some of the causes of variation in results. The original labeled rate for imidacloprid as a basal drench was found to be adequate for small trees (< 12" dbh), but not for larger trees. This was apparently due to a linear relationship between trunk diameter and labeled treatment rates which did not account for the non-linear relationship of trunk diameter to leaf area (a doubling of dbh results in an approximately 5-fold increase in leaf area and vascular tissue). When the amount of imidacloprid applied as a basal drench was doubled for trees exceeding a 12" dbh, efficacy improved. However, even when considering EAB infestation pressure, and size of the test trees, results still varied among test sites.

Long-term study on interaction of tree growth rate and efficacy of insecticide treatments.

In 2005 a test was initiated with 64 green ash planted 4.0 m apart at the Hancock Turfgrass Research Center. Each tree received one of 5 imidacloprid soil treatments: basal drench once per year, basal drench every 2nd year, basal drench every 3rd year, imidacloprid grub treatment under the canopy, or a control. Each treatment was replicated 15 times, with one tree as a replicate. Growth rates were determined for each tree by measuring the dbh annually in July. Canopy thinning ratings were also made in July, according to the scale in figure 1.

Although all the ash trees used in the test were nearly identical when planted, being all of the same cultivar and size, growth rates varied considerably, depending on access to soil moisture and sunlight. Geography of the area and slope of the site created a drainage effect that tended to increase soil moisture on the west side of the planting more than on the east side. In addition, the spacing of trees in a block pattern created more competition of soil moisture and sunlight for trees on the interior of the planting compared with trees on the periphery. This resulted in faster growth rates and a larger trunk dbh for trees located on the outside edges of the plot

Emerald ash borer built-up quickly in a nearby woodlot, causing heavy ash mortality by 2008 in the woodlot. However, the emerald ash borer infestation developed much slower in the experimental planting where approximately half of the trees were receiving effective insecticide treatments. By 2011 most of the ash trees in the control treatments were dead (Table 1). Trees in the drier half of the planting tended to become infested by emerald ash borer more rapidly and declined faster than trees located where the soil moisture was consistently higher. Soil moisture conditions were

reflected by the growth rates of the trees. Trees with a rapid growth rate were better protected by imidacloprid basal drenches than trees that were growing slowly (Table 2).

Conclusion. Although the success of imidacloprid basal drenches clearly depended on tree health as affected by soil moisture and sunlight, the results are preliminary. The data will be analyzed more carefully to determine the relationships between tree health (as indicated by growth rate), insecticide treatment, and canopy thinning due to damage caused by emerald ash borer. A manuscript will be prepared in 2013.

Table 1. Canopy thinning ratings for ash trees receiving different imidacloprid drench treatments.

Imidacloprid								
Treatment	n	2005	2006	2007	2008	2009	2010	2011
Annual Wet	8	0.0	0.0	1.1	11.8	0.0	4.4	4.4 a
Once/2 Yr Wet	8	0.0	0.0	0.5	12.5	0.0	5.6 a	7.5 a
Annual Dry	7	0.0	0.0	0.9	25.0	3.6	21.4 bc	12.1 ab
Grub Rate Wet	7	0.0	0.0	0.8	26.0	13.5	14.3 abc	29.3 abc
Once/2 Yr Dry	7	0.0	0.0	0.5	17.5	4.3	27.1 cd	42.1 bcd
Control Wet	8	0.5	0.0	2.6	20.9	4.4	35.0 cd	49.4 cd
Once/3 Yr Wet	8	0.0	0.0	0.6	14.0	8.1	27.5 bcd	51.2 cd
Once/3 yr Dry	7	0.0	0.0	1.9	35.7	13.5	38.6 cd	50.7 cd
Grub Rate Dry	8	0.0	0.0	1.1	19.1	2.5	41.9 cd	61.3 d
Control Dry	7	11.5	0.0	1.8	28.2	5.7	47.1 d	74.0 d

p = 0.001 p = 0.001

Table 2. Comparison of canopy ratings among trees located in moist or dry soil, when they received the same imidacloprid soil treatment.

Treatment	N	Canopy Rating 2011	SD
Merit drench every year/ Moist	8	4.4	8.2
Merit drench every year/ Dry	7	12.1	13.8
Merit drench every 2 years/ Moist	8	7.5 *	13.9 *
Merit drench every 2 years/ Dry	7	42.1 *	30.1 *
Merit grub rate every year/ Moist	7	29.3*	36.5*
Merit grub rate every year/ Dry	8	61.3*	34.7*

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Forest and Shade Tree Disease Studies-2012
NCERA 224
Colorado State University

Bill Jacobi, Professor

MS Graduate Students: Christy Cleaver

PhD Candidates: Dan West, Javier Mercado

Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins CO 80523

970-491-6927 FAX 970-491-3862 Laboratory

william.jacobi@colostate.edu, betsy.goodrich@colostate.edu

Shade Tree Research Impacts:

- We have a publication in J of Arboriculture and Urban Forestry based on research related to camper movement of firewood in the west. Thirty nine percent of campers bring firewood to National Parks from out of state sources. Several states have increased their inspections at borders (CA) and in many states education efforts have been launched to reduce the movement of firewood across state lines.
- The publication the risk of movement of insects in retail firewood published in J of Economic Entomology. We found >40% of retail firewood have live insects with 15 to 520 insects per bundle. Coleoptera insects accounted for 3,600 of the 4,000 insects collected with a mean of 3 to 60 per each of 24 families. Insects emerged up to 540 days after the wood was purchased. States and the federal government are using this information to formulate management and regulations of firewood movement.
- We are coordinating the National Elm Trial in 16 states and several states (KY, CA) have published reports on the performance of these cultivars.
- We have completed several studies on magnesium chloride ($MgCl_2$) use as a dust suppressant on non-paved roads and found the ions move up to 20 feet from the road, farther in roadside drainages, are detected in low concentrations in roadside streams and have determined foliar concentrations needed to cause mortality in the field and controlled conditions for several tree species. All publications are completed and several counties have modified their use of magnesium chloride in forested regions

Shade Tree Disease Studies:

1. **MgCl₂ Studies:** Salt used for dust control on non-paved roads is common in the west. The salt ions move up to 20 feet away from roads on normal embankments. Roadside culverts and drainages allow salt to move away from roadway and stream sampling indicates low concentrations of salt are detectable in streams. Trees take up chloride and foliar concentrations are correlated with foliar damage. Four manuscripts on these issues are available.
2. **Banded Elm Bark Beetle:** The Dutch elm disease pathogen was successfully isolated from the banded elm bark beetle and a manuscript in Plant Disease is available. Inoculation/vector studies in 2006 and 2007 indicated artificially infested beetles can transport the fungus to feeding wounds.

3. **National Elm Trial:** We have 16 states with 17 sites involved in a trial of 17-19 commercially available elm cultivars. NCR- 193 members and other cooperators are involved.
4. **Firewood and Exotics:** In cooperation with APHIS, we have completed a manuscript on insect infestation of retail firewood purchased in CO, NM, UT, and WY.

Forest Tree Insect/Disease Studies

1. White Pine Blister Rust:

- A manuscript on hazard-rating model for WPBR was submitted this year.
- Small-scale meteorological analysis of the risk of WPBR in the Rocky Mts will be a 2013 project.
- We help coordinate white pine health work via the Central Rocky Mountains White Pine Health Working Group.
- A Research Note from the USDA For. Serv. Rocky Mt Research Station on the techniques and efficiency of pruning limber pine for WPBR management is published.
- Anne Marie Casper, (MS candidate) will submit a manuscript in 2012 on a study of regeneration issues related to natural regeneration and planting limber pine seedlings along the Front Range Mountains of Colorado
- White bark pine regeneration after wild fires in Wyoming. This manuscript will be submitted by the end of the year.

2. **Ponderosa Pine Wood Borers:** Sheryl Costello's last paper from her research is back from the journal with revisions.
3. **Fire, Dwarf mistletoe and Mt Pine Beetles in Front Range Colorado Ponderosa Pine:** Jennifer Klutsch and Russell Beam (MS graduates) have draft manuscripts on the interactions of these three disturbance agents.
4. **Bark Beetle mortality and future fire risk.** Dan West has a draft manuscript from his MS thesis that reports his research on occurrence of wildfires after beetle outbreaks in Colorado's lodgepole pine.
5. **Mountain pine beetle preference for Lodgepole and Ponderosa pine.** Dan West's is finishing his research for his PhD is on Mt Pine Beetle and host preferences. He has exciting results in choice trials in the field and lab and field data from plots along the Front Range Mountains in Colorado.
6. **Spatial relationships of Mountain pine beetle, phoretic mites and fungal associates in front range pine forests.** Javier Mercado (PhD candidate) has made great progress in isolating fungi from beetle and mite and trees. Preliminary results are showing that the dominant species during this time of the epidemic is *Grosmannia clavigera*. This was not expected given that *Ophiostoma montium* has been shown to be better adapted to warmer temperatures, and especially during a warm above average year. Recently, it has been proposed that the *G. clavigera* lineage may be represented by two cryptic species. In the northern range of the epidemic (BC, NW USA see Alamouti et al. 2011) it was found that the species dominant in lodgepole pine could be a new one. In northern Colorado the epidemic has jumped host from lodgepole to ponderosa pine, this could suggest a different epidemic origin of the Colorado and northern epidemic, sustained only by bioassay which indicate better growth of *C. clavigera* from northern Colorado in ponderosa pine.
 The associated phoretic mite fauna of the MPB is not well known in the region, and it is of interest as well. So far, novel associations of phoretic mites and the two expected blue stain fungi in this region have been observed, as well as spatial segregation of the mite associates along an elevation gradient. Using morphological characterization of the fungal fauna, we have recovered *O. montium* only from a species phoretic mite, whereas *G. clavigera* has been recovered from both MPB and other species of mites.
7. **Aspen dieback in Colorado:** Meg Dudley (MS candidate) will complete her manuscript on the health of aspen in Colorado
8. **Aspen insect and disease occurrence Rapid Threat Assessment with WWETEC:** Betsy Goodrich has completed research and report on an analysis if different insect and disease survey types can be combined to

look at large-scale – western U.S. -relationships between aspen health, site and environmental conditions.

9. **Limber pine status and regeneration potential.** Christy Cleaver (MS) has completed a survey of limber pine in CO, WY and MT to determine the impact of Mountain pine beetle and white pine blister rust on adults and if there is regeneration in these areas. She will be analyzing data and writing publications for the next year.

Publications:

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3. Jacobi, W. R., Goodrich, B.A. and Cleaver, C. M. 2011. Firewood transport by National and State Park campers: A risk for native and exotic tree pest movement. Arboriculture and Urban Forestry. 37: 126-138
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6. Goodrich, B.A. and Jacobi, W.R. 2012. Foliar damage, ion content, and mortality rate of five common roadside tree species treated with soil applications of magnesium chloride Water, Air, and Soil Pollution. 223847-862.
7. Jacobi, W. R., Hardin, J. B., Goodrich, B. A. and Cleaver, C. M. 2012. Retail firewood can transport live tree pests. J of Economic Entomology. 105: 1645-1658.

New Disease Issues:

- ◇ Pine wilt nematode killed 15 Scots and Austrian pines this year. We are very worried about this disease since it first appeared in 2005.
- ◇ Death of all walnuts by 1000 cankers is a major concern in Colorado. *Pityophthorus julandis* and *Geosmithia sp.* canker involvement. Drs Tisserat and Cranshaw are heading up this research.
- ◇ Herbicide damage was common this year.
- ◇ Oaks in Boulder CO parks with scale and wood borers and drippy nut disease (bacterial)

New Insect Issues:

- ◇ Mountain pine bark beetles populations are severe now on the northern Front Range forests after attacking limber pine, lodgepole pine in dramatic proportions on the west slope of the continental divide.
- ◇ Leaf mining on elms by European elm leaf weevil was down this year.
- ◇ *Pityophthorus julandis* on walnuts.

Other impacts:

One graduate student graduated in 2012 .

**2012 Ohio Report
NCERA-193**

Pierluigi (Enrico) Bonello
Department of Plant Pathology
The Ohio State University
Columbus, OH 43214
bonello.2@osu.edu

Project: NCERA193: NCR-193: IPM Strategies for Arthropod Pests and Diseases in Nurseries and Landscapes

Ohio Report for the Period: 10/1/11 – 9/30/2012

OUTPUTS / ACCOMPLISHMENTS:

Obj. 3: Pesticide alternatives: Develop management strategies for key pests based on classical biological control (i.e., predators and parasitoids), host plant resistance, and cultural control.

- 1. Systemic induced resistance in the Austrian pine / *Diplodia pinea*.** Collaborators: Patrick Sherwood (Ph.D. student), Dan Herms (Dept. of Entomology), Don Cipollini (Dept. of Biological Sciences, Wright State University).

We tested the integration of the growth/differentiation balance hypothesis (GDBH) and the systemic induced resistance hypothesis (SIRH) using Austrian pine and the pathogenic fungus *Diplodia pinea*. Trees were preconditioned for 1 year under 5-nitrogen fertility levels, controlled by computer irrigation. Plant growth, as measured by stem diameter, plant height, foliar N concentrations and total plant dry mass increased as nitrogen availability increased. Nutrient availability also affected dry matter allocation; as nitrogen levels increased, needle weight ratio increased and root weight ratio decreased, while the stem weight ratio was unaffected. Constitutive secondary metabolism was also affected by resource availability in a tissue and compound specific manner. Four compounds in the stem tissue were significantly impacted by nitrogen availability, while fifteen were affected in the needle tissue. Many of the compounds

affected by fertility conformed to the predictions of the GDBH, displaying a quadratic response to increasing nutrient availability. To assess SIR, trees were inoculated over a temporal gradient with *D. pinea* and then subsequently challenged with another inoculation at distal tissues with the same pathogen. Challenge lesion lengths were significantly impacted by the timing of the induction treatments, but not by fertility. Our results support the SIRH's prediction of enhanced plant resistance following an induction event occurring over a temporal gradient.

In a separate study, we examined the antifungal properties of several previously identified phenolic biomarkers of Austrian pine resistance to *D. pinea*. We found that all compounds exhibited some antifungal properties, but lignin had the greatest individual antifungal activity. Fungistatic growth inhibition was achieved when compounds were tested as clusters (combinations) based on their coordinated metabolic regulation found *in planta*. Significant differences in growth inhibition between constitutive and induced concentrations of individual compounds and the clusters were observed, indicating that the examined phenolics likely play a key role in the expression of the SIR phenotype. This study exemplifies the value of evaluating potential biomarkers of resistance at *in planta* concentrations and the importance of co-regulation of chemical defenses in establishing resistance.

2. Chemistry of coast live oak defense response to *P. ramorum*. Collaborators: Anna Conrad (Ph.D. student), Brice McPherson and David Wood (Dept. of Environmental Science, Policy, and Management, UC Berkeley).

In 2010 we initiated a study in Briones Regional Park (East Bay Regional Park District, Contra Costa Co., California) where we inoculated 150 randomly selected field CLO from 600 completely asymptomatic trees in 2 plots (~300 trees / plot). The Park is currently affected by *P. ramorum* infections in very limited areas. Large areas are thus disease-free, including the ones where we established our plots. As in past work (McPherson et al., under review) trees were inoculated on the main stem, the only reliable way to test resistance of CLO to *P. ramorum*, as trees are killed only by bole cankers. Phloem was collected at the time of inoculation and processed for extraction of soluble phenolics according to published protocols ([Nagle et al. 2011](#)). The 150 inoculated trees were excavated in 2011 to measure subcortical canker lengths as shown in Fig. 1C. Based on the distribution of lesion lengths, phloem phenolic extracts from 10 trees with lesions < 15 cm (classified as resistant) and 10 trees with lesions > 100 cm (classified as susceptible) were subjected to fourier transform-infrared (FT-IR) spectroscopic analysis. Our preliminary data support the ability of IR spectroscopy to provide unique fingerprint information based on at least phenolic profiles of stem phloem, allowing for the identification of CLO resistant to *P. ramorum*.

3. Molecular biology of ash resistance to EAB. Collaborators: David Showalter (Ph.D. student), Sourav Chakraborty (post-doc), Amy Hill (technician), Dan Herms and Om Mittapalli (Dept. of Entomology), Don Cipollini (Dept. of Biological Sciences, Wright State University), Jennifer Koch (US Forest Service, Delaware, Ohio).

Recent studies have looked into constitutive resistance mechanisms in Manchurian ash, concentrating on the secondary phloem, which is the feeding substrate for the insect. In addition to specialized metabolism and defense-related components, primary metabolites and nutritional summaries can also be important to understand the feeding behavior of insect herbivores. Therefore, we compared the nutritional characteristics (water content, total protein, free amino acids, total soluble sugars and starch, percent carbon and nitrogen, and macro- and micronutrients) of outer bark and phloem from black, green, white, and Manchurian ash to determine their relevance to resistance or susceptibility to EAB. Water content and concentrations of Al, Ba, Cu, Fe, K, Li, tryptophan, and an unknown compound were found to separate black and Manchurian ash from green and white ash in a principal component analysis (PCA), confirming their phylogenetic placements into two distinct clades. The traits that distinguished Manchurian ash from black ash in the PCA were water content and concentrations of total soluble sugars, histidine, lysine, methionine, ornithine, proline, sarcosine, tyramine, tyrosol, Al, Fe, K, Na, V, and an unknown compound. However, only proline, tyramine, and tyrosol were significantly different, and higher, in Manchurian ash than in black ash ([Hill et al. 2012](#)).

We are also starting to test some of the compounds we have identified as strongly associated with resistance, particularly phenolics, in artificial diets with the insect. We found that pinoresinol diglucoside, an analog of the pinoresinol hexoside we identified as unique to resistant Manchurian ash ([Whitehill et al. 2012](#)), reduces the survival of EAB larvae.

OUTCOMES / IMPACTS:

Obj. 3: Pesticide alternatives: Develop management strategies for key pests based on classical biological control (i.e., predators and parasitoids), host plant resistance, and cultural control.

Knowing which phenolics are significant in pine interaction with *D. pinea* will allow for their use as selection markers for resistant trees. Knowing that fertility may not be a significant factor in the expression of SIR, at least in containerized plants, reinforces the idea that if inducers of resistance can be identified they may be very useful for the formulation of fungicide-free management strategies for this disease.

Finding that some compounds identified in ash as potential biomarkers of resistance to EAB have actual toxic properties against the insect will allow us to home in on the true traits that make Manchurian ash resistant to EAB and thus move towards a resistant North American ash.

FT-IR spectroscopy of tree phloem may be developed into a practical tool to identify resistant trees in the field.

My program is training 3 PhD students (Anna Conrad, Patrick Sherwood, and David Showalter), 2 undergraduate interns (Michael Falk and Katherine Gambone) and 1 post-doc (Sourav Chakraborty).

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2. Hill AL, Whitehill JGA, Opiyo SO, Phelan P, **Bonello P** (2012) Nutritional attributes of ash (*Fraxinus* spp.) stem phloem and their relationships to resistance against the emerald ash borer. *Tree Physiology*. In press.
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5. Whitehill JGA, Opiyo SO, Koch JL, Herms DA, Cipollini DF, **Bonello P** (2012) Interspecific comparison of constitutive ash phloem phenolic chemistry reveals compounds unique to Manchurian ash, a species resistant to emerald ash borer. *Journal of Chemical Ecology* 38: 499-511.

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USDA Forest Service. 2013-2013. \$51,542. Natural resistance of coast live oak to *Phytophthora ramorum*: Implications for the management of sudden oak death. P. Bonello, D.L. Wood, S. Mori. 1 yr.

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1. Sherwood, P. and **Bonello, P.** 2012. Testing the growth/differentiation balance and systemic induced resistance hypotheses in Austrian pine. 4th International Ecosummit, Columbus, OH, September 30-October 5, 2012.
2. Sherwood, P. and **Bonello, P.** 2012. Austrian pine phenolics, likely contributors to systemically induced resistance in Austrian pine. Ohio Agriculture and Research Development Center, Annual Research Conference, Wooster, OH, April 26, 2012.
3. Sherwood, P. and **Bonello, P.** 2012. Austrian pine phenolics, likely contributors to systemically induced resistance in Austrian pine. APS North Central Division Annual Conference June 13-15, 2012.
4. Chakraborty, S, Hill, A.H., Gambone, K, and **Bonello, P.** 2012. Evaluation of the fluorometric probes dichlorofluorescein (DCFH) and Amplex red for quantitation of reactive oxygen species (ROS) in plants. Ohio Agriculture and Research Development Center, Annual Research Conference, Wooster, OH, April 26, 2012.

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6. Conrad, A.O., McPherson, B.A., Wood, D.L., Opiyo, S.O., and **Bonello, P.** 2012. Metabolite profiling to predict resistance to *Phytophthora ramorum* in natural populations of coast live oak. 4th International Ecosummit, Columbus, OH, September 30-October 5, 2012.
7. Conrad, A., **Bonello, P.**, McPherson, B., Wood, D., Opiyo, S., and Mori, S. 2012. Potential biomarkers of resistance to *Phytophthora ramorum* in coast live oak. 18th Ornamental Workshop on Diseases and Pests, Hendersonville, NC, September 24-28, 2012.
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10. Conrad, A.O., McPherson, B.A., Wood, S.R. Mori, D.L., Opiyo, S.O., and **Bonello, P.** 2012. Metabolite profiling to predict coast live oak resistance to *Phytophthora ramorum*. APS North Central Division Annual Conference June 13-15, 2012.
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- Whitehill JGA, Opiyo SO, Koch J, Herms DA, Cipollini DF, Bonello P (2012) Interspecific comparison of constitutive ash phloem phenolic chemistry reveals compounds unique to Manchurian ash, a species resistant to emerald ash borer. Journal of Chemical Ecology 38: 499–511

Annual Report: Pests of Horticultural Crops in Oklahoma

Incidence of Pierce’s Disease and Bacterial Leaf Scorch in OK. Pierce’s disease (PD) was diagnosed for the first time in Oklahoma in 2008. Samples were submitted from Concord grape collected from a homeowner’s yard in Yukon, OK. In 2009, Oklahoma State University’s Plant Disease and Insect Diagnostic Lab confirmed the presence of the disease in several vineyards located in three Oklahoma counties. Pierce’s disease is caused by *Xylella fastidiosa* subsp. *fastidiosa*, a bacterium that previous climate models predicted could not overwinter in Oklahoma. Pierce’s disease has been found in several more vineyards over multiple years since these early detections (Fig. 1), suggesting *X. fastidiosa* subsp. *fastidiosa* can overwinter in the state.

Bacterial leaf scorch (BLS) is caused by *X. fastidiosa* subsp. *multiplex* and affects a wide variety of ornamental trees including sycamore, mulberry, oak, and elm. Bacterial leaf scorch was first detected in Oklahoma in 2004, and since has been found in many urban street trees in several counties. Once a tree is infected with BLS, it cannot be cured and gradually declines until death. Nursery trees have a high potential for infection from BLS due to plant movement in and out of the nursery and high plant diversity, density, and volume within the nursery (i.e., more potential host plants). Thus, nursery trees are likely an important source of inoculum when introduced into ornamental landscapes.

Both PD and BLS are most efficiently transmitted from plant to plant via xylem-feeding insect vectors, including spittle bugs, sharpshooters, cicadas, and tube-building spittle bugs. My lab is working to: 1) identify key vector species of *X. fastidiosa* in Oklahoma vineyards and nurseries; 2) determine percentage of infective individuals from captured specimens; 3) measure transmission potential of abundant vector species; and 4) identify reservoir host plants of *X. fastidiosa* in Oklahoma vineyards and nurseries.

In 2009-2010, we used 3-inch by 5-inch, double-sided, yellow sticky cards to sample potential insect vectors from several nurseries and vineyards on a weekly basis. Potential vectors were identified to species, removed from sticky cards, and tested for the presence of *X. fastidiosa* in their gut contents using Immunocapture (IC) PCR methods. In vineyards, the dominant vector species was the versute sharpshooter, *Graphocephala versuta*

Table 1. Xylem sap-feeding insects tested for presence of *X. fastidiosa* from vineyards.

Species	2009 and 2010 Results			
	Sampled	Tested	# Positive	% Positive
Hemiptera: Cicadellidae: Cicadellinae: Proconiini				
<i>Paraulacizes irrorata</i>	6	6	0	0
<i>Oncometopia orbona</i>	28	28	3	10.7
<i>Cuerna costalis</i>	5	4	0	0
<i>Homalodisca vitripennis</i>	0	0	0	0
Hemiptera: Cicadellidae: Cicadellinae: Cicadellini				
<i>Graphocephala versuta</i>	2231	946	21	2.2
<i>Graphocephala coccinea</i>	17	17	1	5.8
<i>Graphocephala hieroglyphica</i>	8	7	0	0
<i>Xyphon flaviceps</i>	6	1	0	0
Hemiptera: Cercopidae				
<i>Clastoptera xanthocephala</i>	39	29	0	0

(Table 1). Of the sharpshooter species identified from vineyards, *G. versuta*, *G. coccinea*, and *Oncometopia orbona* were carrying *X. fastidiosa*. Nurseries contained a greater diversity of potential vectors, but the most abundant were *O. orbona*, *G. versuta*, and the spittlebug, *Clastoptera xanthocephala* (Table 2). Species collected from nurseries that contained *X. fastidiosa* are *G. versuta*, *G. coccinea*, *O. orbona*, and *C. costalis*. We are currently sequencing positive samples to determine which *X. fastidiosa* subspecies these vectors are carrying.

Table 2. Xylem sap-feeding insects tested for presence of *X. fastidiosa* from nurseries

Species	2009 and 2010 Results			
	Sampled	Tested	# Positive	% Positive
Hemiptera: Cicadellidae: Cicadellinae: Proconiini				
<i>Paraulacizes irrorata</i>	72	62	0	0
<i>Oncometopia orbona</i>	806	724	21	2.9
<i>Cuerna costalis</i>	39	37	1	2.7
<i>Homalodisca vitripennis</i>	3	0	0	0
Hemiptera: Cicadellidae: Cicadellinae: Cicadellini				
<i>Graphocephala versuta</i>	895	504	17	3.3
<i>Graphocephala coccinea</i>	29	24	1	4.1
<i>Graphocephala hieroglyphica</i>	5	3	0	0
<i>Xyphon flaviceps</i>	6	0	0	0
Hemiptera: Cercopidae				
<i>Clastoptera xanthocephala</i>	299	299	0	0

We are currently processing samples from our transmission study and reservoir host plant study. Our initial results show infective, field-collected *G. versuta* are able to transmit *X. fastidiosa* to our test plants. Of the potential reservoir host plants collected around vineyards, positive samples have been detected from wild grape, *Vitis* sp. Bacterial DNA from these positive samples will be sequenced soon.

IMPACTS: *The positive identification of BLS and PD vectors in Oklahoma will help nursery and vineyard personnel monitor for their presence at key times during the growing season. This information will allow for correct timing of insecticide sprays to manage these pests when they are present in the landscape. Although the number of collected insects carrying X. fastidiosa is low, even a handful of infective vectors can spread leaf scorch diseases and devastate vineyards, nurseries, and landscapes.*

Redbud Leaffolder Control with Foliar- and Soil-Applied Insecticides. In 2012, Oklahoma experienced an outbreak of redbud leaffolder, *Fascista cercerisella*, on our state tree, redbud, *Cercis canadensis*. While this caterpillar pest is not detrimental to healthy hosts, its feeding damage reduces the aesthetic quality of landscape trees. Normally, I advise landscape managers and homeowners to remove dropped leaves in the fall, thereby reducing the number of overwintering pupae in the landscape. However, pest pressure was so high this year that landscape managers requested insecticide options for 2013, anticipating another year of heavy pressure from this insect.

I conducted field work in 2009-2010 to test the efficacy of foliar- and soil-applied insecticides for redbud leaffolder control. The research was performed at Deep Fork Tree Farm in Arcadia, OK, which was experiencing a heavy infestation of redbud leaffolder on Chinese redbud, *Cercis chinensis* 'Avondale'. In 2009, I tested the following foliar treatments:

Acelepryn (chlorantraniliprole) 1.67 SC at 1 fl. oz. per 100 gal.
 Acelepryn 1.67 SC at 2 fl. oz. per 100 gal.
 HGW86 (cyantraniliprole) 1.67 SC at 1 fl. oz. per 100 gal.
 HGW86 1.67 SC at 2 fl. oz. per 100 gal.
 Talstar (bifenthrin) P at 10 fl. oz. per 100 gal.

All treatments were replicated 5 times (i.e., 5 trees) and compared to a non-treated control group in a randomized complete block design. Applications were made on July 23 and evaluations were completed on July 30 (7 DAT). Leaffolder mortality was estimated by opening leaf folds and recording the number of live caterpillars per tree. Percent mortality were arcsine square root (x) transformed to satisfy the condition of normality. Treatment differences were tested using ANOVA (PROC GLM, SAS 9.2) and mean separation testing was performed with Duncan's LSD at $P \leq 0.05$.

The second of three generations of redbud leaffolder was present at the time of insecticide application, and a large number of leaffolder webs contained no larvae. These empty webs were formed by first-generation caterpillars and possibly unknown mortality factors such as predation (e.g., spiders were found in a small number of empty leaffolder webs). On average, percent mortality was greatest with Talstar P and significantly different from background mortality recorded from non-treated controls (Fig. 2). Mean percent mortality was greater than 73% for both Acelepryn and HGW86 applied at 2.0 fl. oz./100 gal.; these average mortality rates were also significantly different than that recorded from non-treated controls (Fig. 2). In contrast, mean percent mortality was only 53.3% and 39.5% for Acelepryn and HGW86 applied at 1.0 fl. oz./100 gal., respectively. These mortality rates were not significantly different from that of non-treated controls (Fig. 2). Excellent control was achieved with Talstar P, resulting in approximately 98% reduction in leaffolder caterpillars relative to the non-treated control (Table 3). For both Acelepryn and HGW86, a higher percentage of caterpillars were controlled when treatments were made at the 2.0 fl. oz./100 gal. rate than the 1.0 fl oz/100 gal rate (Table 3).

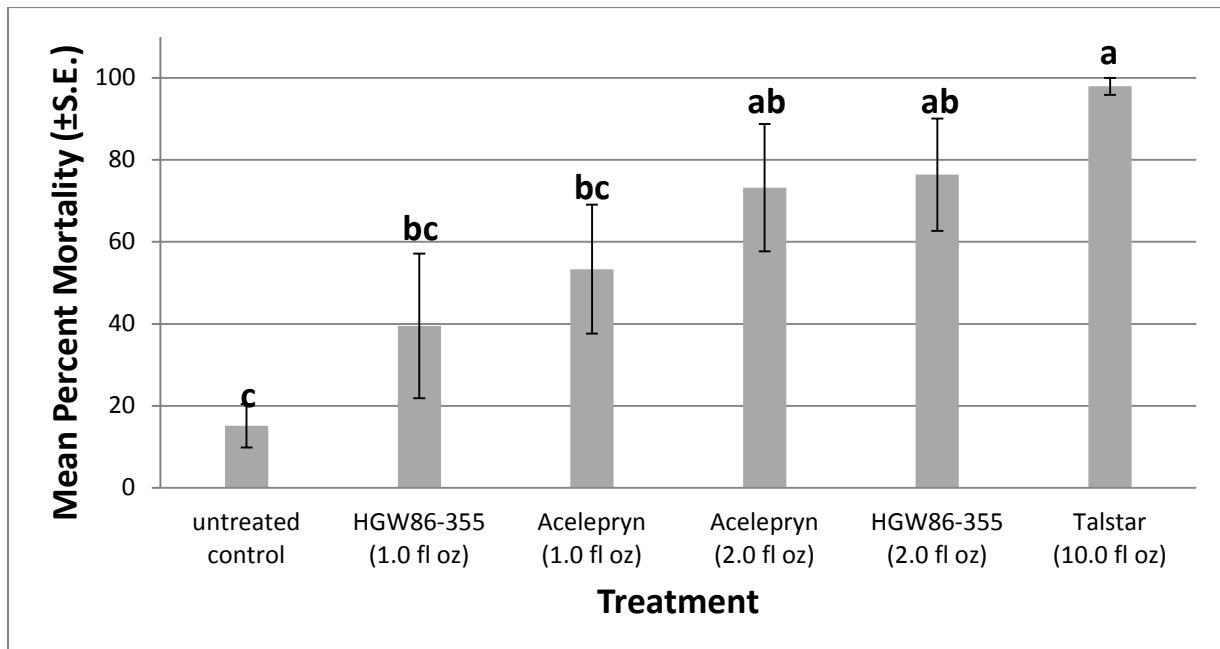


Figure 2. Mean percent mortality (\pm S.E.) of redbud leaffolder, *Fascista cercerisella*, on Chinese redbud, *Cercis chinensis* var. 'Avondale', following foliar applications of insecticides at Deep Fork Tree Farm, Arcadia, OK in 2009. Means marked with different letters are significantly different (Duncan's LSD, $P \leq 0.05$).

Table 3. Mean number of live redbud leaffolder caterpillars recovered per treatment and percent reduction compared to the non-treated control.

Treatment/formulation	Rate (fl oz/100 gal)	Mean number of leaffolders (\pm SE)	Percent reduction
Non-treated check	---	10.4 (3.9)	---
Talstar P 0.67 SC	10.0	0.2 (0.2)	98.1
HGW86-355 1.67 SC	2.0	1.8 (1.6)	82.7
Acelepryn 1.67 SC	2.0	2.6 (2.4)	75.0
Acelepryn 1.67 SC	1.0	3.0 (1.2)	71.2
HGW86-355 1.67 SC	1.0	4.0 (1.6)	61.5

In a separate trial, soil drench applications were made in fall 2009 and spring 2010 to evaluate efficacy of systemic insecticides against redbud leaffolder. I evaluated the following treatments:

- Acelepryn 1.67 SC at 0.25 fl. oz. per foot shrub height (fall application)
- Acelepryn 1.67 SC at 0.25 fl. oz. per foot shrub height (spring application)
- HGW86 1.67 SC at 0.25 fl. oz. per foot shrub height (fall application)
- HGW86 1.67 SC at 0.25 fl. oz. per foot shrub height (spring application)
- Merit 75 WP at 1.9 g per foot shrub height (fall application)
- Merit 75 WP at 1.9 g per foot shrub height (spring application)
- Lepitect (acephate) 97.4 WSP at 5.7 g per foot shrub height (spring application)

All treatments were replicated 5 times (i.e., 5 trees) and compared to a non-treated control group in a randomized complete block design. All treatments were prepared by mixing the product in a bucket of lukewarm water, yielding a solution volume of 32 fl. oz. per foot of shrub height. Prior to application, I removed all bermudagrass, weeds, and mulch from around the base of each shrub to maximize root uptake of each treatment. Treatment solutions were then applied to the soil around each shrub at the root flare. On August 31, 2010, caterpillar abundance was estimated by counting the number of leaf rolls per tree. Web count data were $\log_{10}(x+1)$ transformed to satisfy the condition of normality. Treatment differences were tested using ANOVA (PROC GLM, SAS 9.2) and mean separation testing was performed with Fisher's LSD at $P \leq 0.05$.

On average, caterpillar abundance was lowest on shrubs treated with HGW86 and Acelepryn in fall 2009 and spring 2010 (Fig. 3). In addition, the average number of affected leaves was reduced by greater than 90% for all four treatments (Table 4). Thus, a high level of caterpillar control can be achieved by using either of these products as a soil drench in the fall or spring. Shrubs treated with Merit resulted in 67% and 76% reductions in webs for fall and spring applications, respectively (Table 4). The average number of webs for shrubs treated with Merit and Lepitect was not significantly different from the non-treated control (Fig. 3). Surprisingly, Lepitect had no effect on redbud leaffolder caterpillars and control was less than 1% (Table 4).

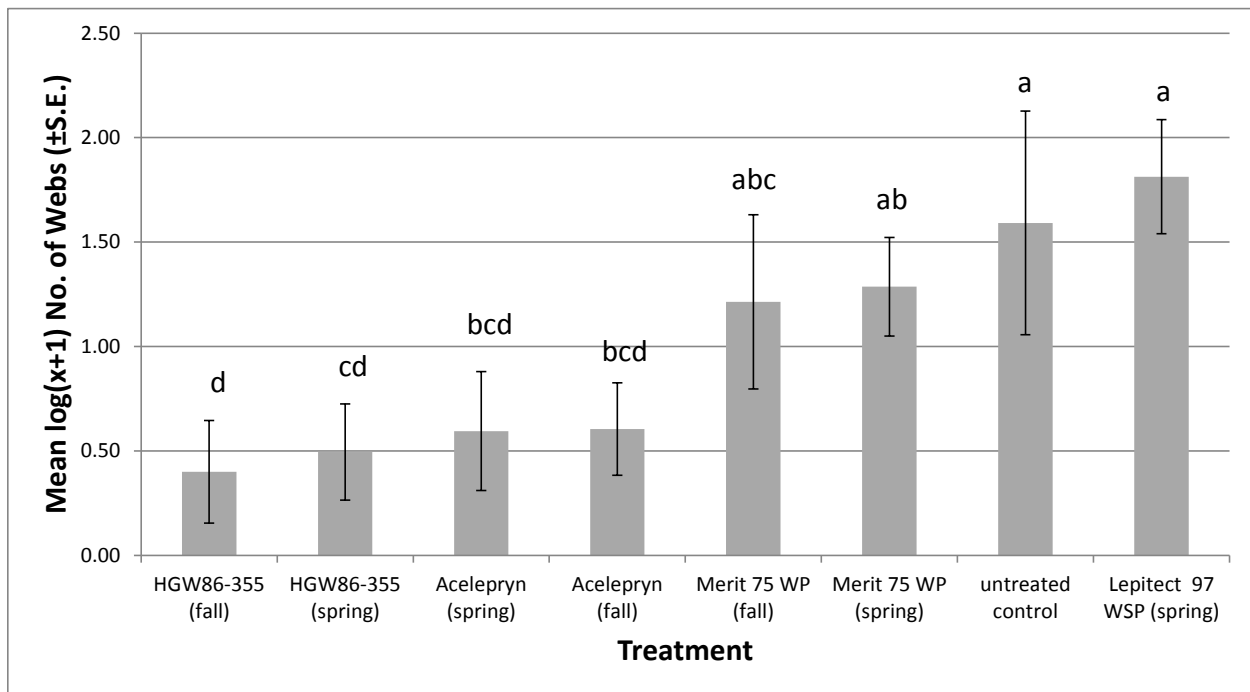


Figure 3. Mean abundance of redbud leaffolder, *Fascista cercerisella*, webs on Chinese redbud, *Cercis chinensis* var. 'Avondale', following soil applications of insecticides at Deep Fork Tree Farm, Arcadia, OK. Means marked with different letters are significantly different (Fisher's LSD, $P \leq 0.05$).

Table 4. Mean number of redbud leaffolder webs recovered per treatment and percent reduction compared to the non-treated control.

Treatment/formulation	Rate (amt./ft shrub ht.)	Mean number of leaffolder webs (\pm SE)	Percent reduction
Non-treated check	---	104.0 (40.9)	---
HGW86 1.67 SC (fall)	7.4 ml	3.0 (2.1)	97.1
HGW86 1.67 SC (spring)	7.4 ml	3.8 (2.5)	96.3
Acelepryn 1.67 SC (fall)	7.4 ml	4.5 (4.2)	95.7
Acelepryn 1.67 SC (spring)	7.4 ml	6.3 (8.8)	93.9
Merit 75 WP (spring)	1.9 g	25.5 (17.7)	75.5
Merit 75 WP (fall)	1.9 g	34.3 (32.9)	67.0
Lepitect 97 WSP (spring)	5.7 g	103.3 (102.6)	0.01

IMPACTS: Current management strategies for webworms and leaffolders involve the use of organophosphates, pyrethroids, and carbamates. Landscape and nursery professionals need alternative chemistries to replace and/or rotate with these conventional chemistries. Products containing chlorantraniliprole and cyantraniliprole show promise for control of leafrollers,

especially when used as a soil drench. They also show promise as environmentally friendly and reduced risk products.

Washington

2012 NCERA-224 State Report
October 29-30, 2012
San Juan, Puerto Rico

Washington State University-Puyallup Ornamental, Christmas Tree, and Nursery Stock Program Personnel, Cooperators and Sources of Support

Project leaders and Staff

Dr. Gary Chastagner, Professor of Plant Pathology (chastag@wsu.edu)
Dr. Marianne Elliott, Research Associate and Sudden Oak Death Education Coordinator
Kathy Riley, Professional Worker I
Andree DeBauw, Ag. Res. Tech. II
Katie Coats, Research Associate
Don Sherry, Professional Worker I
Carly Thompson
Andy McReynolds

Graduate Students

Katie McKeever, Plant Pathology Ph.D. Degree
Anna Leon, Plant Pathology Ph.D. Degree
Lucy Rollins, Plant Pathology MS Degree

2012 Student Interns

Jessica-Michelle A. Corbin, Dominican University, San Rafael, CA
WSU Translational Intern - Nicole Reinsch, Pierce Community College, Puyallup, WA

Location of Cooperators

BLM Sprague Seed Orchard, Merlin, OR
CAL-FIRE, Ben Lomond, CA
Danish Forest and Landscape Research Institute
Michigan State University
Natural Resources Canada, Pacific Forestry Centre, Victoria, BC
North Carolina State University
Norwegian Crop Research Institute
Ohio State University
Oregon Department of Forestry, Salem, OR
Oregon State University

Pennsylvania State University
Starker Forest, Corvallis, OR
University of California Davis
USDA-ARS, Fort Detrick, MD
USDA Forest Service - Dorena Genetic Resource Center, Cottage Grove, OR
USDA Forest Service, Forest Health Protection, Central Point, OR
USDA Forest Service - Umpqua National Forest, Roseburg, OR
Washington Bulb Company
Weyerhaeuser

Major Sources of External Support

USDA Floriculture and Nursery Research Initiative
USDA APHIS
USDA Forest Service
USDA NIFA Specialty Crop Research Initiative (SCRI)
WSDA Specialty Crop Block Grant Program
WSDA Nursery Research Program
Pacific Northwest and Puget Sound Christmas Tree Associations
Washington State Bulb Commission
Northwest Agriculture Research Foundation
PNW Growers

Christmas Trees Research

Developing disease resistant sources of Nordmann and Turkish fir Christmas trees with superior growth and needle retention characteristics (In cooperation with Chal Landgren, Oregon State University, Aurora, OR).

A series of genetic trials have been established in WA and OR to examine the variation in growth and postharvest needle retention among different sources of Nordmann and Turkish fir in an effort to identify locally-adapted sources of these trees with superior postharvest needle retention characteristics. Individual, superior trees can then be used to establish grafted varietal seed orchards for the future production of high quality nursery stock, Christmas trees and greenery products.

Variation in bud break - Bud break data were taken on ~3700 trees in May 2011 at six of the 2004 Nordmann/Turkish fir replicated sites in Washington and Oregon. In addition, a second set of data on ~1700 of those trees was taken at the two WSU-Puyallup sites on June 6th. Each of these sites contained 25 to 50 trees from each of 15 sources of Nordmann fir and 4 sources of Turkish fir.

Results of these evaluations indicate that there was significant tree-to-tree variations in bud break and that bud break also varied by site and rating date. Overall, there were also significant differences in bud break ratings for the different sources of trees in these trials. In general, the Turkish fir tended to break bud earlier than the Nordmann fir.

The bud break ratings for each source were ranked from highest to lowest at each site. A Spearman rank order correlation analysis was then conducted to compare the rankings for each site to the rankings at the other sites. This analysis indicated that there was a highly significant correlation in the bud break ranking from site to site. This means that sources that broke bud earlier at one site, also broke bud early at all of the other sites.

Variation in postharvest needle retention - Needle loss testing was conducted on all of the trees in the two genetic trials at WSU Puyallup, a grower site in southwestern WA to identify sources of trees that exhibit superior needle retention characteristics. In order to determine which of these trees had the best needle retention, two-year-old branches were harvested between mid-October and mid-November. The branches were displayed in a postharvest room at ~64F, with continuous light, and evaluated for needle loss after 10 days of display.

Needle loss ratings varied by species, source, and site. Delaying harvest at the WSU Puyallup site increased needle retention. Overall when branches were harvested on October 18th, only 19.5% of the trees had needle loss ratings of 1 or less, which is the target value for acceptable needle loss. This increased to 61.6% when branches were harvested one month later. There was also a significant effect of site on needle loss ratings. Only 4.7% of the trees at the grower site had acceptable compared to over 60% at Puyallup when branches were harvested in mid-November.

Effectiveness of a Swiss needle cast disease management program in controlling disease development in U.S. Pacific Northwest Douglas-fir Christmas tree plantations.

Swiss needle cast (SNC), caused by *Phaeocryptopus gaeumannii*, has been a major problem on Douglas-fir Christmas trees in the U.S. Pacific Northwest. As a result, in the early 80's a disease management program was developed that was based on monitoring trees in plantations for the presence of infected needles and then making one or two applications of a chlorothalonil-based fungicide during shoot elongation if the disease was present during the 3 to 4-year period prior to harvest. In the 20-year-long period between 1987 and 2007, yearly surveys of Douglas-fir Christmas tree plantations in Oregon were done to determine the effectiveness of this SNC disease management program. This survey found that only 37.7% of the 465 plantations examined had any SNC-diseased trees. The overall incidence of infected trees during this time was 13%, compared to 84% in 1981. These results indicate that the disease management program established in the early 80's has effectively reduced the incidence and severity of SNC on Douglas-fir Christmas trees in the PNW. Although chlorothalonil-based fungicides have consistently been the most effective materials in controlling this disease, recent studies have shown that trifloxystrobin, and caprylic acid also have the potential to provide effective control of SNC.

Quantification of *Fusarium commune* in Douglas-fir nurseries using real-time PCR Anna L. Leon, WSU Plant Pathology Graduate Student

Douglas-fir are often grown in bare-root nurseries as seedlings to decrease mortality and improve overall tree growth and vigor prior to planting in recently harvested forests. Growing the seedlings in an agricultural environment exposes them to pathogens that they would not encounter in the forest, including members of the genus *Fusarium*. Diagnosis of *Fusarium commune* is only done sparingly due to time and technical constraints associated with dilution plating and colony identification. Additionally, *F. commune* and *F. oxysporum* remain morphologically indistinguishable, making it easy for growers to mistake non-pathogenic strains of *F. oxysporum* with *F. commune*. As a result, many growers fumigate unnecessarily or simply fumigate on a scheduled basis without testing the soil for pathogens. The goal of this project is to develop an easy, rapid method of quantifying *Fusarium commune* and distinguishing it from populations of *Fusarium oxysporum* in the field. Real-time quantitative PCR TaqMan primers and probes have been designed for both *F. oxysporum* and *F. commune* and threshold tests with inoculated soil will be conducted to validate this method of pathogen quantification. The data will be compared to traditional dilution plating as well as to a bioassay test in which Douglas-fir seed will be planted directly in soil from the nursery and seedling vigor will be assessed. Using these methods, we hope to develop a comprehensive assay to help interested growers make informed decisions about soil health and chemical use.

Sudden Oak Death

<http://www.puyallup.wsu.edu/ppo/sod/>

An overview of *Phytophthora ramorum* in Washington - *Phytophthora ramorum*, the exotic water mold that causes sudden oak death and Ramorum shoot blight, was first detected in Washington State on ornamental nursery stock in 2003. Since then, all three genetic lineages (NA1, NA2, and EU1) have been detected in a total of 48 nurseries in western Washington. The number of positive nurseries has decreased since a high of 25 in 2004 to 3 in 2011. In 2011, two of the positive nurseries had been positive in previous years. The swimming zoospores of this pathogen are commonly spread via water. In 2006, stream baiting revealed that *P. ramorum* had spread from a nursery in Pierce County into a nearby stream. Subsequent yearly stream baiting has resulted in the detection of *P. ramorum* in a total of 11 drainage ditches and/or streams in five western Washington counties. Genotype analysis indicates that all three lineages of this pathogen have spread into waterways and that contamination of waterways has typically resulted from spread of inoculum from nearby positive nurseries. Stream baiting has also shown that once a waterway becomes infested, it remains infested even after successful mitigation steps have eliminated the pathogen from infested nurseries.

In the spring of 2009, infested ditch water resulted in the infection of salal (*Gaultheria shallon*) plants along the perimeter of another nursery in Pierce County. This represented the first time the NA2 lineage had been detected on plants outside of a nursery in North America. In 2010, additional plants were positive in the nursery, and ditch water continued to be positive along the perimeter of the nursery. Composite soil samples collected from along the ditch were also positive in 2010; making this the first location in Washington with evidence that inoculum had spread from a nursery in water resulting in the contamination of soil and infection of natural vegetation. In addition, positive soil has also been detected at 3 trace forward sites where infected plants from a nursery in Thurston County had been planted in the landscape.

The Washington State Department of Agriculture is continuing to monitor nurseries for *P. ramorum* as required by the Confirmed Nursery Protocol, but as of 2012 will no longer monitor waterways and streams outside of nurseries. Stream baiting efforts are still being conducted by the Washington State Department of Natural Resources (DNR), with initial 2012 sampling being done on 10 watercourses in 5 counties. In addition to leaf baiting, DNR will be working with the USDA Forest Service on “Bottle of Bait” protocols to assay each of the streams for *P. ramorum*.

The spread of *P. ramorum* in water from nurseries is a national problem that increases the risk that this pathogen and its nursery-genotypes (NA2 and EU1) will spread to the landscape. The spread of the NA2 lineage to salal plants and soil in Washington illustrates the importance of this pathway. In some areas, water from infested streams is being used to irrigate a variety of horticultural sites. This also increases the risk that *P. ramorum* will spread onto plants in the landscape. There are a number of challenges associated with efforts to reduce the risk associated with the water pathway of spread. These include the complexity of riparian systems and epidemiological unknowns associated with the biology of pathogen in streams, important hosts in riparian systems, and inoculum thresholds necessary for spread via irrigation water. Regulatory challenges include issues relating to: the regulation of the “Disease” vs. the “Pathogen”, communicating with entities that have water rights for irrigation that their water sources have been contaminated with inoculum, the failure of growers to change practices, a clear understanding of roles and responsibilities among state and federal agencies, and response to wildland detections.

Management of *P. ramorum* in waterways starts at the nursery. Other than treatment of irrigation water, there are limited ecologically acceptable mitigation options to reduce inoculum levels once *P. ramorum* spreads into a stream. Best management education and/or nursery certification programs that change grower practices relating to water management and the spread of this pathogen are needed. There is also a need to increase sampling of nursery water, particularly once the pathogen is initially detected in a nursery. There are currently very few options for treating water leaving the nursery. Water treatment regulations focus mostly on nutrients and vary state-to-state. The environmental acceptability of treating water with algaecides or other chemicals is unclear. Research is needed to develop low-cost biofiltration systems that are effective in removing inoculum of this pathogen from water before it leaves the nursery.

Influence of Nitrogen Fertility on the Susceptibility of Rhododendrons to *Phytophthora ramorum* (In cooperation with Rita L. Hummel and Robert E. Riley) - Growth and susceptibility of container-grown evergreen *Rhododendron* 'English Roseum', 'Cunningham's White', and 'Compact P.J.M.' to *Phytophthora ramorum* in response to nitrogen (N) fertilizer applied twice weekly at rates of 25, 75 and 150 mg N was evaluated during two growing seasons. At the end of both growing seasons, horticultural evaluation of the different plants showed that 150 mg N fertilized cultivars had superior shoot growth, visual quality, leaf color and the highest leaf N content while the 25 mg N cultivars were inferior for these characteristics. Plants fertilized with the 75 mg N rate were typically intermediate to the 150 and 25 mg N plants for the measured characteristics. During the first growing season, nitrogen fertility levels did not effect the number of flower buds that developed on 'Cunningham's White' and 'English Roseum', but increasing nitrogen fertilization did increase the number during the second year. The susceptibility to *P. ramorum* was influenced by N concentration in the most susceptible cultivars, 'English Roseum' and 'Cunningham's White', where lesion size and infection frequency both increased at higher N concentrations. The opposite trend was seen in 'PJM Compact', the most resistant cultivar.

Susceptibility of Larch, Hemlock, and Sitka Spruce to *Phytophthora ramorum* - The recent determination that *P. ramorum* is causing bleeding stem cankers on Japanese larch (*Larix kaempferi* syn *L. leptolepis*) in the UK and that inoculum from this host appears to have resulted in disease and canker development on other conifers, including western hemlock (*Tsuga heterophylla*), Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), and Sitka spruce (*Picea sitchensis*), potentially has profound implications for the timber industry and forests in the U.S. Pacific Northwest (PNW).

An experiment was conducted to examine the susceptibility of new growth on European (*L. decidua*), Japanese, eastern (*L. laricina*) and western larch (*L. occidentalis*), western and eastern hemlock (*T. canadensis*), Sitka spruce and a coastal seed source of Douglas-fir to isolates of the three known lineages (NA1, NA2, and EU1) of *P. ramorum*. Due to the discovery in the UK that *P. ramorum*-infected Japanese larch needles can sporulate heavily in the late summer and early fall, we also conducted an experiment to determine the susceptibility of late-season European, Japanese, eastern and western larch and Douglas-fir foliage to infection by this pathogen.

In the spring 2011 inoculation trial, the percentage of inoculated seedlings with symptoms ranged from 20 to 100%. Symptoms ranged from infection of individual 2011 needles or needle bundles, shoot dieback that extended into the previous year's stems in the form of lesions, to

mortality. All of the conifer species exhibited some level of symptom development, but symptom severity and recovery of *P. ramorum* was most common on the four larch species. Although all of the larches were susceptible, the western larch had the highest level of mortality and percentage of isolation positives for all the genotypes. Of the isolates used, the NA2 isolate caused symptoms on all of the conifers, while symptoms developed on 75% and 50% of the conifers inoculated with the EU1 and NA1 isolates, respectively.

There were minimal symptoms on the late-season inoculated trees and they were the same as those on the controls (e.g., a few gray needles, yellow/brown needle tips, a faint lighter-colored needle banding, single yellow needles, etc). Isolations from symptomatic needles 8 weeks after inoculation resulted in only a single positive recovery of *P. ramorum* from the proximal end and center of a single, partially gray needle from Japanese larch inoculated with the NA2 isolate.

The use of biologically-based substrates to reduce the spread of Phytophthora inoculum in water - Twenty-eight materials have been tested for their ability to suppress the spread of *P. ramorum* inoculum using a baiting technique. The materials tested included fresh bark from 10 species of conifer tree and 18 commercially available products. These products included mulches, composts, and potting mix components. An NA1 isolate of *P. ramorum* was used in the tests. Each material was tested three times and the percent of baits colonized by *P. ramorum* was recorded. Bacteria were isolated from baits that were negative for *P. ramorum*, and *Trichoderma* spp. were isolated when they appeared on bait isolation plates. Electrical conductivity (EC) and pH of bark suspensions were measured.

There was no significant relationship between number of baits positive for *P. ramorum* and EC or pH. The number of *P. ramorum* positive baits was negatively correlated with number of plates with *Trichoderma* spp.. The most suppressive materials were the two mulches RWR Doug-fir Red #2 and RWR Dark aged fines (mixed spp.).

Trichoderma spp. were tested against *P. ramorum* for antagonistic activity in a dual culture experiment. *Trichoderma* isolates from noble fir roots obtained from field-grown trees that were naturally infected with *Phytophthora* spp. were identified using DNA sequence analysis and included as standards. This may be one mechanism by which these materials are suppressive to *P. ramorum*. Isolates of *Trichoderma* have been collected from baits placed in suspensions of Vigoro Rubber mulch (1 isolate), Mountain Magic Western bark nuggets (2), grand fir bark (1), giant sequoia bark (1), peat moss (10), RWR Doug Fir Red #2 (3), RWR dark aged fines (1), and no bark (2).

The results of this screening show the potential for various bark mulches to suppress *P. ramorum*. The variable nature of the results suggests that the mechanism is biological and due to the presence or absence of certain organisms such as *Trichoderma* spp. and bacteria. Addition of large amounts of inoculum of inhibitory organisms to a mulch that is conducive to their growth may be a possible means of controlling *P. ramorum* and other diseases in nursery beds, landscaped areas, and potting mixes. Column tests with the most effective mulch materials and mulches amended with *Trichoderma* spp. and bacteria that are effective biocontrol agents identified in the dual culture experiments are planned.

Examining fungicide resistance among lineages in *Phytophthora ramorum* - In 2011 we completed screening 85 isolates of *P. ramorum* collected from 12 nurseries in Washington, one in Oregon, and one Christmas tree farm in California. All isolates were grown on media amended with 0, 0.01, and 1 ppm mefenoxam (Subdue Maxx) and colony diameter after 10 days was measured. Isolates were considered to be sensitive to the fungicide if there was scant or no growth at 1 ppm a.i. and resistant if there was significant growth at 1 ppm. Representative isolates were chosen and grown at a range of concentrations between 0 and 100 ppm a.i. to determine EC50 and EC90 values. In an earlier study EC50 for mefenoxam for *P. ramorum* was determined to be 0.01 ppm (Elliott et al. 2009).

Only a few of the isolates tested showed resistance to mefenoxam, and these were all of the EU1 lineage and originated from one nursery (nursery #41) and its trace-forwards. A subset of 19 representative isolates was selected for further study. EC50 and EC90 values for these isolates were calculated. These isolates will be used in studies of temperature growth rates and sporulation potential in the second year of the project.

***Phytophthora ramorum* projects at the National Ornamental Research Site at Dominican University California (NORSUDUC)** – We currently are conducting the following projects at NORSUDUC:

- Determining the risk that fungicide applications will mask symptom development on rhododendrons under commercial production practices and determine how long suppression of symptom development lasts following the cessation of fungicide treatments.
- Examining the root-to-root spread of *P. ramorum* on *Viburnum tinus*.
- Effect of fungicides and biocontrol agents on inoculum production and persistence of *Phytophthora ramorum* on nursery hosts.

Ornamental Bulb Crops

Develop protocols to quantify threshold levels of *R. tuliparum* inocula in soil and bulbs -

Tests are currently underway to confirm the effectiveness of a new primer and probe set in detecting *R. tuliparum* in artificially-infested soil and bulbs. We have collected and analyzed initial emergence and plant health data on field and pot trials that are examining the effect of inoculum levels in the soil on the development of Rhizoctonia gray bulb rot on tulips and iris. In both trials, inoculum consisted of sclerotia that were grown on sterilized rice grains. This was then passed through a 0.25" sieve and 0 to 33 grams of the inoculum mixture per foot of row was added to the soil covering the bulbs at the time of planting. No disease developed on any of the iris or tulips grown in the field or pots when the soil was not infested with inoculum. The numbers of iris and tulips that emerged varied with inoculum level. Data analysis showed that there was a highly significant correlation between the number of emerged and/or healthy iris and tulips plants and inoculum level. The highest inoculum levels resulted in a three-fold reduction in the number of tulips that emerged and reduced the number of healthy iris plants by one half.

Identification of viruses in small-farm lily and dahlia cut flower crops in western

Washington (In cooperation with Hanu R. Pappu) - During July through October 2011, virus surveys were conducted at 10 lily and 10 dahlia farms in western Washington State to identify

viruses present at farms that mainly supply cut flowers to local markets. At each site, information collected included general farming practices, virus and pest management practices, treatments used, farm location and history, origin and history of the planting stock, cultivar name, flower color, description of symptom(s), and incidence of symptom(s).

A total of 226 lily samples were collected, 114 from “symptomatic” plants and 112 from “randomly” selected plants. Each lily sample was tested individually by ELISA for the following six viruses: Cucumber mosaic virus (CMV), Impatiens necrotic spot virus (INSV), Lily symptomless virus (LSV), Tomato aspermy virus (TAV), Tobacco ringspot virus (TRSV), and Tomato spotted wilt virus (TSWV). Samples were also tested for the presence of potyviruses using a potyvirus group test that would detect members of this group, such as Tulip break virus (TBV).

At the dahlia farms, leaf samples were generally designated either as “symptomatic” (from one plant or a composite sample from several plants with the same symptoms) or “symptomless” (composite sample from multiple plants). A total of 111 samples were collected, 81 symptomatic and 30 symptomless. Each of the dahlia samples were tested individually by ELISA for the following four viruses: CMV, INSV, Tobacco streak virus (TSV), and TSWV. Samples were also tested using a potyvirus group test. Each sample was additionally tested by conventional PCR for the presence or absence of caulimovirus strains associated with dahlia mosaic: Dahlia mosaic virus DMV-D10, Kentucky, and Dahlia common mosaic virus (DCMV).

Approximately one-third of the lily plants sampled were positive for LSV, CMV, and/or a potyvirus. LSV was the most common virus found, occurring in 30.5% of the samples. The incidence of a potyvirus (8%) and CMV (1.3%) samples were much lower. Overall, almost three quarters of the dahlia plants sampled were positive for one or more virus. The most common virus was one or more of the caulimoviruses. DMV-D10 was detected in 49.5% of the samples, followed by DCMV (19.8%) and Kentucky (7.2%). TSV and TSWV were each detected in 26.1% of the samples. Two or more viruses were detected in 5.3% of the lily samples and 36% of the dahlia samples. No INSV, TRSV, TAV, or TSWV was detected in any of the lily samples and all of the dahlia samples were negative for CMV, INSV, and potyviruses.

These results suggest that viruses, particularly in dahlias, are a widespread problem. It is clear that additional work is needed to better understand the factors that are contributing to the persistence and spread of viruses at these farms. Educational programs that demonstrate the impact of these viruses on flower production and the postharvest vase life of cut flowers are also needed.

Pacific Madrone

<http://www.puyallup.wsu.edu/ppo/madrone/>

Range-wide genetic variability in Pacific Madrone (*Arbutus menziesii*). (In cooperation with Alan Kanaskie Oregon Department of Forestry, Salem, OR; Richard Sniezko USDA Forest Service - Dorena Genetic Resource Center, Cottage Grove, OR; and Jim Hamlin USDA Forest Service - Umpqua National Forest, Roseburg, OR) - Pacific madrone (*Arbutus menziesii* Pursh,

Ericaceae) is an important evergreen hardwood species in Pacific Northwest (PNW) forests that provides food and habitat for wildlife and has high value in urban environments. Pacific madrone provides habitat for numerous wildlife species, especially cavity-nesting birds. Its evergreen foliage provides browse, especially in the winter, for a number of animals. The berries are an important food for deer, birds, and other small mammals because they are produced in large quantities and may persist on the tree in winter when alternative food sources are limited. This species also provides excellent erosion control and slope stabilization and is highly prized as an ornamental species for its crooked beauty, colorful bark, showy flowers, and brightly colored fruits. Pacific madrone is relatively drought tolerant, which makes it desirable in urban habitats. Native American tribes have also used various portions of this tree for food, utensils, and medicinal purposes.

Very little is known about the range of genetic variability in Pacific madrone. A Canadian study found that genetic diversity was low within populations in the northern part of the range. In 2011-12 common garden plantings consisting of 105 families collected from 7 ecoregions were planted in 4 locations in California, Oregon, and Washington. A separate sowing is planned for a planting site in British Columbia. The specific objectives of these plantings include: 1) screen for resistance to multiple pathogens such as *P. ramorum*, *P. cinnamomi*, and endemic canker and foliar pathogens. 2) examine variation in growth and adaptive traits in the nursery and at multiple field sites. 3) identify seed sources or populations that may contain individuals that are best adapted to climate change and for urban and restoration plantings.

Transplanting Madrone Seedlings - Pacific madrone has a reputation for being difficult to transplant since its roots react poorly to disturbance. Before transplanting into the common garden sites, a study was undertaken to determine the best methods for planting madrone seedlings. Two-year-old seedlings from three families were subjected to five treatments based on methods used in Hummel et al. (2008) and planted in December 2010. Seedlings were examined in May 2011. Treatments that involved washing the roots resulted in the most seedling mortality. Seedling mortality was between 5 and 10 percent in the unwashed treatments and 30 to 50 percent in the two treatments that involved washing the roots. Resprouting was observed on some seedlings that had died back, indicating that the root system may still be functioning. Total mortality and final measurements of height, stem diameter, and dry weight will be taken after two growing seasons.

Variation in susceptibility to *P. ramorum* - Pacific madrone is a host for the invasive pathogen *Phytophthora ramorum*. Seedlings grown from seed collected from the range of the species were inoculated with *Phytophthora ramorum* to examine susceptibility to this pathogen within the species. Seedlings grown from 81 seed sources from 36 sites within 7 ecoregions in the range of Pacific madrone were used. Zoospore inoculum was prepared from one isolate from each of the three clonal lineages (NA1, NA2 and EU1) of *P. ramorum* and sprayed on six-month-old seedlings in plug trays. Seedlings were incubated under optimum conditions for infection, and disease incidence and severity was rated after 8 days.

There were significant differences in both incidence and severity of foliar infection caused by each of the three isolates of *P. ramorum*. The NA2 isolate was the most aggressive, EU1 intermediate, and NA1 least aggressive. Cluster analysis of incidence and severity ratings for

seed sources of Pacific madrone formed three groups: resistant (24%), intermediate (40%), and susceptible (36%). There was no interaction between *P. ramorum* isolates and susceptibility of madrone seedlings: if plants from a given seed source were resistant to one isolate of *P. ramorum*, they were resistant to the others. There was no relationship between susceptibility and the ecoregions where seed was collected, but there were differences in disease incidence and severity on seedlings among individual sites.

A new leaf blight disease on Pacific Madrone (*Arbutus menziesii*) caused by *Phacidiopycnis washingtonensis* in western Washington and Oregon (In cooperation with Parama Sikdar, Washington State University, and Chang-Lin Xiao, USDA ARS) - A leaf blight, consisting of brown, desiccated leaves occurring mainly in the lower canopy has been observed on Pacific madrone throughout its range and generally occurs during periods of wet spring weather. In May 2009 and 2011 severe outbreaks occurred in western Washington and Oregon. A fungus was isolated from blighted foliage, leaf spots on emerging foliage, lesions on green shoots, and from the petiole and leaf blade of dead, attached leaves. The ITS region of the fungus was identical to the type isolate of *Phacidiopycnis washingtonensis* Xiao & J.D. Rogers, which has been reported to cause rots of stored apple and persimmon fruit and cankers and twig dieback of apple and crabapple trees, but has not been reported on other plant species.

Pathogenicity tests suggest that *P. washingtonensis* is able to cause foliar blight on Pacific madrone when leaves are predisposed to cold stress, and that the fungus host range includes madrone in addition to apple, crabapple, pear, and persimmon. Increased disease severity on madrone observed in winter 2010-2011 in Washington and Oregon may have been due to predisposition of foliage by extreme cold temperatures in November 2010 and February 2011.

WSU Puyallup Plant Clinic Report

Jenny Rebecca Glass, Plant Diagnostician, WSU Puyallup Plant & Insect Diagnostic Laboratory. glass@puyallup.wsu.edu, <http://www.puyallup.wsu.edu/plantclinic/>

- Boxwood blight, caused by *Cylindrocladium pseudonaviculatum* has become a serious disease problem in some states. Based on WSU Plant Clinic samples and nursery inspection by the WSDA, this disease has not yet been detected in Washington.
- Downy mildew on impatiens was detected on samples for home landscapes in the south King/north Pierce county area.
- *Pustula (Albugo)* rust, which is an unusual disease, was detected on *Echinacea angustifolia* in the Ephrata area of eastern WA.
- We had a first report of lily leaf beetle (*Lilioceris lili*) in King County in May. <https://sharepoint.cahnrs.wsu.edu/blogs/urbanhort/Media/Red%20lily%20leaf%20beetle.pdf>
- It has been a very bad year for Mt. Ash sawfly, with numerous reports all over western WA. http://oregonstate.edu/dept/nurspest/mountain_ash_sawfly.htm
- The southwest counties (Clark/Skamania) are seeing scattered outbreaks of Brown Marmorated Stinkbug on trees such as holly and maple. <http://cru.cahe.wsu.edu/CEPublications/FS079E/FS079E.pdf>

Publications

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- Chastagner, G., Elliott, M., Dermott, G., Kanaskie, A., Sniezko, R., Hamlin, J., and Russell, J. 2012. Monitoring a Range-Wide Seed Collection of Pacific Madrone (*Arbutus Menziesii*) for Disease Resistance and Adaptive Characteristics in a Common-Garden Planting . Forest Health Monitoring Work Group Meeting Tucson AZ April 16-19 2012.
- Elliott, M., Chastagner, G., Dermott, G., Kanaskie, A., Sniezko, R., and Hamlin, J. 2012. Range-wide Genetic Variability in Pacific Madrone (*Arbutus menziesii*): Examining Disease Resistance, Growth, and Survival in a Common Garden Study. Proceedings of the 4th International Workshop on Genetics of Host-Parasite Interactions in Forestry. General Technical Report PSW GTR-240 (in press).
- Elliott, M., Chastagner, G., DeBauw, A., Dermott, G., and Sniezko, R. 2012. A First Look at Genetic Variation in Resistance to the Root Pathogen *Phytophthora cinnamomi* using a Range-wide Collection of Pacific Madrone (*Arbutus menziesii*). Proceedings of the 4th International Workshop on Genetics of Host-Parasite Interactions in Forestry. General Technical Report PSW GTR-240 (in press).

Book Chapters

- Chastagner, G., and T. Miller. 2012. Ornamental Flower Bulb Crop Research in the Pacific Northwest (2). RHS Daffodil, Snowdrop and Tulip Yearbook 2011, p 30-33.
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- Copes, W. E., B. Barbeau, and G. A Chastagner. In Press. Chlorination – Chlorine Dioxide. In: Hong, Chuan (Ed.). Waterborne Plant Pathogens: Biology, Detection and Management'. APS Press.

Extension Publications

- Hart, J., C. Landgren, R. Fletcher, M. Bondi, B. Withrow-Robinson, and G. Chastagner. Christmas Tree Nutrient Management Guide: Western Oregon and Washington. EM 8856-E. (Received the 2012 Extension Education Materials of Excellence Award).

**2012 North Dakota Report
NCERA-193**

Jared M. LeBoldus
Department of Plant Pathology
North Dakota State University
Fargo, ND 58108
Jared.leboldus@ndsu.edu

Project: NCERA193: NCR-193: IPM Strategies for Arthropod Pests and Diseases in Nurseries and Landsapes.

North Dakota Report for the Period: 10/1/11 – 9/30/2012.

Statement of context: Jared M. LeBoldus began his appointment at NDSU August 16, 2011. It is a 60% Research, 20% Teaching, and 20% Extension appointment in the areas of Tree, Nursery Plant, and Turfgrass Pathology. This report consists of projects initiated following appointment at NDSU related to the NCERA-193 objectives.

OUTPUTS / ACCOMPLISHMENTS:

Obj. 3: Pesticide alternatives: Develop management strategies for key pests based on classical biological control (i.e., predators and parasitoids), host plant resistance, and cultural control).

1. Understanding disease resistance to Septoria canker in hybrid poplar. Collaborators: Ruqian Qin (M.S. Student), and Glen Stanosz (University of Wisconsin – Madison).

We tested the relationship between field and greenhouse resistance to Septoria canker by conducting non-wounded inoculations of 15 genotypes of hybrid poplar using the protocol developed by LeBoldus *et al.* 2010. These genotypes have known levels of resistance to Septoria canker (Low, intermediate, and High, canker length, and % girdle – provided by Dr. Stanosz). Following inoculation several disease severity parameters were measured (lesions/cm, % necrotic area, lesion number). Preliminary analysis suggested that correlations between these parameters and (canker length and % girdle) field resistance were relatively low ($R^2 = 0.2$). However, logistic regression using % necrotic area as a predictor of susceptibility category (low, intermediate, and high) indicated that the non-wound inoculation protocol was a good predictor of the most resistant and susceptible genotypes under field conditions.

A second study characterizing the mode of infection of *Septoria musiva* into host tissue is being conducted. A resistant genotype, a susceptible genotype, and an intermediate genotype were selected. Inoculations were conducted as described by LeBoldus *et al.* 2010. Stem tissue was collected 6 h, 12 h, 24 h, 72 h 1 week, and 3 weeks post inoculation. Scanning electron microscopy and histology will be used to examine pathogen development over the series. This

will provide information on the mode of infection and potentially improve resistance screening protocols

2. Riparian Forest Health along the Souris and Missouri Rivers following 2011 flooding.

Collaborators: Larry Kotchman (North Dakota Forest Service), Aaron Bergdahl (North Dakota Forest Service), Jim Walla (Research Associate), Odaliz Faria (Undergraduate Student – Puerto Rico), Mike Kangas (North Dakota Forest Service), and Bill Haase (North Dakota game and Fish Department).

The objective of this study was to determine what, if any are the long term impacts of flooding on Riparian ecosystems. Five sites, two in the Souris River drainage and three in the Missouri River drainage will be selected in the spring of 2012. Selection will be based on two criteria: (1) evidence of flooding from the 2011 floods; and (2) contiguous forest cover. At each of the sites, five (7.6m) permanent sample plots, will be established. Species composition, diameter at breast height (DBH), live/dead ratio, and presence of diseases will be recorded. The understory will also be evaluated for species composition, percent cover, and depth of siltation. The sites will be evaluated on a yearly basis to determine how the flood events of 2012 have affected Riparian health. In the fifth year of the study each tree taller than 1.3 m will be cored. These tree cores will be used to estimate changes in growth rate which may have been associated with the 2011 flood. In the case of older trees changes in growth rate related to historical flooding events will also be recorded. In the summer of 2011 the permanent sample plots were established and baseline data, as described above, was collected.

OUTCOMES/IMPACTS:

Obj. 3: Pesticide alternatives: Develop management strategies for key pests based on classical biological control (i.e., predators and parasitoids), host plant resistance, and cultural control).

The ability of the greenhouse screening protocol to detect the most resistant and susceptible genotypes of hybrid poplar will allow forest companies to focus resources for expensive and time consuming field testing on the most resistant genotypes.

The study characterizing the mode of infection of Septoria canker into hybrid poplar stems will allow us to improve our understanding of this pathosystem and improve current disease resistance screening strategies.

My program is training: One M.S. student (Ruqian Qin), One Ph.D.student (Kelsey Dunnell), and one undergraduate intern (Odaliz Faria).

PUBLICATIONS: (1)

LeBoldus, J.M. Zhang, Q. and Kinzer, K. (2012). First report of dollar spot caused by *Sclerotinia homoeocarpa* on *Agrostis stolonifera* in North Dakota. *Plant Disease* 96: 1071.

FUNDING:

USDA Forest Service – North Dakota Forest Service. \$45,000. Riparian Forest Health Assessment. 3 yrs.

USDA NIFA. 2012-2015. \$98,190. Integrated Pest Management of Septoria Canker on Hybrid Poplar Farms in the North Central Region. J.M. LeBoldus, G.R. Stanosz. 3 yrs.

REFERENCES:

LeBoldus, J.M., Blenis P.V., Thomas B.R. (2010) A method to induce stem cankers by inoculating nonwounded *Populus* clones with *Septoria musiva* spore suspensions. *Plant Disease* 98: 1238-1242.

NCERA -224: Arthropod Pests & Diseases of Ornamentals: Delaware & SE PA

University of Delaware Co-operative Extension:

Brian Kunkel, Nancy Gregory, Bob Mulrooney,

Longwood Gardens:

Casey Sclar & Mike Leventry

Regional Weather:

Winter 2011 – 2012 was milder and drier than previous years and precipitation was less compared to the historical averages of each county.

Table 1. Winter precipitation for 2011 – 2012 (December, January, February)

County	Precipitation (inches)	Departure from Normal
New Castle	9.4	0 – 25% below
Kent	8.9	26 – 50% below
Sussex	6.8	26 – 50% below

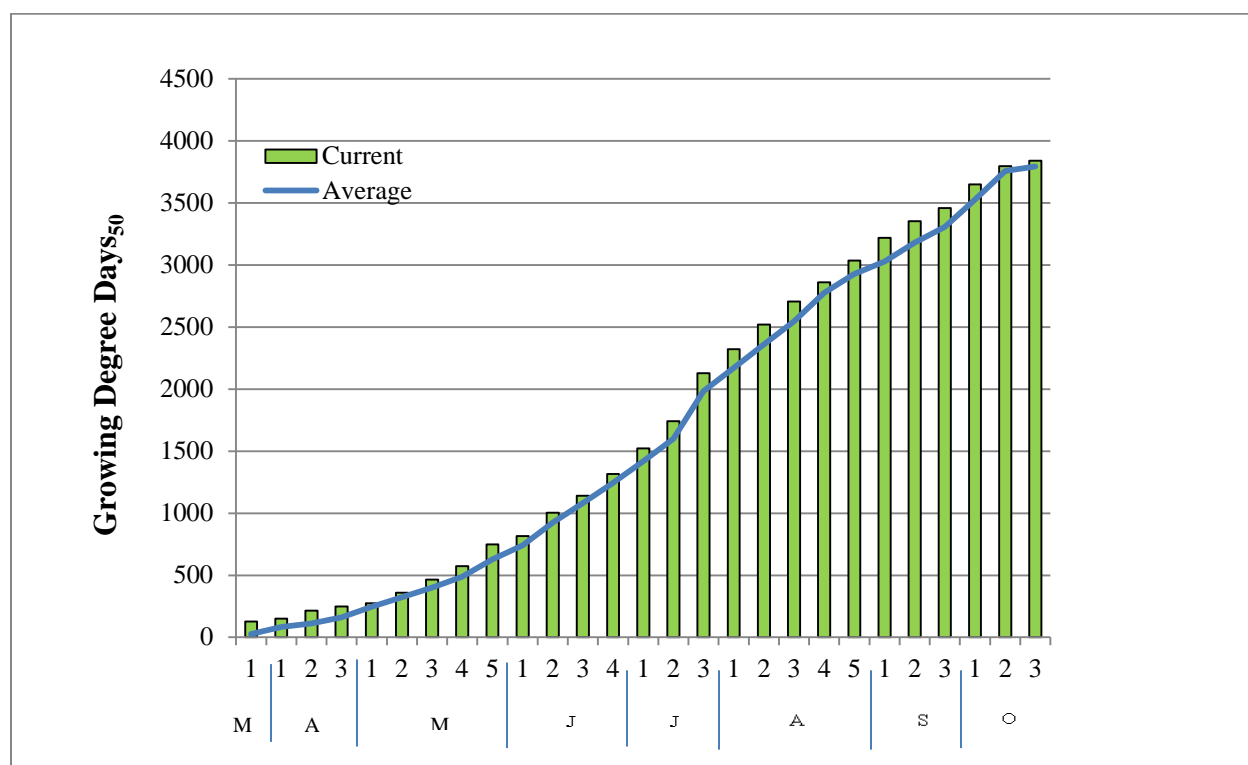
Lack of precipitation was common throughout much of the year with October 2012 being the exceptional month where each county received greater than 75% of normal rainfall due to Hurricane Sandy. Yearly precipitation totals for each county are below normal (0 – 25%). Temperatures were above historical averages throughout the state as illustrated in the graph below using accumulated growing degree days (GDD₅₀) starting on 1 March 2012 in New Castle County (Fig. 1). March through early May saw GDD about two weeks earlier than average and the remainder of the year saw GDD values about one week earlier than average. Insect activity was typically a week to two weeks earlier than normal for most species. Drought like conditions experienced in Delaware were not as severe as neighboring states, however, drought stressed plants did lead to a greater number of arthropod pest problems.

Table 2. Spring – Summer precipitation for 2012 (March – August)

County	Precipitation (inches)	Departure from Normal
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New Castle	15.2	26 – 50% below
Kent	13.3	26 – 50% below
Sussex	16.1	26 – 50% below

Figure 1. GDD₅₀ for 2012 compared against historical average GDD₅₀ (2006 – 2011)



Insect and Disease Highlights:

Insects

Some of the more common caterpillar pests encountered this year were *Malacosoma americanum* (**Eastern tent caterpillars**), *Podosesia syringae* (**Lilac/Ash borer**), *Podosesia aureocincta* (**Banded ash clearwing**), *Synanthedon pictipes* (**Lesser peachtree borer**), *Synanthedon exitiosa* (**Peachtree borer**), and *Hyphantria cunea* (**Fall webworms**). **Tobacco budworms**, *Helicoverpa virescens*, were frequently encountered in SE PA and in DE *this spring through early fall*. **Bagworms**, *Thyridopteryx ephemeraeformis*, were numerous *this spring and summer*, and had a *delayed and lengthened egg hatch period compared to previous years*.

Previous research suggests this was due to the warmer than average winter experienced in 2011 – 2012.

Soft scales such as *Ceroplastes ceriferus* (**Indian wax scale**), *Toumeyella liriodendri* (**Tuliptree scale**), *Eulecanium cerasorum* (**Calico scale**) and *Pulvinaria* scales (**Cottony Taxus/Camellia Scale, Cottony Maple Scale**) were sent to the lab for identification this year. Based upon samples turned into extension offices or phone calls, soft scales appeared to be more abundant this spring and summer. Tuliptrees on the research farm in Sussex County had dying branches heavily infested with tuliptree scale. The most common problem reported was the abundance of honeydew and sooty mold on or in undesired places (i.e., cars, patio furniture, etc...). Most of these problems were reported by individual homeowners or homeowner associations.

Many armored scale samples were diagnosed this summer including *Chionaspis pinifoliae* (**pine needle**), *Fiorinia externa* (**elongate hemlock**), *Aspidiotus cryptomeriae* (**cryptomeria**), *Pseudaulacaspis prunicola* (**white prunicola**), *Unaspis euonymi* (**euonymus**), *Lepidosaphes pallida* (**Maskell**), *Melanaspis tenebricosa* (**gloomy**), *Pseudaulacaspis pentagona* (**white peach**), *Carulaspis juniperi* (**juniper**), *Lepidosaphes ulmi* (**oystershell**) and *Lopholeucaspis japonica* (**Japanese maple**) scales. Many landscape companies reported multiple cases of white prunicola scale and claimed this pest was more abundant than recent years. Japanese maple scale continues to be one of the most common armored scales encountered in nurseries and landscapes.

Other sucking insect pests such as **whiteflies**, **aphids** and **mealy bugs** were minor problems reported during the year. **Azalea lace bugs** were abundant in some areas and caused significant damage when found. **Apple mealybug** continued to be problematic on native azaleas, *Kalmia* and *Fothergilla*. **Boxwood psyllids** were minor pests in northern DE and SE PA.

Cool and warm season spider mites were more abundant this year compared to previous years. Many Christmas tree farms reported issues with *Oligonychus ununguis* (**spruce spider mites**) this spring and summer. Nurseries in the mid-Atlantic continue to struggle with **redheaded flea beetle** control and also reported another chrysomelid feeding on azaleas (species still undetermined). **Roseslug** and **dogwood sawflies** were prevalent in landscapes this year causing considerable damage. Other minor pests encountered this year include, various **ant species** and **millipedes** invading homes, **thrips**, **boxwood leafminer**, **woolly aphids** and **European hornets**. **Japanese beetle** populations were more abundant this summer and emerged about seven to 10 days earlier than previous years. **White grub** management was of greater concern this year for nurseries, landscape contractors and homeowners – possibly due to the precipitation in late summer – early fall. **Cicada killers** and *Scoliid* wasp populations were not as abundant as previous years.

Select Invasive species

Scouting for **emerald ash borer** continues in Delaware and none have been found to date. **Brown Marmorated Stink Bug** (*Halyomorpha halys*) populations were very low from March through late July. Their numbers increased dramatically in August into fall as a number of nurseries reported BMSB on trees and other woody plants. This pest continues to be the most abundant household invader in the fall. Common ornamental plants this pest was reported to be found on this summer include: **elms, redbud, Hibiscus, London plane tree, Pawlonia, tree lilac, ash and maples** among others (see table below).

Diseases

Of the 370 ornamental plant samples, physiological or **environmental stress** was predominant in **104 samples**, while insects were the problem or a factor in 58 samples. Many landscape ornamentals newly planted in the past 2 or 3 years suffered from environmental stress during establishment, often leading to further issues with disease organisms and insects. Herbaceous perennials had *Pythium* or **Phytophthora root rot** in rooting and/or early production beds for large nursery or professional garden situations. **Heat and drought** were severe in July across the state, followed by wet and rainy weather in August and September.

New Reports - There were boxwood samples submitted, but the only **boxwood blight** positive detection was several miles over the state line in **Maryland**. **Impatiens downy mildew** was confirmed on *Impatiens walleriana* and *Impatiens balsamina*. A **downy mildew** was confirmed on *Veronica*, caused by a *Peronospora* sp. A **Phyllosticta leaf spot** on Golden Club (*Orontium aquaticum*) was identified. Some residual herbicide damage was suspected on landscape trees on sites where Imprelis had been used two years prior.

Pathogens of regulatory significance – **Chrysanthemum white rust** was identified in a retail location in Sussex County and plants were destroyed. Several plants sampled as a part of a trace forward for *Phytophthora ramorum* were **negative** for *P. ramorum*. **Swiss needlecast** has become a predominant problem on Douglas fir in Christmas tree and landscape plantings. **Bacterial leaf scorch** on oak and **Hypoxylon canker** continue to cause decline in oaks and other hardwoods stressed due to the environment.

Commonly encountered diseases this year included: **peony blotch, frog-eye leaf spot, black spot, cedar quince rust, powdery mildew, cedar apple rust, apple scab and Septoria leaf spot**. Common diseases found on turf were **red thread** and **brown patch**. **Sycamore anthracnose** was very prevalent on susceptible London planetree and American sycamore. **Crown gall**, *Agrobacterium tumefaciens*, was found on euonymus this summer and **Botryosphaeria** and **Cytospora cankers** were common diseases on stressed plants. **Bacterial leaf scorch** was frequently encountered in the landscape this summer. **Rose rosette**, previously only found on multiflora rose, was confirmed on cultivated roses in DE landscapes.

2011-2012 Publications & Notables

Kunkel, Mulrooney, Gregory, and Sclar contributed weekly columns on insects and diseases to *Ornamentals Hotline*, a grower newsletter published and distributed by University Delaware Cooperative Extension to over 150 subscribers. We changed the blog site for the pictures corresponding to Hotline this year. Kunkel contributed articles to the local newspaper on insect pests, and participated in TV and radio interviews about insect pests. Kunkel and Gregory continue to work on updating and creating new fact sheets for professionals in the mid-Atlantic region.

The Ornamentals Task Force at the University of Delaware continues to offer training sessions for green industry professionals at their business. The disease training is conducted by Gregory and Kunkel provided entomology training. Kunkel provided ‘hands-on’ workshops this spring aimed at landscape maintenance personnel in addition to typical workshops and pest walks. The ‘hands-on’ workshop provided green industry professionals the opportunity to closely examine scale samples under the microscope and to ask questions regarding these and other pests. Kunkel and Gregory continue to work with Delaware Christmas tree growers on a project evaluating new Christmas tree variety susceptibility to insects and diseases of the area.

Master Gardener training was also conducted by Mulrooney, Kunkel, and Gregory.

2012 Research Highlights:

Summary of Brown marmorated stink bug (BMSB; *Halyomorpha halys*) project:

- Searched herbaceous perennials and woody ornamentals at greenhouses, nurseries, production areas at public gardens in DE, MD, PA, NJ for BMSB eggs, nymphs and adults
- BMSB populations were seldom encountered until late July, then typically on woody ornamentals
- Observed native and non-native plants for BMSB populations this year – greater populations were found on natives vs non-natives
- 5 – 10% of ‘wild’ (i.e., not sentinel/placed) BMSB egg masses encountered at nurseries experienced predation or some level of parasitoid activity
- IR-4 trials found: Aloft, Flagship, Talstar, Tristar, Onyx, Safari and Scimitar provided >70 control of BMSB early in trials; however residual activity quickly diminished by 16 DAT.

Table 3. BMSB were found on native and non-native plants

Natives	Non-native
White pine	Burning Bush

Red Maple	Tree-of-Heaven
Winged Sumac	Crape Myrtle
Chokeberry	Autumn Olive
Rose	European Ironwood
Hackberry	Mock Orange
Green Ash	Forsythia
Black Cherry	Bradford Pear
Sycamore	Chinese Privet
Red Cedar	Norway maple
Sweet Gum	Kousa dogwood
River Birch	Ornamental Cherry
	Linden Arrowhead
	Asian Azalea
	Ginko
	Asian Cherry

Summary of redheaded flea beetle (*Systema frontalis*) project:

- First generation larvae were active 275 – 481 GDD₅₀ or when Black locust and Chinese fringetree are in full bloom
- First generation adults began emerging 590 – 593 GDD₅₀ or when Magnolia grandiflora flower buds were swelling or *Ilex verticillata* and *Hydrangea macrophylla* were in bloom to full bloom
- Second generation larvae activity was noted from 1818 – 1856 GDD₅₀ or when ‘Miss Molly’ butterfly bush were just after peak full bloom, ‘Hopi Pink’ crape myrtle was in bloom to full bloom, *Hisbiscus* in bloom
- Second generation adult activity was observed to start around 2100 – 2240 GDD₅₀
- Entomopathogenic nematodes demonstrated potential in laboratory trials with *Steinernema carpocapsae* providing >70% control; however control in field settings were not sufficient. High temperatures in nursery containers may contribute to low nematode viability in field situations.
- Host plant ‘no-choice’ and ‘choice’ trials revealed redheaded flea beetle feeds on a variety of different host plants and no preferences have been detected.

2012 Impact statements

Information regarding early spring activity of larvae and adults of redheaded flea beetles will enable nurseries dealing with this pest to better time pesticide applications; thus potentially reducing applications by 25 – 30%. (Research)

Pesticide efficacy testing provides nursery operations tools to manage BMSB when they migrate into production areas and begin to feed on various plants. Many nurseries and greenhouses have

few problems with BMSB currently; however about 10% of the nurseries in surrounding area are using these recommendations. (Research)

Cooperative BMSB projects with colleagues at the University of Maryland are investigating potential herbaceous host plants and will benefit many of the greenhouses in the mid-Atlantic. (Research)

Eighty percent of survey respondents claimed Co-operative extension's phenological indicator and demonstration garden in each county will provide useful tools for them regarding management of pests in the landscape. (Outreach)

The multi-lingual extension fact sheets will be beneficial to 20 – 35% of green industry professionals in DE and surrounding areas. (Outreach)

Ninety percent of survey respondents felt the workshops, short-courses and other education events helps them manage disease and insect pests encountered in the landscape. (Outreach)

**NCERA-193 Maintaining Plant Health:
Managing Insect Pests and Diseases of Landscape Plants
2012 State Report - Colorado/Entomology**

Whitney Cranshaw, Rachael Fithian, Emily Peachey & Ned Tisserat
Department of Bioagricultural Sciences and Pest Management
Colorado State University
Ft. Collins, CO 80523

Walnut Twig Beetle/Thousand Cankers Disease (TCD)

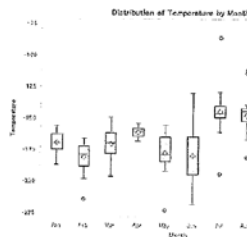
This insect vectored fungal disease continues to spread in Colorado and now includes all the major metropolitan areas along the Front Range. The most recent urban areas with confirmed first cases of TCD were Pueblo (2012), Canon City (2011), Fort Collins (2011) and Greeley (2012). Tree losses greatly increased in 2012 across the state, compared to 2011, likely due to weather differences.

Lines of study related to this disease that are being pursued at Colorado State include evaluations of methods to disinfest TCD-affected logs, determination of temperature limits of the walnut twig beetle, genetics of *Geosmithia morbida* (Tisserat lab). Some key findings include:

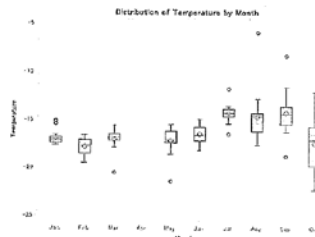
Genetics. The genetics of *Geosmithia morbida* are proving very complex. In general, it indicates that there are two primary groups, a ‘western’ group from southern California (presumably native to *Juglans californica*) the ‘southwestern’ group from Arizona/New Mexico (native to *Juglans major*). The genetics of those from the Pacific states are uniformly of the ‘western’ type, as are samples from Tennessee. Those in Colorado are a mixture of the two, suggesting multiple introductions.

Lower temperature thresholds of the walnut twig beetle. Based on liner regression models, the LLT₅₀ and LLT₉₉ for WTB adults was -16.7°C and -22.97°C respectively ($R^2 = 0.917$) and for larvae was -16.9°C and -25.19°C respectively ($R^2 = 0.915$). The beetles are more cold hardy going into the winter as compared to summer as shown in the figures below.

Adults through October



Larvae



The preliminary comparison between males and females shows a significant difference between their supercooling points with females being more cold hardy.

Log disinfestation studies. Heat treatment of logs at 56°C for 30 minutes kills all beetles. The potential for reinfestation of logs after heat treatment is being studied. However, it appears that some reinfestation is possible.

Deep freeze treatment (-23 to -26 C for six days) killed all beetles in logs. The potential for reinfestation of logs following deep freezing is a trial in progress.

Chipping greatly reduced (90-95%) the number of beetles emerging from wood over the course of two months. Beetles emerged from chipped wood only for 3 weeks; whole logs may sustain breeding for two years.

Insecticide applications to logs were incompletely effective. In evaluations of carbaryl and bifenthrin neither completely eliminated subsequent beetle emergence, but bifenthrin was most effective: 21,473 beetles emerged from the control / 7147 carbaryl / 3662 bifenthrin (apx. 6 / 2 / 1). Permethrin, imidacloprid and biodiesel were previously demonstrated to be ineffective in preventing beetle emergence.

Water soaking of logs with surfactant for one week did not work eliminate beetles. Soaking in 75% ethanol did eliminate beetles (115 beetles emerged in control / 105 water / 0 ethanol)

Educational/Outreach Activities related to Thousand Cankers Disease. A web site of materials useful for education related to thousand cankers is found within the Department of Bioagricultural Sciences and Pest Management at Colorado State University, under "Extension and Outreach":

<http://www.colostate.edu/Depts/bspm/extension%20and%20outreach/thousand%20cankers.html>

This site includes a multi-page Pest Alert fact sheet on thousand cankers disease, Questions and Answers about Thousand Cankers Disease, and Diagnosing Thousand Cankers Disease. These are regularly updated, most recently in September 2012. A (free!) poster is now also available on *Diagnosing Thousand Canker Disease*.

Elm Bark Beetle "Hand-off"

The banded elm bark beetle, *Scolytus schevyrewi*, first came to national attention with its 2003 identification in Aurora, Colorado. Subsequently, preexisting museum records were discovered from several western states (CO, OK, NM, CA) with the earliest being from 1994 (Denver, Colorado). The insect is presently common and widely distributed throughout the western United States.

In Colorado, the banded elm bark beetle appears to have largely, if not entirely, supplanted the previous present invasive elm bark beetle, *Scolytus multistriatus* (smaller European elm bark beetle). Lindgren funnel trap collections over the past decade indicate increasing proportion of

the elm *Scolytus* to be *S. schevyrewi*, and in 2011 it was the sole species found in collections. (The 2012 collections are being processed, but so far are entirely *S. schevyrewi*.)

This likely is due to competitive displacement of *S. multistriatus* by *S. schevyrewi*. Both species seem to occupy the same niche but the latter emerges earlier during both annual generations.

The impact of this on elms is unclear. *Scolytus schevyrewi* appears to be slightly more aggressive than *S. multistriatus*. Some *S. schevyrewi*-caused tree kills involving transplants has been noted and branch die back may be more extensive with this new species. Both appear to be potential vectors of *Ophiostoma novo-ulmi*.

Apparent European Elm Scale Resistance to Neonicotinoids

Anecdotal reports of neonicotinoid failures for control of European elm scale have been received from the Denver area for the past four years. In the past two years similar reports have become widespread in the Denver Metro area and are now also being reported in Larimer County. Although resistance measures have not been conducted, it is clear that neonicotinoids are no longer effective against some European elm scale populations.

Use of imidacloprid to control European elm scale in Denver and Larimer Counties was one of the first uses of imidacloprid on shade trees (work by Casey Sclar, summarized in 1995 M.S. Thesis) and it has been used continuously for almost two decades in some sites. The great majority of American elms in Colorado very regularly (typically annually) receive imidacloprid or chlothianidan applications to control this insect.

Drippy Blight of Red Oaks and Kermes Scale

Extensive dieback of red oaks and pin oaks in the Boulder and Denver metro areas has been observed for over a decade. At points where twig die back occurs, a kermes scale (*Allokermes galliformis*, tentatively) is always present. However, often around the feeding point of the scale is a clear gum.

Investigations in 2010 identified *Brennaria quercina* as the causal agent for the gumming reaction. This bacterium has previously been reported to produce “drippy acorn” syndrome in oak in California; it is also reported from oak in Spain.

The interaction of the two organisms in producing “drippy blight” of oak in Colorado is unclear. External gummosis only develops around feeding points of the kermes scale and die back of twigs only occurs on kermes infested twigs. However, the bacteria are present throughout symptomatic twigs and causes dieback a considerable distance from the scale feeding site.