# NCERA 224 Report from Michigan Nov. 22, 2013, D. Smitley

Factors influencing success of nicotinoid insecticides applied basally for systemic protection against emerald ash borer (Coleoptera: Buprestidae). A long term study (2005 – 2012) investigating the importance of frequency of treatment, trunk diameter and environmental stress on efficacy of nicotinoid insecticides used to protect trees from emerald ash borer, was concluded in 2012. The study was conducted at the Michigan State University Hancock Turfgrass Research Center. Seventy-eight Fraxinus pennsylvanicus 'Marshall Seedless' were planted at the HTRC in 1996 for a turfgrass shade study. This project concluded in 2002. In 2005 we initiated a longterm study using imidacloprid as a basal drench to protect trees from emerald ash borer. The stand of 78 trees was divided into two blocks of 39 trees to account for the drainage slope along the west side of the stand that tended to be moist throughout the summer. Imidacloprid basal drenches were applied at the label rate in late May every year, every 2<sup>nd</sup> year, every 3<sup>rd</sup> year, or at the grub control rate each year, for a total of 5 treatments with 15 replicates per treatment. Each tree was rated for canopy thinning and dieback in July of each year. Trunk diameter was measure annually, in July. When trunk diameter data is mapped onto a plot diagram another spatial effect can be seen: tree in the outer ring tend to be the largest, and trees in the the inner-most ring tend to be the smallest. This placement effect, apparently caused by competition for light and soil moisture, was investigated by giving each tree a spatial placement number of 1 to 5, corresponding the outermost layer of trees (1) to the inner-most layer of trees (5).

## Results

Overall emerald ash borer developed slowly in this stand of 78 ash trees when compared with ash trees in a nearby woodlot. In the woodlot ash trees died 2 to 3 sooner than did control trees in our research plots, probably because of a group effect where treated trees were suppressing the overall population within the stand. Annual basal drenches of imidacloprid applied at the original label rate gave excellent protection of these young ash trees with a diameter of 10 to 30 cm at the end of the study in 2012. In more marginal treatments where imidacloprid drenches were made every 2<sup>nd</sup> year, every 3<sup>rd</sup> year, or every year at the grub rate, the canopy thinning resulting from emerald ash borer attack was much greater for trees located in the east block compared with the west block, indicating that environmental factors such as soil moisture and soil type can be important factors (Table 1, Table 2, Figure 2).

Table 1. Protection of ash trees with basal drenches of imidacloprid applied: once per year, once every 2 years, once every 3 years, or once per year at the grub control rate. Data are canopy thinning and dieback ratings made in July of each year.

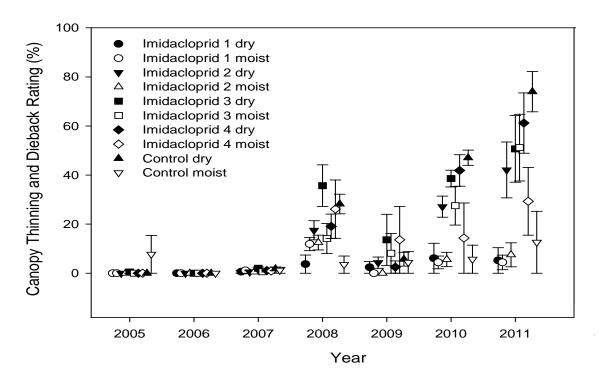
Imidacloprid	Canopy thinning and dieback rating (%)								
treatment	n	2005	2006	2007	2008	2009	2010	2011	
Annual Wet	8	0.0	0.0	1.1	11.8	0.0	<b>4.4</b> a	<b>4.4</b> a	
Once/2 Yr Wet	8	0.0	0.0	0.5	12.5	0.0	<b>5.6</b> a	<b>7.5</b> a	

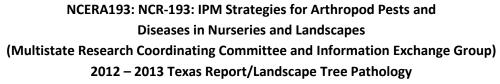
Annual Dry	7	0.0	0.0	0.9	25.0	3.6	<b>21.4</b> bc	<b>12.1</b> ab
Grub Rate Wet	7	0.0	0.0	0.8	26.0	13.5	<b>14.3</b> ab	<b>29.3</b> abc
Once/2 Yr Dry	7	0.0	0.0	0.5	17.5	4.3	<b>27.1</b> cd	<b>42.1</b> bcd
Control Wet	8	0.5	0.0	2.6	20.9	4.4	<b>35.0</b> cd	<b>49.4</b> cd
Once/3 Yr Wet	8	0.0	0.0	0.6	14.0	8.1	<b>27.5</b> bcd	<b>51.2</b> cd
Once/3 yr Dry	7	0.0	0.0	1.9	35.7	13.5	<b>38.6</b> cd	<b>50.7</b> cd
Grub Rate Dry	8	0.0	0.0	1.1	19.1	2.5	<b>41.9</b> cd	<b>61.3</b> d
Control Dry	7	11.5	0.0	1.8	28.2	5.7	<b>47.1</b> d	<b>74.0</b> d
							p = 0.001	p = 0.001

Table 2. Comparison of canopy thinning ratings in trees located in the drier east block ('dry') with the same for trees located in the more moist west block ('moist').

Merit drench every year 'Moist'	8	4.4
Merit drench every year 'Dry'	7	12.1
Merit drench every 2 years 'Moist'	8	7.5 *
Merit drench every 2 years 'Dry'	7	42.1 *
Merit grub rate every year 'Moist'	7	29.3*
Merit grub rate every year 'Dry'	8	61.3*

Figure 2. Canopy thinning and dieback ratings (0 is healthy, 10 is dead) of ash trees treated with an imidacloprid basal drench once per year in late May (imidacloprid 1), once every 2 years (imidacloprid 2), once every 3 years (imidacloprid 3) or once per year at the turf grub control rate (imidacloprid 4). Half of all the ash trees in each treatment were in block 1 ('dry' east half of stand) and half of the trees in each treatment were in block 1 ('dry' east edge of the stand bordered a drainage slope that kept the soil moist throughout the summer.





Prepared by

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

Introduction

The historic drought of 2011 continued to dominate tree health issues in Texas landscapes during 2012. As anticipated, such contributing factors as *Biscogniauxia atropunctata* and *Ganoderma applanatum* were commonly found killing trees. *Xylella fastidiosa* is another commonly found pathogen in Texas landscapes, probably due to the drought as well. Other typical tree disease issues, not related to the drought, include the highly destructive oak wilt, caused by *Ceratocystis fagacearum*. Although there have been no systematic surveys, cotton root rot caused by *Phymatotrichopsis omnivora* was increasingly detected during 2012 on trees and other landscape plants by the Texas Plant Disease Diagnostic Laboratory in College Station, TX.

Another common theme in disease of nurseries and landscapes in Texas has been the influx of invasive species. As reported last year, Huanglongbing of citrus, caused by *Candidatus Liberibacter asiaticus*, is the cause of great concern due to the potential for losses in the valuable commercial citrus producing region of the Rio Grande Valley of South Texas. Following the discovery of the pathogen in two adjacent citrus groves in San Juan, TX, in January 2012, a few additional locations have been recently found nearby in

dooryard trees. Also, contaminated Asian Citrus Psyllids, the vector in the HLB syndrome, continue to be found in the Valley. However, no positive finds in trees or psyllids have been found during survey of dooryard trees in the upper Gulf Coast. Increasing attention is being paid to another invasive pathogen of landscape plants in Dallas, TX, where rose rosette virus is causing losses of roses.

The total economic impact of the Green Industry in Texas was estimated to be \$16.9 billion in 2010. This impact includes 192,565 jobs and \$10.1 billion in value added. The Texas nursery industry is an integral, if not essential, part of this vital enterprise. There are a number of recent factors preventing growth in the Texas nursery industry, including a suppressed economy and climatic extremes. Therefore, there is a low tolerance for additional factors that might be a detriment to productivity and economic sustainability. Sudden Oak Death (SOD), caused by the invasive pathogen Phytophthora ramorum, is a disease with the potential to significantly impact the health of the Texas nursery industry. SOD is caused by a highly visible, nationally recognized exotic pathogen that has destroyed vast acreages of oaks (Quercus spp.) in California and Oregon. In addition to threatening the economic health of nurseries, the threat the pathogen might pose to the valuable natural resources of Texas must also be considered. There is a risk of introducing P. ramorum into Texas because of the tens of thousands of nurseries receiving and distributing potentially contaminated plant materials from commercial operations on the West Coast where the pathogen is known to exist. Due to the prevalence of information regarding SOD on the internet, (see www.suddenoakdeath.org/, www.aphis.usda.gov > Plant Health > Pest Information, and www.na.fs.fed.us/sod/ for examples), homeowners, landowners, landscape consultants, and natural resource managers confuse the symptoms of SOD with other prevalent tree problems in Texas such as oak wilt and drought. For these reasons, there is a need for regular and sustained monitoring to determine whether the SOD pathogen is being introduced into Texas. The remainder of this report focuses on the results of recent surveys for the detection of *P. ramorum*.

#### Summary of SOD Surveys

Texas has participated in SOD surveys for over a decade. Survey sponsors, objectives, and methods have varied during that period. In 2004, a USDA APHIS "pilot survey" was initiated in which plants with SOD were found in 11 of 114 "trace forward" nurseries. These nurseries were randomly selected from a list of over 1,000 nurseries in Texas that had received shipments of plants from contaminated nurseries in California. Subsequent surveys in following years consisted of sampling plants, soil, and water within boundaries of those same 11 infected nurseries as well as on the perimeters and in external streams and waterways nearby. Most of the detection methods were bait-based protocols. Streams were baited with rhododendron leaves floating in net bags for a week. A similar baiting method for soil was used by soaking samples in water and baiting the soil slurry with leaves, and pears, in the lab. The rhododendron leaves, or pear tissues, were then processed on semi-selective media for the isolation of *P. ramorum*. In some cases, enzyme linked immunosorbent assay (ELISA) was used to supplement isolation procedures. All suspected positives were verified by USDA APHIS and USDA Forest Service labs. Plant samples in the nurseries were collected from high risk hosts such as *Camellia* spp. and *Viburnum* spp., as well as any plants exhibiting symptoms typical of the foliar blight caused by *P. ramorum*. Perimeter surveys focused on *Quercus* spp. and any native species related to natural hosts of the pathogen on the West Coast.

#### Survey Results

Prior to 2012, no samples yielded a positive detection for *P. ramorum* from any of the surveyed nurseries. There were, however, numerous other *Phytophthora* spp. found on a variety.

Phytophthora species	Plant host (no. positives)	Scientific Name	Family
P. parasitica (Fig. 4)	Azalea (6)	Rhododendron spp.	Ericaceae
	Honeysuckle(1)	Lonicera spp.	Caprifoliaceae
	Indian Hawthorne (5)	Raphiolepis spp.	Rosaceae
	Nandina (3)	Nandina spp.	Beridaceae
P. palmivora (Fig. 5)	Lioriope (1)	Liriope muscari	Liliaceae
P. drechsleri (Fig. 6)	Pyracantha(1)	Pyraeantha coocinea	Rosaceae
P. cinnamomi	Live oak roots (1)	Quercus virginiana	Fagaceae
P. megasperma (Fig. 7)	Azalea (4)	Rhododendron spp.	Ericaceae

Table 1. Typical Phytophthora spp. found during survey of nurseries in Texas for detection of P. ramorum.

of host plants (Table 1). *Phytophthora* spp. are common in the nursery trade, and often require routine treatment to minimize damage to valuable nursery stock. However, in June of 2012, a soil sample was collected within a nursery in Seabrook, TX, that yielded a positive detection of *P. ramorum*. The sample was collected in the immediate vicinity of Camellias acquired from a large nursery wholesale operation in California. In December of 2012, a stream outside of the same nursery in Seabrook was found to be positive for *P. ramorum* as well. These positive finds were followed by positive stream detection at a separate location outside a different nursery in Webster, TX, in February, 2013.

#### Discussion

The positive detection of *P. ramorum* in nursery soils and streams in Texas raises many questions regarding the epidemiology of the pathogen and potential for future damage from SOD. Based on risk mapping, Texas has not been considered to be a likely location for the persistence and spread of *P. ramorum*. This conclusion was based largely on unfavorable climatic conditions. Evidently, there are sufficient periods of cool, moist conditions in the nurseries to allow for survival of the pathogen. Also, the stream environments also appear to be favorable in spite of the long periods of extraordinarily high temperatures. The question of when the pathogen was first introduced in the nurseries is also intriguing. Both nurseries were found to contain positive plants in the pilot survey of 2004. Having found the pathogen again 8 years later in soil and nearby streams leads to a few different potential scenarios. The most likely is that *P. ramorum* was re-introduced relatively recently, because both locations have been periodically sampled since the originals detection in 2004 with negative results. If true, then efforts to contain SOD through regulatory means have been unsuccessful. Investigations are ongoing to address these issues and will be further discussed in future reports.

## 2013 Ohio Report NCERA224

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

# Project: NCERA224: NCR-193: IPM Strategies for Arthropod Pests and Diseases in Nurseries and Landscapes

**Ohio Report for the Period**: 10/1/12 – 9/30/2013

### **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. Resistance mechanisms in the Austrian pine / Diplodia pinea pathosystem. Collaborators: Patrick Sherwood (Ph.D. student), Dan Herms (Dept. of Entomology), Don Cipollini (Dept. of Biological Sciences, Wright State University).

The molecular basis of the systemic induced resistance (SIR) phenotype known to occur in Austrian pine (*Pinus nigra* J.F. Arnold) in response to the tip blight and canker pathogen *Diplodia pinea* (Desm.) remains unclear. Specialized metabolites like phenolics are considered to be an important component of plant defense, including SIR, but the antimicrobial activity of many of these putative defensive chemicals remains untested at realistic concentrations and in conjunction with each other. Here, we examined the anti-*Diplodia* activity of several previously identified Austrian pine phenolics associated with SIR by comparing the diameters of fungal colonies grown on media amended with ferulic acid, coumaric acid, taxifolin, pinosylvin, pinosylvin monomethyl ether, and lignin. All compounds were tested both individually and as clusters (combinations) previously determined to occur *in planta* in a co-regulated fashion. Both the individual compounds and clusters were tested at constitutive concentrations and pathogen-induced concentrations linked to a SIR phenotype. Lignin possessed the strongest antifungal activity individually, and clusters at the SIR concentrations had the greatest antifungal effects, achieving fungistasis. This study exemplifies the value of evaluating potential biomarkers of resistance at *in planta* concentrations that are associated with the systemically resistant phenotype.

In a separate study, we examined mechanisms of enhanced susceptibility to Diplodia tip blight in droughtstressed Austrian pine. Water limitation is an important abiotic factor which can predispose plants to pathogen infection. However, the molecular mechanisms of this induced susceptibility are poorly understood in non-model plants like most tress. Here we studied the effects of drought on *Pinus nigra* susceptibility to the shoot blight pathogen *Diplodia pinea*. Drought increased host susceptibility and resulted in the accumulation of proline and reactive oxygen species (ROS) in the shoots. When trees were both water stressed and infected, there was a significant reduction in ROS levels, but an increase in free proline concentrations. Proline has several roles in plant physiology including protein synthesis, osmoregulation, oxidative protection during stress events, and serving as a nitrogen storage source. In particular, the ability of proline to scavenge ROS and act as a nutrient source may be contributing to *D. pinea*'s success in Austrian pine. We found that proline can enhance *in vtiro* growth of *D. pinea* by functioning as a preferred N source while also mitigating H<sub>2</sub>O<sub>2</sub> damage. *D. pinea* was also examined for enzymatic ROS scavenging ability by growing the fungus on media amended with H<sub>2</sub>O<sub>2</sub>. Catalase and peroxidase activity increased with increasing concentrations of H<sub>2</sub>O<sub>2</sub>, but no superoxide dismutase activity was detected. Chemistry of coast live oak defense response to *P. ramorum*. Collaborators: Anna Conrad (Ph.D. student), Brice McPherson and David Wood (Dept. of Environental Science, Policy, and Management, UC Berkeley).

Putative phenolic biomarkers of resistance have been identified in CLO. To test whether constitutive phenolics can be used to predict CLO resistance, 600 trees were selected from a naïve population. Constitutive phenolics were quantified in the phloem of each tree. A subset of trees (N=154) was then artificially inoculated with *P. ramorum* to determine resistance level. A logistic regression analysis using individual phenolic compounds as predictor variables was used to predict CLO resistance; resistance was estimated based on external canker length and beetle presence assessed approximately one year after inoculation. The model correctly classified 73% of resistant trees in our training set (N=60). No other methods currently exist for identifying resistant CLO before *P. ramorum* infection. Identification of resistant CLO ahead of the disease front will be useful in CLO management, including breeding efforts aimed at preserving this endemic California oak. Finally, similar methods could be developed for other important forest pathosystems.

3. Molecular biology of ash resistance to EAB. Collaborators: David Showalter (Ph.D. student), Sourav Chakraborty (post-doc), Caterina Villari (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).

The invasive emerald ash borer (EAB) beetle is a significant threat to the survival of North American ash. In previous work we identified putative biochemical and molecular markers of constitutive EAB resistance in Manchurian ash, an Asian species coevolved with EAB. More recently, we employed high throughput HPLC-PDA-MS to characterize the induced response of soluble phloem phenolics to EAB attack in resistant Manchurian and susceptible black ash under conditions of either normal or low water availability, and the effects of water availability on larval performance. Total larval mass per tree was lower in Manchurian than in black ash. Low water increased larval numbers and mean larval mass overall, but more so in Manchurian ash. Low water did not affect levels of phenolics in either host species but six phenolics decreased in response to EAB. In both ashes, pinoresinol A was induced by EAB, especially in Manchurian ash. Pinoresinol A and pinoresinol B were negatively correlated with each other in both species. The higher accumulation of pinoresinol A in Manchurian ash after attack may help explain the resistance of this species to EAB, but none of the responses measured here could explain increased larval performance in trees subjected to low water availability.

## **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* and how droght stress actually is linked to increased susceptility to the same pathogen will allow for a more rational approach to the management of this and similar diseases.

Non-adaptation/maladaptation of north American ash to EAB is reflected in differential expression of several constitutive and inducible resistance traits relative to Manchurian ash. Such maladaptation may be further exacerbated by environmental conditions that favor susceptibility. For example, global warming will likely lead to more frequent and intense, extreme weather events, including prolonged droughts and excessive precipitation. It is expected that these weather extremes will negatively impact trees both directly and indirectly, via reduced resistance to pests, including wood boring beetles such as EAB. Finding

that some compounds identified in ash as potential biomarkers of resistance to EAB are not affected by water availability will allow us to focus on different traits that are instead affected by, for example, drought.

Understanding the phenotype that characterizes CLO resistant to *P. ramorum*, both at the whole plant and at the chemical levels, will allow for a rational approach to the identification and protection of resistant genotypes ahead of the infection front.

My program has trained 3 PhD students (Anna Conrad, Patrick Sherwood, and David Showalter), 3 undergraduate interns (Michael Falk, Katherine Gambone, and Elizabeth Roche) and 2 post-doc (Sourav Chakraborty and Caterina Villari).

# **PUBLICATIONS: (3)**

- 1. Sherwood P, **Bonello P** (2013) Austrian pine phenolics are likely contributors to the systemic induced resistance phenotype. Tree Physiology 33: 845-854.
- Muilenburg VL, Phelan PL, Bonello P, Loess PF, Herms DA (2013) Characterization of wound responses of stems of paper birch (*Betula papyrifera*) and European white birch (*Betula pendula*). Trees – Structure and Function. Trees 27: 851-863.
- 3. McPherson BA, Erbilgin N, **Bonello P**, Wood DL (2013) Fungal species assemblages associated with *Phytophthora ramorum*-infected coast live oaks following bark and ambrosia beetle colonization in northern California. Forest Ecology and Management 291: 30-42.

# NEW FUNDING IN CALENDAR YEAR 2012:

USDA Forest Service. 2013-2014. \$22,000. 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.

# ABSTRACTS: (14)

- Chakraborty, S., Whitehill, J.G.A., Hill, A.L., Opiyo, S., Cipollini, D.F., Herms, D.A., and Bonello, P. 2013. Water stress decreases ash resistance to *Agrilus planipennis* without affecting phloem phenolics. ClimTree 2013 – Climate Change and Tree Responses in Central European Forests. Zurich, Switzerland, September 1-5, 2013.
- Bonello, P., Herms, D.A., Cipollini, D.F., and Mittapalli, P. 2013. Mechanisms of ash resistance to emerald ash borer, *Agrilus planipennis*. European COST Action FP1103 FRAXBACK 4<sup>th</sup> MC Meeting & Workshop "Frontiers in ash dieback research", 2013, Malmö, Sweden, September 4-6, 2013.
- 3. Roche, L., Conrad, A.O., and **Bonello, P.** 2013. Effect of water stress on northern red oak constitutive defenses and disease resistance to *Phytophthora cinnamomi*. Fall Undergraduate Research Day, Summer Undergraduate Research Institute, The Ohio State University, September 20, 2013.
- 4. Sherwood, P., Gambone, K., and Bonello, P. 2013. Assessing the Reactive Oxygen Species Scavenging Activity of *Diplodia pinea*. Annual Meeting of the American Phytopathological Society, Austin TX, Aug. 10-14, 2013.
- 5. Conrad, A.O., McPherson, B.A., Wood, D.L., and Bonello, P. 2013. Can constitutive phenolic biomarkers be used to predict coast live oak resistance to *Phytophthora ramorum*? Annual Meeting of the American Phytopathological Society, Austin TX, Aug. 10-14, 2013.
- Chakraborty, S., Hill, A.L., Shirsekar, G., Afzal, A., Wang, G.-L., Mackey D., and Bonello, P. 2013. Quantification of reactive oxygen species in plants using the fluorimetric probe Amplex Red. Annual Meeting of the American Phytopathological Society, Austin TX, Aug. 10-14, 2013.
- 7. Falk, M., Showalter, D., Hill, A., Chakraborty, S., Phelan, P., Herms, D.A., and **Bonello, P.** 2013. Do free amino acids in Asian and North American ash species explain patterns of induced resistance to the

emerald ash borer? Denman Undergraduate Research Forum, The Ohio State University, Columbus, OH, March 28, 2013.

- 8. Gambone, K., Sherwood, P., and **Bonello, P.** 2013. Assessing the reactive oxygen species scavenging activity of *Diplodia pinea*. Denman Undergraduate Research Forum, The Ohio State University, Columbus, OH, March 28, 2013.
- 9. Gambone, K., Sherwood, P., and **Bonello, P.** 2013. Assessing the reactive oxygen species scavenging activity of *Diplodia pinea*. College of Food, Agricultural, and Environmental Science, Undergraduate Research Forum, The Ohio State University, Columbus, OH, Feb. 26, 2013.
- Showalter, D., Muilenburg, V., Herms, D.A., Bonello, P. 2013. Effects of methyl jasmonate trunk injection on phloem phenolics of *Fraxinus americana* and *F. pennsylvanica* and associated emergence of the emerald ash borer (*Agrilus planipennis*). Gordon Research Conference – Plant-Herbivore Interactions, Ventura, CA, February 28-March 1, 2013.
- Rigsby, C.M., Muilenburg, V.L., Whitehill, J.G.A., Amstutz, E.M., Tarpey, T., Yoder, J.A., Bonello, P., Herms, D.A., Cipollini, D.F. 2013. Determinants of successful dispersal and establishment of the emerald ash borer. Gordon Research Conference – Plant-Herbivore Interactions, Ventura, CA, February 28-March 1, 2013.
- Lieurance, D., Chakraborty, S., Whitehead, S., Powell, J., **Bonello, P.**, Bowers, D., and Cipollini, D.F. 2013. Chemical defense profiles in native and non-native *Lonicera* species: partitioning variance and associations with herbivore resistance. Gordon Research Conference – Plant-Herbivore Interactions, Ventura, CA, February 28-March 1, 2013.
- Chakraborty, S., Whitehill, J.G.A., Hill, A.L, Opiyo, S.O., Cipollini, D.F., Herms, D.A., and Bonello,
   P. 2013. Are phloem phenolics involved in ash induced resistance to the emerald ash borer? Gordon Research Conference Plant-Herbivore Interactions, Ventura, CA, February 28-March 1, 2013.
- Herms, D.A., Koch, J.L., Mittapalli, O., Cipollini, D.F., Knight, K.S., Poland, T.M., and Bonello,
   P. 2013. An overview of our quest for resistance to emerald ash borer. 24<sup>th</sup> USDA Interagency Research Forum on Invasive Species, Annapolis, MD, January 8-11, 2013.

# **REFERENCES:**

- 1. Sherwood P, **Bonello P** (2013) Austrian pine phenolics are likely contributors to the systemic induced resistance phenotype. Tree Physiology 33: 845-854.
- Muilenburg VL, Phelan PL, Bonello P, Loess PF, Herms DA (2013) Characterization of wound responses of stems of paper birch (*Betula papyrifera*) and European white birch (*Betula pendula*). Trees – Structure and Function. Trees 27: 851-863.
- 3. McPherson BA, Erbilgin N, **Bonello P**, Wood DL (2013) Fungal species assemblages associated with *Phytophthora ramorum*-infected coast live oaks following bark and ambrosia beetle colonization in northern California. Forest Ecology and Management 291: 30-42.
- 4. Kleczewski NM, Herms DA, **Bonello P** (2012) Nutrient and water availability alter belowground patterns of biomass allocation, carbon partitioning, and ectomycorrhizal abundance in *Betula nigra*. Trees-Structure and Function 26: 525-533.
- Villari C, Battisti A, Chakraborty S, Michelozzi M, Bonello P, Faccoli M (2012) Nutritional and pathogenic fungi associated with the pine engraver beetle trigger comparable defenses in Scots pine. Tree Physiology 32: 867-879.

# South Carolina State Report

Project: NCERA-224: IPM Strategies for Arthropod Pests and Diseases in Nurseries and Landscapes State: South Carolina

# **Report period:** 10/1/2012 – 9/30/2013

Principal Investigator: Juang-Horng "JC" Chong

Associate Professor and Extension Specialist

Clemson University, Pee Dee Research and Education Center, 2200 Pocket Road, Florence, SC 29506

Tel. (Office): 843-662-3526 x 224; E-mail: juanghc@clemson.edu

## **OUTPUTS/ACCOMPLISHMENTS:**

**Objective 1**: New and emerging pests: Investigate detection methods, biology, and management of new and emerging pests

Study 1. Life cycle, distribution and host plants of an armored scale insect, Melanaspis deklei.

Collaborator: Ernesto Robayo Camacho (Ph.D. student)

*Melanaspis deklei* is an armored scale insect species that is new to South Carolina. This study was initiated in 2009 and completed in 2013 to document the life cycle, host plant and distribution of *M. deklei* in GA, SC and NC. 74 locations were surveyed and *M. deklei* was found in the urban landscapes (but not nurseries) of NC (new state record), SC and GA (new county record). *Melanaspis deklei* only infested and damaged wax myrtles (*Myrica cerifera*), although other known suitable host plant species (e.g., *Agave* sp., *Ilex* spp., *Sambucus* sp., *Ditrysinia* (= *Sebastiania*) sp. and *Viburnum* spp.) were present in the same landscapes. In SC, three overlapping generations of *M. deklei* were detected annually in 2009 and 2010, with the majority of individuals overwintered as adults. Crawlers were present from May to December with the peak emergence occurred in June, August and October-November. Information on management of M. deklei was published by Chong et al. (2009).

**Objective 2**: Pesticide technology development: Evaluate effectiveness of reduced–risk pesticides, biopesticides, new and novel chemistries, and application technologies for control of key disease and arthropod pests of landscapes, nurseries, and Christmas trees

Study 2: Efficacy of cyantraniliprole and chlorantraniliprole against cabbage looper and beet armyworm in outdoor and greenhouse ornamental plant productions

Collaborator: Lucas Dent (Syngenta)

Cyantraniliprole and chlorantraniliprole are reduced-risk insecticides in the anthranilic diamide chemical class (IRAC Group 28) with high efficacy against caterpillars and selected arthropod pests. Several experiments were conducted to evaluate the efficacy of the two compounds against the cabbage looper and the beet armyworm, two of the most commonly encountered and damaging caterpillar pests of ornamental productions. Results suggested that cyantraniliprole at application rate as low as 1 fl oz/100 gallons was effective in significantly reduced the caterpillar populations, and the efficacy was comparable to chlorantraniliprole and lambda-cyhalothrin (an industry standard). Drench and foliar applications of the two compounds were equally effective in reducing the caterpillar populations.

# **Objective 3**: Pesticide alternatives: develop management strategies for key pests based on classical biological control, host plant resistance and cultural control

Study 3: Field acute and residual toxicity of cyflumetofen against the predatory mites

Collaborators: James Bethke (University of California Cooperative Extension), Kathie Kalmowitz (BASF), Lance Osborne (University of Florida – Apopka)

Cyflumetofen is a new miticide in the benzolacetonitrile chemical class (IRAC Group 25) with high efficacy against twospotted spider mites. This study was conducted at three locations in the country to investigate

the residual and acute toxicity of cyflumetofen against two of the most frequently used predatory mite species (*Phytoseiulus persimilis* and *Amblyseius swirskii*). The acute toxicity was tested by treating plants with the two predatory mite species 1 day after the release. Toxicity of 1-, 3-, 7- and 14-day residue was also tested by releasing the predatory mites on treated plants at the prescribed intervals. The results on *P. persimilis* were inconclusive because of the low survivorship (likely related to mite quality) of the predatory mites. Cyflumetofen applied at 6.5 and 13 fl oz/100 gallons appeared to be compatible with *A. swirskii* where survival rates on treated plants were similar to those on the water-treated plants.

**Objective 4**: Technology transfer: Develop and deliver science-based educational materials focused on management of key pests through outlets such as mass media, publications and fact sheets, eXtension.org and social media

Study 4: Development of a handbook of wood boring insects of ornamental trees and shrubs of the eastern US

Collaborators: Karla Addesso (Tennessee State University), Joshua Basham (Tenessee State University), Alicia Bray (Central Connecticut State University), Emily Dobbs (University of Kentucky), Steven Frank (North Carolina State University), Dan Gilrein (Cornell University Cooperative Extension), Matt Ginzel (Purdue University), Frank Hale (University of Tennessee), Dan Herms (Ohio State University), Bill Klingeman (University of Tennessee), Joseph Lampley (Tennessee State University), Jonathan Larson (University of Kentucky), Deb McCullough (Michigan State University), Venessa Muilenbeurg (Ohio State University), Jason Oliver (Tennessee State University), Randy Ploetz (University of Florida), Dan Potter (University of Kentucky), Christopher Ranger (USDA-ARS), Laurie Reid (SC Forestry Commission), Sarah Vanek (University of Kentucky), Andrew Young (University of Tennessee), Nadeer Youssef (Tennessee State University)

A handbook that will assist growers, landscape care professionals, arborists, ground managers and extension personnel in diagnose, identify, monitor and manage common wood boring insects of ornamental trees and shrubs in the eastern United States is being prepared. About 45 most commonly encountered species and various chapters on IPM, monitoring, pheromone and kairomone communications, disease and invasive species are included in the handbook. Currently, 26 out of the 46 chapters have been prepared. The project was initiated in 2012 and will continue until the handbook is completed and published (the Entomological Society of America was identified as a potential publisher).

## OUTCOMES/IMPACTS:

The armored scale *M. deklei* is a newly found species to SC but it has caused considerable damage to wax myrtles planted as privacy fence or prominent landscape features in the urban landscapes in SC. As demonstrated in Study 1, the distribution of this armored scale species is restricted largely to the urban landscapes of SC, southern NC and GA. Chong et al. (2009) reported that applications of systemic insecticides via soil drench application were largely ineffective against *M. deklei*. The most effective approach was to apply horticultural oil, insect growth regulators (buprofezin and pyriproxyfen) and dinotefuran as foliar application. For foliar application to be effective, it has to be applied during the time of crawler emergence. Study 1 reported three generations within a year, with the crawlers emerging over a one-month period in June, August and October-November. Practitioners should focus the management of *M. deklei* during the crawler emergence periods by applying effective insecticides targeting the crawlers.

The evaluation of cyantraniliprole against caterpillar pests of ornamental plant productions had provided empirical and practical information on the use rates, application methods and residual longevity of the chemical. The information will be very useful in developing an IPM program that incorporate the novel chemical compound or product into the comprehensive arsenal against arthropod pests in the ornamental plant production systems.

Cyflumetofen was shown to be a compatible chemical for the predatory mite *A. swirskii*. The results suggested that in greenhouse and nursery operations where *A. swirskii* was used in biological control programs against whiteflies and thrips, cyflumetofen can be used as a compatible chemical for managing twospotted spider mite when infestations occur without significant negative impact on the efficacy of the predatory mites against whiteflies and thrips. Therefore, cyflumetofen can be safely incorporated into a comprehensive, multiple-target IPM system in ornamental plant production systems. Results from the study on *P. persimilis* were inconclusive.

The handbook on wood boring insects of ornamental trees and shrubs of the eastern US is expected to become an important reference for producers and managers of ornamental plants, as well as extension personnel. There are few, if any, comparable source of information or publication, particularly considering the inclusion of many useful and informative photographs.

## **PUBLICATIONS:**

Refereed:

Chong, J.-H. and E. R. Camacho. 201\_. Distribution, host plants and life cycle of *Melanaspis deklei* (Hemiptera: Diaspididae) in South Carolina, U.S.A. Submitted to Acta Zoologica Bulgarica.

Extension:

Chong, J. H. 2013. Scale insects: the banes of ornamentals. South Carolina Nurseryman September/October 2013: 38-39.

# 2013 North Dakota Report NCERA-224

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

Project: NCERA224: IPM Strategies for Arthropod Pests and Diseases in Nurseries and Lansdscapes.

North Dakota Report for the Period: 10/1/12 - 9/30/2013.

## **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 nonwounded 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). Logistic regression using % necrotic area and lesion number 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 genotypes under field conditions.

A second study characterizing the mode of infection of *Septoria musiva* into host tissue is being conducted. Two experiments were conducted using a resistant (DN 34) and a susceptible (NC11505) genotype. For both experiments inoculations were conducted as described by LeBoldus *et al.* 2010. In experiment 1 Stem tissue was collected 6 h, 12 h, 24 h, 72 h 1 week, and 3 weeks post inoculation. Scanning electron microscopy was used to examine the infection biology of the pathogen a determined that the majority of new infections occur via lenticels and that the pathogen appears to penetrate host tissue within 12 h of inoculation. In the second experiment trees were harvested at 3 weeks, 5 weeks, and 7 weeks fixed, embedded, sectioned and stained. Responses between the resistant and susceptible genotypes will be compared.

2. IPM strategies for Septoria canker on hybrid poplar farms in the north central region. Collaborators: Kelsey Dunnell (Ph.D. student), Nivi Abraham (Ph.D. Student), Achala Nepal (Research Specialist), Bernie McMahon (NRRI University of Minnesota – Duluth), and William Bergeson (NRRI University of Minnesota – Duluth).

In order to characterize the variability in *Septoria* canker resistance, fifty genotypes of *P. nigra*, a common parent in hybrid poplar breeding programs, were inoculated with six isolates of *S. musiva*. Three weeks following inoculation the number of cankers cm<sup>-1</sup> and a disease severity rating (1 resistant - 5 susceptible) were recorded. A wide range of variation from completely resistant to highly susceptible was detected among genotypes and among provenances; with some provenances showing on average more resistance. A second experiment was conducted to determine the relationship between disease severity and mortality. Thirteen genotypes representing the full range of disease severity ratings were inoculated with a bulk spore suspension of six isolates of *S. musiva*. Disease severity and cankers cm<sup>-1</sup> were evaluated at three weeks as described above. Subsequently, trees were evaluated for mortality on a weekly basis. The mean disease severities of each genotype were similar between the two experiments (R<sup>2</sup> = 0.8798) and trees assigned a disease severity of 4 and 5 had a high probability of mortality.

In the spring of 2013 a field trial evaluating the relative tolerances of 75 different genotypes of *Populus* (40 *P. deltoides* x *P. nigra* hybrids and 35 *P. nigra*) to Septoria canker was established. The experimental design is a randomized complete block design with 5 blocks. Trees were planted at 3m spacing and the entire trial was surrounded by two rows of border trees. Genotypes were randomly arranged within each block as two tree plots. In the spring of 2014 one tree in each paired plot will be wound inoculated with a virulent isolate of *S. musiva*. Growth and survival will be monitored at the beginning and end of each growing

season. This information will be used to compare the relative tolerances of each genotype to Septoria canker.

A quantitative real time PCR assay is also being developed to quantify *S. musiva* growth in host tissue following inoculation. This is a multiplexed assay using the Beta-tubulin gene in the fungus and Initiation factor gene in the pathogen. To date this assay has been demonstrated to be both sensitive and specific. A time course experiment (0h, 24h, 48h, 72h,1wk, 3wk, 5wk, Control) is currently being conducted, using four genotypes of hybrid poplar (1 resistant, 1 susceptible, and 2 intermediate), to determine the best time point to distinguish between resistant and susceptible genotypes. This assay will be used to phenotype both leaf spot and stem canker symptoms.

Research to characterize the genetics of pathogen populations has been limited by the availability of adequate genome resources and species characterization at a molecular level. To overcome these bottlenecks, a non-resource prohibitive method of deep coverage genotyping of pathogen populations is required. Here we describe the use of a two-enzyme genotype-by-sequencing (GBS) method adapted for Ion Torrent sequencing technology. Utilizing this method, 2,373 and 5,960 unique loci," sequence tags", containing 9,992 and 17,193 SNPs were identified and characterized from natural populations of Septoria musiva and Pyrenophora teres f. maculata, respectively. S. musiva is a necrotrophic fungal pathogen that causes leaf spot and stem canker of poplar hybrids and *P. teres* f. maculata is a necrotrophic fungal pathogen that causes spot form net blotch of barley. Based on the estimated sizes of the S. musiva and P. teres f. maculata genomes, ~30 and 34 Mb, respectively, this GBS analysis placed a SNP marker on average approximately every 12.6 to 5.7 kb in the respective fungal genomes. This high-density of markers is adequate for association mapping in natural populations and positional cloning efforts in bi-parental fungal populations. Blast searches of the sequence tags generated from the S. musiva genome revealed that ~95% represent predicted genes, providing ~23% coverage of the total predicted genes present in the genome. This genotyping can be done at a relatively low cost per fungal isolate or progeny making it an option for researchers with limited resources and/or working on pathogens with no or limited genome information available.

**3. 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 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 and the qPCR assay will allow us to improve our understanding of this pathosystem and improve current disease resistance screening strategies.

Disease tolerance is an often overlooked tool which could be used to manage disease. The field experiment will be used to evaluate the potential of disease tolerance as a management tool for Septoria canker of hybrid poplar.

The low cost genotyping assay for the pathogen will be used to identify virulence factors and further improve our understanding of host pathogen interactions and disease resistance in *Populus*. This information will be used to improve isolate selection for screening assays.

**My program is training:** One M.S. student (Ruqian Qin), Two Ph.D. students (Kelsey Dunnell and Nivi Abrabham), one post-doctoral fellow (Achala Nepal).

# **REFEREED 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.

# ABSTRACTS (5):

**LeBoldus JM**, Stanosz GR, Qin R, and Faria O. 2012 Screening hybrid poplar for resistance to *Septoria musiva*: Greenhouse to field correlations using a novel inoculation procedure. Oak Ridge, TN.

Brueggeman R, Friesen T, and **LeBoldus JM**. 2012. Genotype by sequencing and Marker Assisted Selection: Breaking the Bottleneck. Proceedings of the National Fusarium Head Blight Forum. Orlando, FL.

Dunnell KL, Nepal A, and **LeBoldus JM**. 2013. A medium thouput greenhouse phenotyping assay of *Populus* spp. for Septoria canker resistance. Southern Forest Tree Improvement Conference. Clemson, SC.

Nepal A, Friesen T, **LeBoldus JM**. 2013. Polyethylene Glycol (PEG) mediated transformation of *Septoria musiva*. North Central - American Phtypathological Society Regional Meeting. Manhattan, KS.

Qin R, Stanosz GR, **LeBoldus JM**. 2013. Non-wound greenhouse screening of hybrid poplar trees with *Septoria musiva*. America Phytopathological Society. Austen TX.

Walla, J., **LeBoldus J.M.**, and Bergdahl A. 2013. The old and the New: Two needle diseases of spruce in North Dakota. F1680.

**LeBoldus, J.M.**, Bergdahl, A., Knodel, J., and Zeleznik, J. 2012. Dutch Elm Disease in North Dakota: a new look. PP1635.

## 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, and B. Berguson. 3 yrs. USDA NIFA. 2013-2016. \$61,120. North Dakota eIPM – CS Program. Knodel J, Beauzay P, LeBoldus JM.

# NCERA 224 Meeting, Santa Barbara, CA October 14-15, 2013 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 william.jacobi@colostate.edu

#### **Current Shade Tree Disease Studies:**

- National Elm Trial: We have 16 states with 17 sites involved in a trial of 17-19 commercially available elm cultivars. NCERA 224 members and other cooperators are involved. In Colorado all American elm cultivars are hit hard by European elm scale. Impact: The National Elm Trial web site now reports what each state site is finding in performance.
- 2. Pinewood nematode: In cooperation with Dr. Tisserat we are sampling recently dead urban and forest pines for the pinewood nematode to help determine management options for this disease. Pine wilt is now killing both Scots and Austrian pine in urban areas of Colorado and we are worried that this disease will become an epidemic on our exotic Austrian and Scots pines. The first infected tree was found in 2006, and in 2012 we had over 15 scots and Austrians killed. The slightly cooler summer of 2013 may have been the reason we had fewer pine wilt trees this year.

## **Current Forest Tree Insect/Disease Studies**

#### 1. White Pine Blister Rust:

- Small-scale meteorological analysis of the risk of WPBR in the Rocky Mts. will be a 2013-2014 project.
- We help coordinate white pine health work via the Central Rocky Mountains White Pine Health Working Group.
- Anne Marie Casper (MS) will submit a manuscript in 2013 on planting limber pine
- White bark pine regeneration after wild fires in Wyoming. This manuscript will be submitted in 2013.
- 2. Fire fuels and Dwarf mistletoe and Mt Pine Beetles in Front Range Colorado Ponderosa Pine: Jennifer Klutsch has submitted a manuscript on the interactions of these three disturbance agents.
- 3. **Bark Beetle mortality and future fire risk.** Manuscript by Dan West from his MS is back from Journal for revision.
- 4. **Mountain pine beetle preference for Lodgepole and Ponderosa pine.** Dan West will submit four manuscripts on his findings.
- 5. Spatial relationships of Mountain pine beetle, phoretic mites and fungal associates in Front Range pine forests. Javier Mercado (PhD candidate) is isolating fungi from beetles, mites and trees. Preliminary results are

showing that the dominant species during the current epidemic is Grosmannia clavigera.

- 6. **Aspen dieback in Colorado:** Meg Dudley (MS) will submit her manuscript on the health of aspen and occurrence of diseases and bark beetles in Colorado in 2013.
- 7. Aspen insect and disease occurrence Rapid Threat Assessment with WWETEC: Betsy Goodrich has completed research and the report on an analysis of large-scale western U.S. -relationships between aspen health, site and environmental conditions.
- 8. 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, dwarf mistletoe and white pine blister rust on adults and regeneration. Papers will be submitted spring of 2014

### Publications:

- 1. 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.
- 2. Jacobi, W. R., Koski, R. D, Negron, J. F. 2013. Dutch elm disease pathogen transmission by the banded elm bark beetle *Scolytus schevyrewi*. Forest Pathology: Doi:10.111/efp.12023
- 3. Kearns, H. S. J., Jacobi, W. R., Reich, R. M., Flynn, R. L., Burns, K. S. and Geils, B. W. 2013. Risk of white pine blister rust to limber pine in Colorado and Wyoming, USA. Forest Pathology. DOI: 10.1111/efp.12065
- 4. Costello, S. L., Jacobi, W. R, and Negron, J.F. 2013. Emergence of Burprestidae, Cerambycidae, and Scolytinnae (Coleoptera) from mountain pine beetle-killed and fire-killed ponderosa pine sin the Black Hills, South Dakota, USA. The Coleopterists Bulletin 67: 149-154

### New Disease Issues:

Winter drying, spring freeze caused leaf bud damage on ash and shrubs and major winter desiccation of drought stressed blue spruce. Road salt continues to stress trees.

**Insect Issues**: Mountain pine bark beetles populations are dropping now on the northern Front Range forests. Leaf mining on elms by European elm leaf weevil was down this year.

# NCERA 224 meeting Oct 14 Santa Barbara, CA at Best Western Peppertree Inn <u>(1-805-687-2245)</u> Vera Krischik, University of Minnesota, 612.625.7044; <u>krisc001@umn.edu</u>

Neoni	Neonicotinyl insecticide use in US							
	Imidacloprid lbs (ai)	Clothianidin lbs (ai)	Thiamethoxam lbs (ai)					
MN	52,048	43,663	68,876					
CA	348,247	3,182	30,687					
US	700,000	1,200,000	990,000					
US	In 2009 143/442 million a	acres use a neonicotinyl inse	cticide					
	83 million acres of corn h	83 million acres of corn have seed treatments of neonicotinyls						
	and honeybees rely on c	orn for pollen						

One of the major deficits in knowledge is how much neonicotinyl insecticide is found in pollen and nectar of neonicotinyl-treated plants, besides seed-treated crops. A canola seed is covered with 0.11 mg and a corn seed 0.625 mg imidacloprid that results in imidacloprid residues of 4 ppb nectar and 7 ppb pollen in canola (EFSA 2013). Field crops have a limit of 4 mg/sgft each year. Squash has residues of imidacloprid of 12 ppb nectar and 14 ppb pollen and of thiamethoxam of 11 ppb nectar and 10 ppb pollen (Stoner and Eitzer 2012)

In urban landscapes, where bees forage for pollen and nectar, a soil surface application of imidacloprid can be applied to a plant (300 mg) and tree (67 g). We calculate that a 609,000 times greater amount of imidacloprid is applied to basswood trees compared to a canola seed.

#### Stoner and Eitzer 2012, field rate, summer squash

	Imida	cloprid	Thia	methoxam
	Tissue (ppb)	Soil Drip (ppb	) Tissue (ppb)	Soil Drip (ppb)
Whole Plant	47	218	154	362
Female Flower	Bases 10	31	10	22
Synandria	15	46	19	31
	Imida	acloprid	Thiar	nethoxam
	Pollen (ppb)	Nectar (ppb)	Pollen (ppb)	Nectar (ppb)
Mean	14	12	10	11
Min	6	5	5	5
Max	28	14	35	20

#### Dively and Kamal 2012 pumpkin, field crop rate

-	Imida	cloprid	Thiamethoxam		
	Pollen (ppb)	Nectar (ppb)	Pollen (ppb)	Nectar (ppb)	
Mean	31	9	24	11	
Min	23	7	17	9	
Max	44	16	33	15	

#### Byrne et al, citrus field crop rate

imidacloprid, Bakersfield 12 ppb 1X, 25 ppb 2X

#### Larson et al 2013, turf rate, Clover nectar in a lawn

Clothianidin, 171 ppb range 89–319; n= 5

#### Paine et al 2011, tree-landscape rate, Eucalyptus nectar 5 mo after soil drench,

imidacloprid 286 ppb, 660 ppb all, n=5

#### Krischik 2013, tree landscape rate, soil drench of imidacloprid to golf course linden trees

Applied May 7 2012; June 20 flowers, leaves, soil collected; Aug 30 leaves, soil collected; 1X application on 25 cm (around 9.7 in) DBH=26 g/tree

 June (43 DAT) ppb
 August (70 DAT) ppb

 leaves
 554
 1,023

 soil
 15,436
 5,956

 flowers
 30 (3.6 to 72 ppb, n=8 trees)

Systemic neonicotinyl insecticides (imidacloprid, clothianidin, dinotefuran, and thiamethoxam) are widely used due to low toxicity to humans, but they are very toxic to bees and maybe birds as addressed in two new review papers by the Xerces Society (2012) and American Bird Conservatory (2013). Visit the 2010 LCCMR pollinator website for research and outreach products at <a href="https://www.entomology.umn.edu/cues/pollinators/index.html">www.entomology.umn.edu/cues/pollinators/index.html</a>.

To understand how little kills a bee, let us think of a heart healthy aspirin that is 80 milligrams = 80,000 micrograms = 80,000,000 nanograms (ng). A bee that eats 4-40 ng imidacloprid can be killed and 1- 3 ng reduce the bee's ability to forage, navigate, and return to the hive. Research showed that bee brains have 40x more nicotinic receptors compared to other insects, as bees perform higher brain functions dealing with memory, spatial orientation, and learning.

On June 18 2013, 25,000 bumblebees were killed at a Target store in Wilsonville, Oregon when the bees fed on nectar from linden trees treated with the neonicotinyl insecticide dinotefuran (label Safari). The incident was documented by the Oregon Department of Agriculture which covered the treated trees with netting and a 6 mo. ban on using dinotefuran was started.

Krischik 2013, Table 1,The ppb in a solution of nectar or sugar syrup can be related to the ng that a bee consumes and the published LD50 values by using this table. The greyed areas in the first 3 columns are the published LD50 values based on a bee consuming a 10 or 20 microl solution of 50% sugar syrup. Balderrama et al. 1992 states that a resting bee needs 5.5 to 14.6 mg sugar and while a foraging bee uses 33 to 88 mg of sugar. Since bee colonies in this study were foraging, the greened areas in the last 4 columns predicts the ng of imidacloprid that is ingested that is around an LD 50 of 21 ng/bee. This table predicts that a 185-200 ppb solution fed to bees may cause mortality, which is the value that Bayer uses (Schmuck et al. 2001, Fischer and Chalmers 2007).

	LD50	LD50	Suchail et al. 2001	Frazier et al. 2011	Byrne et al. 2013 , Balderrama et al. 1992 50% sugar syrup			
				10 mg nectar for 100 mg bee	resting bee 5.5 mg (0 load)	es 14.6 mg (50%load)	6 hrs fora 33 mg	ging 88 mg
ppb solutio n	10 ul ng /bee	20 ul ng/bee	10 ul ng/bee	ng/bee	(11 ul) ng/bee	(29.3 ul) ng/bee	(66.5 ul) ng/bee	(176 ul) ng/bee
0	0	0	0	0	0	0	0	0
10	0.10	0.20	1	1	0.132	0.292	0.66	1.75
20 ( <b>1)</b>	0.20	0.40 alters behavior	2	2	0.264	0.594	1.32	3.50
50	.50	1.00	5	5	0.660	1.45	3.3	8.75
100	1.00	2	10	10	1.32	2.92	6.6	17.5
185 <b>(1)</b>	1.85	3.70 LD50	18.5	18.5	2.4	5.4	12.21	32.37
200	2	4	20	20	2.6	5.8	13.2	35
300	3	6	30	30	3.9	6.7	19.8	53
400	4	8	40	40	5.3	11.6	26.4	70
500	5	10	50	50	6.5	14.6	33	87
600	6	12	60	60	7.8	17.52	39	105

# 2011 Imidacloprid residue plants

Dose in mg/soil	Dead bees on Agasatche	Agastachespp. nectar ppb	Asclepias spp. nectar ppb	Esperanza spp. nectar ppb	Rosa spp. pollen ppb
0	0.6b	6b	3c	0c	26b
25	0.6b	52b	80c	8c	36b
50	0.5b	133b	175bc	21c	30b
300 1X 3 gal pot	1.1ab	1973b	1568bc	106c	95b
600 2X 3 gal pot	2.4a	5265ab	2950b	276b	332b
1200	2.4a	9335a	8337a	9162a	720a
	F=3.2, 0.01	F=3.7, 0.017	F=25.8, 0.0001	F=166, 0.0001	F=5.7, 0.0025

# 2009, 2010, 2011 Imidacloprid residue

Dose in mg/soil Marathon 1%G	Rose 2009 field	Rose 2010 GH	Rose 2011 field
0	9d	0c	26b
25	na	5c	36b
50	na	7c	30b
Homeowner 1X 270 mg	812c	na	na
Homeowner 2X 270 mg	1648a	na	na
300 1X 3 gal pot	1175b	32bc	95b
600 2X 3 gal pot	na	161ab	332b
1200	na	268a	720a
	F=256, 0.0001	F=4.9,0.0045	F=5.7,0.0025

2005, 2007, 2011 Imidacloprid residue buckwheat, milkweed

Dose in mg/soil Marathon 1%G	2005 Nectar ppb	Nectar ppb	2011 Milkweed Nectar ppb	The Car
0			3c	
25	na	na	80c	
50	na	na	175bc	
Homeowner 1X 270 mg	na	na	na	
Homeowner 2X 270 mg	na	na	na	
300 1X 3 gal pot	6000	6000	1568bc	
600 2X 3 gal pot	12000	12000	2950b	
300 21 days later	na	20000	na	
600 21 days later	na	34000	na	
1200	na	na	8337a	
	F=25.86, (2,22) 0.001	F=22.72, (2,6) 0.0016	F=25.8, 0.0001	

# 2012 Imidacloprid residue canola pollen

Dose in mg/soil	April 5 2010, E June 1 flowers 1 app April	May 19, 2010, W July 2 flowers 1 app May	July 2, 2010, E August 18 flowers 2 app April+July	July 29, W Sept 15 flowered 1 app May	and the second
Black w aust		0		0	
Poncho blue invigor 601		0		0	
Gaucho red invigor 701		0		0	
0	0c	0b	0b	0b	
4	0c	0b	313b	5b	
8	14c	7b	179b	8b	]
80	461b	15b	342b	24b	
160	2072a	341a	3860a	162a	
	F=410, 0.0001	F=271,0.001	F=7.5, 0.0002	F=70.6, 0.0001	

# 2012 Imidacloprid residue canola pollen

Dose in mg/soil	April 5 2010, E June 1 flowers 1 app April	May 19, 2010, W July 2 flowers 1 app May	July 2, 2010, E August 18 flowers 2 app April+July	July 29, W Sept 15 flowered 1 app May	
Black wi aust		0		0	AX I
Poncho blue invigor 601		0		0	
Gaucho red invigor 701		0		0	
0	0c	0b	<b>0</b> b	0b	
4	0c	0b	313b	5b	
8	14c	7b	179b	8b	
80	461b	15b	342b	24b	
160	2072a	341a	3860a	162a	
	F=410, 0.0001	F=271,0.001	F=7.5, 0.0002	F=70.6, 0.0001	

# Efficacy and duration imidacloprid in <u>field grown poplars</u> at 12 mo

Treatment	1 mo	10 mo	12 mo
Control	90.0±3.0 a	100.0±0.0 a	91.2±4.3 a
Granular 0.12x	3.3±2.6 ef	33.3±16.7 bc	55.6±6.7 bcd
Granular 0.25x	0.0±0.0 f	16.7±16.7 bc	41.7±7.3 cd
Granular 0.5x	0.0±0.0 f	0.0±0.0 c	0.0±0.0 e
Drench 0.25x	30.8±8.5 cd		
Drench 0.5x	0.8±0.8 f	25.0±17.1 bc	36.1±8.9 d
Drench 1x	3.3±2.6 ef	0.0±0.0 c	24.1±8.2 de
Tablet 0.12x	15.0±7.2 def	16.7±16.7 bc	68.5±9.3 abc
Tablet 0.25x	20.8±9.3 cdef	0.0±0.0 c	2.8±2.8 e
Tablet 0.5x	54.2±9.7 b	0.0±0.0 c	2.8±2.8 e
Stick Soak 0.5x	55.0±8.6 b		
Stick Soak 1x	42.5±8.8 bc		
Stick Soak 2x	22.8±9.2 cde		
F (df), P ANOVA	17.6 (12, 182), <0.001	10.1 (12, 65), <0.001	21.8 (12, 216), <0.001

Efficacy and duration of imidacloprid in container grown poplars at 12

mo			
Treatment	1 mo	8 mo	12 mo
Control	88.8±2.79 a	76.2±8.8 a	86.4±7.0 a
Granular 0.12x	0.0±0.0 c	0.0±0.0 b	0.0±0.0 c
Granular 0.25x	0.0±0.0 c	0.0±0.0 b	0.0±0.0 c
Granular 0.5x	0.0±0.0 c	0.0±0.0 b	0.0±0.0 c
Drench 0.5x	0.0±0.0 c	0.0±0.0 b	0.0±0.0 c
Drench 1x	0.0±0.0 c	0.0±0.0 b	0.0±0.0 c
Drench 2x	0.0±0.0 c	0.0±0.0 b	0.0±0.0 c
Tablet 0.25x	27.9±8.0 b	0.0±0.0 b	0.0±0.0 c
Tablet 0.5x	13.9±7.3 bc	0.0±0.0 b	0.0±0.0 c
Tablet 1x	13.9±6.1 bc	0.0±0.0 b	0.0±0.0 c
Stick Soak 0.5x	0.0±0.0 c	0.0±0.0 b	20.8±11.4 bc
Stick Soak 1x	0.7±0.7 c	0.0±0.0 b	20.8±9.6 bc
Stick Soak 2x	0.0±0.0 c	0.0±0.0 b	8.3±5.6 c
Root Dip 1x	0.0±0.0 c	0.0±0.0 b	39.4±13.2 b
Root Dip 2x	0.0±0.0 c	0.0±0.0 b	7.1±7.1 bc
Root Dip 4x	0.0±0.0 c	0.0±0.0 b	5.0±5.0 c
F (df), P ANOVA	49.8 (15, 269), <0.001	62.6 (15, 81), <0.001	15.3 (15, 165), <0.001

Efficay and duration in containers of rose

Treatment	reatment % Mortality ±SEM		Mean flip time ±SEM (s)		% Leaf eaten area ±SEM (cm <sup>2</sup> )	
	2006	2007	2006	2007	2006	2007
Control	2.6±0.4 e	0.7±0.7 e	30.4±2.e	29.5±2 e	100.0±0.0 a	89.4±1 a
GR 1x	35.9±4 b	25.0±4 bc	101.9±3 bc	74.4±7 cd	3.4±0.3 cd	12.1±1 de
GR 3x	42.7±4 b	37.5±5 b	106.0±3 ab	61.5±7 d	2.7±0.cd	11.8±1 de
DR 1X	30.1±3 bc	2.1±2 de	98.6±3 bc	57.6±4 d	2.1±0.3 cd	38.6±6 b
Tab 1X	9.1±2de	20.8±3 cd	82.0±3 d	109.7±2 a	11.8±2 b	7.5±1 de
Tab 2X	10.6±2.de	22.2±3 bcd	95.5±3 bcd	100.9±3 ab	7.1±1 bcd	5.0±0.3 de
IRt Dip, 3.1 ml	21.3±3cd	36.1±3 b	95.9±3bcd	106.9±3 a	2.3±0.3 cd	23.9±3 c
Discus	83.3±2.a	75.0±4a	120.0±0 a	112.7±2 a	0.2±0.1 d	2.9±0.7 e
F (df), P treatment	66.3(9, 218), <0.0001	41.7 (9, 302), <0.0001	72.5 (9, 218), <0.0001	47.4 (9, 302), <0.0001	631.4 (9, 218), <0.0001	180.1 (9, 302), <0.0001

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

# University of Delaware Co-operative Extension: Brian Kunkel & Nancy Gregory

## **Regional Weather:**

Overall the total precipitation for each county for Delaware was slightly above normal this past year. The wettest months of the year were June, July and August.

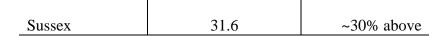
County	Precipitation (inches)	Departure from Normal (inches)
New Castle	46.6	+2.5
Kent	48.5	+4.5
Sussex	49.7	+4.8

Table 1. Total precipitation for Delaware over past year (November 2012- November 2013)

The abundance of rainy days provided challenges for managing pests because there were fewer opportunities to make applications during key times in the growing season (Table 2).

Temperatures were below historical averages throughout the state as illustrated in the graph below using accumulated growing degree days (GDD<sub>50</sub>) starting on 1 March 2013 in New Castle County (Fig. 1). March through early June saw GDD about a week to ten days behind the average and after approaching normal GDD around mid-July, the year remained cool and behind a week or so.

County	Precipitation (inches)	Departure from Normal
New Castle	31.1	~30% above
Kent	30.8	~30% above



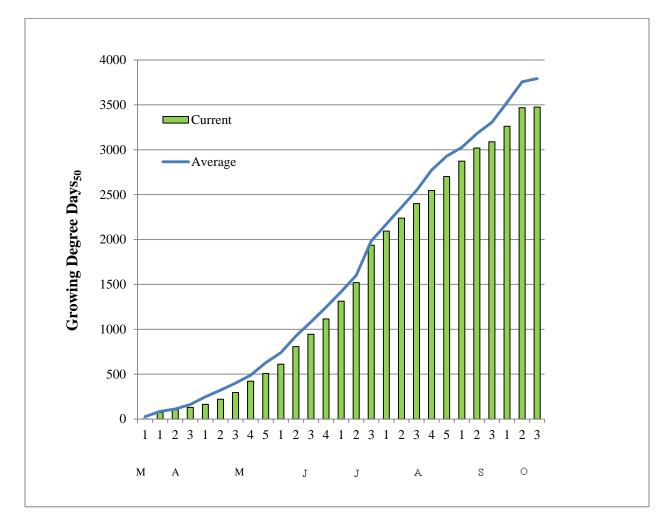


Figure 1. GDD<sub>50</sub> for 2013 compared against historical average GDD<sub>50</sub> (2006 – 2011)

# Insect and Disease Highlights:

Insects

This year was a fantastic year for many non-pest caterpillar species such as: Actias luna (Luna moth), Acharia stimulea (Saddleback moth), Hemaris diffinis (Snowberry clearwing), Hemaris thysbe (Hummingbird clearwing), Orgyia leucostigma (White-marked tussock moth), Pyrrharctia isabella (Isabella Tiger moth; larvae called woolly bears), and Eumorpha pandorus (Pandorus sphinx). Caterpillar pests such as Malacosoma americanum (Eastern tent caterpillars) and Hyphantria cunea (Fall webworms) were not as common as the past few years. Podosesia syringae (Lilac/Ash borer) emergence holes were confused to be emerald ash borer attacks on ash trees (New Castle and Sussex counties). We still have <u>not found</u> emerald ash borer in Delaware. Bagworms, Thyridopteryx ephemeraeformis, and Manduca quinquemaculata (Five-spotted hawk moth; larvae called tomato hornworms) were commonly found this past growing season in all counties.

Soft scales such as *Ceroplastes ceriferus* (Indian wax scale), *Toumeyella liriodendri* (Tuliptree scale), and *Pulvinaria* scales (Cottony Taxus/Camellia Scale, Cottony Maple Scale),

*Parthenolecanium corni*, *P. fletcheri*, and *P. quercifex* (European fruit lecanium, Fletcher, and oak lecanium respectively) were sent to the lab for identification this year. *Ceroplastes* spp. and *Pulvinaria* spp. scales were submitted as late as early November this year. We are uncertain if this is an increase in abundance or if professionals are finally recognizing which hosts they are most frequently found. Samples brought into the diagnostic lab, extension offices or phone calls, suggested scale activity was later than previous years for most scale species and was probably the result of cooler temperatures.

Many armored scale samples were diagnosed this summer including *Chionaspis pinifoliae* (**pine needle**), *Fiorinia externa* (**elongate hemlock**), *Aspidiotus cryptomeriae* (**cryptomeria**), *Pseudaulacaspis prunicola* (**white prunicola**), *Lepidosaphes pallida* (**Maskell**), *Pseudaulacaspis pentagona* (**white peach**), *Carulaspis juniperi* (**juniper**), and *Lopholeucaspis japonica* (**Japanese maple**) scales. Landscape companies continue to report white prunicola scale and Japanese maple scale are among their most common and difficult pests to manage.

Other sucking insect pests such as **whiteflies**, **aphids** and **mealy bugs** were minor problems reported during the year. Greenhouse managers still encounter high populations of *Frankliniella occidentalis* (**Western flower thrips**). **Apple mealybug** continues to be a challenge to manage on native azaleas, *Kalmia* and *Fothergilla*, especially when these hosts are planted near streams or are located in low lying areas with high water tables.

Nurseries in the mid-Atlantic continue to struggle with **redheaded flea beetle** control; however this year beetle populations decreased sharply after mid-July and may be related to abundant rainfall. *Atomacera decepta*, **hibiscus sawfly**, *Macremphytus tarsatus* (**dogwood sawflies**) **and roseslug sawflies** were very common in landscapes this year causing considerable damage on their respective hosts. Other minor pests encountered this year include, **boxwood leafminer**, and **carpenter bees**. Bark beetles were reported at various times during the year throughout Delaware. **Bark beetles** included *Xylosandrus germanus*, *X. crassiusculus*, *Ips* among others. **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 landscape contractors and homeowners.

## 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 until mid-August.

## Diseases

Ornamentals constituted over 50 % of the total of approximately 675 samples. Of the 370 ornamental plant samples, **physiological** or **environmental stress** was a factor in 170 samples, while insects were the problem or a factor in 68 samples. Many landscape ornamentals newly planted in the past 2 or 3 years suffered from environmental stress, often leading to further issues with disease organisms and insects. Excessive rains in the spring and early summer led to **saturated soils** and problems with **root rot** and **establishment of good root systems**. Holly and juniper with *Pythium* or **Phytophthora** root rot were common. **Anthracnose** was common on hardwoods in the spring, but trees leafed out again in a few weeks. **Gymnosporangium** rusts were severe on Rosaceous hosts such as hawthorn and serviceberry.

<u>New Reports</u> – Numerous boxwood samples were submitted. **Boxwood blight** caused by *Cylindrocladium* was positively confirmed, first in a nursery site where all plants were destroyed, but later in a residential setting. **Impatiens downy mildew** continued to be a problem on

*Impatiens walleriana* and *Impatiens balsamina*. A **downy mildew** was confirmed on *Viburnum*, caused by a *Plasmopara sp*. **Stigmima lautii** was confirmed on spruce.

<u>Pathogens of regulatory significance</u> – **Chrysanthemum white rust** was identified in two retail locations in Dealware and plants were destroyed. Several plants sampled as a part of two trace forwards for *Phytophthora ramorum* were tested serologically and were negative for *P. ramorum*.

**Swiss needlecast** has become a predominant problem on Douglas fir in Christmas tree and landscape plantings. **Phytophthora root rots** have been widespread due to excessive rains and saturated soils in the spring and summer. **Rose rosette disease** continues to spread to cultivated and hybrid rose plantings.

Some common turf diseases included **red thread** and **brown patch** during the summer. Other diseases reported during the growing season included: **Forsythia blight**, *Phomopsis* and *Kabatina* **tip blights**, *Monolinia* **blight**, **fairy ring**, **daylily leaf streak**, **azalea leaf gall** and **bacteria leaf scorch**.

# 2010-2011 Publications & Notables

Kunkel and Gregory 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. Kunkel was interviewed by local newspapers about various 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 and entomology workshops are provided to Delaware green industry professionals in addition to "pest walk" tours in New Castle and Sussex counties. 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. Guest speakers were brought to a spring workshop for additional perspective on common Christmas diseases.

Master Gardener training was also conducted by Kunkel and Gregory.

# 2012 -2013 Research Highlights:

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

- Searched herbaceous perennials nurseries and production areas at public gardens in DE, MD, and PA for BMSB eggs, nymphs and adults
- BMSB populations were seldom encountered until mid-July; populations on plants after mid-July were still very low (average < 5/plant inspected)
- Observed native and non-native plants for BMSB populations this year
- Little evidence of predation or parasitism on the few egg masses or nymphs found on herbaceous perennials this summer

# 2012 Impact statements

About 40% of professionals attending a recent ornamentals workshop stated they learned more about reduced risk products and the new product labels being released regarding bee health.

Turf professionals (~12%) attending a recent turf workshop stated they intended to begin incorporating IPM practices into their business model as a result of the new information. Another 20% claimed to already utilize IPM practices for turf, and still found new useful information regarding beneficial arthropods and cultural practices.

All Christmas tree workshop attendees answering a post-workshop survey felt they learned a great deal about Christmas tree diseases and would like to receive a greater in depth review of insects important to Christmas trees. About 80% of survey respondents stated they intended to change some of their management practices based upon information provided at the workshop.

NCERA 224 2013 Report from Nebraska: Gerard Adams, Plant Pathology University of Nebraska Lincoln gadams3@unl.edu

**Disease conditions:** Nebraska agriculture and natural resources have sustained unprecedented extremes in climate that will have longer term consequences on the health of the urban and native trees and landscape plants.

<u>2011</u>: Flooding along the Missouri river corridor was the most extreme flooding event in greater than 100 years in Nebraska and contiguous states. Significant damage occurred to the majority of the native forests of Nebraska which are primarily located along the eastern river corridors. Especially damaging was the long period of saturated soil conditions experienced by the flora caused by a months-long delay in drainage.

*Quantifying and qualifying damage*: The state and federal forest services were unable to map and estimate extent and nature of damage by aerial photography and sketch mapping, due to lack of operational funding.

*Response*: Nebraska NCERA member wrote and submitted two grant proposals for multistate (7 state and 2-state proposals) to obtain funding for cooperative evaluation of tree mortality, damage, and the eventuality of subsequent decline. Regional USFS funding and State funding of the proposals was not realized. North Dakota was the sole state capable of funding an adequate research response.

<u>2012</u>: Drought state-wide was the most extreme experience in greater than 100 years.

*Quantifying and qualifying damage*: The state and federal forest services were unable to study the extent and nature of damage to urban forests, parks and recreational areas, and native and planted forests due to lack of operational funding.

*Response*: Nebraska NCERA member wrote and submitted a grant proposal to study extent and nature of damage and impact on trees but regional forest health monitoring funds were not awarded.

<u>2013</u>: State-wide climate is of well-distributed abundant precipitation and moderate normal temperatures.

*Response*: Nebraska NCERA member wrote and submitted an innovative grant proposal to the internal McIntire-Stennis program for study of the resilience to climate change of the urban forest (in Omaha, Lincoln and surrounding communities; an area of residence for 98% of state's population). Proposal receives recognition and is remodeled with Administration to initiate an urban forest program led through the School of Natural Resources. Long term program funding through McIntire-Stennis is awaiting USFS review and approval. Program funding of graduate students will be aimed for NSF funding emphasizing the ecosystem services of the urban forest and modeling future resiliency during predicted climate change scenarios.

# Diseases of unusual nature:

1. "Black xylem wilt of Aspen"-- aspen in a stand mixed with maple were wilting and dying in a location within the Lincoln urban forest. Symptoms resembled Verticillium wilt but poplars are not susceptible to the pathogen whereas maples succumb commonly to Verticillium wilt. Isolation from the stained xylem yielded either sterile cultures or a *Melanconium* sp. Koch's Postulates have not yet been fulfilled and a question on the level of stress experienced by the trees is not yet resolved to facilitate recreation of possible predisposition.

2. "Sudden death of Eastern white pine"—Stately specimen trees, often several feet DBH and over 50 years old, throughout the parks and urban forests of Lincoln and Omaha died suddenly in great numbers during the drought of 2012. The mortality of hundreds of stately white pine in one major urban park was featured in prominent news articles. The species showed exceptional vigor up to this specific drought and mortality was unexpected. Dendroecological studies are being initiated to develop predictive models of the species response to climate change in Nebraska's future.

3. Pine Wilt—the recently arrived disease continues to be the greatest agent of mortality in the urban forest for large trees, even though Scots pine is the only species generally affected. Mortality of Scots pine during the drought of 2012 did not increase notably. Outreach education was needed in 2012 to clarify the causes of mortality of white pine versus Scots pine. The general public is cognizant of continuing mortality of "pines".

4. Entomosporium leaf spot—In 2013 weather conditions favored a local epidemic of severe leaf spot on ornamental *Pyrus* in Lincoln, NE.

5. *Leveillula* powdery mildew on tomatoes – increasing incidences of infection of greenhouse tomato crops with this recalcitrant powdery mildew is requiring education on management with strobilurin fungicides.

6. "Walnut mystery root disease" – Old trees (>25yrs) in planted Black walnut stands in Nebraska occasionally have decorticated areas at the base of the trunks that may extend to half the diameter. Uniquely, a region of open space is observed beneath the tree sufficient for a flat palm to be inserted under the tree at the soil line. The symptoms appear to be the result of some type of root rot, perhaps *Phytophthora*, that kills a portion of the tree and subsequently wood decay, perhaps *Ustulina*, that digests the woody material at the soil line, resulting in open space beneath a considerable portion of the tree. The trees remain productive, but hazardous, for many years.

# Research results and publications:

*Alder Phytophthora*— The devastating epidemic disease has caused widespread mortality of several alder species in riparian forests through northwestern Europe. The riparian alders commonly originate as nursery stock planted for erosion control, but also as natural establishment. The epidemic mortality is caused by a putatively exotic invasive pathogen,

*Phytophthora alni*. As conclusion of a 6 year effort, we have shown that the pathogen population in Europe is of exotic origin based on our population genetic studies (Aguayo, Adams et al 2013). Additionally, following our discovery of the Alder Phytophthora in America, we have

shown that the pathogen population in Alaska is native, but unlikely the source of the introduction(s) to Europe. More extensive population-genetic studies in our lab at Michigan were disrupted and irreparably destroyed by the political corruption of science.

Aguayo J, **Adams GC**, Halkett F, Catal M, Husson C, Nagy ZÁ, Hansen EM, Marçais B, Frey P. 2013. Strong genetic differentiation between North American and European populations of *Phytophthora alni* subsp. *uniformis*. Phytopathology 103: 190-199.

# MLG = Multi-Locus Genotype

*Physiological leaf scorch of Quercus*— The causes of Leaf scorch in hardwoods in North America can be the xylem-limited bacterial pathogen *Xylella fastidiosa* or unknown environmental stresses. Following a 9-state North Central & Plains state survey of bacterial leaf scorch using qPCR detection and quantification, a NCERA-193 cooperative research project (Adams et al 2012), we continued to study the predominant leaf scorch in the northern tier of north central states, physiological leaf scorch.

\*Adams GC, Catal M, Walla, J, Gould AB. January 2012. Bacterial leaf scorch distribution and isothermal lines (project NC-EM-08-02). Chapter 10, p 108-114. *In*: Potter, KM, Conkling BL. Forest health

monitoring 2011 national technical report. US Forest Service. <u>http://fhm.fs.fed.us/pubs/misc/draft\_FHM\_2011\_National\_Technical\_Report\_FHM\_Web.pdf</u>

\* Bold letters are authors of NCERA-193 membership. NCERA-193 contributors also included Megan **Kennelly** and others.

Our research on physiological leaf scorch included seven research plots consisting of planted landscape stands of *Quercus rubra* of 23-30 years of age, each plot contained trees that showed no scorch and trees with scorch on shared soil type, planting date and climate. Six of the 7 research plots were studied with 3 years of replicated data collection, as follows: Site **BR1** with 11 trees, Site **BR2** with 12, Site **FR** with 6, Site **SW** with 10, Site **GR** with 7, and Site **LB** with 6 Oaks.

Factors measured and statistically tested for significant correlation to presence of scorch: Planting depth, amount of girdling roots, foliage nutrition, soil bulk density, soil texture, soil oxygen profile, and soil compaction, soil pH and CEC, buried rubble, efficiency of photosynthesis system II, transpiration rate, movement of CO<sub>2</sub> through stomata, hydraulic conductivity, DBH, canopy health (FHM crown condition protocol), and remnants and effect of planting hardware (wire baskets,nylon rope girdling).

**CONCLUSION:** Leaf scorch was highly significantly associated with <u>planting depth</u>. Planting depth was highly significantly correlated with girdling roots. No other factors were significantly associated with leaf scorch at all plots. At one plot, depth of top soil overlaying a sand berm was negatively correlated with leaf scorch; at another plot, soil bulk density above the theoretical root penetration limit was associated with leaf scorch. Complex spatial patterns of variation in soil oxygen content were discovered with the use of steel rod rust-accumulation profiles. This technique was highly revealing of buried debris and other soil structure problems.

Developing a host index for alder— During study of widespread mortality of alder species in the southern Rocky mountains and southcentral and interior Alaska, it was discovered that the national records and literature of pathogens on alder was deficient especially for the Alaska region. Study of fungi present on diseased above ground stems of alder,

and oomycetes present in the rhizosphere beneath alders showing dieback and mortality, was undertaken. Isolated fungi from diseased tissues were identified and those belonging to potentially pathogenic genera were evaluated in Koch's Postulate tests of inoculated replicated trials. Trials were located near Fairbanks and in the Kenai Peninsula which have different climates. Description and identification of the fungi, and inoculation trials continue. Wood decay fungi are also being identified and recorded. Some results, below:

*Western Hemlock Inoculation Project (*WHIP)--- Location: Tungass National Forest, Ketchikan & Prince of Wales Island, Alaska.

A new occurrence of a mysterious branch dieback and mortality of Western hemlock is being studied by this NCERA\_224 member to determine what fungal pathogens may be

involved in the disease. Other parameters such as site factors are being studied separately. The damage has been noticed to occur periodically and in different stands over the last 30 years. Several different Discomycetous fungi in the Helotiales and Dermataceae ("cup fungi") have been physically isolated from the advancing canker margins and identified through DNA sequencing of pure cultures. Additionally, direct sequencing of canker margin woody material, using fungal-specific primers and PCR, is being used as a diagnostic tool. This method is new

and is dependent upon removal of complex compounds inhibitory to PCR reactions during DNA

extraction of crushed wood particles.

Pyrosequencing of the DNA extractions of canker margin wood is being used for the first time to study canker pathogens and has been contracted to identify all fungi present including non-cultureable fungi. Replicated inoculation trials located in three GPS mapped areas of Prince of Wales Island are being evaluated for completion of Koch's Postulates. The study is funded for two years, currently.

The pathogenic fungus most frequently isolated from the cankers and the only fungus observed (very rarely) fruiting on cankered material is *Coccomyces heterophyllae*. Inoculation trials will be evaluated next spring and new inoculation trials established.

## Washington

# 2013 NCERA-224 State Report October 14-15, 2013 Santa Barbara, CA

# Washington State University-Puyallup

# **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
- Andy McReynolds, Plant Technician II
- Katie Coats, Research Associate
- Don Sherry, Professional Worker I
- Carly Thompson

# **Graduate Students Supervised**

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

# 2013 Undergraduate Student Interns

• Monica Gallucci – Washington State University

# **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
- Oregon Department of Forestry, Salem, OR
- Oregon State University
- Pennsylvania State University
- Starker Forest, Corvallis, OR
- University of Alaska, Fairbanks
- 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

# **2013 Sources of External Support**

• USDA NIFA SCRI - \$421,387. Development and use of genomic tools to improve firs for use as Christmas trees.

- WSDA Specialty Crop Block Grant Program \$117,243. Management of an emerging adelgid pest on Nordmann fir Christmas trees.
- WSDA Nursery Research Program \$24,381. Steaming as a method of eradicating *Phytophthora ramorum* and associated *Phytophthora* species in nursery field soil.
- APHIS Farm Bill Suggestion \$152,376. Steaming as a method of eradicating *Phytophthora ramorum* and associated *Phytophthora* species in nursery field soil.
  - **APHIS NORS-DUC** \$35,000. Determination of *Phytophthora ramorum* threshold inoculum levels in irrigation water needed for infection of nursery hosts.
  - USDA Floriculture and Nursery Research Initiative (FNRI) \$58,423.
  - Improved pathogen detection and management of bulb diseases.
- **PNWCTA -** \$7,500. Christmas tree disease and keepability research.
  - Northwest Agricultural Research Foundation (NARF) \$10,000. Management of diseases on ornamental bulbs and cut flowers.
- UC Davis (IR-4 program) \$26,250. Fusarium and Botrytis disease management trials.
  - **USDA Forest Service** \$5,000. Biological control of tanoak and bay laurel resprouts using *Chondrostereum purpureum* and other wood decay fungi.
- Gift grants \$12,000. Syngenta Crop Protection
- In-Kind Support PNW Growers

# **Christmas Trees Research**

**Initiation of a multi-institution Christmas Tree Research Project** (In collaborations with Bert Cregg, Michigan State Univ.; David Neale and Jill Wegrzyn, U.C. Davis; Joireman, Jeffrey, Washington State Univ.; John Frampton, Ross Whetten, and Lilian Matallana, North Carolina State Univ.; Rick Bates, Pennsylvania State Univ.; and Rick Dungey, National Christmas Tree Association).

A new five-year, \$1.3 million grant from the U.S. Department of Agriculture's Specialty Crop Research Initiative (SCRI) was awarded to Christmas tree researchers from Washington State University (WSU), North Carolina State University (NCSU), University of California at Davis, Michigan State University, Pennsylvania State University, and the National Christmas Tree Association (NCTA). This project will develop genetic markers to identify true firs (*Abies* spp.) that have superior needle retention and resistance to Phytophthora root rot as well as augment our understanding of consumer perspectives on real Christmas trees. The project began in September 2012 and builds on the success of previous industry-supported research.

Needle loss is one of the biggest concerns for families purchasing real Christmas trees. Decades of postharvest needle retention work at WSU has identified genotypes of Douglas-fir, balsam, Canaan, Nordmann, and Turkish firs that have excellent needle retention as well as those that tend to exhibit extensive shedding within a few days of drying. Over the past 15 years, scion wood from tested trees has been used to establish about 3 acres of clonal holding blocks at WSU-Puyallup. The SCRI project will identify genetic indicators, or markers, for needle retention, by isolating RNA from needles of Fraser, balsam and Canaan fir trees that have a history of either excellent or poor needle retention. The "RNAseq", or RNA sequencing, process will hopefully

reveal regions of transcribed genetic material that are exclusively shared by trees that have excellent needle retention. Markers will then be developed that allow scientists to quickly identify trees, including those in existing seed orchards, with the desirable needle retention genes.

Similar techniques will be used to identify trees that are resistant to Phytophthora root rot. This disease plagues growers in all regions where true firs are grown as Christmas trees and often

limits where consumer-desirable species, such as noble and Fraser fir, can be grown. There is no effective control for this disease, so the best way to tackle the problem is to find resistant tree species and strains. Considerable work on Phytophthora root rot has been done at WSU and NCSU.

Compared to the growth in the number of artificial trees used today, the actual number of real Christmas trees sold in the U.S. has remained relatively static for decades. To address the stalled market for real Christmas trees, the SCRI grant will also support Jeff Joireman, a consumer behavior scientist at WSU, who will research consumer preferences in collaboration with Rick Dungey of NCTA.

A combination of telephone and online surveys and focus groups to gather relevant information for this project will be used.

- We will collect and analyze data from growers to better understand the challenges they face in growing, harvesting and distributing fresh, high quality Christmas trees.
- We will review earlier studies and talk with retailers to better understand the reallife conditions under which real trees are stored before going home with the customers.
- And we will survey consumers to learn more about their experiences with real Christmas trees, including how they actually care for the tree, their satisfaction with the product and what attributes would improve their real tree experience.

Consumer preferences for Christmas trees are varied. Color, size, taper, density, needle length, strength of branches and aroma all impact individual buying decisions. Research conducted on behalf of NCTA has shown that consumers identify messiness, inconvenience and the fear of fire hazards as barriers to purchasing a real Christmas tree. Consumers have also noted concerns about pesticide residues and the environmental impact of cutting trees as barriers. As a part of this project, we seek to further explore consumer preferences that may transcend the differences between species and ultimately genetically improve some, and perhaps eventually all species to become more consumer-friendly.

# Mid-rotation growth and postharvest needle retention characteristics of balsam fir grown in western Washington

In 2008, a replicated common garden field trail was established at the Washington State University Research and Extension Center in Puyallup, WA to evaluate the growth and postharvest characteristics of 26 provenances of balsam fir (*A. balsamea*) and eight progeny collections of 'bracted' balsam fir (*A. balsamea var. phanerolepis*). A single source of Fraser fir (*A. fraseri*) was included in the trial as a standard. Seed was obtained from the Canadian Forest Service's National Tree Seed Center and P+2 seedlings were out-planted in February of 2008 in a 1.08 acre plot at 6 ft x 6 ft spacing. The plot design was a randomized complete block with five blocks. Five trees of each source were planted in a row within each block. To obtain information on the mid-rotation growth characteristics; tree heights were measured, bud break growingdegree days (GDD) were calculated, and data were collected on the color and form of the trees during the past two years. In 2012, tree heights ranged from 133 to 191 cm. All of the balsam sources broke bud prior to Fraser fir and the bud break GDD ranged from 208 to 437 among the balsam sources. Color was rated on a scale of 1 (poor) to 3 (excellent) and ranged from 1.7 to

2.7. Form was rated on a scale of 1 (poor) to 5 (excellent) and ranged from 2.3 to 4.1. Branches were harvested for trees and displayed dry in early October to also obtain preliminary information on differences in needle retention among the sources of trees. Needle loss was rated on a scale of 0 (none) to 7 (91-100% loss) and ranged from 1.3 to 4.8. These and previous early-rotation growth data indicates that there are significant growth and postharvest needle retention differences among the 34 sources of balsam fir in this trial.

# Observation on the inspection of Christmas trees in Hawaii

Insects are commonly found by Hawaii quarantine inspectors on Christmas trees, which are imported from the Pacific Northwest. To reduce the risk of importing yellowjacket (Vespula *spp.*) queens, other insects, and slugs, an inspection and tree shaking certification program was initiated in 1991, requiring exporters to shake all trees at destination using a mechanical shaker, or shake 10% of trees manually in the presence of an inspector, who would then require 100% shaking if yellowjackets were observed. For the period 1993-2006, the percentage of shipping containers rated by Hawaii quarantine inspectors as moderately or highly infested with insects following manual and mechanical shaking was 17.0% and 2.6%, respectively. Live yellowjacket queens and slugs continue to be intercepted in Hawaii from containers certified as manually shaken and from containers certified as mechanically shaken. During the past couple of years, the Hawaii Department of Agriculture (HDA) has given importers the option of treating trees in containers with intercepted regulated pests with a hot water shower treatment that was originally developed to treat plants to control the spread of the coqui frog, an invasive pest that is already present on a number of island in Hawaii. In an effort to better understand the inspection process and hot water treatment option, the HDA inspection of containers of PNW-grown Christmas trees was observed during November 2012. Mechanical shanking of trees is an accepted procedure to reduce the risk of regulated pests being intercepted in exported trees. Observations indicate that growers may be able to reduce the risk of pests on exported trees by improving the shaking process to remove more dead needles prior to bailing.

# An emerging adelgid pest on Nordmann fir Christmas trees in western Washington

During the past 10 to 15 years there has been an increased interest in growing Nordmann and the closely-related Turkish fir as Christmas trees in the Pacific Northwest (PNW). Research has shown that these species have excellent postharvest moisture and needle retention when displayed in water and are tolerant or have limited susceptibility to Phytophthora root rot, Annosus root rot, spider mites, and balsam woolly adelgid. Since 2004, an average of 500,000 Nordmann/Turkish firs has been planted per year in Oregon. Although data are not available, a similar increase has taken place in western Washington and the Inland Empire. Currently, these species are the third most widely planted Christmas trees in the PNW. Most of the Nordmann and Turkish fir are being planted in areas where true firs, such as noble fir, can't be grown because of Phytophthora root rot. About three years ago an unidentified adelgid was detected on Nordmann and Turkish fir trees in regional genetic trials located at WSU Puyallup. Large numbers of crawlers attack the foliage, which leads to discoloration and severe distortion of the needles on infested shoots. In Europe where Nordmann fir is widely grown for Christmas trees, the silver fir woolly adelgid [Adelges (Dreyfusia) nordmannianae] is a serious pest on this host.

Although this adelgid is not known to occur in the PNW, the damage to trees in Puyallup is very similar to what has been reported for this adelgid in Europe.

A series of trials were initiated in 2013 to obtain information on the distribution of this adelgid, its rate of spread, its life cycle in western Washington, the susceptibility of different North American and European species of true firs to this pest, and the effectiveness of insecticide applications in controlling this pest.

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

Douglas-fir are 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 fungi in 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 from F. commune. As a result, many growers fumigate unnecessarily or 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 (qPCR) TaqMan primers and probes have been designed for both F. commune and F. oxysporum to be run in a single, multiplex assay. This method of pathogen quantification is being validated using inoculated soil from greenhouse and field pathogenicity threshold tests, by comparing the results from the qPCR assay to traditional soil dilution plating (Figure 1). Using these methods, we hope to develop a comprehensive assay to help interested growers make informed decisions about soil health and chemical use.

Figure 1. Correlation between observed qPCR Ct value and average inoculum concentration (CFU/g) of soil inoculated with *Fusarium commune* and *Fusarium oxysporum*.

# Sudden Oak Death http://www.puyallup.wsu.edu/ppo/sod/

# Mitigation of soil and water contaminated with Phytophthora ramorum

# **Biofilters**

Results of laboratory baiting tests have shown that several materials harbor biocontrol organisms that are effective in reducing inoculum of *Phytophthora* spp., in aqueous suspensions. Suspensions with low pH (4.5-6.0) were the most effective. Of the *Phytophthora* spp. tested, *P. ramorum* had the best survival rate in all suspensions. This suggests that *P. ramorum* is more adapted to the aquatic environment than other common nursery *Phytophthoras* used in the tests. Based on these results, several materials were chosen for further testing. These include peat moss, composted bark, and composted yard/food waste.

We are partnering with a local biotech company, Fungi Perfecti LLC, Shelton, WA, to construct biofilters using materials colonized by suitable strains of their basidiomycete fungi. Challenge tests are underway with isolates of basidiomycetes and *Trichoderma spp.* vs. *Phytophthora* 

*ramorum* and surrogate species. The results of some of the in-vitro challenge tests and baiting of colonized substrates indicate that the common white-rot fungus *Irpex lacteus* has potential to control *P. ramorum* in a biofilter. The interactions of suppressive *Trichoderma* spp. with these fungi are being investigated for possible synergistic effects.

Column tests with the most effective mulch materials and mulches amended with *Trichoderma* spp., basidiomycete fungi, and bacteria that are effective biocontrol agents identified in the dual culture experiments are planned. The most suppressive combination of biocontrol agents and substrates will be selected for use in biofilters.

A pilot study of a slow sand filter and a biofilter using organic media is being constructed at a local nursery that has *P. ramorum*-positive pond water. Presently the organic media will be peat, but results of column tests will suggest other possibilities that may be even more effective at removing *Phytophthora* inoculum. These materials will also be used to treat runoff from the nursery.

# <u>Steam</u>

Experimentally-verified steaming methods will be employed in trials to eradicate *Phytophthora* from field soils with a focus on *P. ramorum*-infested field soils in affected Washington nurseries. These studies and parallel studies in California and Oregon will result in a collection of data on 1) steaming efficacy, 2) the long-term efficacy of post-steam soil treatments, and 3) field soil types and longitudinal soil climatic data of the major West Coast growing regions. These data will facilitate the development of scientifically-based steaming standards for field soils in nurseries and will further contribute to the development of soil mitigation measures for other pests and pathogens of quarantine significance.

# Examining fungicide resistance among lineages in Phytophthora ramorum

In 2012 we completed screening of 141 isolates of *P. ramorum* collected from 13 nurseries in Washington, one in Oregon, and one Christmas tree farm in California. NA1 isolates from Oregon and California forest hosts were also included. 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).

Seventeen out of the 141isolates screened (12%) 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 from all lineages was selected for further study. EC50 and EC90 values for these isolates were calculated. These isolates were used in studies of temperature growth rates and sporulation potential in the second year of the project. Phenotypic data (pathogenicity, sporulation, fungicide resistance, temperature growth rates) will be examined to determine whether there are trade-offs between fitness and fungicide resistance and whether there are phenotypic differences among the three main clonal lineages. Testing of fungicide-resistant isolates identified in the laboratory will be carried out on rhododendrons treated with fungicide, to determine whether these isolates can infect plants under field conditions.

# Phytophthora ramorum projects at the National Ornamental Research Site at Dominican

# University California (NORS-DUC)

We currently are conducting the following projects at NORS-DUC:

- Determination of *Phytophthora ramorum* threshold inoculum levels in irrigation water needed for infection of nursery hosts.
- Testing biological control agents for suppression of *Phytophthora ramorum* in potting mixes in a simulated nursery environment.

These projects have been completed:

- 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*.

# **Quantifying** *Phytophthora ramorum* **inoculum in water** (Lucy Rollins, WSU Plant Pathology Graduate Student)

*Phytophthora ramorum*, a fungal-like pathogen that causes the disease sudden oak death (SOD), has caused substantial mortality in tanoak (*Litocarpus densiflorus*) in some West Coast forests. In nursery settings, the pathogen causes leaf blight and has led to the mandatory destruction of host plants such as *Rhododendron*, *Camellia* and *Viburnum* spp. Nationally, several streams and

rivers near positive nurseries have tested positive for *P. ramorum* creating concern that the pathogen may spread from infested waterways to farms, nurseries and other entities that use stream or river water for irrigation. Currently, the level of inoculum in streams where water is used for irrigation and the inoculum threshold required for disease onset in plants irrigated with infested stream water are both unknown. My research project involves simultaneously testing several methods currently used to determine *P. ramorum* presence and concentration in water in order to correlate the number of zoospores each method can detect to the number of zoospores in a serial dilution. This information will help identify the inoculum levels in infested streams and rivers and assist scientists and regulatory agencies in assessing the risk of using *P. ramorum* infested water for irrigation.

I have run two simultaneous tests in the lab to compare the sensitivity of direct plating of zoospores, filtration, baits (D'anjou pear and *Rhododendron x 'Nova Zembla'* leaves and leaf disks) and quantitative polymerase chain reaction (qPCR) in detecting *P. ramorum* zoospores in water. There were nine targeted zoospore concentrations ranging from 0, 25, 50, 100, 250, 500, 1,000, 10,000 and 100,000 zoospores per liter of deionized water. I found that direct plating and filtration colony forming units (CFUs) represented between 10-30% of the zoospores counted by hemacytometer. Results showed that all bait types had 100% infection at the 10,000 and 100,000 target zoospore concentrations (25-50 zoospores/L). Wounded leaf baits in containers by themselves appeared to be more sensitive at detecting P. *ramorum* zoospores than pear baits or when leaf disks and wounded leaf baits were combined in the same container. The qPCR process has been able to detect zoospore DNA for all target concentrations and there is a highly significant correlation between cycle threshold values and zoospore concentrations. The above tests will be performed using environmental water samples in the near future.

# Risk of *Phytophthora ramorum* to North American larch

The recent determination that *Phytophthora ramorum* is causing bleeding stem cankers on Japanese larch (*Larix kaempferi*) in the UK and that inoculum from this host appears to have resulted in disease and canker development on several other conifers has raised questions about the potential risk *P. ramorum* poses to larch in North America. To examine this risk, a series of experiments were conducted to determine the susceptibility of western (*L. occidentalis*) and eastern (*L. laricina*) larch to *P. ramorum*. European (*L. decidua*) and Japanese larch were included for comparison. Container-grown plants were used in all experiments. Plants of each species were inoculated with a single isolate of three genotypes (NA1, NA2, and EU1) of *P. ramorum* by spraying the foliage with a zoospore suspension  $(10^5/ml)$ . Plants sprayed with water alone served as the control. Plants were encased in individual plastic bags for 5-6 days and placed in a biocontainment unit maintained at 19-20C with 24 hour light. After bag removal, plants were monitored for up to 8 weeks and isolations were done to confirm the presence of *P. ramorum*. Due to the discovery in the UK that *P. ramorum*-infected Japanese larch needles can sporulate heavily in the fall, we conducted inoculation trials in both the spring when new growth was emerging and in early fall.

Symptoms observed on the spring-inoculated plants initially appeared on individual needles or needle bundles, sometimes leading to shoot dieback that extended into the previous year's stems. Plants were killed when lesions girdled the stems. In the 2011 spring-inoculation trial, the number of infected plants varied by isolate of the pathogen and host. Western larch tended to have the largest lesions. Although the number of plants that became infected varied by isolate, there was little difference in average size of the lesions that developed. In 2012, there was no significant difference among the three isolates of *P. ramorum* with respect to the overall percentage of symptomatic bundles, number of lesions per seedling, lesion size, and mortality on the spring-inoculated larch species. The data from the spring-inoculated trials confirmed that western, eastern, European, and Japanese larch were susceptible to *P. ramorum*. Western larch tended to have the highest percentage of symptoms of all types and eastern larch had the lowest. Limited symptoms developed on the fall-inoculated plants and isolations from the few symptomatic needles resulted in only a single positive recovery of *P. ramorum* from one Japanese larch seedling inoculated with the NA2 isolate.

Results from these trials indicate that all of the larch species tested exhibited some level of susceptibility to *P. ramorum*. The highest recovery and symptom severity of *P. ramorum* occurred on western larch. Also, the NA1 and NA2 genotypes appear to have as much potential as the EU1 genotype to cause disease on newly-emerging growth. Virtually no infection occurred on fall-inoculated larch. Ongoing sporulation studies indicate that western larch supports very high levels of sporulation of *P. ramorum* on foliage.

## **Ornamental Bulb Crops**

**Identification of** *Botrytis* **spp. causing gray mold on peonies in Alaska and Washington** (In collaboration with Patricia S. Holloway, Georgeson Botanical Garden, University of Alaska Fairbanks)

Gray mold, caused by *Botrytis paeoniae* or *B. cinerea*, is the most important disease of peonies. This fungal disease can occur as a blight of emerging shoots and a leaf spot or blight on the foliage throughout the growing season. It can also be a major issue during the postharvest storage and shipment of cut flowers. To improve the management of *Botrytis* on peonies, a collaborative research project involving Alaska and Washington was initiated in 2012. We are working to:

• Determine the identity of specific *Botrytis* species causing gray mold on peonies in

Alaska and Washington.

- Determine if there are shifts in the prevalence of *B. paeoniae*, *B. cinerea* and other species during the growing season.
- Determine the persistence of primary inoculum and identify conditions that favor initial infection and secondary spread of the disease.
- Determine if *Botrytis* populations in grower fields are resistant to the fungicides currently being used to control this disease.
- Obtain efficacy data for newer fungicides and biocontrol agents to control gray mold during the growing season and the postharvest storage of cut flowers.

## Summary of Initial 2012 Studies

To identify *Botrytis* species in production fields, isolates of *Botrytis* were obtained from samples of diseased tissue from eight fields in Alaska and three fields in Washington near the end of the growing season. DNA was extracted from each isolate and used for identification purposes. The G3PDH region of the DNA was amplified and sequenced. The sequence data were then compared to the global GenBank database of DNA sequences of *Botrytis* species utilizing a MegaBlast search technique. This region of DNA was not specific enough to differentiate *Botrytis* species.

The G3PDH sequences were used to produce a genetic tree that shows how closely the *Botrytis* isolates from each farm and state are related to each other or to previously described *Botrytis* species (Figure 2). Based on these tests, *B. cinerea* was isolated more frequently from the samples than *B. paeoniae* in both Alaska and Washington. The molecular data also suggest that there is considerable variation in isolates compared to known isolates of *B. paeoniae* and *B. cinerea* that are in GenBank and that other *Botrytis* species may be present in some fields.

#### Ongoing activities

A survey of diseases and samples were collected from 25 farms in Alaska during 2013. We are currently sequencing isolates to obtain a clearer picture of the importance of *B. paeoniae*, *B. cinerea*, and other *Botrytis* spp. in the development of gray mold.

#### Efficacy of fungicides in controlling foliar diseases on peonies

An IR-4 trial targeting Botrytis leaf blight was conducted during 2013. A total of 16 fungicides were included in this trial (Table 1). This field trial was located at a commercial grower's field in the Puyallup River Valley near Sumner, WA. The planting was a 4-year-old mixed peony planting with field rows spaced 5' apart and 850' long. Plants were spaced on 2' centers. This trial consisted of 17 treatments with 5 replications. Each replication was 15' long and contained 4 to 5 plants or crowns per planting cell. Weeds were initially controlled with a spot application of a 2% Ranger Pro solution on March 19, 2013 using a Solo backpack sprayer equipped with a 8002 Tee-Jet nozzle. Subsequent weed management was done by hand cultivating or with a tractor. The first fungicide application was made on March 22, 2013 and the last application was made on June 17, 2013. Fungicides were applied with a CO<sub>2</sub> sprayer at 15 psi in 100 gallons of water. Sprayer was equipped an 8002 Tee-Jet nozzle. Plants were sprayed to the point of drip. A non-treated check was included in each block and disease development was the result of naturally occurring inoculum in the field. On average each plant received 50 mls of fungicide solution per application.

Initial emergence data was taken on March 21, 2013 just prior to first fungicide application. On July 2, 2103 data were collected on *Cladosporium* red spot, irregular shaped Botrytis leaf lesions and Botrytis shoot blight, and visible fungicide residue. Isolations from symptomatic plant tissue were collected throughout the trial. Although *Botrytis paeoniae* and *Botrytis cineria* were associated with irregular shaped leaf lesions and a shoot blight/dieback on plants during this trial, insufficient *Botrytis* developed to evaluate the control of this pathogen (Table 2). However, we were able to evaluate the effectiveness of the fungicide treatments in controlling red spot or blotch (Figure 3), which is caused by *Cladosporium paeoniae*. The ANOVA analysis of data in this test indicate that there was a significant (P<0.05) treatment effect on the severity of red spot that developed on leaves. Average treatment severity ranged from 0.2 to 5.5. All of the treatments, except Medallion, Prestop, Chipco 26019, SP2770 and Proud 3 reduced the severity of diseases compared to the non-treated check (Table 2). Torque, Pageant, Disarm, Daconil WeatherStik, Kocide, Trinity, Palladium, and SP2773 were the most effective materials tested. Kocide and Daconil Weather Stik were the only products that results in visible residues on the plants (Table 2).

#### Figure 3. Red blotch caused by *Cladosporium paeoniae*

PRODUCT/CODE	COMMON NAME	MANUFACTURER
Pageant	pyraclostrobin (12.8). boscalid (25.2)	BASF
Chipco 26019 N/G	iprodione	OHP
Decree 50 WDG	fenhexamid	Arysta
Disarm 480SC	fluoxastrobin	OHP
F9110	extract of Lupinus	FMC
Palladium 62.5 WG	cyprodinil & fludioxinil	Syngenta
Torque 3.6 SC	tebuconazole	Cleary Chemicals
Medallion 50 WDG	fludioxonil	Syngenta
Trinity 2 SC	triticonazole	BASF
Prestop	Gliocladium catenulatum	AgBio
Proud 3	thyme oil	BioHumanetrics
SP2770 10WP	proprietary	SePRO
SP2773	proprietary	SePRO
V-10135	proprietary	Valent
Daconil WeatherStik	chlorothalonil	Syngenta
Kocide DF	copper hydroxide	Griffin Corp

	Table 1. Product(s)	applied during	experiment (	(including	treatments.	, fertilizers,	etc):
--	---------------------	----------------	--------------	------------	-------------	----------------	-------

Table 2. Effect of foliar fungicides on severity of Red Spot, Botrytis leaf lesions and shoot blight, and fungicide residues on a mixed variety planting of peonies on July 2, 2013<sup>1</sup>.

				Severity Ratings <sup>2</sup> Botrytis		
				Leaf	Shoot	Fungicide
TRT	TRT/PRODUCT	Rate/100 gal	Red Spots	Lesions	Blight	Residue <sup>3</sup>
7	Torque 3.6 SC	8 oz	0.2 d	0.4 a	0.2 b	0.0 c
10	Pageant 38WG	14 oz	0.2 d	0.7 a	0.5 ab	0.0 c
9	Disarm 480SC	0.08g + 1oz	1.2 cd	0.3 a	0.2 b	0.0 c
16	Daconil WeatherStik	1.4 pints	1.4 cd	0.4 a	0.7 ab	2.3 b
17	Kocide DF	1 lb-	1.6 cd	0.1 a	0.3 ab	3.0 a
11	Trinity 2SC	12 oz	2.2 cd	0.2 a	0.2 ab	0.0 c

3	Palladium 62.5 WG	6 oz	2.3 bc	d 0.2 a	0.2	b	0.0 c
6	SP2773	1.66 lbs	2.4 bc	d 0.5 a	0.1	b	0.0 c
2	F9110	24 oz	2.6 bc	0.6 a	0.1	b	0.0 c
13	Decree	1.5 lbs	2.6 bc	0.5 a	0.2	b	0.0 c
8	V10135 SC	16 oz	2.8 bc	0.0 a	0.2	b	0.0 c
14	Medallion 50 WDG	8 oz	3.3 ab	c 0.1 a	0.3	ab	0.0 c
15	Prestop	0.50%	3.3 ab	c 0.1 a	1.0	а	0.0 c
12	Chipco 26019 N/G	16 oz	3.5 ab	c 0.0 a	0.4	ab	0.0 c
5	SP2770 10WP	2.66 lbs	4.6 ab	0.4 a	0.3	ab	0.0 c
4	Proud 3	4 quarts	5.2 a	0.7 a	0.8	ab	0.0 c
1	Non-sprayed check	-	5.5 a	0.0 a	0.4	ab	0.0 c

<sup>1</sup>Numbers in each column followed by the same letter are not significantly different, P=0.05, Tukey's Studentized Range Test.

<sup>2</sup>Rated on a scale of (0-10) where: 0 = none, 1 = 1-10%, 2 = 11-20%,...10 = 91-100% foliage exhibiting symptoms of red leaf spot or Botrytis leaf lesions. Botrytis shoot blight was rated on a 0-10 scale: where: 0 = none, 1 = 1-10%, 2 = 11-20%,...10 = 91-100% of the shoots on the plants exhibited symptoms of shoot dieback.

<sup>3</sup>Fungicide residue rating (0-3) where 0 = none, 1 = slight, 2 = moderate, 3 = severe fungicide residue.

# The effectiveness of reduced risk and new biocontrol products in controlling *Botrytis* on lilies

Fungicides are an important tool used in the management of *Botrytis elliptica* on lilies. During 2012 and 2013, trials were conducted to determine the effectiveness of several reduced-risk and biocontrol fungicides in controlling Botrytis on field-grown 'London' Asiatic hybrid lilies. In 2012, treatments included foliar applications of Chipco 26019 (iprodione), Daconil Weather Stik SC (chlorothalonil), Disarm 480 SC (fluoxastrobin), Medallion 50WP (fludioxonil), Palladium 62.5 WG (cyprodinil + fludioxonil), Pageant 38 WG (pyraclostrobin), Proud 3 (thyme oil), Regalia (Extract of Reynoutria sachalinensis), Trinity 2SC (triticonazole), Torque 3.6SC (tebuconazole), Tourney 50 WDG (metconazole), V10135 SC (fenpyrazamine), and ZeroTol (hydrogen dioxide) along with a non-treated control. Treatments were applied on a 7, 10, or 14 day intervals. Daconil Weather Stik SC, Disarm 480 SC, Medallion 50WP, Palladium 62.5 WG, Pageant 38 WG, and Proud 3 with a non-treated control were included in the 2013 trial. All of these treatments were applied on a 14 day interval. Treatments were applied with a CO<sub>2</sub> sprayer equipped with an F110/0.4/3 Braber (2013) or 8002 LP TEE-Jet (2012) nozzle at 1.1 kg/cm<sup>2</sup> in the equivalent of 935.4 liters of water/ha. Applications were made throughout the growing season and the experimental design was a randomized complete block with each treatment applied to a 0.9 m long section of row in each of five blocks. Limited disease developed in 2012. Disease incidence was rated on a 0 to 10 scale, where 0 = none and 10 = 91 to 100% of the plants with some disease. Final disease incidence data taken on September 19, 2012 indicated that only the plants sprayed with Palladium and Proud 3 had significantly lower disease incidence than the checks. Disease pressure was very high in 2013 and disease severity was rated on a scale of 0 to 10 scale, where 0 = none and 10 = 91-100% of the foliage killed by *Botrytis*. All of the fungicides, except Disarm and Proud significantly reduced disease severity levels. Plants treated with Pageant or Daconil Weather Stik had the lowest levels of damage.

# Identification of viruses in small-farm lily and dahlia cut flower crops in western Washington (In cooperation with Hanu R. Pappu)

Virus surveys were conducted at 27 grower sites in 2011-2012 to identify viruses present at lily and dahlia farms that mainly supply cut flowers to local markets. Samples were collected from a total of 259 lily plants and 194 dahlia plants. Samples were tested individually by ELISA (lily and dahlia) and PCR (dahlia) for viruses. Approximately one third (36.3%) of the lily plants sampled were positive for a least one virus. Lily symptomless virus (LSV) was the most

prevalent (32.4%) virus detected. The incidence of a potyvirus (7.3%) and Cucumber mosaic virus (CMV) (3.5%) were much lower. No Impatiens necrotic spot virus (INSV), Tomato aspermy virus (TAV), Tobacco ringspot virus (TRSV), or Tomato spotted wilt virus (TSWV) was detected in any of the lily samples. The overall prevalence of viruses in dahlias was much higher (80.9%). The most common virus was one or more of the caulimoviruses associated with dahlia mosaic: the endogenous plant para-retroviral sequence DvEPRS was the most prevalent (65.6%), followed by Dahlia common mosaic virus (33.5%) and Dahlia mosaic virus (11.9%). TSWV and TSV were each detected in 22.7% and 19.6% of the samples, respectively. All of the dahlia samples were negative for CMV, INSV, and potyviruses. These results suggest that viruses, particularly in dahlias are a widespread problem.

## **National Elm Trial**

#### Ice damage to elms at WSU-Puyallup from January 2012 storm

An ice storm during January 2012 in western Washington provided a unique opportunity to examine the severity of ice damage to the elm cultivars in this trial. The period of 14-19 January 2012 featured some heavy snowfall and freezing rain in the lowlands of western Washington. The freezing rain was especially severe south of the Seattle metro area. The heavy ice load caused breakage of tree branches and widespread power outages were the result. Elms in the trial planting at WSU-Puyallup were surveyed for damage shortly after the storm. A rating scale where 0 = no breakage, 1 = < 1%, 2 = 1-5%, 3 = 5-25%, and 4 = >25% of canopy with broken branches was used on each tree (Figure 4). Percent of trees in each damage category varied among elm cultivars. Cultivars that had no damage on any of the trees were 'Frontier' (*U. carpinifolia x U. parvifolia*) and 'New Horizon' (*U. pumila x U. japonica*). Most of the cultivars had between 0 = 5% breakage. The most heavily damaged was 'Pioneer' (*U. glabra x U. carpinifolia*) (Table 3). Based on this information, some of the elm cultivars included in this trial may not be adapted to areas where ice storms occur.

Table 3. Percent of trees in each ice damage category for 17 elm cultivars planted at WSU uyallup.

2012				Ice damage category, % of trees				
WSU#	Cultivar	Parentage	No breakage	< 1% of	1 - 5%	5 - 25%	> 25%	
				canopy				
1.	'Clone D'	U. propinqua	60	40	0	0	0	
2.	'Emer II' Allee	U. parvifolia	80	0	20	0	0	
3.	'Frontier'	U. carpinifolia x U. parvifolia	100	0	0	0	0	
4.	'Homestead'	U. glabra x U. carpinifolia x U. pumila	25	0	25	0	50	
5.	'Morton Glossy' Triumph	U. pumila x U. japonica x U. wilsoniana	60	0	40	0	0	
6.	'Morton Plainsman' Vanguard	U. pumila x U. japonica	20	20	20	0	40	
7.	'Morton Red Tip' Danada Charm	U. japonica x U. wilsoniana	20	0	20	20	40	
8.	'Morton Stalwart' Commendation	U. carpinifolia x U. pumila x U. wilsoniana	80	0	0	0	20	
9.	'Morton' Accolade	U. japonica x U. wilsoniana	80	0	0	0	20	
10.	'New Horizon'	U. pumila x U. japonica	100	0	0	0	0	
11.	'Patriot'	(U. glabra x U. carpinifolia x U. pumila) x U. wilsoniana	60	20	20	0	0	
12.	'Pioneer'	U. glabra x U. carpinifolia	0	0	0	25	75	
13.	'Prospector'	U. wilsoniana	50	50	0	0	0	
14.	'Valley Forge'	U. americana	20	20	0	0	60	
15.	'Princeton'		60	20	20	0	0	
16.	'New Harmony'		40	40	20	0	0	
17.	'Prairie Expedition'		80	0	0	0	20	

## **Publications, Presentations, Workshops and Field Tours**

## **2013 Publications**

## Journal:

• Elliott, M., G. Chastagner1, K. Coats, A. DeBauw, K. Riley, and J. Griesbach. Variation in susceptibility of Rhododendron species and cultivars to foliar infection by *Phytophthora ramorum*. Plant Disease (In review)

• Hummell, R. L., M. Elliott, G. Chastagner, R. E. Riley, K. Riley, and A. DeBauw. 2013. Influence of nitrogen fertility on the susceptibility of rhododendrons to *Phytophthora ramorum*. HortScience 48:601-607.

## **Extension publications:**

• Mulvey, R. L., D. C. Shaw, G. M. Filip and G. A Chastagner. 2013. Swiss Needle Cast. Forest Insect and Disease Leaflet 181. USDA Forest Service. 16 p.

## **Proceedings:**

• Chastagner, Gary. A., Katie Coats, and Marianne Elliott. 2013. An Overview of *Phytophthora ramorum* in Washington State. p. 14-15. In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M.; Alexander, J.M. tech. coords. 2013. Proceedings of the Sudden Oak Death Fifth Science Symposium. Gen. Tech. Rep. PSW-GTR-243. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 169 p.

• Elliott, Marianne, Gary Chastagner, Katie Coats, and Gil Dermott. 2013. Determining the Risk of *Phytophthora ramorum* Spread From Nurseries Via Waterways. p. 55-59. In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M.; Alexander, J.M. tech. coords. 2013. Proceedings of the Sudden Oak Death Fifth Science Symposium. Gen. Tech. Rep. PSW-GTR-243. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 169 p.

• Chastagner, Gary, Kathy Riley, and Marianne Elliott. 2013. Susceptibility of Larch, Hemlock, Sitka Spruce, and Douglas-fir to *Phytophthora ramorum*. p 77-79. In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M.; Alexander, J.M. tech. coords. 2013. Proceedings of the Sudden Oak Death Fifth Science Symposium. Gen. Tech. Rep. PSW-GTR-243. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 169 p.

• Elliott, Marianne, Gary Chastagner, Simon Shamoun, Grace Sumampong, Ellen Goheen, and Alan Kanaskie. 2013. Biological Control of Tanoak and Bay Laurel Resprouts Using the Fungus, *Chondrostereum purpureum*. p134-136. In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M.; Alexander, J.M. tech. coords. 2013. Proceedings of the Sudden Oak Death Fifth Science Symposium. Gen. Tech. Rep. PSW-GTR-243. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 169 p.

• Tjosvold, Steve, David Chambers, Gary Chastagner, and Marianne Elliott. 2013. Effect of Fungicides and Biocontrol Agents on Inoculum Production and Persistence of *Phytophthora ramorum* on Nursery Hosts. p 136. In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M.; Alexander, J.M. tech. coords. 2013. Proceedings of the Sudden Oak Death Fifth Science Symposium. Gen. Tech. Rep. PSW-GTR-243. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 169 p. • Chastagner, G. 2013. Challenges associated with the spread of *Phytophthora ramorum* in water from nurseries. P.4-5. In: McManus, K. A., and K. W. Gottschalk. 2013. Proceedings, 23rd U.S. Department of Agriculture interagency research forum on invasive species 2012. Gen. Tech. Rep. NRS-P-114. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 126 p.

• Elliott, M., Chastagner, G.A., Coats, K.P., DeBauw, A., & Riley, K. 2013. Volunteer stream monitoring for invasive *Phytophthora* species in Western Washington. P. 8. In: McManus, K. A., and K. W. Gottschalk. 2013. Proceedings, 23rd U.S. Department of Agriculture interagency research forum on invasive species 2012. Gen. Tech. Rep. NRS-P-114. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 126 p.

• Elliott, M., G. Chastagner, and S. Shamoun. (In Press). An overview of Sudden Oak Death (SOD) disease caused by *Phytophthora ramorum*: Research results and challenges for the Pacific Northwest of North America. International Symposium on Oak Forest Preservation. August 27-29, 2012, Korean Forest Research Institute, Seoul, Korea

## **Book Chapters:**

• Copes, W. E., B. Barbeau, and G. A Chastagner. In Press. Chlorination – Chlorine Dioxide. In: Hong, C. and G. Moorman (Eds.). Waterborne Plant Pathogens: Biology, Detection and Management, APS Press. (In Press)

## Abstracts:

• Chastagner, G. A., and K. Riley. 2012. Effectiveness of a Swiss needle cast disease management program in controlling disease development in U.S. Pacific Northwest Douglas-fir Christmas tree plantations. Phytopathology 102 (Suppl. 6): S6.8.

• Dungan, F. M., S. L. Lupien, C. M. Vahling-Armstrong, G. A. Chastagner, B. K. Schroeder. 2013. Host range of *Penicillium* spp. (blue mold) rotting bulb crops. Phytopathology 103 (Suppl. 2): S3.

• Chastagner, G. A., K. P. Coats, A. DeBauw, H. R. Pappu. 2013. Identification of viruses in small-farm lily and dahlia cut flower crops in western Washington. Phytopathology

103 (Suppl. 3):S3.12.

• Talgø, V., G. Chastagner, A. Dobson, A. Stensvand, and I. M. Thomsen. 2013. Use of shade netting strongly reduces current season needle necrosis (CSNN). IUFRO International Christmas Tree Research Conference. Truro, Nova Scotia, August 10-15, 2013.

• Chastagner, G., A. McReynolds, and K. Riley. 2013. Mid-rotation growth and postharvest needle retention characteristics of balsam fir grown in western Washington. IUFRO International Christmas Tree Research Conference. Truro, Nova Scotia, August 10-15, 2013.

## **Technical reports:**

- Gary Chastagner, Katie Coats and Annie DeBauw. 2013. Disease Management Update. 2013 Bulb/Flower Grower Meeting, Wilbur-Ellis University, January 22, 2013, Auburn, WA. 8 p.
  - Gary A Chastagner, Annie DeBauw, and Katie Coats. 2013. Management of diseases on ornamental bulbs and cut flowers. Annual NARF Research Report. 10 p.

• Gary A Chastagner, Annie DeBauw, and Katie Coats. 2013. Management of virus and soilborne fungal diseases on cut flower bulb crops. WSDA Specialty Crop Block Grant Program Annual Performance Report (October 1, 2011 – September 30, 2012). 17 p.

• Gary Chastagner and Kathy Riley. 2013. Developing disease resistant sources of Nordmann and Turkish fir Christmas trees to sustain future markets. WSDA Specialty Crop Block Grant Program Annual Performance Report (October 1, 2011 – September

30, 2012). 13p.

## Industry

• Chastagner, G. 2013. Recent Christmas Tree Research Grants Aim to Solve Major Problems for Industry. Lookout 46(1): 25-26.

• Landgren, C. and G. Chastagner. 2013. An update on Turkish/Trojan fir collaborative trial. Lookout 46(1): 27-28.

## Web sites

- Elliott, M., Chastagner, G.A., & Dart, N.L. (2013). WSU Sudden Oak Death website. http://www.puyallup.wsu.edu/ppo/sod/.
- Elliott, M., Chastagner, G.A., Hamlin, J., & Sniezko, R. (2013). Pacific Madrone Research website. http://www.puyallup.wsu.edu/ppo/madrone/.

## 2013 Presentations

• Emerging adelgid problem on Nordmann fir, Swiss needle cast, and Phytophthora root rot. Wilbur-Ellis University, Auburn, WA. January 22, 2013.

• Management of diseases on ornamental bulb and cut flower crops. Wilbur-Ellis University, Auburn, WA. January 22, 2013.

• Update of virus surveys on lilies and dahlias at cut flower farms in western Washington. Wilbur-Ellis University, Auburn, WA. January 22, 2013.

• WSU projects at NORS-DUC. NORS-DUC Steering Committee Meeting. San Rafael, CA, March 11, 2013.

- Tree keepability: Improving the postharvest quality & safety of cut trees.
- Inland Empire Christmas Tree Assn. Spring Meeting. Spokane, WA. April 6, 2013.

• Overview of new research projects: Nordmann, Turkish and Trojan firs; adelgid; needle necrosis; needle retention and Phytophthora root rot; and more. Inland Empire Christmas Tree Assn. Spring Meeting. Spokane, WA. April 6, 2013.

• New tools to help manage diseases on ornamental bulb crops. Bulb Grower Field Day. Puyallup, WA. May 15, 2013

• Risk Phytophthora ramorum poses to North American larch. IUFRO Shoot and Foliage Disease Conference. Brno, Czech Republic. May 20-25, 2013.

• Identification of viruses in small-farm lily and dahlia cut flower crops in western Washington. Joint APS Pacific and Caribbean Division Meeting. Tucson, AZ. June 17-19, 2013.

• An emerging adelgid problem on Nordmann and Turkish firs and new needle retention and Phytophthora root rot research. PNWCTA Summer Meeting. Portland, OR. June 21, 2013.

- Diagnosis and management of needle diseases of Christmas trees. PNWCTA Summer Meeting. Portland, OR. June 21-22, 2013.
- Disease diagnosis and management. Alaska Peony Grower Field Tour. Fairbanks, AK. July 15, 2013.
- Disease diagnosis and management. Alaska Peony Grower Field Tour. Soldona, July20, 2013.
- Disease diagnosis and management. Alaska Peony Grower Field Tour. Homer, AK. July 22, 2013.
- Update on postharvest needle retention research. SCRI Christmas Tree Genomics Project meeting. St. Louis, MO. July 30, 2013.
- Observations on the inspection of Christmas trees in Hawaii. IUFRO International Christmas Tree Research Conference. Truro, Nova Scotia, August 10-15, 2013.

• An emerging adelgid pest on Nordmann fir Christmas trees in western Washington. IUFRO International Christmas Tree Research Conference. Truro, Nova Scotia, August 10-15, 2013.

• Nordmann and Turkish fir: Phytophthora root rot, CSNN, bud break, and postharvest needle retention evaluations. IUFRO International Christmas Tree Research Conference. Truro, Nova Scotia, August 10-15, 2013.

- Relationship of inoculum levels to the development of gray bulb rot on tulips and iris.
- 5th International Symposium on Rhizoctonia. Zhengzhou, Henan, China, August 22-24, 2013.
- Relationship of inoculum levels to the development of gray bulb rot on tulips and iris.
- 10th International Congress of Plant Pathology (ICPP 2013). Beijing, China. August 25-30, 2013.

• The U. S. Christmas Tree industry. WSU CB Marketing 368 ~Marketing Research~ class. Pullman, WA. September 16, 2013.

## 2013 Workshops/Field Days/Tours Organized or Co-organized

- Bulb and Cut Flower Section of the 2013 Wilbur-Ellis University, January 22, 2013. Auburn, WA.
- WSU Bulb Growers Field Day, May 15, 2013, WSU Puyallup. Puyallup, WA
- Integrated Pest Management for Christmas Trees. OSU North Willamette Research and Experiment Station. Aurora, OR
- PMWCTA Summer Meeting Farm Tour. La Center, WA. June 22, 2013.
- Alaska Peony Grower Field Tour. Fairbanks, AK. July 15, 2013.
- Alaska Peony Grower Field Tour. Soldotna, AK. July 20, 2013.
- Alaska Peony Grower Field Tour. Homer, AK. July 22, 2013.

## 2013 State Report for Pennsylvania NCR-193: IPM Strategies for Arthropod Pests and Diseases in Nurseries and Landscapes

Gregory A. Hoover Department of Entomology Pennsylvania State University 543 ASI Building University Park, PA 12/3/13

A total of 35 invited lectures were given in three states; approximately 2,800 green industry stakeholders were in attendance at these lectures -- who knows the additive effect of these presentations on client groups. The total time spent lecturing to these green industry client groups was approximately 82.0 hours. Preparation and travel time is not included in this lecture time.

### Update on Some Arthropod Pests of Woody Ornamental Plants in Pennsylvania

The armored scale insect called **"Japanese maple scale"**, *Lopholeucaspis japonica*, has been frequently submitted for diagnosis during the last several years. Most of these submissions are coming from southeastern and central Pennsylvania counties. I've seen this relatively new pest on the twigs and branches of Japanese maple, red maple, dogwood, Japanese zelkova, pyracantha, redbud, holly, crabapple, and Bradford pear. I believe this diaspidid produces one generation each year in Pennsylvania.

The impact of the armored scale insect known as the **elongate hemlock scale**, *Fiorinia externa*, continues to cause mortality of hemlock in both the landscapes and forests in eastern and central Pennsylvania. Soil applications of dinotefuran are performing well against this key pest of hemlock, true fir, and spruce.

The **emerald ash borer**, *Agrilus planipennis* is now confirmed in 47 of 67 counties in Pennsylvania. The state order of quarantine was lifted on April 15, 2011. Releases of natural enemies of this pest have been made in Allegheny, Union, Huntingdon, and Bedford Counties in Pennsylvania.

The **hemlock woolly adelgid**, *Adelges tsugae* is known from 56 of the 67 counties in Pennsylvania. Trees infested with this pest in landscapes and nurseries are being protected with soil applications of registered formulations of dinotefuran.

A relatively new pest called the **Japanese cedar longhorned beetle**, *Callidiellum rufipenne* has been detected both in ornamental nurseries and landscapes on arborvitae, cryptomeria, Chinese juniper, *Juniperus chinensis* (the cultivars 'Blue Hetz'; 'Sea Green'), eastern redcedar, *Juniperus virginiana*, Leyland cypress, *Cupressocyparis leylandii*, and Hinoki falsecypress in several southeastern and south central Pennsylvania counties. This species produces one generation each year. It overwinters as an adult in a gallery formed by the mature larva deep in the wood of the host plant. Treatment timing and products need to be evaluated for this pest.

The **viburnum leaf beetle**, *Pyrrhalta viburni* has moved very quickly from northern to southern counties in Pennsylvania near the Maryland border during the past five years. There is a need to identify additional active ingredients that are effective against the adult stage of this leaf beetle. Mortality of arrowwood, *Viburnum dentatum*, was observed by PA Game Commission biologists who are concerned about the mortality caused by this insect and the potential impact it may have on non-game as well as game bird populations.

## Short Courses and Inservice Training

Arborist Short Course: Integrated Management of Woody Ornamentals, Urban and Community Forestry Council, Pennsylvania State Cooperative, Montgomery County, Creamery, PA.

Arborist Short Course: Integrated Management of Woody Ornamentals, Urban and Community Forestry Council, Pennsylvania State Cooperative, Luzerne County, Forty Fort, PA.

### **Graduate Student Committees**

I've served on six graduate student committees during this period of time. One is a student in the Department of Entomology (Ph.D. candidate), a second is an (M. S. candidate) in the Department of Entomology. The third, fourth, fifth, and sixth graduate student committees are in the School of Forest Resources (three are M. S. candidates and the other a Ph.D. candidate).

## PUBLICATIONS

### **Article in Refereed Publication**

Rosa, C., L. MacCarthy, K. Duong, G. A. Hoover, and G. W. Moorman. 2013. First Report of the Spittlebug, *Lepyronia quadrangularis* and the Leafhopper *Latalus* sp. As Vectors of the Elm Yellows Associated Phytoplasma, *Candidatus Phytoplasma ulmi*. Plant Dis.: (in press)

## **Articles in Nonrefereed Publications**

Hoover, G. A. 2012. Pest Profile: Gypsy Moth, Tree Farmer, Guide to Sustaining America's Family Forests 31: 31.

Hoover, G. A. 2012. A Look at Some Trout Stream Insects in Pennsylvania, Pennsylvania Forests 103: 21-23.

Hoover, G. A. 2013. Japanese maple scale, Pennsylvania State Univ., Dept. Entomol., Entomol. Note, TS-40, 2 pp.

The pdfs of nine EAB publications continued to be used by ornamental horticulture extension educators, urban forester extension educators, and green industry professionals to educate their stakeholders regarding this wood-boring pest in Pennsylvania.

## SCHOLARSHIP

## **Grants Received**

2012 – USDA Forest Service, Urban/Community Forestry and Wildlife, \$300,000.00/yr. (with many collaborators at Pennsylvania State University).

## **OTHER ACTIVITIES**

#### **Emerald Ash Borer (EAB)**

Distributed the museum replicas of the adult and larval stages (20X the actual size) of the EAB and a 12" X 15" replica of ash bark with larval galleries and a D-shaped emergence hole to the ornamental horticulture county extension educator in Bucks County. I've received unsolicited feedback (USDA-Forest Service) on the effectiveness of these in educating stakeholders regarding the EAB in Pennsylvania.

The Pennsylvania Forest Pest Task Force (formerly the PA Emerald Ash Borer Task Force) completed the Action Plan for this exotic pest. More than 4,000 copies of the color, tri-fold publication titled, "What is the Emerald Ash Borer?" that targets the public and focuses on prevention of the movement of this pest in firewood were provided to many Penn State Extension offices. This color publication is also being distributed statewide by the Penn State, College of Agricultural Sciences, Publications Distribution Center. I also provide this publication at my ornamental entomology lectures and other entomology extension venues. The EAB is now found in 47 of the 67 Pennsylvania counties. I continued to assist with ground surveys for the EAB in eastern Pennsylvania.

I continued updating of the Web site on the EAB in Pennsylvania during the period from 2012-2013. This Web site contains images of the EAB and its symptoms and signs, native wood borers of ash, and EAB look alikes, US and Pennsylvania distribution maps, pdfs of publications on the EAB, and changes in the orders of quarantine are some examples of the sources of information on the EAB for stakeholders in Pennsylvania.

#### Website -- Ornamental Extension Entomology

The arthropod data regarding growing degree day and plant phenological information collected by the members of the Penn-Del IPM Research Group may soon be available as an App to be accessed at my Penn State Ornamental Extension Entomology web site for the green industry stakeholders in Pennsylvania as well as the US. Links at my ornamental entomology web site have been made to other helpful sites for green industry stakeholder groups. This web site also has Entomological Notes on woody ornamental arthropod pests with both line illustrations and color images

# **Development of an Integrated Pest Management Program for the American Elms at University Park Campus – Dutch Elm Disease / Elm Yellows**

Continued to conduct an IPM program designed to protect the health of almost 250 American elms that are 80-130 years old on the University Park Campus of the Pennsylvania State University. The switch to Astro Insecticide (permethrin) on the University Park Campus of Penn State has been a positive one. Protection of the health of the residual elms on main campus from Dutch elm disease continues to be realized. This pest management project is in cooperation with members from the Office of the Physical Plant (arborists). Monitoring and identification of both the native elm bark beetle and smaller European elm bark beetle populations was conducted by me for the past 22 years. This unique plant health care program continued to receive both state and national interest.

Mortality of elms caused by elm yellows (a phytoplasma vectored by leafhoppers) has declined continued around campus and in the Centre Region during this past year. More American elms were removed from campus including the east side, matching American elm at the Old Main Administration Building. As a member of the University Tree Commission, I'm involved in planning a new landscape design for this significant part of campus when the west side, matching American elm needs to be removed.

## **Testimony Before Committee in the United States House of Representatives**

Presented oral and written (10 pp.) testimony before the US House of Representatives Committee on Agriculture, Subcommittee on Conservation, Energy, and Forestry regarding the impact of invasive arthropod pests on managing and maintenance of forest health. I provided 150 copies of my written testimony for those in attendance (approx. 90) that was entered into the Congressional Record.

### **Media Contacts**

May 19, 2012 – Brood I of the Periodical Cicadas in southwestern Pennsylvania (Washington, Fayette, and Greene Counties), Observer-Reporter, Washington, PA.

July 3, 2012 – Why are Fireflies Abundant in 2012?, Tribune-Review, Pittsburgh, PA.

March 19, 2013 - Brood II of the Periodical Cicadas, WOR-TV, New York City, NY.

April 8, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, Scranton Times-Tribune, Scranton, PA.

April 9, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, The Daily Item, Somerset, PA.

April 15, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, WTAJ-TV, State College, PA.

April 15, 2013 – Brood II of the Periodical Cicadas, Cape May Magazine, Cape May, NJ.

April 15, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, York Daily Record, York, PA.

April 15, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, Lewistown Sentinel, Lewistown, PA.

April 17, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, The Patriot News, Harrisburg, PA.

May 6, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, Radio Pennsylvania, Harrisburg, PA.

May 8, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, Lancaster Farmer, Lancaster, PA.

May 8, 2013 – Methuselah of the Insect World to Emerge in Eastern Pennsylvania. The Periodical Cicada (Brood II), News Release, Pennsylvania State University, College of Agricultural Sciences, Agricultural Information Services, University Park, PA.

May 9, 2013 – Brood II of the Periodical Cicadas in Pennsylvania, Altoona Mirror, Altoona, PA.

May 24, 2013 – Brood II of the Periodical Cicadas in the Eastern US, Time Magazine, New York, NY.

May 25, 2013 - Brood II of the Periodical Cicadas in Pennsylvania, Butler Eagle, Butler, PA.

August 19, 2013 - Dog-Day Cicadas Emerge, 17-Year Cicadas Depart, Accuweather, accuweather.com

September 4, 2013 – Do Woolly Bear Caterpillars Predict the Weather in Pennsylvania?, Radio Pennsylvania, Harrisburg, PA.

## Biocontrol / Natural Enemies of Pests in Nurseries and Landscapes - a DVD

The video titled, *Insects and Spiders and Mites, OH My! : Recognizing Beneficials in the Nursery and Landscape* that received first place recognition in the education category awarded by the Broadcast Education Association is now available in DVD format from San Luis Video Publishing, Los Osos, CA for \$49.95. This 39-minute video covers the identification of natural enemies of key arthropod pests that feed on trees and shrubs. The video project was the result of a cooperative effort between Pennsylvania Department of Agriculture entomologists, a nursery inspector, the grounds manager at Longwood Gardens, Ag Information Services, and myself.

## SERVICE

## **Committee Assignments**

University Tree Commission, Pennsylvania State University, Member, 1992 - present.

Pennsylvania Task Force on Asian Longhorned Beetle, Member, 1998-present.

Pennsylvania Task Force on Emerald Ash Borer, Member, 2004-present.

Penn State University Integrated Pest Management Technical Committee, 2001-present. Consumer Horticulture Center, Penn State University, Department of Horticulture, member, 2002-present.

Regional Ornamental Research Committee, NCERA-193, Plant Health: Managing Insects and Diseases of Landscape Plants, member, 2005-present.

National Pest Detection Network (NPDN) Entomology Committee, member, 2006-present.