Introduction to the Emerald Ash Borer
*Agrilus planipennis* and the Latest Research

D. Barry Lyons
Canadian Forest Service
Sault Ste. Marie, Ontario
Federal Government Roles in Forest Invasive Alien Species

**Science-Based Policy Organization**

**Emerald Ash Borer Science Committee**
(Barry Lyons, CFS-GLFC, Chair)

**Natural Resources Canada**
Canadian Forest Service

**Canadian Food Inspection Agency**

**Regulatory Agency**
Plant Protection Act

**Science-based advice**

**Biology/ Signs & Symptoms**
Survey/Monitoring (Development of Detection Tools)
Chemical Control (Systemic Insecticides)
Biological Control (Parasitoids and Pathogens)

**Quarantines/Regulations**
Outreach/Communications
Cultural Controls (Sanitation)
Host Range (*Fraxinus* spp. – ashes)

**China**
- *Fraxinus chinensis* var. *chinensis*
- *F. chinensis* var. *rhynchophylla*
- *F. mandshurica*
- *F. velutina*

**Japan (*A. planipennis ulmi*)**
- *F. mandshurica* var. *japonica*
- *Juglans mandshurica* var. *sieboldiana*
- *Pterocarya rhoifolia*
- *Ulmus davidiana* var. *japonica*

**Northeastern North America**
- *F. pennsylvanica* – green ash (red ash)
- *F. nigra* – black ash
- *F. americana* – white ash
- *F. profunda* – pumpkin ash
- *F. quadrangulata* – blue ash ???

**Europe**
- *F. excelsior* – European/common ash
Life Cycle
Tree Anatomy
Adult Emergence - 2003

Number of adults observed (28 May)

- Males
- Females

Number of adults observed:

- 28 May - 4 June: 0
- 4-11 June: 0
- 11-19 June: 0
- 19-27 June: 0
- 27 June - 3 July: 0
- 3-8 July: 0
- 8-17 July: 0
- 17-24 July: 0
- 24-30 July: 0
Female

Male

[Image of two beetles, one labeled 'Female' and the other labeled 'Male.']
Eggs (after Yu 1992)

- eggs laid in sunny bark crevices and on the base of the trunk
- only one egg at each site
- cream-colored -> yellowish brown
- oblate, 1.0 by 0.6 mm slightly protruding in center, with reductus (fold) extends radially toward edges

TOO SMALL TO DETECT DURING SURVEY
Larva

Diagram showing the different segments of a larva, including:
- Head
- Prothorax
- Mesothorax
- Metathorax
- Abdominal segments 1-10
- Urogomphi

Illustration of four larval stages labeled I, II, III, and IV, with a scale of 5 mm.
mature larva

head

urogomphi

frass
Overwintering Stage  
(n = 2909)
Freeze-intolerant prepupae
Extremely low SCPs (-30 °C) in midwinter
High Conc. Glycerol
SCP is likely to be lower lethal temperature
Warm snaps affect ability to supercool
Decrease in supercooling ability not fully reversible


Elements of an Emerald Ash Borer Management Plan

1. Survey/Monitoring
2. Outreach/Communications
3. Quarantine/Regulations
4. Cultural Controls/ Sanitation
5. Biological Controls
6. Chemical Controls

Emerald Ash Borer Dynamic Management Plan

Action?

Yes

No

DEAD ASH TREES
Assessing Canada’s Urban Jungle
- A street tree survey to aid in alien species research
  J. Pedlar (CFS-GLFC) et al.

Survey Overview
• Most urban centres have thousands of trees within their boundaries
• Our approach surveys only a sample of the trees in an urban centre
• Participants walk a number of routes (0.5 km in length) that have been randomly located throughout their urban centre, identifying trees as they go
• In total, the routes cover about 10% of the total length of roads in each urban centre
Results so far...

- numerous surveys carried out by the Ontario Stewardship Rangers this summer
- interest shown by the Ontario Field Naturalists as well
- surveys also carried out by GLFC employees when possible

<table>
<thead>
<tr>
<th>City</th>
<th>Province</th>
<th>% Ash</th>
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<tbody>
<tr>
<td>Chatham</td>
<td>Ontario</td>
<td>0.6</td>
</tr>
<tr>
<td>Bracebridge</td>
<td>Ontario</td>
<td>0.0</td>
</tr>
<tr>
<td>Guelph</td>
<td>Ontario</td>
<td>6.3</td>
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<tr>
<td>Huntsville</td>
<td>Ontario</td>
<td>1.5</td>
</tr>
<tr>
<td>London</td>
<td>Ontario</td>
<td>3.3</td>
</tr>
<tr>
<td>Meaford</td>
<td>Ontario</td>
<td>8.2</td>
</tr>
<tr>
<td>Owen Sound</td>
<td>Ontario</td>
<td>6.7</td>
</tr>
<tr>
<td>Parry Sound</td>
<td>Ontario</td>
<td>19.8</td>
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<tr>
<td>Porcupine</td>
<td>Ontario</td>
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<td>Sault Ste Marie</td>
<td>Ontario</td>
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<td>Sudbury</td>
<td>Ontario</td>
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<tr>
<td>Timmins</td>
<td>Ontario</td>
<td>0.4</td>
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<tr>
<td>Bathurst</td>
<td>NB</td>
<td>4.0</td>
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<tr>
<td>Oromocto</td>
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<td>0.4</td>
</tr>
<tr>
<td>Fredericton</td>
<td>NB</td>
<td>0.0</td>
</tr>
<tr>
<td>Moncton</td>
<td>NB</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td><strong>3.7</strong></td>
</tr>
</tbody>
</table>
Exposed Galleries (cracks, windows, peelings)

Bark Deformities (stains, cracks, swellings)

Emergence Holes

Woodpecker Feeding Holes

Epicormic Shoots (root, bole, crown)

trap tree

visual inspection
Dispersal Study (Lyons/Jones)

Monitoring - Detection/Delimitation Surveys
Monitoring - Detection/Delimitation Surveys

- 84,000 traps
- 1.5 mile spacing
- manuka oil lures
- 100 mile band
Monitoring - Detection/Delimitation Surveys

GC-EAD – G. Grant
Monitoring - Detection/Delimitation Surveys

EAD (mV) (Mean ± SE)

- **Male**
- **Female**

- **GC**
  - 142
  - 3
  - 5
  - 6
  - 7
  - 8

- **EAD**
  - 4

- 2: Ald
- E2-6: Ald
- Z3-6: OH
- E2-6: OH
- 6: OH
- Z3-6: Ac
- 6: Ac

- **D**: b
- **E**: ab
Monitoring - Detection/Delimitation Surveys

Semiochemical Testing – Grant/Ryall/Silk/Lyons
Experiment G2: Green Canopy Traps

Mean number of adults (n = 10)

Lures

Aspects of the Pheromone Chemistry of the Emerald Ash Borer, Agrilus planipennis
P. Silk (CFS-AFC) & K. Ryall (CFS-GLFC) et al.

- Difficult to detect early infestation
- Trees asymptomatic for several years
- Need for effective lure to deploy with traps

- Improve understanding of the chemical ecology of EAB
- Develop chemical lure for better monitoring and detection tool for EAB
  1. Test female-produced lactone pheromone for biological activity in the field
  2. Identify contact pheromone and test for biological activity in the field

Monitoring - Detection/Delimitation Surveys

Hypothesized host volatiles necessary to synergize attraction for EAB males, similar to brown spruce long-horned beetle (Silk et al. 2007)

* significant increase in male captures with (3Z)-lactone

** significant interaction (increase in male captures with (3E)-lactone + (3Z)-hexenol

Sampling urban trees for EAB: developing an early-detection
K. Ryall, J. Fidgen and J. Turgeon

- Sample **two** branches per tree
  - open grown, semi-mature tree
  - 20 -50 cm DBH
  - minimum 5-8 cm dia
  - one 50-cm sample per branch
  - from any crown level or aspect
- **Mid crown, south aspect**
  - carefully dissect bark
Monitoring - Detection/Delimitation Surveys
K. Ryall (CFS-GLFC) et al.

Delimitation surveys

Sault Ste Marie EAB Pilot Detection Survey
Overview of Survey Results

Primary Study Area

No Ash  EAB present
No Data  No EAB detected

Kilometers 0 1 2 3 4 5
Comparing efficacy of early detection methods

- Trap and branch negative (30%)
- Trap and branch positive (46%)
- Inconsistent results (23%)
Chemical Controls

- Foliage/Trunk Sprays
- Systemic Trunk Injections

- Confidor
- Ecoprid/Ecoject System
- TreeAzin/Ecoject System
- ACECAP 97

Product? Efficacy? Public Concern?
Chemical Control

- natural product insecticide
- CFS proprietary formulation
- registered in US for organic production on greenhouse and outdoor food crops
- temporary registration of Neemix 4.5 in 2000 for control of sawflies by aerial application
- low risk of impact on non-target organisms
- no bioaccumulation
EcoJect™ System for Pest Management

- Simple to use;
- Minimum exposure risk;
- Light weight;
- Moderate pressure:
  - rapid injection times
  - reduced damage to host.
Dutton, Ontario Service Centre (Hwy 401) - 2007

- three dosages, 0.05, 0.1 and 0.2 g azadirachtin/cm dbh
London, Ontario
• TreeAzin™ 50 mg/mL or 5% total azadirachtins (A+B)
• Treatment rate = 0.2 g a.i./cm dbh
• Standard protocol
• 4 injection ports per tree
• 8 mL EcoJect cannisters; 8 per tree
• All trees injected June 26, 2007
• Foliage sampling throughout growing season 2, 7, 14, 29, 43, 56, 70 and 83 as well as 365 DAT
• Rapid uptake
• In all cases, foliar residues declined significantly with time ($P < 0.0001$)
• Dissipation via exponential kinetics
• $DT_{50}$ ranged from 5.1 to 12.3 d
• $DT_{90}$ ranged from 15.6 to 44.1 d
• At the time of leaf senescence, foliar residues levels ~ LOQ (0.01 mg/kg f.w.).
• Minimal residues at leaf fall – no non-target effects

EAB Eggs Laid Per Treatment of Everbearing Ash Averaged over 5 Reps

Days after Start of Assay

# of Eggs

H2O
Form Blank
0.01 ppm
0.1 ppm
1.0 ppm
5.0 ppm
10.0 ppm

< 50% of eggs viable @ 0.1 ppm
No viable eggs at concentration > 1 ppm

Double whammy effect – may explain two year protection
• Assess risk of non-target impacts from systemic insecticides
• Imidacloprid, at realistic concentrations, likely to inhibit leaf litter breakdown processes
• Negative implications for organic matter processing, nutrient cycling
• Know these risks; use accordingly
• Azadirachtin (TreeAzin): not so….

Microcosm experiments
D. Kreutzweiser (CFS-GLFC) et al.
Chemical Control

TreeAzin received an Emergency Registration in 2008, 2009 and 2010
Treatment reduces populations of emerald ash borer larvae and the damage they cause, but may not provide control of this pest.
Chemical Control – United States


<table>
<thead>
<tr>
<th>Insecticide Formulation</th>
<th>Active Ingredient</th>
<th>Application Method</th>
<th>Recommended Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merit® (75WP, 75WSP, 2F)</td>
<td>Imidacloprid</td>
<td>Soil injection or drench</td>
<td>Mid-fall and/or mid- to late spring</td>
</tr>
<tr>
<td>Xytec™ (2F, 75WSP)</td>
<td>Imidacloprid</td>
<td>Soil injection or drench</td>
<td>Mid-fall and/or mid- to late spring</td>
</tr>
<tr>
<td>IMA-jet®</td>
<td>Imidacloprid</td>
<td>Trunk injection</td>
<td>Early May to mid-June</td>
</tr>
<tr>
<td>Imicide®</td>
<td>Imidacloprid</td>
<td>Trunk injection</td>
<td>Early May to mid-June</td>
</tr>
<tr>
<td>Pointer™</td>
<td>Imidacloprid</td>
<td>Trunk injection</td>
<td>Early May to mid-June</td>
</tr>
<tr>
<td>TREE-äge™</td>
<td>Emamectin benzoate</td>
<td>Trunk injection</td>
<td>Early May to mid-June</td>
</tr>
<tr>
<td>Inject-A-Cide B®</td>
<td>Bidrin®</td>
<td>Trunk injection</td>
<td>Early May to mid-June</td>
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<tr>
<td>Safari™ (20 SG)</td>
<td>Dinotefuran</td>
<td>Systemic bark spray</td>
<td>Early May to mid-June</td>
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<tr>
<td>Astro®</td>
<td>Permethrin</td>
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<tr>
<td>Onyx™</td>
<td>Bifenthrin</td>
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<td>Tempo®</td>
<td>Cyfluthrin</td>
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<tr>
<td>Sevin® SL</td>
<td>Carbaryl</td>
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</tbody>
</table>

**Professional Use Products**

- Bayer Advanced™ Tree & Shrub Insect Control
  - Imidacloprid
  - Soil drench
  - Mid-fall or mid- to late spring

**Homeowner Formulation**
Biological Control

- Augmentative Biological Control
  - Local Exploration
    - Parasitoids
    - Pathogens

- Classical Biological Control
  - Foreign Exploration
    - Parasitoids
    - Pathogens
Classic Biocontrol/Foreign Exploration
USDA-FS and USDA-APHIS

- **Spathius agrili** Yang
  (Hymenoptera: Braconidae)

- **Tetrastichus planipennisi** Yang
  (Hymenoptera: Eulophidae)

- **Oobius agrili** Zhang and Huang
  (Hymenoptera: Encyrtidae)

USDA-APHIS biocontrol production laboratory - Brighton, Michigan (full time operation January 2009)
Surveys for EAB Natural Enemies in Michigan
(Liu et al. 2003)

Larval/Pupal Parasitoids (0.7% parasitism)

- *Spathius similimus* Ashmead (Braconidae) (= *S. floridanus* Ashmead?)
- *Heterospilus* sp. (Braconidae)
- *Phasgonophora sulcata* Westwood (Chalcididae)
- *Balcha* sp. (Eupelmidae) (= *B. indica* Mani & Kaul)) – exotic species
- *Eupelmus* sp. (Eupelmidae)

Egg Parasitoid (>6000 eggs reared – remains only)

Parasitoids of EAB in western Pennsylvania (Duan et al. 2009)

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Individuals</th>
<th>Relative abundance (%)</th>
<th>Parasitism† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Balcha indica</em> (Eupelmidae)</td>
<td>32</td>
<td>82.0</td>
<td>2.9</td>
</tr>
<tr>
<td><em>Eupelmus pini</em> (Eupelmidae)</td>
<td>1</td>
<td>2.6</td>
<td>0.1</td>
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<tr>
<td><em>Dolichomitus vitticrus</em> (Ichneumonidae)</td>
<td>2</td>
<td>5.1</td>
<td>0.2</td>
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<tr>
<td><em>Orthizema sp.</em> (Ichneumonidae)‡</td>
<td>1</td>
<td>2.6</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Cubocephalus sp.</em> (Ichneumonidae)‡</td>
<td>3</td>
<td>7.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

† 1091 EAB  
‡ could not be associated with EAB life stages  
- only female *B. indica* observed, associated with larval, prepupal and pupal remains  
- both eupelmids successfully reared on late instar larvae, prepupae and pupae  
- both reproduced via thelytokous parthenogenesis

Leluthia astigma (Ashmead) (Hymenoptera: Braconidae: Doryctinae) (Kula et al. 2010)

- Delaware Co., Ohio
- 2567 EAB larvae/prepupae were found – 45 parasitoid cocoons, 10 parasitoid larvae – 2.1% parasitism
- F₁ larvae observed feeding externally on non-feeding EAB larvae – idiobiont ectoparasitoid

Literature records and/or specimens examined from Canada; QC: United States; AZ, CA, IN, IA, KS, MD, NY, OH, NC, OK, PA, TX, UT, VA, WV, WY: Mexico; JA, SO.

Augmentative Biological Control/Local Exploration

*Atanycolus cappaerti* Marsh and Strazanac (Hymenoptera: Braconidae)

- parasitism rates of 9 to 71%
- bivoltine
- adults long lived (mean female = 31.7 d)
- apparent synchrony problem: at least the first generation of wasps will die before new generation are large enough for parasitization (i.e., they’re stuck with the EAB in 2nd of 2-year life cycle)
- also develop on *A. liragus* and *A. bilineatus*


Augmentative Biological Control/Local Exploration

### Methods

- **Red oak** (*Quercus rubra*) infested with *Agrilus bilineatus*
- **White birch** (*Betula papyrifera*) infested with *Agrilus anxius*
- **Trembling aspen** (*Populus tremuloides*) infested with *Agrilus liragus*
- **Green ash** (*Fraxinus pennsylvanica*) infested with *Agrilus planipennis*

#### Table: Species of Hymenopterous Parasitoids

<table>
<thead>
<tr>
<th>Species</th>
<th>Host Tree</th>
<th>Fraxinus</th>
<th>Betula</th>
<th>Populus</th>
<th>Quercus</th>
<th>Agrilus host</th>
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</thead>
<tbody>
<tr>
<td><strong>Braconidae</strong></td>
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<td></td>
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<tr>
<td><em>Ailorus stictopleurus</em> Martin</td>
<td>U</td>
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<td></td>
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<tr>
<td><em>Atanycolus disputabilis</em> (Cresson)</td>
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<td></td>
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<tr>
<td><em>Atanycolus hiconiae</em> Shenefelt</td>
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<td>VC</td>
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<td><em>Atanycolus longicauda</em> Shenefelt</td>
<td>C</td>
<td>C</td>
<td></td>
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<tr>
<td><em>Atanycolus cappaerti</em> Marsh and Strazanac</td>
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<td><em>Bassus</em> sp</td>
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<td><em>Chelonius</em> sp</td>
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<td><em>Coeloides roseus</em> b. <em>Betulae</em> Mason</td>
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<tr>
<td><em>Doryctes rufipes</em> (Provancher)</td>
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<td><em>Leluthia astigma</em> (Ashmead)</td>
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<td><em>Macrocentrus marginator</em> (Nees)</td>
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<td><em>Spathius similimus</em> Ashmead</td>
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<td><strong>Ichneumonidae</strong></td>
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<td><em>Dolichomitus irritator</em> (Fabricius)</td>
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<tr>
<td><em>Dolichomitus messor</em> (Gravenhorst)</td>
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<td>U</td>
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<tr>
<td><em>Rhyssella nitida</em> (Cresson)</td>
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<tr>
<td><em>Xorides humeralis</em> (Say)</td>
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<tr>
<td><strong>Pteromalidae</strong></td>
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<tr>
<td><em>Holcaeus</em> sp</td>
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<td><em>Platyergus algorega</em> (Girault)</td>
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<tr>
<td><strong>Chalcididae</strong></td>
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<tr>
<td><em>Phasgonophora sulcata</em> Westwood</td>
<td>VC</td>
<td>U</td>
<td></td>
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<td></td>
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<tr>
<td><strong>Eupelmidae</strong></td>
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<tr>
<td><em>Metaelma spectabilis</em> Westwood</td>
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<td></td>
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<td><em>Baicha indica</em> (Mani &amp; Kaul)</td>
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<td><strong>Eulophidae</strong></td>
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<tr>
<td><em>Barystatus</em> sp</td>
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<tr>
<td><strong>Aulacididae</strong></td>
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<td></td>
<td>C</td>
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<td>no</td>
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</tr>
</tbody>
</table>

*U = uncommon, C = Common, VC = very common*
Augmentative Biological Control/Local Exploration

**Phasgonophora sulcata**
Westwood
(Hymenoptera: Chalcididae)

**Balcha indica** (Mani & Kaul)
(Hymenoptera: Eupelmidae)

### Rearing (Essex Co. site 1 - 2006)
54 *P. sulcata*
9 *B. indica*
6 *Atanycolus* spp.
146 *A. planipennis*
Parasitism = 32.1%

### Rearing (Essex Co. site 2 - 2006)
8 *P. sulcata*
0 *B. indica*
3 *Atanycolus* spp.
648 *A. planipennis*
Parasitism = 1.2%

### Sticky Band Captures (Essex Co. site 1 - 2007)
407 *P. sulcata*
600 *A. planipennis*
Parasitism = 40.7%
Augmentative Biological Control/Local Exploration

Atanycolus spp.

A. disputabilis
A. hicoriae
A. cappaerti
**Sex Ratio**

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Atanycolus</em> sp.</td>
<td>55</td>
<td>69.1*</td>
</tr>
<tr>
<td><em>A. planipennis</em></td>
<td>645</td>
<td>48.1</td>
</tr>
<tr>
<td><em>P. sulcata</em></td>
<td>355</td>
<td>73.5*</td>
</tr>
</tbody>
</table>

* significantly different from 1:1 (chi-square)

**Parasitism Rates**

<table>
<thead>
<tr>
<th>Species</th>
<th>Parasitism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Atanycolus</em> sp.</td>
<td>5.2</td>
</tr>
<tr>
<td><em>P. sulcata</em></td>
<td>33.6</td>
</tr>
<tr>
<td>Overall</td>
<td>38.9</td>
</tr>
</tbody>
</table>

**Emergence Data McKeough Dam - 2010**

- **Mean time to emergence (days)**
  - **Species**: Atanycolus sp., A. planipennis, P. sulcata
  - **Temp**: 24°C
  - **Males, females**

**Note:**
- The data shows a significant difference in the sex ratio for *Atanycolus* sp. and *P. sulcata* compared to the 1:1 ratio (chi-square test).
- Parasitism rates vary significantly among the species, with *P. sulcata* having the highest rate.

**Graphically:**
- Bar graph showing mean time to emergence for males and females of each species at 24°C.
Augmentative Biological Control/Local Exploration

Life Stages of *Phasgonophora sulcata*

- Eggs dissected from female
- Early instar larva dissected from host
- Late instar larva in host
- Pupa dissected from host
- Adult visible within host

- solitary
- endoparasitoid
- koinobiont?

**Life Stages of *Phasgonophora sulcata***
Conclusions

- many species of native parasitoids have made the host switch from native *Agrilus* species to *A. planipennis*
- potential biological control agents are Braconidae and Chalcidoidea
  - *P. sulcata* is capable of building to high population densities on *A. planipennis* but only in declining populations
  - *P. sulcata* is synchronized with larval stages of *A. planipennis*
- species is solitary koinobiont endoparasitoid
  - *P. sulcata* has bred in laboratory but effective rearing techniques need to be developed
- mating conditions for *P. sulcata* unknown
Biological Control – Native Entomopathogens
George Kyei-Poku
Mycosed larva

Fungus growing on frass scooped from galleries
Metarhizium anisopliae growing on EAB
Virulence of *Beauveria* spp. against EAB

Fungus inoculated male (0-1 days p.i.) mating with a naïve female

Conidia contamination

Horizontal transmission studies of *Beauveria* spp. against EAB
Black Intercept™ Panel Trap

Green Intercept Panel Trap

Green Prism Trap

uncoated or coated with Insect-a-Slip Barrier (Fluon – fluoropolymer resin)
Biological Control – Entomopathogens
Development of an Autocontamination Trap

- no fungus – salt solution
- all baited with Z-(3)-hexenol
- young green ash plantation
- complete random block design
- n = 10

\[ F = 152.149 \quad P < 0.001 \quad df = 4 \]
Biological Control – Entomopathogens
Development of an Autocontamination Trap

*Beauveria bassiana* (strain CFL-INRS)
Biological Control – Entomopathogens
Development of an Autocontamination Trap

Mortality Rate for EAB Adults in Panel Trap

- Days post exposure
- Mortality (%)
- Exposed to B. bassiana
- Not exposed to B. bassiana
The End

Questions?