Current Research at the European Biological Control Laboratory

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St. Johnswort (Hypericum perfoliatum)

- Released 4 insects in 1940s-50s,
- extensive control
- still continuing
Classical Biological Control

- Origin of target (weed or pest)
- Discover potential BC agents
- Identify them
- Send live agents to USA
- Test them for safety (host specificity)
- Test them for potential efficacy
- Release agents
- Measure impact on target

Research Areas

- Entomology, Botany, Microbiology
- Taxonomy (plants, arthropods, microbes)
- Molecular genetics
- Ecology (abiotic & species interactions)
- GIS (geographic information systems)
- Behavior
- Physiology
- Computer modeling; statistics
### Scientific Staff

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<th>Country</th>
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### Invasive Alien Species

#### Classical Biological Control:
- **insects** – *Anoplophora, Bactrocera*
- **plants** – *Centaurea, Genista, Lepidium*
- **ticks** – *Rhipicephalus annulatus*

#### Vector Control:
- **mosquitos**
- **sand flies (phlebotomine)**
Quarantine for insects and pathogens

- Insects
- Pathogens

Limited access

Distribution of *Genista monspessulana* in USA

http://plants.usda.gov, 4 Aug 2011
World Distribution of French Broom

**Figure 3:** distribution de *Genista monspessulana* et du climat méditerranéen dans le monde (adaptation de : Ecosystems of the world, Vol. II, Mediterranean-Type Shrublands ; DiCastri, Goodall et Specht 1981).

Distribution of French broom in Europe

**Figure 2:** localisation de *Genista monspessulana* (Fabaceae) en Europe dans le bassin Méditerranéen.
Genotypes of invasive plants have not been found in "native" range.

12 nuclear microsatellite markers
**French broom**  
(*Genista monspessulana*)

- **psyllid**  
  *Arytinnis hakani*

  Evaluated for release in Australia.  
  Kills Fr. broom in Australia.  
  Can develop on some lupines.  
  Ongoing host specificity testing.

- **seed-feeding weevil**  
  *(Lepidapion nr argentatum)*  
  Larvae feed inside seed pods.  
  Adults eat flowers and pollen.  
  Only found attacking French broom

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**French broom killed by psyllid**  
(*Arytinnis hakani*) **in Australia**
Is there a pathogen involved?

*Candidatus Liberibacter europea* reported in Scotch broom (*Cytisus scoparius*) in New Zealand [Thompson et al. 2013. New Disease Reports 27: 6.]

Not detected in California

Rapid plant death in French broom psyllid lab colony

French broom weevil
*Lepidapion argentatum* (Col.: Apionidae)

Develops in seeds ... and in stem galls

R. Sforza (EBCL), T. Thomann (CSIRO)
French broom weevil
*Lepidapion argentatum*
(Col.: Apionidae)

Life cycle of a native population of *Lepidapion argentatum* emerged from galls and pods on *Genista monspessulana* (Sheppard et al. 2013, Carré 2014).
Gall formation occurs from spring to summer on the same year. Females from pods cannot induce galls in the same year.
Psylliodes chalcomera
3-Way Choice Experiments

Host plant in field
Onopordum acanthium

Prefer to eat same host plant in laboratory as collected from in the field

Cryptic populations of *Psylliodes chalcomera* collected on different host plants

*Carduus nutans* in field
*Centaurea solstitialis* in the field

On *Onopordum acanthium* in Russia

On *Carduus* in Italy

Based on 500 bp of COI (DeBiase, Antonini & Audisio 2003)
Interaction of weevil and pathogen for weed biological control
M-C. Bon (EBCL) / A. Caesar (NPRL, MT)

- **Hypothesis**: Would the combined effect of a root galling weevil (*Ceutorhynchus assimilis*) and a pathogen (*Rhizoctonia solani*) better suppress *Lepidium draba sp. draba* than the effects of a single natural enemy?
- Already one example of direct **synergistic interaction** between Leafy Spurge (*Euphorbia esula*) / root weevil (*Aphthona* spp.) / fungi (*Rhizoctonia solani & Fusarium*) (Caesar 2003).

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**American/European Targets**

- **Brown marmorated stink bug**
  - *Halyomorpha halys*
- **Spotted-wing drosophila**
  - *Drosophila suzukii*
- **Olive fruit fly**
  - *Bactrocera oleae*
- **Citrus Longhorned Beetle (CLB)**
  - *Anoplophora chinensis*
  - Asian Longhorned Beetle (ALB)
  - *Anoplophora glabripennis*
Brown Marmorated Stink Bug
*(Halyomorpha halys)* (Hemiptera: Pentatomidae)

Native to subtropical and temperate areas in East Asia
Highly polyphagous pest that feeds on ornamental plants; fruit trees; field and vegetable crops
Established in Pennsylvania in the mid-1990’s; spread to 39 states

- COII, 12S
- Eastern US population from Beijing area in China.
- Lower genetic diversity in US compared to native populations
  - 2 mitochondrial haplotypes in 55 US specimens
  - 43 haplotypes in 77 native specimens

**Brown Marmorated Stink Bug**

- 4 haplotypes found in the Western US (mainly Oregon),
- One matching a haplotype found near Nanjing, and one in Japan
- More diversity on the West Coast than on the East Coast
- Multiple introductions

**Trissolcus spp.** (Scelionidae: Hymenoptera)

— egg parasitoids of Hemiptera.

Taxonomy not well known

Using molecular genetic and morphology to identify specimens reared from hosts collected in East Asia

Nondestructive extraction of DNA; morphological analysis (M. Buffington)
• CO1 & ITS2
• 9 Terminal taxa correspond to 9 species

• Bayesian and MP phylograms congruent in topology

• The bayesian phylogram was overall better resolved

• No evidence of geographical or host structure for the three species (T. japonicus, T. plautiae, and T. cultratus)

• Existing phylogenies are being completed with 18S and wingless sequence data sets

Specimens originating from eggs of different hosts
Biological control of Asian and citrus longhorned beetle
Franck Hérard
European Biological Control Laboratory, USDA, ARS, Montferrier-sur-Lez, France

Asian Longhorned Beetle (ALB) Anoplophora glabripennis
Citrus Longhorned Beetle (CLB) Anoplophora chinensis

Invasive tree pests (Coleoptera: Cerambycidae, Lamiinae) both native to eastern Asia

Signs and damage to plants

Bark of young shoots is chewed by the beetles
Female chews a hole in bark and deposits an egg under bark (ALB), or within bark tissues (CLB)
Young larvae tunnel under bark
Adult exit hole
Large larvae tunnel deeper in wood

F. Hérard, European Biological Control Laboratory, USDA, ARS
**ALB introduction pathway**

- Seaborne trade
- Wood packing material
- Larva
- Pupa
- Pre-emerging adult

**CLB introduction pathway**

- Importation of bonsai and ornamental plants (many maple trees) from Asia
- Bonsai of apple tree infested with *A. chinensis*
Anoplophora glabripennis infestations in North America

- New Jersey (2008)
- Toronto (Canada)
- Worcester, MA (2011)

2003 = year of finding
+ = eradicated

Anoplophora spp. infestations and recent interceptions in Europe

- Italy
  - Parabiago (2000)
  - Assago (2006)
  - Milano (2006)
  - Montichiari (2006)
  - Gussago (2007)
  - Corbeil (2007)
  - Cornuda (2009)
  - Maser (2010)

- France

- England
  - Paddock Wood (2004)
  - Kent (2012)

- Belgium
  - West Flanders (2006, 2007)

- Germany
  - Almere (2010)
  - Bornheim (2005)

- Austria
  - Braunau (2001)

- Switzerland
  - Braunfels (2011)

- Croatia
  - Zadar (2007)

- Italy

- France
  - Soyons (2003)

- Germany
  - Almere (2010)
  - Bornhem (2005)

- Austria
  - Braunau (2001)

Interceptions: 
- interceptions

Infestations: 
- infestations

Eradicated: 
- +
Early stage parasitoids of ALB in Europe (northern Italy)

- All are polyphagous (known from local xylophagous hosts)

- Spathius erythrocephalus (Hym.: Braconidae)
  - Eurytoma melanoneura (Hym.: Eurytomidae)
  - Calosota agrili (Hym.: Eupelmidae)
  - Sclerodermus sp. (Hym.: Bethylidae)

- Cleonymus brevis (Hym.: Pteromalidae)
  - Trigonoderus princeps (Hym.: Pteromalidae)

ALB larva

Early stage parasitoids of CLB in Europe (northern Italy)

- All larval parasitoids are polyphagous (known from local xylophagous hosts)

- Spathius erythrocephalus (Hym.: Braconidae)
- Eurytoma morio (Hym.: Eurytomidae)
- Eurytoma melanoneura (Hym.: Eurytomidae)
- Eupelmus aloysii (Hym.: Eupelmidae)
- Calosota agrili (Hym.: Eupelmidae)
- Trigonoderus princeps (Hym.: Pteromalidae)
- Cleonymus brevis (Hym.: Pteromalidae)
- Sclerodermus sp. (Hym.: Bethylidae)

- Aprostocetus anoplophorae (Hym.: Eulophidae)
  - New species (described in 2004)
  - Specific to CLB
  - Originating from Asia (?)
2014 RESULTS IN SOUTH KOREA:

- an ALB egg parasitized by nematodes

- an ALB egg parasitized by an unidentified species (biomolecular identification of it is in progress).

- 2 CLB with larval parasitoid (Spathius sp. n., Braconidae)

F. Hérard, European Biological Control Laboratory, USDA, ARS

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**Improvement of Olive Fruit Fly parasitoid rearing technology**

– taking advantage of insect behavior and chemical ecology

- *Psyttalia lounsburyi* (Kenya and South Africa)
- *Psyttalia ponerophaga* (Pakistan)
- *Utetes africanus* (Namibia)

These species are very challenging to rear
Labor intensive, low overall production, inconsistent sex ratio

Livy Williams, Michelangelo La Spina
EBCL Entomopathogen collection

- >1,200 strains in liquid N₂ (collected by Guy Mercadier since 1988)
- ARS (Peoria, Illinois) screening for pathogenicity to mosquitos
- Mélanie Tannières (new to EBCL) will test *Beauveria* strains on olive fruit fly (*Bactrocera oleae*)

EBCL Entomopathogen collection

- Characterization of the collection through morphology and ITS sequencing
- Good start, but not sufficient
**EBCL Entomopathogen collection**

**First phase:**
Biolog OmniLog Identification System
- uses automated biochemical methods.
  - e.g., test a microorganism's ability to utilize or oxidize a panel of 95 carbon sources.
  - produce a unique biochemical pattern or “fingerprint” for each strain.

Matrix Assisted Laser Desorption / Ionisation system
- analyses the protein “fingerprint” of organisms by mass spectrometry.
  - For each strain, a unique MALDI biotype is produced.

**Second phase:**
Bioassays to screen microbial biocontrol agents that kill eggs of *Aedes aegypti*, *Ae. albopictus*, *Ae. japonicus*, and *Ae. vexans*.

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**Available Positions**

- Immediately available:
  postdoc for phylogeographic study.
  Contact mcbon@ars-ebcl.org

- Dominique Coutinot retired 2 Feb. 2015.
  Position soon available for Entomologist / Quarantine Officer