Current Research at the European Biological Control Laboratory

Lincoln Smith, Center Director
U.S. Department of Agriculture
Agricultural Research Service
LSmith@ars-ebcl.org

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

France

- **Marie-Claude Bon** – geneticist
- **Dominique Coutinot** – quarantine officer
- **Franck Hérard** – entomologist
- **René Sforza** – entomologist
- **Mélanie Tanniéres** – microbiologist
- **Lincoln Smith** – Director / entomologist
- **Livy Williams** – insect physiologist

Greece

- **Alexandra Chaskopoulou** – entomologist
- **Javid Kashefi** – exploration

Invasive Alien Species

Classical Biological Control:

insects – *Anoplophora, Bactrocera*

plants – *Centaurea*, *Genista*, *Lepidium*

ticks – *Rhipicephalus annulatus*

Javid Kashefi

Vector Control:

mosquitos

sand flies (phlebotomine)

Alexandra Chaskopoulou

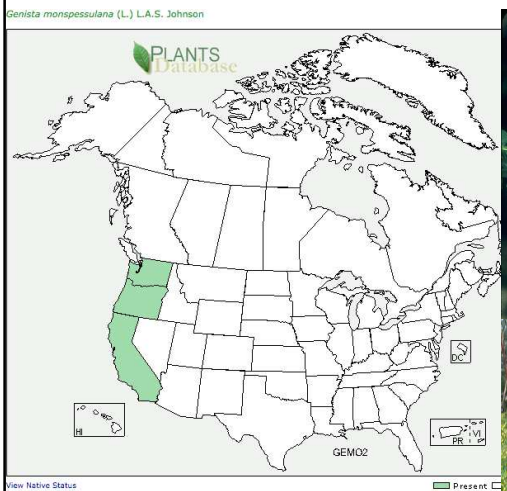
Thessaloniki, Greece

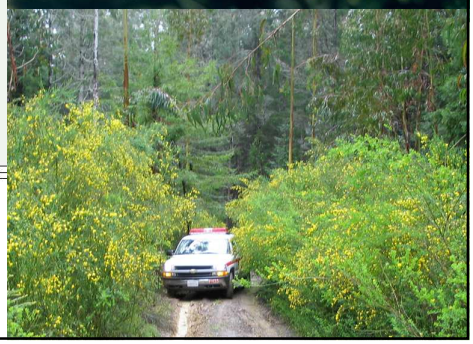
Quarantine for insects and pathogens

Limited access

- **Insects**
- **Pathogens**

Distribution of *Genista monspessulana* in USA





http://plants.usda.gov, 4 Aug 2011

World Distribution of French Broom

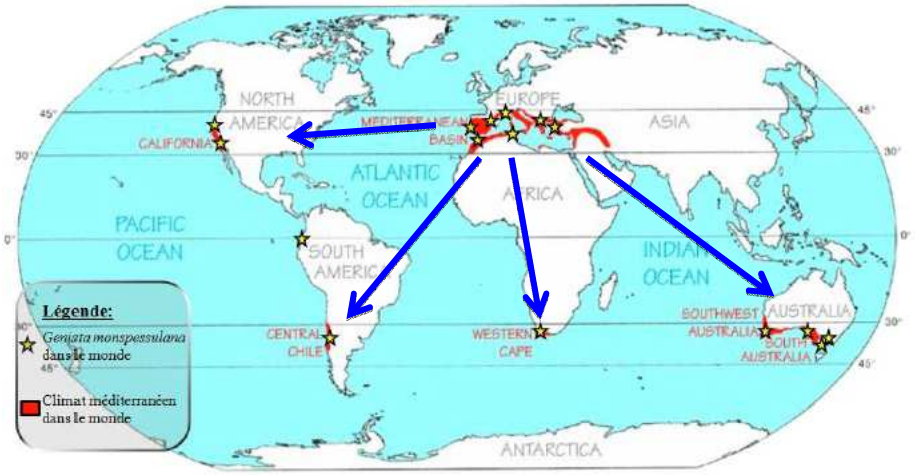


Figure 3: distribution de *Genista monspessulana* et du climat méditerranéen dans le monde (adaptation de : Ecosystms of the world, Vol. II, Mediterranean-Type Shrublands ; DiCastri, Goodall et Specht 1981).

Thierry Bernard (2012) Stage de Master 2

Distribution of French broom in Europe

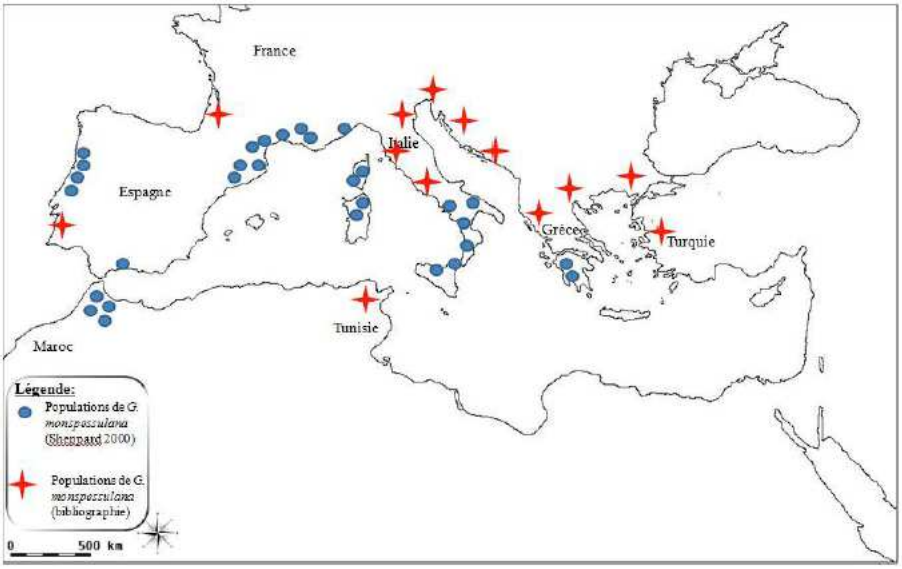
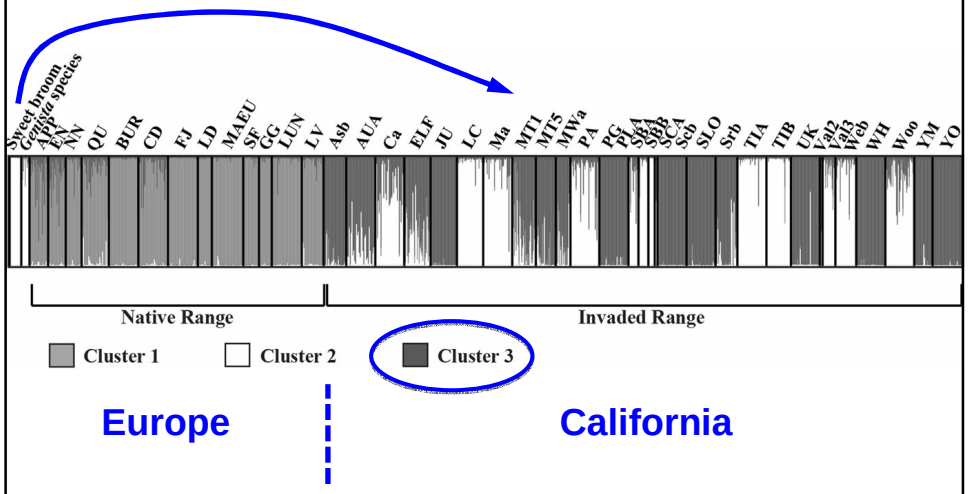


Figure 2 : localisation de *Genista monspessulana* (Fabaceae) en Europe dans le bassin Méditerranéen

Thierry Bernard (2012) Stage de Master 2

STRUCTURE analysis of French broom

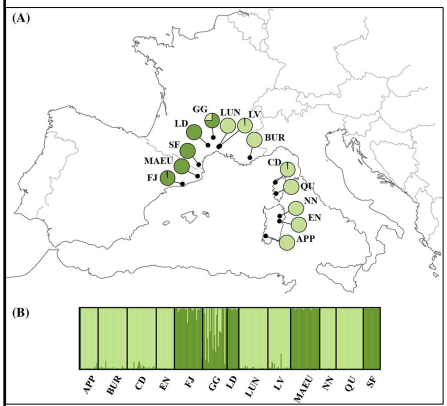


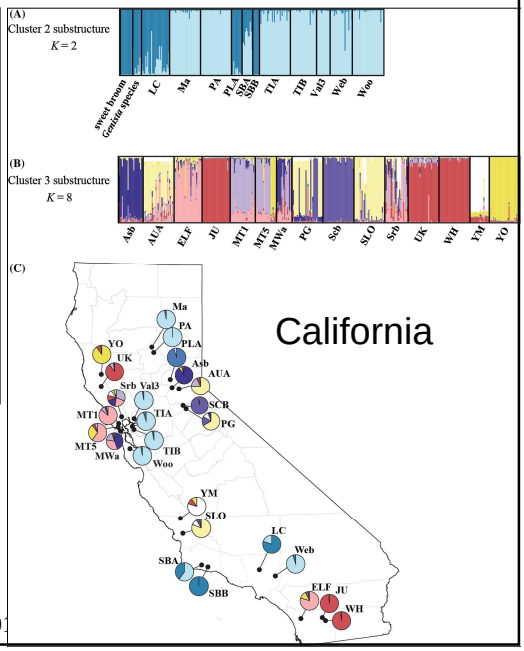
Europe | **California**

Genotypes of invasive plants have not been found in "native" range.

12 nuclear microsatellite markers
Kleist et al. 2014. Biol. Invasions 16: 887-901.

STRUCTURE analysis of French broom





Kleist et al. 2014. Biol. Invasions 16: 887-90

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

image from:
http://www.gonhs.org/Lepidapionargentatum.htm

French broom killed by psyllid (*Arytinnis hakani*) in Australia

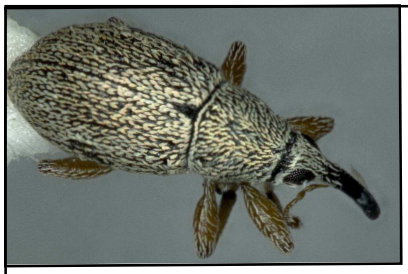
4 Sept. 2009, A. Sheppard, CSIRO

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

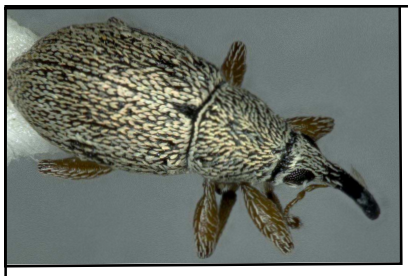


French broom weevil *Lepidapion argentatum* (Col.: Apionidae)

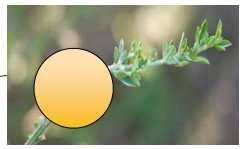
R. Sforza (EBCL), T. Thomann (CSIRO)

Develops in seeds ...

and in stem galls

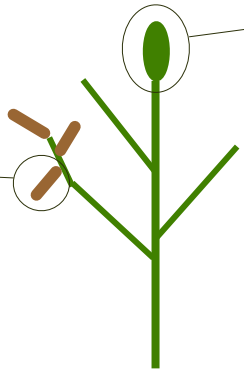


French broom weevil *Lepidapion argentatum* (Col.: Apionidae)



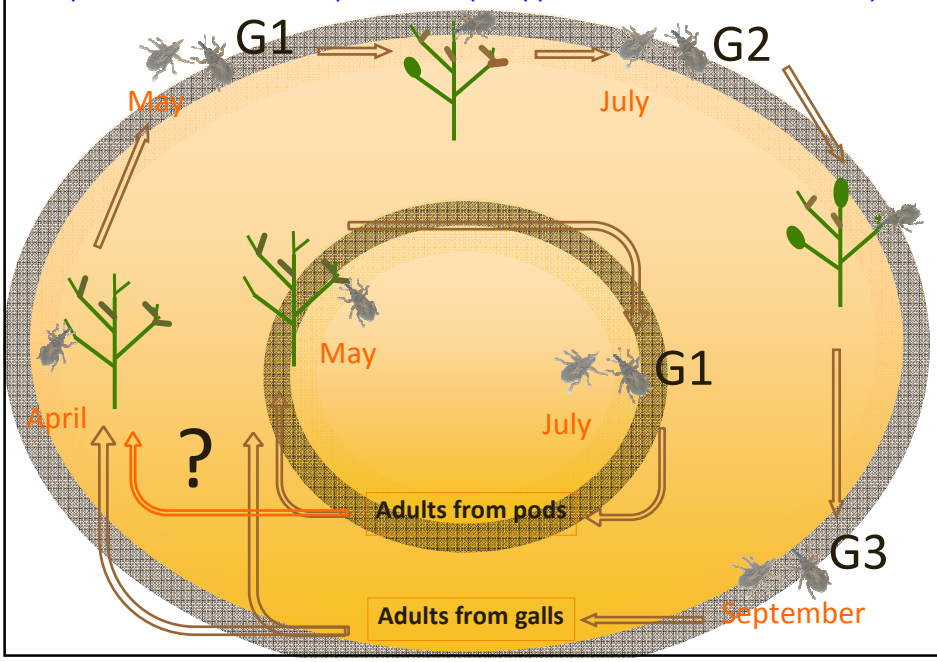
galls in stems

seed pods



French broom

Life cycle of a native population of *Lepidapion argentatum* emerged from galls and pods on *Genista monspessulana* (Sheppard et al. 2013, Carré 2014).



pod / stem	Origin	Galls induced
	Mixed population (2014)	✓
	Pod population (2013)	✓
	Gall population (2014)	✓
	Pod population (2014)	✗

Gall formation occurs from spring to summer on the same year.
Females from pods cannot induce galls in the same year.

Cryptic Species

3-Way Choice Experiment

Psylliodes chalcomera
(Chrysomelidae)

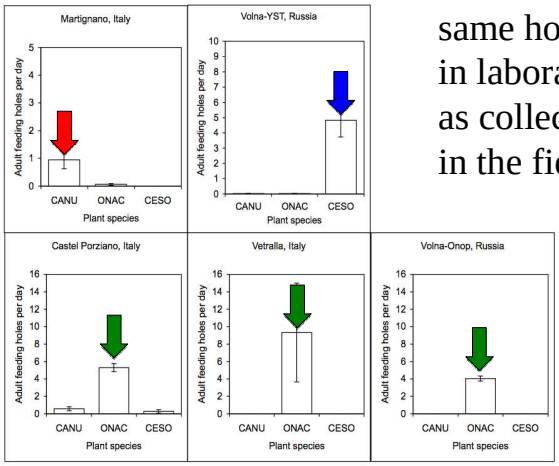
Onopordum acanthium ***Carduus nutans*** ***Centaurea solstitialis***

Psylliodes chalcomera
3-Way Choice Experiments

Host plant in field

Onopordum acanthium

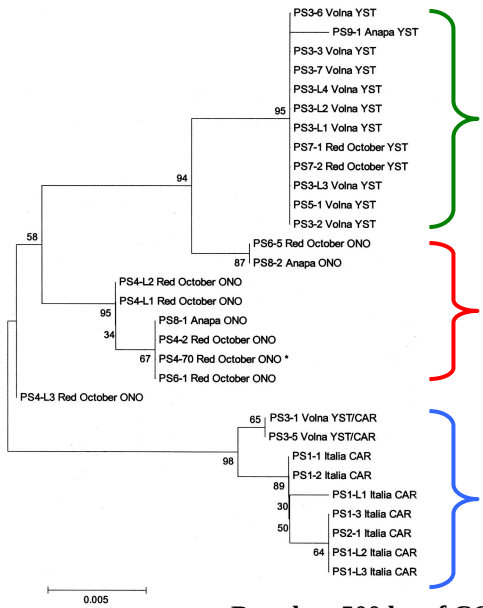
Carduus nutans | *Centaurea solstitialis*



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

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

Fig 1. Neighbor Joining Tree on the K2P distances matrix



on YST in Russia

on *Onopordum* in Russia

on *Carduus* in Italy

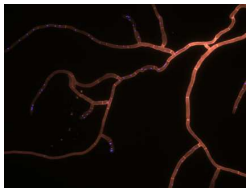
Based on 500 bp of COI (DeBiase, Antonini & Audisio 2003)

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).






USDA ars

Solutions from Nature

EBCL

American/European Targets

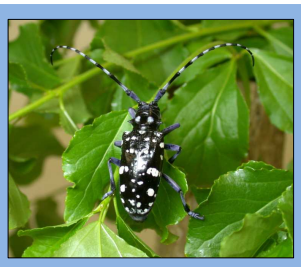
Brown marmorated stink bug
Halyomorpha halys

Olive fruit fly
Bactrocera oleae

Africa

Spotted-wing drosophila,
Drosophila suzukii

Asia

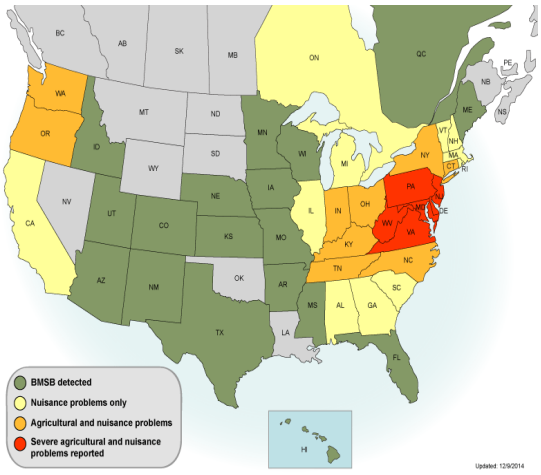


Citrus Longhorned Beetle (CLB)
Anoplophora chinensis
Asian Longhorned Beetle (ALB)
Anoplophora glabripennis

Brown Marmorated Stink Bug

(*Halyomorpha halys* (Hemiptera: Pentatomidae)

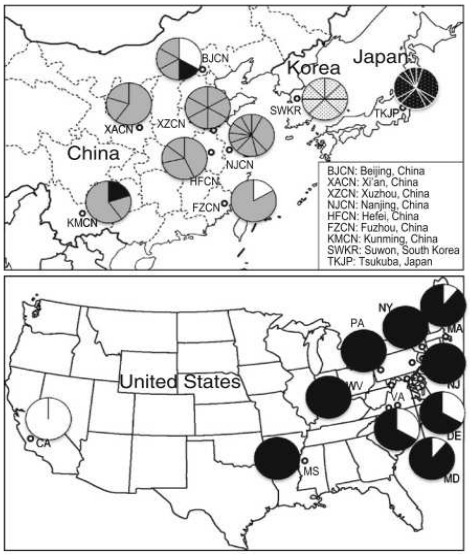
- Native to subtropical and temperate areas in East Asia
- Highly polyphagous pest that feeds on ornamental plants; fruit trees; field and vegetable crops
- Established in Pennsylvania in the mid-1990's; spread to 39 states



Brown Marmorated Stink Bug

(*Halyomorpha halys* (Hemiptera: Pentatomidae)

- 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

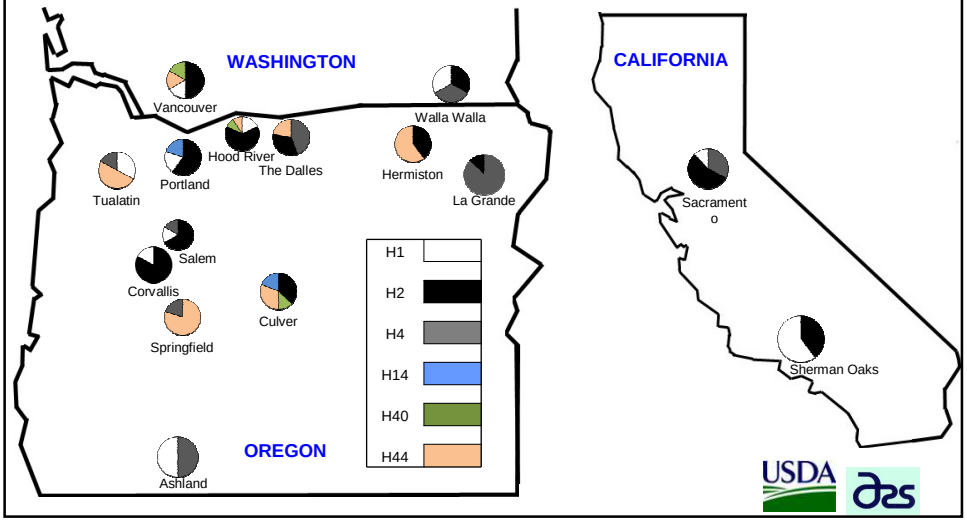


Xu et al. 2014. Biological invasions 16(1): 153-166.

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

Bon et al., 2015, (in preparation)



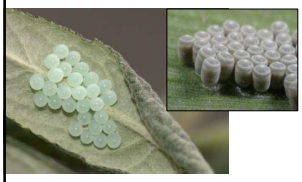
Trissolcus spp. (Scelionidae: Hymenoptera) — egg parasitoids of Hemiptera.

M-C. Bon (EBCL)

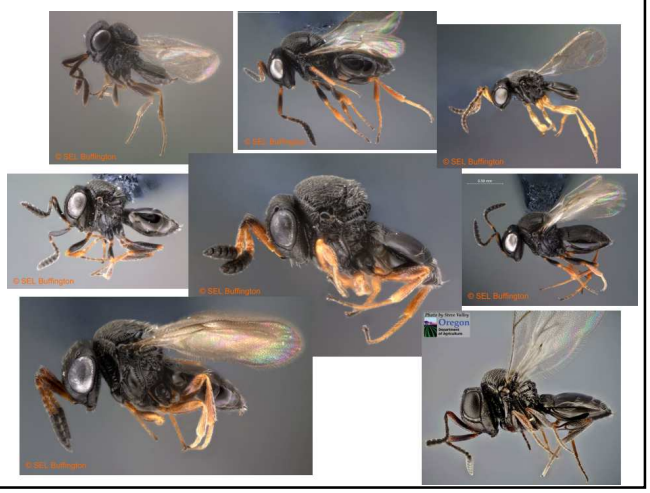
Taxonomy not well known

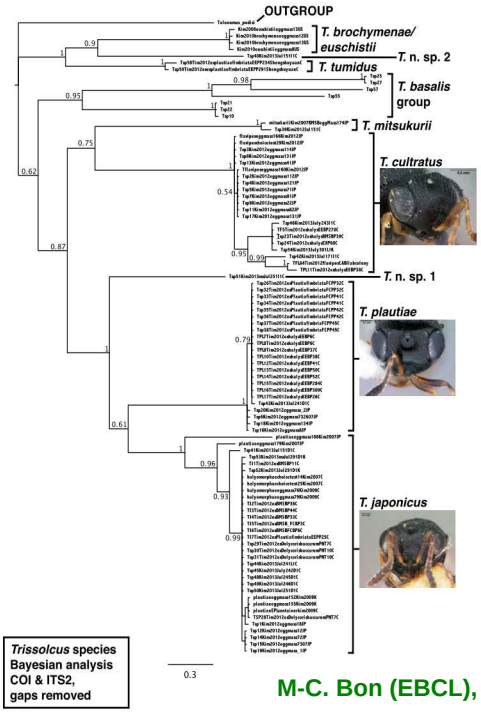
Using molecular genetic and morphology to identify specimens reared from hosts collected in East Asia

Nondestructive extraction of DNA; morphological analysis (M. Buffington)



collected by EBCL, CABI





Trissolcus species
Bayesian analysis
COI & ITS2,
gaps removed

- **CO1 & ITS2**
- **9 Terminal taxa correspond to 9 species**

- Bayesian and MP phylograms congruent in topology

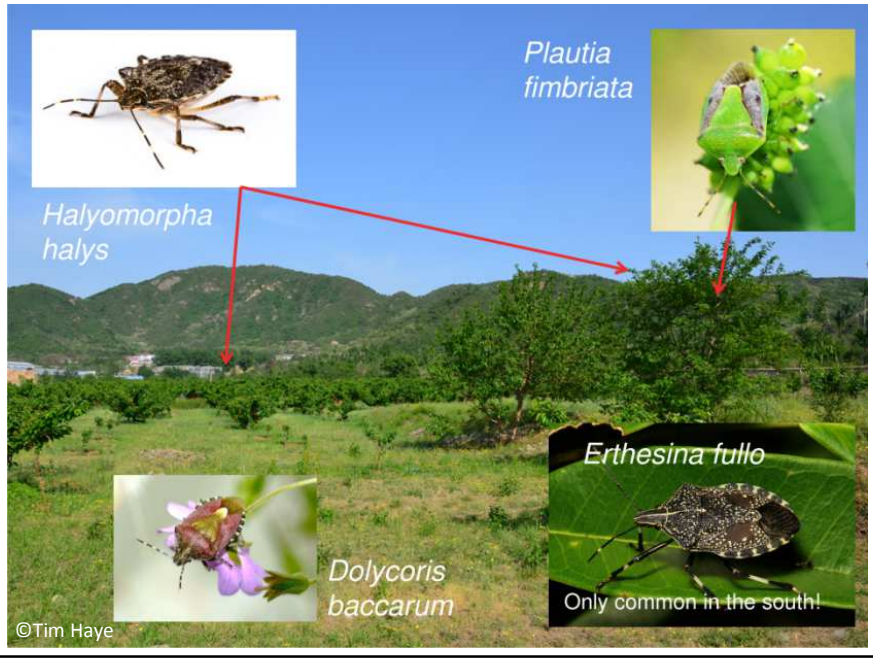
- The bayesian phylogram was overall better resolved

- 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

M-C. Bon (EBCL), M. Buffington (ARS)

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

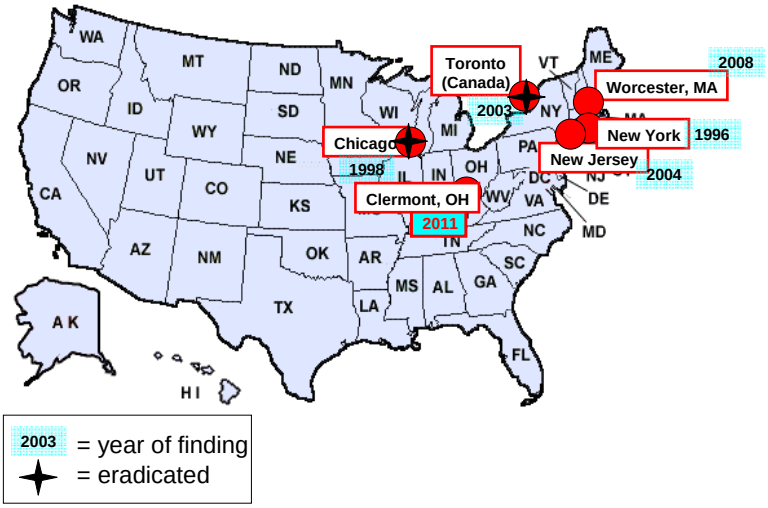
Pre-emerging adult

CLB introduction pathway

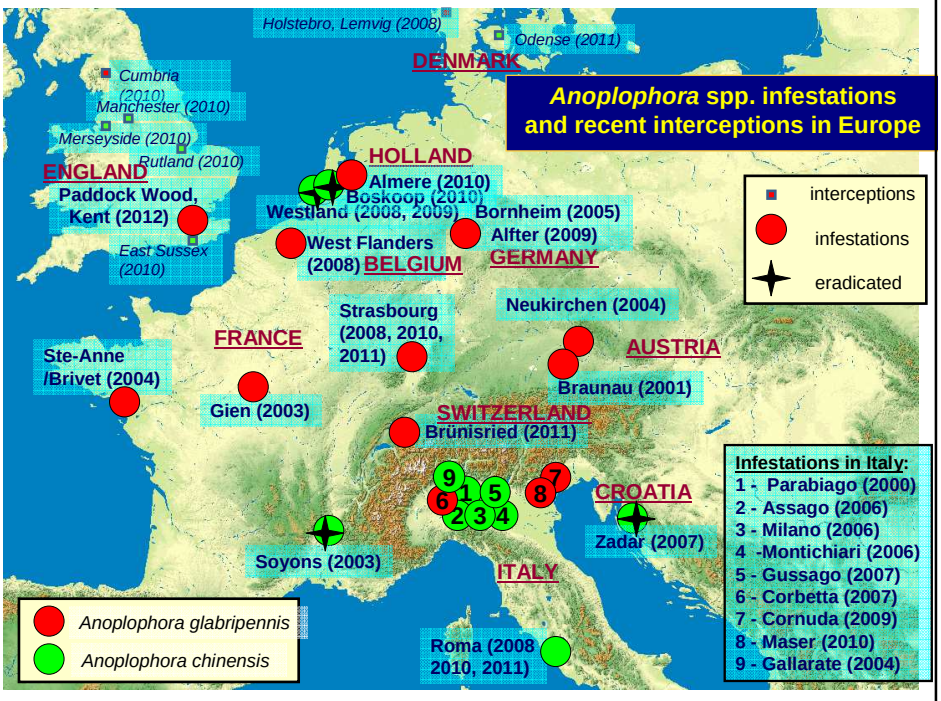
Importation of bonsai and ornamental plants (many maple trees) from Asia

Bonsai of apple tree infested with *A. chinensis*

Anoplophora glabripennis infestations in North America



Anoplophora spp. infestations and recent interceptions in Europe



Early stage parasitoids of ALB in Europe (northern Italy)

ALB larva

NO egg parasitoid

All are **polyphagous** (known from local xylophagous hosts)

Spathius erythrocephalus (Hym.: Braconidae)

Eurytoma melanoneura (Hym.: Eurytomidae)

Eurytoma morio (Hym.: Eurytomidae)

Eupelmus aloysii (Hym.: Eupelmidae)

Cleonymus brevis (Hym.: Pteromalidae)

Trigonoderus princeps (Hym.: Pteromalidae)

Calosota agrili (Hym.:Eupelmidae)

***Sclerodermus* sp.** (Hym.: Bethylidae)

Early stage parasitoids of CLB in Europe (northern Italy)

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

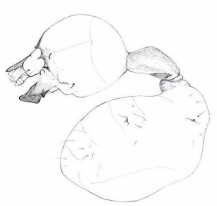
Spathius erythrocephalus (Hym.: Braconidae)
Eurytoma morio (Hym.: Eurytomidae)
Eurytoma melanoneura (Hym.: Eurytomidae)
Eupelmus aloysii (Hym.: Eupelmidae)
Calosota agrili (Hym.:Eupelmidae)
Trigonoderus princeps (Hym.: Pteromalidae)
Cleonymus brevis (Hym.: Pteromalidae)
***Sclerodermus* sp.** (Hym.: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

Gregarious egg parasitoid

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)

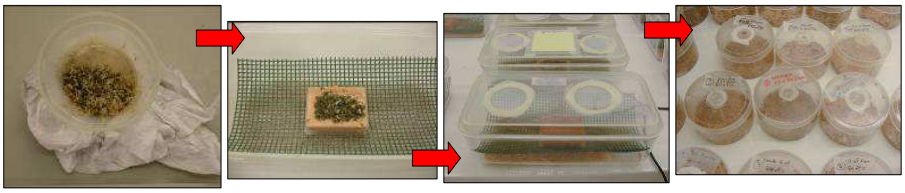
F. Hérard, European Biological Control Laboratory, USDA, ARS

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)

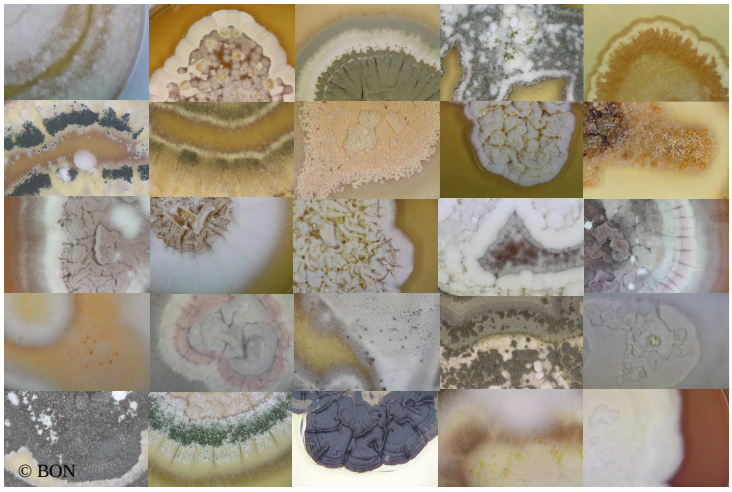
These species are very challenging to rear
Labor intensive, low overall production, inconsistent sex ratio



Livy Williams, Michelangelo La Spina

EBCL Entomopathogen collection

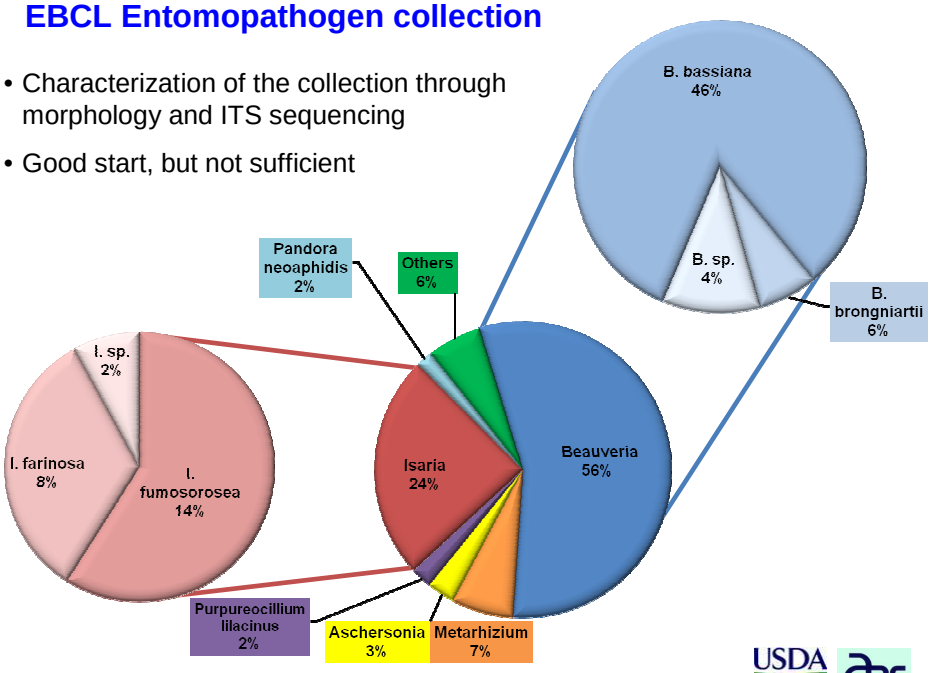
- >1,200 strains in liquid N_2 (collected by Guy Mercadier since 1988)
- ARS (Peoria, Illinois) screening for pathogenicity to mosquitos
- Mélanie Tannières (new to EBCL) will test *Beauveria* strains on olive fruit fly (*Bactrocera oleae*)



USDA ars

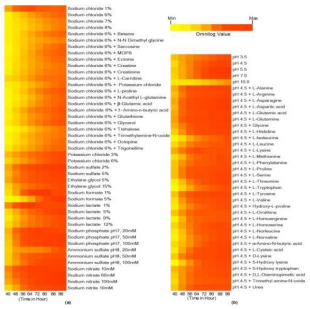
EBCL Entomopathogen collection

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



USDA ars

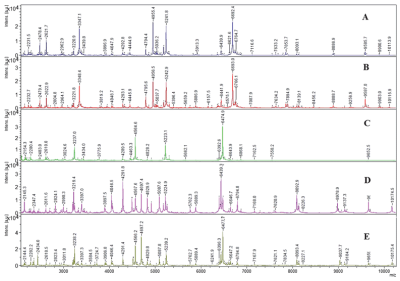
EBCL Entomopathogen collection



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*.

USDA ARS

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