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Edited by Dawn Gundersen-Rindal

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Introduction

OBCL is a group of overseas laboratories that support the domestic research carried out by USDA-ARS with the aim of "finding solutions to agricultural problems that affect Americans every day from field to table".

The **Australian Biological Control Laboratory (ABCL)** is based in Brisbane, Australia. The facility is run through a Specific Cooperative Agreement between USDA-ARS and Australia's Federal research body, CSIRO.
Contact: Matthew Purcell, matthew.purcell@csiro.au

The **European Biological Control Laboratory (EBCL)** is based in Montpellier, France, and has a satellite laboratory in Thessaloniki, Greece. Contact: Dawn Gundersen-Rindal, dawn.gundersen-rindal@ars.usda.gov / www.ars-ebcl.org

The **Foundation for the Study of Invasive Species (FuEDEI)** is based in Hurlingham, Argentina and is operated as a nonprofit research organization. Contact: Guillermo Cabrera Walsh, gcabrera@fuedei.org / www.fuedei.org

The **Sino-American Biological Control Laboratory (Sino-ABCL)** is based in Beijing, China.
Contact: Liu Chenxi, liuchenxi@caas.cn

Previous newsletter editions are available at:
www.ars-ebcl.org

Sino-American Biological Control Laboratory - Sino-ABCL

30th Anniversary Celebration of the Establishment of the USDA Overseas Sino-American Biocontrol Laboratory held in Vancouver, BC, Canada

by Chenxi Liu

On November 12th, 2018, the USDA-ARS Office of National Programs (ONP) and Office of International Research Programs (OIRP) held a celebration of the 30th anniversary of the establishment of the Sino-American Biological Control Laboratory (Sino-ABCL) during the 2018 joint meeting of the Entomological Society of America (ESA), Entomological Society of Canada (ESC) and Entomological Society of British Columbia (ESBC), held in Vancouver, BC, Canada.

Figure 1. Dr. Chenxi Liu delivered a retrospective research review marking the 30th anniversary of the establishment of the Overseas Sino-American Biological Control Laboratory (Sino-ABCL).

Mr. Bryan Norrington, Director of the USDA OIRP, presided over the meeting, which included all USDA Overseas Biological Control Laboratory (OBCL) Directors, as well as research scientists from USDA-ARS experimental stations and laboratories, and OBCL stakeholders. Dr. Chenxi Liu delivered a retrospective review entitled "Research achievements of the Sino-American Biological Control Laboratory during the past 30 years", where he reviewed the history of China-US cooperation in research conducted by the Sino-ABCL (Fig. 1).

The Sino-ABCL is based in Beijing, China. The agreement for the establishment of the Sino-ABCL in the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS), was signed by the USDA-ARS and CAAS in November, 1988, and represented the first formal governmental agreement in the history of China and United States agricultural cooperation (Fig. 2).

Figure 2. In November 1988, an Agreement for establishment of the Sino-American Biological Control Laboratory was signed by USDA-ARS and CAAS. It was the first cooperative agreement in the history of China and United States agricultural research cooperation.

The primary mission of the Sino-ABCL is to search for, identify, and evaluate the potential of natural enemies of pest insects, weeds, and plant diseases that affect Chinese and United States agriculture, and to develop biological control technologies which can be used to suppress invading weeds and insect pests for both countries. During the past 30 years, the Sino-ABCL carried out sixteen invasive species biological control programs for serious problems such as wheat stem sawfly, soybean aphid, Asian longhorn beetle, brown marmorated stink bug, and leafy spurge, to name a few (Fig. 3). Effective biological agents against each of these invasive species were identified during the past 30 years, with survey for biological control agents covering 25 provinces including at least 150 sites in China. The Sino-ABCL also organized local collaborators from more than ten research institutes and Universities, including Institute of Grassland Research in Inner Mongolia, Yunnan Agricultural Academy of Sciences in Kunming, Nanjing Agricultural University and more, helping with both collection and research.

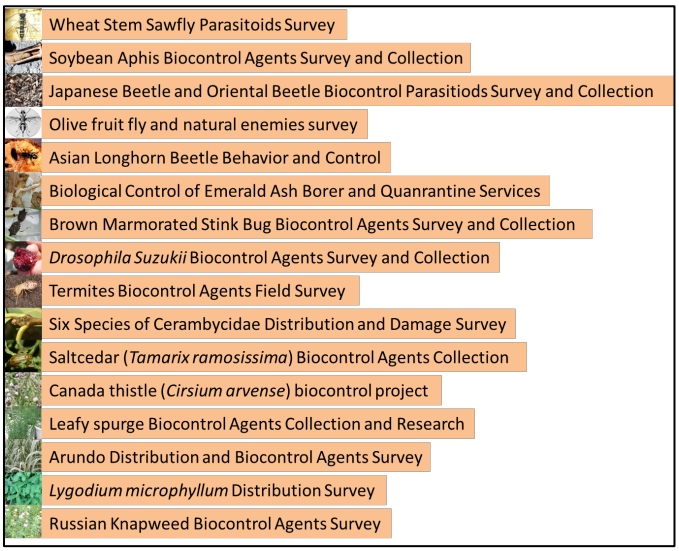

Figure 3. During the past 30 years, the Sino-ABCL carried out research for sixteen invasive species biological control programs.

Living materials for use in biocontrol have been shipped to the United States more than 140 times (Fig. 4). Over the past 30 years, more than 120 researchers have been sent to the United States for cooperative training and 260 US research scientists and governmental officials have visited the Sino-ABCL (Fig. 5).

Figure 4. Living materials have been shipped from the Sino-ABCL to the United States more than 140 times since 1988.

Figure 5. USDA Under Secretaries, Administrators, and other officials have visited the Sino-ABCL over the years.

Recently, the Sino-ABCL expanded research interests into nutrigenomics, biochemical and molecular mechanisms of reproduction, and diapause of beneficial insects. The Sino-ABCL also collaborates with scientists in many countries including Canada and Italy to explore the biodiversity of natural enemies and promote international collaboration on biological control of invasive species (Fig. 6).

Figure 6. USDA-ARS and Sino-ABCL research scientists collect biological control agents in China.

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Australian Biological Control Laboratory - ABCL

The first potential biological control agent for earleaf acacia shipped to Florida

by Ryan Zonneveld, Christine Goosem, John Schatz, Matt Purcell, and M. Smith

ABCL surveys to date have discovered 92 herbivores on earleaf acacia, *Acacia auriculiformis*. This includes 66 species of Lepidoptera, four Coleoptera, two Hemiptera, one Hymenoptera, two Diptera, one Acari and one pathogen that are known to complete their full development on this host, as well as a further 15 insect species that have been recorded feeding as adults.

The first potential biocontrol agent for earleaf acacia was sent to the USDA ARS Invasive Plant Research Laboratory (IPRL), Ft. Lauderdale, in early November. Fifty-two pupae of *Calomela intemerata* (Coleoptera: Chrysomelidae) (Fig. 7) that originated from the Northern Territory in Australia were packaged and sent to the US where adult beetles emerged and began laying eggs in quarantine. These developed and are now at the prepupal stage and adults continue to lay heavily. Once F1 generation adults emerge, host-range testing will begin as will studies looking at the effects of leaf litter on pupation.

At ABCL, life history research has been completed. Data on the fecundity, longevity and larval instar durations has been determined. The total egg to adult development time is approximately 23 days. Early results have shown that adults usually live for 2-8 months on cut plant material in the laboratory at 25°C. Females lay on average ~50 eggs per week throughout their lifespan.

An experiment being planned and undertaken by Muhammad Nawaz (PhD student) along with the ABCL team, aims to examine the importance of matching weed and herbivore provenances in biological control programs. For this research, we will be using two geographically isolated and genetically distinct populations of *C. intemerata* collected from the Northern Territory and Queensland in Australia. The experiment will examine adult and larval feeding and female

Figure 7. The leaf-feeding Chrysomelidae beetle, *Calomela intermerata*, a potential biological control agent of earleaf Acacia, *Acacia auriculiformis*.

fecundity of the two *C. intemerata* populations on plants of *A. auriculiformis* from the Northern Territory, Queensland, Papua New Guinea and Florida. The results will hopefully show which population of *C. intemerata* will feed more heavily and reproduce more successfully on the Florida genotype of *A. auriculiformis* which is thought to have originated from the Northern Territory. A pilot study began in November and will be completed early in the New Year. The full experiment will commence in February 2019.

Calomela intermerata from the Northern Territory differ genetically by 10% (C01) from populations found in Queensland yet they interbreed in lab cultures. In July of this year, a team of six including ABCL and IPRL staff members along with collaborators from the University of Queensland travelled across northern Australia on a 10-day survey between Cairns and Darwin to see if the two genetically distinct populations of *A. auriculiformis* and *C. intermerata* in the Northern Territory and Queensland occur in sympatry (Fig. 8). It aimed to elucidate whether or not there are two distinct isolated populations separated by the Carpentarian Gap, a biogeographical barrier. The findings suggest a disjunction does exist in Northern Australia. This information may influence where potential agents are collected, favoring herbivores from the same provenance as the introduced weed in Florida; the Northern Territory.

Figure 8. The team on the Cairns-Darwin field trip. (Left to right: Gio Fichera, Gimme Walter, Ryan Zonneveld, Melissa Smith, Raghu Sathyamurthy, Muhammad Nawaz).

Foundation for the Study of Invasive Species - FuEDEI

Stink Bugs

by Willie Cabrera Walsh

The brown marmorated stink bug (BMSB, *Halyomorpha halys*) is an East Asian species now established across North America and Europe. It has also established in Chile, and probably Brazil. It is expected to inevitably enter other South American countries soon, as there do not seem to be climatic limitations to its dispersal. It is a polyphagous pest that affects several fruit crops, ornamentals, legumes, and vegetables. It is also considered a structural nuisance as it aggregates inside buildings and homes to overwinter. Cross-attraction in stink bug pheromones is not infrequent, and BMSB is attracted to the aggregation pheromone of *Plautia stali* (MDT), as well as its own aggregation pheromone (BMSB lure). Synthetic versions of both pheromones could be used for control (e.g. in crop-trapping) and monitoring of the BMSB, however, it is important to know the species range of a pheromone to evaluate its safety as a management tool. This can be of some concern especially if they capture large numbers of beneficial insects, such as predatory

stink bugs or bug parasitoids. Both pheromones were deployed with sticky traps in a field with clear, 30x15 cm sticky traps, hung at 150 - 180 cm from the ground with wire ties (Fig. 9). The traps were distributed in random positions in four blocks of four treatments each: MDT lures, BMSB lures, MDT+BMSB lures, and controls (sticky trap with no lures). The traps were 50 metres apart within blocks, with at least 100 metres between blocks.

Trapping was performed at conserved lands in Mercedes, province of Buenos Aires (S 34° 33' 48"; W 59° 21' 57"), and at the Botanical Gardens in the city of Buenos Aires (S 34° 34' 58"; W 58° 25' 02"). The first site was chosen because of the security of no interference with the traps, and the proximity of wild lands and fruit tree groves; and the latter was chosen because due to its proximity to the port of Buenos Aires and its profusion of suitable BMSB host trees, it was deemed to be the first place where the BMSB could show had it entered Argentina. The traps were inspected every week when weather allowed, and continued for four months. Lures were changed every 12 weeks.

Figure 9. Sticky trap hanging from fence showing both types of lures exposed together.

The BMSB was not captured during this trapping effort, suggesting this species has not entered the country yet. However, several species of stink bugs were consistently attracted to the MDT and MDT+BMSB lures. The specimens captured have been sent to the specialist Dr. Gimena Dellape for identification, but preliminary identifications indicate that two species in the genus *Banasa* were the most attracted (Fig. 10). Other common agricultural pest species were also captured, although in lower numbers. Also, the BMSB lure traps captured a similar spectre of species, but in much lower numbers. However, the fact that no bugs at all were captured on the control traps suggests some degree of attraction operated in every case.

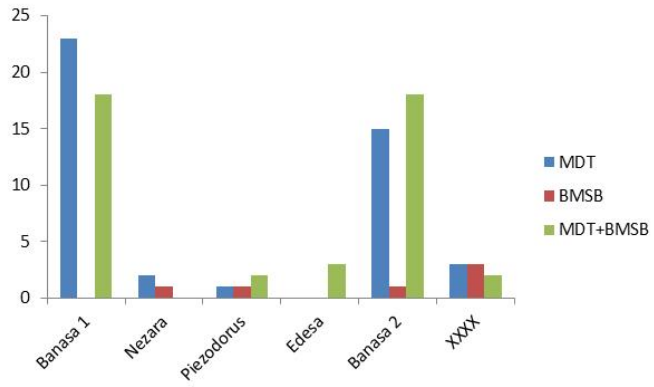

Figure 10. Total number of stink bug species captured per lure.

European Biological Control Laboratory - EBCL

Consumption but no predation: *Trichoribates trimaculatus* has no value for biological control of the viburnum leaf beetle *Pyrrhalta viburni*

by Gaylord Desurmont, Marie-Claude Bon, Elven Kerdellant, and Fatiha Guermache

The viburnum leaf beetle *Pyrrhalta viburni* (Coleoptera: Chrysomelidae) is a specialist *Viburnum* leaf- feeder that is native to Eurasia and invasive in the United States and Canada. It defoliates viburnum shrubs in gardens, managed landscapes, and natural areas, and can lead to plant death after 2-4 years of complete defoliations. Investigations for natural enemies of *P. viburni* in Europe have not yet led to the introduction of a biological control agent in North America.

High densities of the oribatid mite *Trichoribates trimaculatus* have been recently found in egg masses of *P. viburni* in southern France (Fig. 11). This mite is known to occur in mosses and on the bark of diverse shrubs or trees. The abundance of *T. trimaculatus* in *P. viburni* egg masses prompted the question: can this mite impact *P. viburni* egg survivorship? Our main working hypotheses were that *T. trimaculatus* may directly impact *P. viburni* by feeding on its eggs and/or may

indirectly impact *P. viburni* by physically altering the egg masses and reduce egg overwintering success. In order to test these hypotheses, laboratory experiments and molecular analyses of body content using quantitative PCR were developed at EBCL in 2017 and 2018, as well as a manipulative field experiment where twigs containing *P. viburni* egg masses were enclosed in sleeve nets with and without mites during the overwintering period of *P. viburni*.

The integrity and survivorship of *P. viburni* eggs were not impacted by the presence of mites in all laboratory experiments, providing no evidence of an active predation of mite eggs. However, traces of *P. viburni* egg DNA were detected in the body of mites exposed to *P. viburni* eggs, indicating that they consumed either fragments of the chorion or fragments of the "egg cap" secreted by *P. viburni* females to protect the eggs. In the field, the overwintering success of *P. viburni* was similar in sleeve nets with and without mites (35% and 36% egg survivorship, respectively), and live mites were found in most twigs from the mite treatment at the end of the experiment (18 out of 21 twigs).

Collectively, our results showed that *T. trimaculatus* does not directly or indirectly impact *P. viburni* eggs and does not have value for biological control. It is likely that this mite feeds primarily on wood and plant debris and finds in *P. viburni* egg masses a microhabitat favorable for its development. The fact that mites did contain traces of *P. viburni* egg DNA indicates that consumption does not necessarily equal predation. These results may serve as a cautionary tale for biological control scientists attempting to reconstruct trophic interactions using molecular methods.

Figure 11. Left: *Pyrrhalta viburni* egg masses along a *Viburnum tinus* twig. Eggs are laid in cavities excavated by the females and covered by the "egg cap", a frass-like secretion (only egg caps are visible on this picture). Right: An oribatid mite *T. trimaculatus* wandering on *P. viburni* eggs. Note that the "egg cap" secretion surrounds the eggs and sticks them together.

A new rearing method for *Cryptonevra nigritarsis*, a potential biological control agent of the giant reed *Arundo donax*

by Gaylord Desurmont, Yaël Escobar, and Elven Kerdellant

The giant reed *Arundo donax* is a perennial rhizomatous grass native to the Mediterranean basin and India. It is highly invasive in different areas of the world, including 14 US states. In the US, *A. donax* causes environmental damage in riparian ecosystems, reduces water availability, displaces native flora, and creates environmental conditions facilitating the spread of another invader, the cattle fever tick. Biological control efforts against *A. donax* in the US have been initiated with the release of a galling wasp, *Tetramesa romana* and the arundo scale *Rhizaspidiotus donacis*, both native to the Mediterranean basin. The chloropid fly *Cryptonevra nigritarsis* has been considered as a potential additional biological control agent, triggering studies on its biology and ecology at EBCL in 2017 and 2018.

Rearing *C. nigritarsis* under controlled conditions has proved a challenging task. The fly attacks *A. donax* shoots when they are young (< 75 cm) and larvae develop in the decaying shoots. Under laboratory or greenhouse conditions, rearing efforts have traditionally been made by using potted *A. donax* plants and creating conditions favorable for *C. nigritarsis* attack and development. The process involves cutting sections of arundo rhizomes containing buds, planting them in individual pots with some soil mix, waiting for the shoots to start growing, exposing them to *C. nigritarsis* adults once the shoots have reached the desired size, and waiting for the development of the next generation. Theoretically, this process can lead to the production of a F1 generation of *C. nigritarsis* within a month after exposure. The problem faced at EBCL using this method was that often the vast majority of *A. donax* shoots exposed to *C. nigritarsis* adults remained uninfested, which hindered efforts to mass-rear this insect. Water and soil humidity management are also critical for successful development of *C. nigritarsis*: shoots that are water-stressed are more susceptible to *C. nigritarsis* attack, but larvae need the shoot tissues to remain humid in order to successfully develop, and young shoots that dry up too quickly are not suitable for fly development.

A new method was designed at EBCL in August-September 2018 to overcome some of the challenges associated with *C. nigritarsis* production (Fig. 12). Instead of using potted sections of arundo rhizomes, freshly cut *A. donax* shoots collected in the field were used and planted in supports filled with water agar. The advantage of using cut shoots was that they did not remain too vigorous to prevent *C. nigritarsis* attack, and the water agar allowed plant tissues to remain humid for the duration of larval development. Using cut shoots in water agar instead of pieces of rhizomes in soil also greatly decreased the amount of work, material, and space needed to expose arundo shoots to flies, and allowed exposure of many shoots simultaneously in a reduced space. For tests of this method, we planted 2-3 cut *A. donax* shoots per support and exposed either 1 support (in glass bottles), or 3 supports (in cages with a thin mesh) to *C. nigritarsis* females. This new method of rearing was successful: about a third of the shoots exposed (12 out of 37) yielded 1-14 *C. nigritarsis* pupae (total: 64 pupae) after dissection of the shoots.

Results were highly encouraging for future research with *C. nigritarsis*. The setup developed can be used not only for mass rearing but also for non-choice and choice tests as well as manipulative experiments to investigate the effects of different parameters on the developmental success of *C. nigritarsis*.

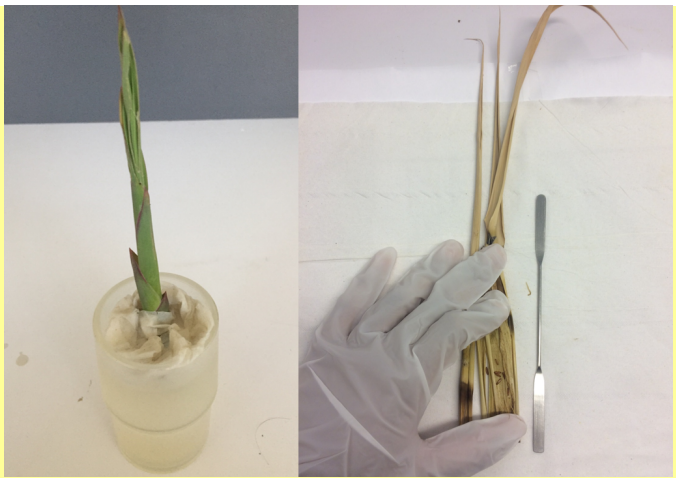

Figure 12, Rearing method. Left: young shoot of *Arundo donax* planted in a layer of water agar. The basis of the shoot is covered with a paper towel to avoid direct contact between *Cryptonevra nigritarsis* adults and water agar. The support container is made of glass. Right: dissection of a shoot after exposure to *C. nigritarsis* adults, showing the *C. nigritarsis* pupae.

Photoattractants for monitoring and population control of pest and disease vector insects (Sabbatical program research project conducted at EBCL-Thessaloniki)

by Lee Cohnstaedt and Alexandra Chaskopoulou

As part of EBCL's sabbatical program, Dr. Lee Cohnstaedt, a USDA-ARS Research Entomologist from the USDA Center for Grain and Animal Health in Manhattan, Kansas, conducted collaborative research during a three-month period (June- August) in Thessaloniki, Greece. He used light emitting diodes to evaluate the effects of light intensity and wavelength (color) on positive insect phototaxis.

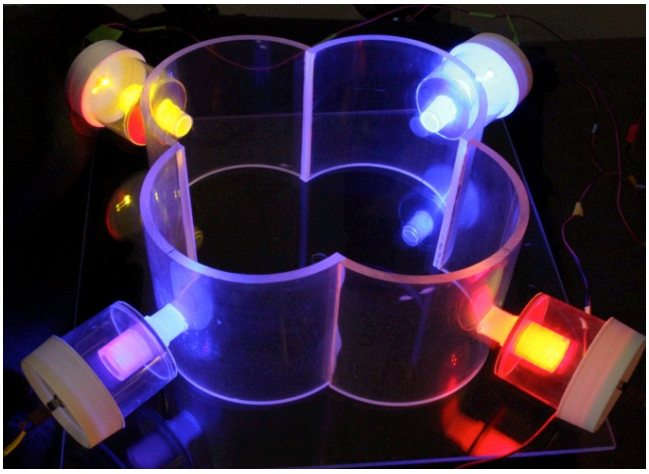

Figure 13. Bioassay arena used to test the photo attraction of Olive fruit flies and sand flies. The arena uses light emitting diodes and resistors to control color and intensity.

Some insect surveillance and pest management methods require traps to be visually attractive. Therefore, optimizing the visual attractiveness by altering color and light intensity can improve the collection efficiency of the traps. To find the optimal attractive conditions, a clover leaf photoattraction bioassay arena was used to evaluate lab and field collected insects. The bioassay arena (Fig. 13) used light emitting diodes and resistors to control color and intensity. The arena was used to test the photo attraction of both Olive fruit flies (agricultural pests) and sand flies (insect vectors). A methods paper and video description of the bioassay arena was published in the Journal of Visual "*Key Elements of Photo Attraction Bioassays for Insect Studies or Monitoring Programs*" (http://www.jove.com/video/57445).

The main purpose of the bioassay arena was to reduce the costly and time intensive field trials needed to evaluate and optimize visual-attractants in the field.

Figure 14. The olive fruit fly (*Bactrocera oleae*), a serious pest in the cultivation of olives.

The first major project was to determine the photo attraction of the olive fruit fly [*Bactrocera oleae* (Rossi 1790)], a serious pest of olives (Fig. 14). A strong positive attraction to green light was observed, which was logical given that immature olives are green. Longer-term field studies are required to make green targets stand out in a green olive grove. Additional findings for the olive fruit fly were the use of time lapse photos to determine the fly's active period. The diurnally active flies were most active during the mornings, however activity could remain high if the humidity remained elevated. No activity was observed at night or during the crepuscular night time hours. Field tests are needed to confirm these findings.

European Society for Vector Ecology (E-SOVE) conference, Palermo, Italy, 2018

by Alexandra Chaskopoulou

E-SOVE is the European branch of the Society for Vector Ecology (SOVE), an international organization that promotes research on suppression of nuisance organisms and disease vectors through environmental management, biological control, public education, and appropriate chemical control technology. The Society publishes the Journal of Vector Ecology, quarterly Newsletters, and holds annual/bi-annual conferences.

This year's E-SOVE conference was held October 22nd to 26th, 2018, in Palermo, Sicily, and was one of the most well attended SOVE conferences ever. Greater than 200 participants from 36 countries provided 175 abstracts; 82 exceptional speakers covered important topics on vector ecology and behavior, novel vector control tools, vector pathogen interactions, invasive vector species, and emerging and re-emerging vector-borne diseases, just to name a few.

The chair of the conference was Dr. Alexandra Chaskopoulou (EBCL-Greece), current president of E-SOVE, who in collaboration with the SOVE Advisory Scientific Committee and the support of the local organizing institute "Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri" built a highly informative scientific program. EBCL scientists were active during the meeting, presenting their research relating to vector control and/or chairing scientific sessions. For example, Javid Kashefi presented his research on Cattle Fever Tick Biological Control and Ioannis Giantsis chaired the session entitled "Vector Ecology & Behavior" (Fig. 15).

For more information on the scientific program of the conference please visit the society's website at:http://www.sove.org/European%20SOVE%20folder/greecescientificprogramme.html

Figure 15. Top: Javid Kashefi present cattle tick research, and Bottom: Ioannis Giantsis chairing session on "Vector Ecology & Behavior" during the E-SOVE conference in Palermo, Sicily, 2018

Recent Publications by ABCL

McCulloch, G.A., Hereward, J.P., Lake, E.C., Smith, M.C., **Purcell, M.F**. and Walter, G.H. 2018. The complete chloroplast genome of the invasive fern *Lygodium microphyllum* (Cav.) R. Br. Mitochondrial DNA Part B: Resources 3(2): 746-747.

McCulloch, G.A., **Makinson, J.R**., **Zonneveld, R.**, **Purcell, M.**, **Raghu, S.** and Walter, G.H. 2018. Assessment of genetic structuring in the Lygodium fern moths *Austromusotima camptozonale* and *Neomusotima conspurcatalis* in their native range: implications for biological control. Biological Control 121: 8-13.

Wheeler, G.S., Dyer, K., Broggi, E., Ding, J., **Purcell, M.** and Madeira, P. 2018. Molecular comparisons of native range collections of *Gadirtha fusca*, a potential biological control agent of Chinese tallowtree. Biocontrol Science and Technology 28(8): 796-804.

Williams, D.A., Harms, N.E., Grodowitz, M.J., and **Purcell, M.** 2018. Genetic structure of *Hydrilla verticillata* L.f. Royle in eastern China and the Republic of Korea: Implications for surveys of biological control agents for the invasive monoecious biotype. Aquatic Botany 149:17-27

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