OBCL Newsletter

December 2019

Overseas Biological Control Laboratories
US Department of Agriculture, Agricultural Research Service

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.
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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.
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Foundation for the Study of Invasive Species - FuEDEI

FUEDEI's quest for reactivating weed biocontrol in South America

by Willie Cabrera Walsh, Alejandro Sosa, Fernando McKay and Mariano Maestro

Invasive alien species are considered the second most important threat to biodiversity, and among them invasive plants rank the highest. Classical weed biocontrol (CWB) is a technique for controlling widespread exotic plants by releasing specific natural enemies (arthropods and pathogens) from the plant's native range. CWB was first used toward the end of the 19th century, but picked up momentum as from the 1950s, with a peak in biocontrol agent releases between 1990 and 2000 (Fig. 1). Argentina made auspicious incursions into

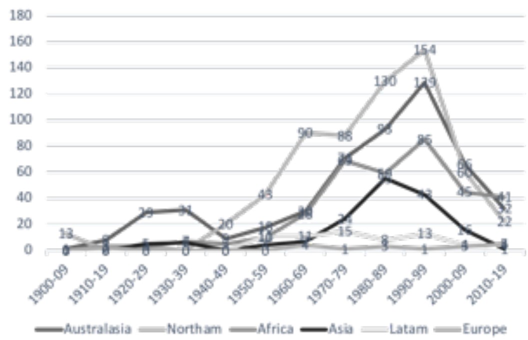

Figure 1. Number of CWB releases per continent. Note Europe and South America show remarkably lower levels than North America, Australasia and Africa.

CWB to control American and Eurasian weeds in the 70s and 80s, but the research teams were mostly dismantled in the 90s. On the other hand, CWB research for South American plants invasive in the US, Australia and South Africa remains pretty well developed in Argentina, Brazil and Mexico. FuEDEI, together with other local scientists, are trying to rekindle interest for CWB in South America by organizing the next International Symposium of Biological Control of Weeds, and CWB to control American and Eurasian weeds in the 70s and 80s, but the research teams were mostly dismantled in the 90s. On the other hand, CWB research for South American plants invasive in the US, Australia and South Africa remains pretty well leading several projects of national and international interest (see previous reports).

The reason why CWB is not easily accepted in Latin America, compared to other continents, is baffling. A revision of over 3,500 publications of the last 30 years on weed management from all over the world reveals that scientific perception of CWB has been consistently favourable during the last decades, with lower acceptance levels in Latin America (Fig 2). On the other hand, Latin America, essentially Mexico, Brazil and Argentina, have provided more biocontrol agents to the world than any other continent, followed by Europe (Fig. 3), contradictorily, the regions with lowest CWB application (Fig. 2).

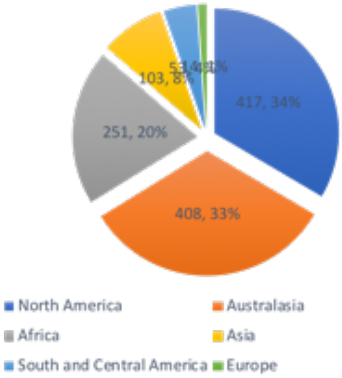

Figure 2. Releases (in numbers) of CWB agents around the world.

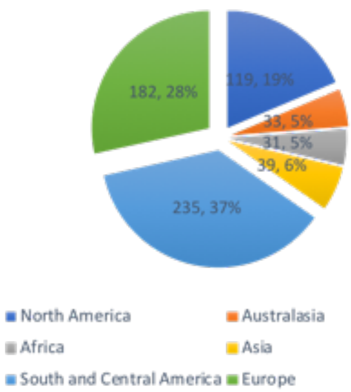

Figure 3. Origin (in numbers) of the CWB agents used around the world.

However, support for CWB is not equal across scientific disciplines. In theoretical ecology and multidisciplinary scientific journals there were 1.75 favourable articles for each unfavourable one. This quotient increased to 2.9 for management and agriculture journals, and 13.6 for taxonomy and experimental biology journals. Results suggest that ecological academia is more hostile toward weed biocontrol than scientists in applied and experimental biology.

The number of weed biocontrol agents released around the world has fallen drastically in the last twenty years, or so, suggesting that the exotic weed management community could be disconnected from the opinion of science. This slump is probably related to lower investment levels, shorter financial cycles, difficulties to obtain collection and export permits, and lower tolerance for less than categorical specificity test results. However, it is possible that administrators may be influenced by negative reports from the less specialized but more widely read multidisciplinary scientific journals, where biocontrol is frequently criticized.

Biological control of Palo Verde

by Fernando Mc Kay

Parkinsonia aculeata L. is a thorny leguminous shrub native to the hot and dry regions of North, Central and South America. In Australia, where the plant was accidentally introduced, its dense thorny thickets are injurious to the environment and agriculture. A biological control program was initiated by Australia in 1983, with extensive surveys for biological control agents conducted in North America, resulting in the release into Australia of three insects, a mirid (Hemiptera) and two bruchids (Coleoptera). None of them have caused significant population-level impacts, so native-range surveys were recommenced in 2008 in South America. Since then, two species of Geometridae, *Eueupithecia cisplatensis* Peout and *E. vollonoides* Hausmann have been released from Argentina and Paraguay. At the moment, CSIRO researchers are tidying up some loose ends of the *Eueupithecia* project but are also evaluating the suitability of another Parkinsonia biocontrol agent for Australia.

Field surveys conducted in North-central Argentina on *P. aculeata* L. between 2008 and 2011 revealed the presence of the stem-galling midge *Neolasioptera aculeatae* Gagné (Diptera: Cecidomyiidae) (Fig. 4; Fig. 5). The biological attributes of *N. aculeatae* and its restricted field host range suggests that it could be a promising biological control agent for *P. aculeata*. Building on FuEDEi's previous *N. aculeatae* work, CSIRO (Commonwealth Scientific and Industrial Research Organisation) and FuEDEI are initiating a co-operative biological control program on *Parkinsonia*. FuEDEI researchers will conduct field collection of *N. aculeatae* and characterization of the biology of *N. aculeatae* and its associated parasitoid community, field host-associations of *Neolasioptera,* and development of a rearing methodology to establish a colony of *N. aculeatae*.

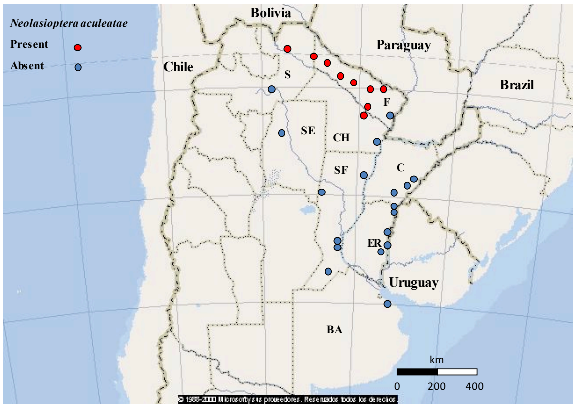

Figure 4. Main collecting sites of *N. aculeatae* in Argentina.

Figure 5. *Neolasioptera aculeatae* galls on *Parkinsonia aculeata*.

Invasive crazy ants

by María Belén Fernández, Luis Calcaterra

Nylanderia fulva is an ant native to South America which has spread throughout Central and North America during the past few years. Its introduction affects native ant biodiversity and is a nuisance in urban environments and agriculture. *N. fulva* originated in South America, presumably in the Rio de la Plata basin, being native to Argentina and Brazil. Due to recent systematic changes, the species list of the genus had to be revised, and the range of *N. fulva* determined for the southern distribution of the genus (Fig. 6a). The objective was to assess the native and introduced ranges, addressing questions such as: Where did introduced populations come from? What characteristics make this species so invasive? Does it have natural enemies which could be potential biocontrol agents?

The province of Misiones, in northeastern Argentina, holds the southernmost region of the Atlantic Forest, a biodiversity hotspot, and hosts both *N. fulva* and its parasitoid phorid specie *Pseudacteon convexicauda*. Phorids were found throughout this province in several campaigns, but only in two other sites within the whole native range.

During collection surveys in that province, we also found one population of the old world species, *Paratrechina longicornis,* ant with dubious previous records in Argentina, thus probably being the first record in this country. The longhorn crazy ant has spread across six continents and lives in some 120 countries, including Brazil and Paraguay. We believe this population might have arrived to Argentina through a commercial route from Brazil, where the ant has been registered in 2010 (Fig. 6b). As this is considered a tramp species, its discovery should raise concern for local biodiversity.

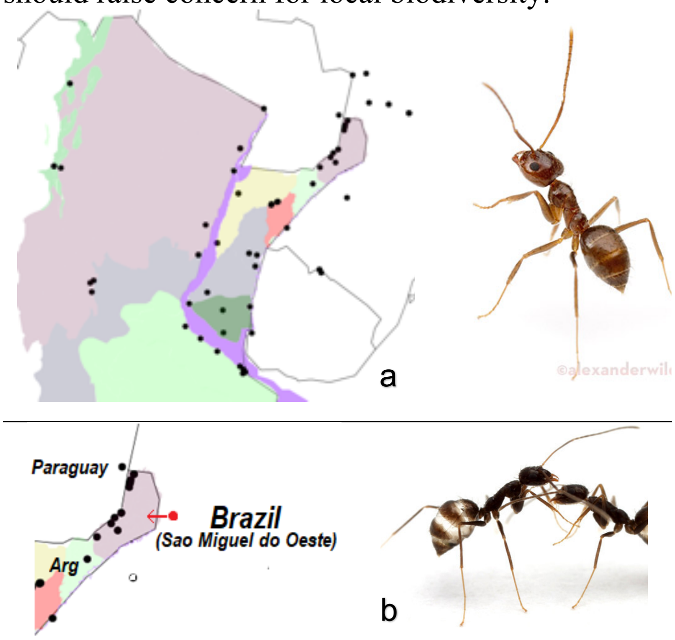

Figure 6.a) Colonies of *Nylanderia fulva* found throughout its native range in Argentina, Brazil, Uruguay and Paraguay; b) Possible introduction route of *Paratrechina longicornis* from Brazil into Argentina (red). Photographs of workers from each species by Alex Wild.

After studying a fragment of the COI gene in the surveyed populations, we discovered a possible cryptic species in the *N. fulva* taxon, together with a clade that could correspond to one of the other previously registered species for Argentina (Fig. 7). We have found no obvious morphological or behavioral differences between the two cryptic clades so far, so a species delimitation study could help to better understand the status of the species. Additionally, phorid flies known to be specific of *N. fulva* (*P. convexicauda*) have been found in nests of both clades (*N. fulva* and its cryptic species). Morphology of the closely related species *N. silvestrii*, native to Argentina, is being studied from museum specimens with morphometric analyses and scanning electron microscopy in order to solve the identity of the samples grouped in the sister clade to *N. fulva* and *N. steinheili* (Fig. 7, uppermost clade).

As usual in biological invasions, *N. fulva* experienced a genetic bottleneck during its invasion to the US lowering its genetic diversity by 60%. Besides, population structure analyses performed for invasive populations showed that *N. fulva* has a supercolonial structure, colonies being of up to 2000 km wide (Eyer et al. 2018), but for the native populations this might not be the case. Multicoloniality seems to be the form of population organization in the native range, which is being confirmed with both population structure analyses and aggression tests performed between distanced colonies in Argentina, where colony sizes were only up to 5 km. Several, up to hundreds, of reproductive queens, each being mated with a single male, were found in the introduced range, whereas only a maximum of nine queens and dozens of males were found in native nests. Preliminary results show that there is a mixed reproductive system, involving sexual and clonal reproduction as well as presence of diploid males in populations of Argentina. Edward Vargo, our cooperator at University of Texas A&M, is working in resolving the reproductive system of this species through analyzing microsatellite and rad-seq *loci*.

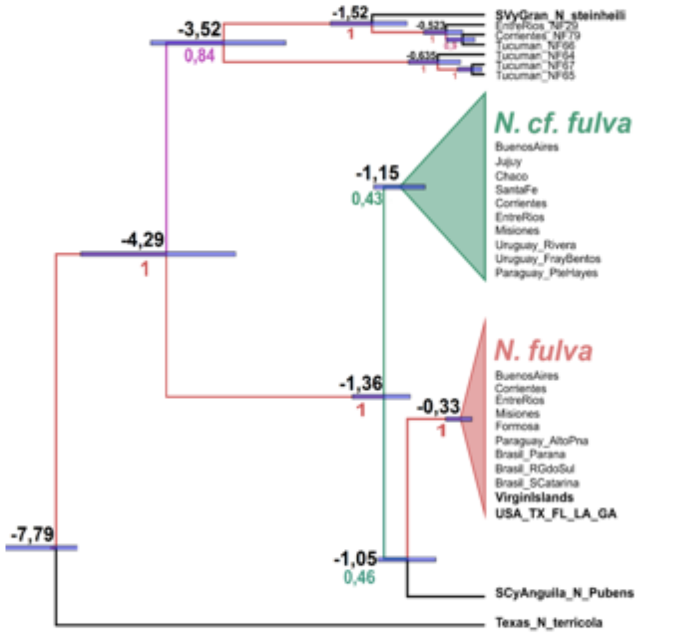

Figure 7. Phylogenetic relationships between *Nylanderia* species in southern South America. Clades represented in red and green correspond to *N. fulva* and its cryptic species, respectively. Samples in the uppermost clade grouped with *N. steinheili*.

Grant from Perez-Guerrero Trust Fund (PGTF) of the United Nations

by by Alejandro Sosa and Tomás Righetti

A consortium formed by FuEDEI and the National University of Hurlingham, based in Hurlingham, Buenos Aires province, Argentina, was granted US$20,000 by the United Nations to support education in biological control at high school level. The National Autonomous University of Mexico (UNAM, based in Mexico City), and the Center for Biological Control of the Rhodes University, Grahamstown, South Africa, also participated in the initiative. The project is called "A proposal for capacities development and strengthening in the educational communities to manage invasive species in aquatic systems: the Argentine, Mexican and South African experience in biological control and biodiversity". The objective of the project is to organize awareness workshops based on research for the participating institutions, and prepare a good-practices manual on water preservation through biological control. This is an integrated proposal for research, science and community participation for the preservation of biodiversity.

Figure 8. FuEDEI scientist Alejandro Sosa speaking in front of a crowd at a science fair. The slide says “Are scientists and educators communicated?”.

Opening lecture of the biological control session during Argentine Meeting of Parasitoidologists

by Laura Varone

Laura Varone gave a lecture to open the biological control session during the VII Argentine Meeting of Parasitoidologists, held in the city of Santa Rosa, La Pampa, on September 11-13, 2019 (Fig. 9). The conference was titled "Historical and current situation of biological control through the use of parasitoids in Argentina".
Biological control is one of the disciplines used in integrated management programs. It uses natural enemies of pests to reduce their impact, reducing the use of chemicals to control them. Parasitoids amount to about 80% of natural enemies used in biological control programs. In Argentina there have been records of biological control projects since the beginning of the 20th century; however, excessive pesticide reliance as from the middle of last century, led to an interruption of such programs. At first, the programs were essentially classical biological control -reuniting an introduced pest with their natural enemies from the place of origin-. Argentina provided parasitoids to Africa, Australia, Canada and the United States; and in turn, it received numerous species for the control of exotic pests. By the end of 1990, a change of course was considered to reduce pesticide use. This, coupled with the growth of ecological awareness in recent decades and the demand for organic products, are generating new impulse for old control alternatives, thus, biological control is once again positioned as a sustainable tool beneficial to the environment. There are currently numerous research groups in Argentina that carry out their activities mainly related to the exploration, development and application of all biological control techniques, i.e. augmentative, inundative, conservation, classical and neoclassical. The current state of the biological control programs in Argentina was explored, as well as the export of natural enemies to other countries around the world. In this context, the four main projects on biological control of arthropods developed at FuEDEI were presented: research and exportation of decapitating flies to control imported fire ants in U.S.; discovery of cryptic species of mymarid egg parasitoids during exploration for natural enemies to control glassy wing sharp shooters in U.S.; host range studies, interspecific interactions and exportation of encyrtid parasitoids of *Harrisia* cactus mealybug, pest of columnar cactus in Puerto Rico; and survey, rearing, host specificity and exportation of a braconid wasp to control cactus moth in U.S.

Figure 9. Attendees of the VII Argentine Meeting of Parasitoidologists, Santa Rosa, La Pampa, Argentina.

Australian Biological Control Laboratory - ABCL

Exploration for biological control agents of roundleaf toothcup

by Matt Purcell, Brad Brown, Phil Tipping and Greg Wheeler

Roundleaf toothcup, *Rotala rotundifolia* (Lythraceae) is an emerging weed in ponds and irrigation channels in south Florida and has the potential to be a more serious aquatic weed than both hydrilla, *Hydrilla verticillata* and Alligator Weed, *Alternanthera philoxeroides*. A project to find potential biological control agents in Asia is being supported by the Florida Fish and Wildlife Conservation Commission (FWC). *Rotala rotundifolia* grows on the margins of water bodies and across open water. The open water mats can be so dense as to support the weight of land managers. The submerged and emergent forms of the plant differ morphologically. Stem fragments can become new plants and disturbance through manual or mechanical control can exacerbate infestations. The plants also form viable seeds enhancing dispersal and persistence at field sites. In the native range this species occurs in Southeast Asia and into cooler regions of China and therefore it has the potential to spread north and westward in the US.

Thus far, biological control agents under evaluation have included *Altica spp.* beetles (Coleoptera: Chrysomelidae) from mainland China as well as tip binding moth species. More recently a gall forming weevil (Fig. 10) was discovered in a stream in Hong Kong at sites in streams in Hong Kong. The galls were found on fully submerged plant stems in which were found larvae, pupae and fully formed adults. Identification by Dr Miguel A. Alonso-Zarazaga (Museo Nacional de Ciencias Naturales) and Dr Wang Zhiliang (Museum of Beijing Forestry University) has revealed they are likely Nanophyinae weevils, the same or similar to species known from *Rotala* in Xishuangbanna in Yunnan, southern China. We plan to ship these weevils to quarantine facilities at the USDA ARS IPRL in Fort Lauderdale, Florida.

Figure 10. Galls formed by Nanophyinae weevils on roundleaf toothcup in Hong Kong.

Earleaf Acacia and indigenous engagement

by Ryan Zonneveld, Jon Schatz, Christine Goosem, Melissa Smith and Carey Minteer

Earleaf Acacia, *Acacia auriculiformis*, is native to northern Australia, Papua New Guinea and Indonesia. It was introduced into Florida in 1932 and is becoming a serious invasive weed in the US. A biological control project has been funded by the South Florida Water Management District and the FWC.

In June 2019, staff of ABCL and US researchers, Melissa Smith (USDA ARS IPRL) and Carey Minteer (UFL), surveyed field sites across Arnhem Land in the Northern Territory, Australia. Arnhem Land is a culturally significant region for the indigenous Australians and permission from the traditional owners is required for traveling and conducting research in this region. Genetic analysis of *A. auriculiformis* discovered that this region is also likely the genetic origin of the invasive *A. auriculiformis* in Florida, making it a particularly significant area to survey for potential biocontrol agents. During this survey we were escorted and allowed access to restricted areas by the local indigenous rangers who have a deep understanding of the native flora and fauna. They lead us to stands of *A. auriculiformis* where we shared our knowledge of insect collection and identification. This field survey proved valuable for initiating collaboration and building trusting with the traditional owners of this region to facilitate future surveys for biocontrol agents.

In October 2019, Jon Schatz and Ryan Zonneveld visited the Tiwi Islands, 80 km north of Darwin, where they met with the local indigenous rangers. Being isolated islands, we are hoping to discover a different suite of insect herbivores on the native *A. auriculiformis*. After returning to Darwin, Jon and Ryan also visited rangers from Arafura Swamp and Warddeken on the mainland to provide feedback and results from a survey conducted in these areas earlier in the year.

The traditional owners are passionate about protecting the biodiversity and cultural heritage of their lands. As researchers, ABCL acknowledges and respects this position and by sharing our knowledge through conversation and visual presentations of the benefits of using insects as biological control agents, we have been granted access to areas for surveys. Staff had the opportunity to work with both men and women rangers who had a keen eye for insects and insect damage and we look forward to further collaboration with these communities.

Figure 11. Indigenous rangers, ABCL staff, and US Scientists conducting surveys of Earleaf Acacia on traditional lands in the Northern Territory, Australia.

European Biological Control Laboratory - EBCL

Molecular phylogeny for *Trissolcus* parasitoids associated with Brown Marmorated Stink Bug

by Marie-Claude Bon and Fatiha Guermache

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae), (Fig. 12), has emerged as a very damaging invasive insect pest in North America in the 1990s. Native to eastern Asia, this highly polyphagous pentatomid (>120 different host plants) is spreading rapidly worldwide, notably through human-mediated activities. In the mid-Atlantic region of the USA, BMSB has become one of the most significant pests in apple production, causing >$37 million in losses in 2010 (Fig. 13). Aside from being a severe agricultural and horticultural pest, BMSB has become well known as a nuisance pest, as adults often invade human-made structures to overwinter inside protected environments. Among the management strategies developed to limit the spread and prevent the economic impact of BMSB, classical biological control shows strong potential, and research on its egg parasitoids including *Trissolcus* species has increased accordingly. While most research is focusing on foreign exploration in the native range, experimental assessments of host ranges in quarantine, and surveys to assess parasitism in the field, present knowledge on *Trissolcus* parasitoids is inadequate to identify species and to facilitate their molecular diagnostics for detection and monitoring survey in the field. In this context, a sound taxonomy together with a molecular phylogeny of *Trissolcus* that includes all species reared from live BMSB eggs is critical to the future of the biological control of BMSB. Through a collaboration between EBCL and taxonomists of the Florida Department of Agriculture and Consumer Services and the USDA/ARS Systematic Entomology Laboratory of the Smithsonian Institution, Washington DC, and entomologists/biocontrol practitioners of BMSB of USDA/ARS Beneficial Insects Introduction Research, Newark, DE and CABI (Commonwealth Institute of Biocontrol, Switzerland), a molecular phylogeny based on several markers that includes all species of *Trissolcus* reared from live BMSB eggs is published for the first time. In this study, the species names of 20 *Trissolcus* with corresponding molecular data are provided with the highest level of confidence possible, given that the specimens were identified in the context of the most recent and thorough taxonomic treatments and with direct comparison to primary types. This study will facilitate molecular diagnostics for detection of these *Trissolcus* and monitoring survey in the field. This work has been published and maybe downloaded at the following link: https://jhr.pensoft.net/article/39563/

Figure 12. Adult BMSB, a pest that attacks valuable crops and plagues homeowners (photo by Matt Rourke).

Figure 13. BMSB damages to apple (photo by Matt Rourke).

Bagrada hilaris, an exception among phytophagous stinkbugs

by Mélanie Tannières and René Sforza

In most insects, the digestive system consists of the foregut, the midgut and the hindgut. The majority of plant-sucking species represented by diverse stinkbugs of the Pentatomorpha infraorder harbor crypt-associated symbiotic bacteria in the posterior midgut. The midgut of Pentatomorpha is often divided into four distinct sections. It has been suggested that the M1 section serves for transient food storage and digestion, the M2 and M3 perform food digestion and absorption, and the M4 is specialized for harboring the symbiotic bacteria. Usually a single bacterial species dominates in the crypts of the midgut fourth section. Recently, a constricted region between the M3 and the M4 regions of the midgut has been identified in different stinkbug species harboring midgut crypts. This constricted region selectively allows passing of the specific bacterial symbiont but blocks food fluid indicating that the M4 region does not contribute to the food flow. However, it has been shown in different stinkbug species that the symbiont is vertically transmitted by different post-hatch transmission mechanisms and that elimination of the symbiont causes retarded growth and increased mortality of the host, indicating important biological roles of the symbionts.

Surprisingly, dissection of the alimentary tract of *Bagrada hilaris* revealed a very short midgut M4 section and the absence of midgut crypts (Fig. 14) whereas *Eurydema rugosa*, a member of the tribe Strachiini to which *Bagrada hilaris* belongs possess the crypts which are filled with a gammaproteobacterial symbiont. Additionally, no other symbiotic organs such as bacteriocytes or bacteriomes have been observed in *Bagrada hilaris*. Midgut crypts are conserved across diverse stinkbug families, and some symbiotic bacteria are stably maintained across closely related host species. Therefore, it has been hypothesized that this symbiotic organ evolved in an ancestor of the Pentatomorpha in association with the evolutionary transition from predatory to plant sucking lifestyle. It would also imply the loss of midgut crypts in *Bagrada hilaris*, raising questions about the evolutionary advantage of the absence of midgut crypts in some phytophagous stinkbugs. In any case, absence of a symbiotic organ and of a specific bacterial symbiont did not prevent *Bagrada hilaris* from becoming an important agricultural pest and rapidly spreading across the world.

Furthermore, insects that feed on restricted diets, such as phloem sap, usually host symbiotic microorganisms which can detoxify plant toxins and provide essential nutrients. Thus, despite the absence of crypts in the posterior midgut of *Bagrada hilaris*, some bacteria colonize the anterior and posterior midgut, which could play a crucial role in nutrition and other biologically important traits. Further work will assess the bacterial communities associated with *Bagrada hilaris* to gain better understanding of their role on the fitness of their host, which may provide new possibilities for biological control.

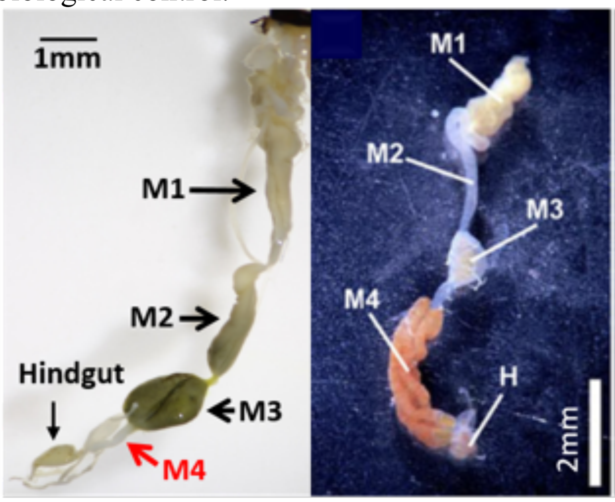

Figure 14. Comparison of dissected alimentary tracts of adult females of *Bagrada hilaris* (left) and *Eurydema rugosa* (right), both belonging to the pentatomid tribe Strachiini. M1 midgut first section, M2 midgut second section, M3 midgut third section, M4 midgut fourth section. Crypts are absent in *B. hilaris* and present in the fourth section of *E. rugosa*.

Optimizing use of braconid parasitoids in pest management of the olive fruit fly *Bactrocera oleae*: role of visual cues for parasitoid foraging and application of the augmentorium concept to the olive production system (sabbatical research conducted at EBCL, France)

by Gaylord Desurmont and Nicholas Manoukis

As part of EBCL's sabbatical program, Dr. Nicholas Manoukis, a USDA-ARS Research Leader from the Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center in Hilo, Hawai'i, conducted a collaborative research project during a three-month period (July- September 2019) in Montpellier, France. Dr. Manoukis' project focused on improving the use of braconid parasitoids against the olive fruit fly *Bactrocera oleae*, the main pest of olives worldwide. A number of parasitoids have been studied over the years for their potential as biological control agents, and two of them are currently reared at EBCL Montpellier: *Psyttalia lounsburyi* and *Psyttalia ponerophaga*. *P. lounsburyi* has been approved for introduction in the United States as a biological control agent and released in California since 2005, where it has successfully established. *P. ponerophaga*, on the other hand, is still being evaluated for its host specificity. Working with these two species as well as with native parasitoids of *B. oleae* found in France, Dr. Manoukis investigated two main experimental questions: 1) Are visual cues important for parasitoids to locate infested olives?, and 2) Is if possible to apply the augmentorium concept to the olive production system?

To investigate the first question, two experiments were conducted. In the first one, larvae placed under disks of four different colors (blue, black, green, and red) were presented to parasitoids in a choice-test setting, and the frequency of visits of each disk was recorded for one hour. The disks were made of water agar colored with commercial food colorants, and the disks were inserted into a background also made of water agar. Third instar larvae of the host used for mass-rearing the parasitoids, *Ceratitis capitata,* were placed between the water agar and a thin layer of parafilm (Fig. 15). Adult parasitoids were then placed in the box for one hour. The arena was monitored via a video camera, and videos were later analyzed to reveal the preferences of the parasitoids. An attraction to a particular color may relate on host fruit (and by extension host fly) preference. In the second experiment, parasitoids were placed in a similar arena with larvae placed between a layer of water agar and a thin layer of parafilm either with black visual cues (round shapes emulating the shape of olives) or without visual cues in a non-choice setting. The parasitoids (15 females + 5 males) were left in the arenas for 4 hours. At the end of the experiment, the larvae were recovered and monitored until emergence of adult flies or parasitoids in order to compare parasitism rates in presence of absence of visual cues. These experiments have been conducted with both *P. lounsburyi* and *P. ponerophaga* and are currently ongoing with several generations of parasitoids. Parallel experiments are now being initiated in Hilo to enable comparisons with parasitoids used in the biological control of tephritids other than the olive fruit fly.

To investigate the second question, interviews were conducted with olive growers from the Montpellier area to understand the modalities of olive production and the pertinence of applying the augmentorium concept to this system. This is critical because this method is an adaptation of the technique, originally developed in Hawaii by ARS, and successfully applied in La Reunion by CIRAD. The augmentorium is a field device where infested, non-marketable fruits (i.e. olives infested by *B. oleae*) can be deposited at the site of production (i.e. an olive field): the augmentorium is built with a mesh material that is small enough to prevent pests emerging from infested fruits to escape from the augmentorium, but large enough to allow the parasitoids that may emerge from infested fruits to exit the augmentorium and disperse in the field. In other words, the augmentorium is a low-cost and easy to use device to enhance the densities of parasitoids present in cultivated fields and increase their impact on pest populations. In the case of olive production in France, timing plays a critical role: olive fruit flies have several successive generations in olives as they mature from August to December, and most of the harvests occur in October-November. In order to have an impact on *B. oleae* populations over the course of a production season, the augmentorium needs to release natural enemies early enough to impact *B. oleae* populations before the main harvest. Early olive varieties could be potentially used in order to produce parasitoids early in the season and protect late-season olives. Another critical factor in designing an augmentorium is mesh size and shape. An experiment was conducted at EBCL to test the efficacy of different meshes within the size range of 0.5-2 mm. Olives naturally infested with *B. oleae* were collected in olive fields in the south of France and were placed in boxes with different mesh sizes (Fig. 16). Each box was placed in a larger box, and the boxes were monitored to count the number of flies and parasitoids that remained trapped or managed to escape through the mesh (Fig. 17). This experiment was also conducted under quarantine conditions with the two species of *Psyttalia* parasitoids and *C. capitata*. Experiments are still ongoing but the first results clearly indicate that some mesh sizes are inappropriate for building an augmentorium, as they let too many flies pass through. Surprisingly, some mesh sizes that were seemingly smaller than the size of an adult olive fruit fly still let some flies escape: newly-emerged adults apparently took advantage of their soft cuticle to squeeze themselves through small mesh openings. Parasitism by native parasitoids was disappointingly low in the field, and larger-scale testing will be needed in order to collect enough parasitoids to run these tests next year.

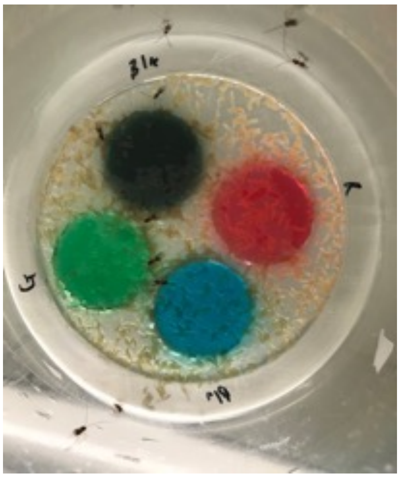

Figure 15. Bioassay arena used to test the effects of visual cues on the foraging behavior of parasitoids of the olive fruit fly in a choice-test setting. The color disks and the background were made of water agar. Host larvae were placed between the water agar and a thin layer of parafilm. Parasitoids were released in the arena for one hour, and the frequency of visits of each color disk was recorded. Water agar allowed host larvae to remain contained but also active during the time of the experiment, which increased their attractiveness to parasitoids (photo by Nicholas Manoukis).

Figure 16. Boxes with olives naturally infested by *B. oleae* used for mesh size tests for the augmentorium sub-project. Five different mesh sizes were tested (photo by Marie Roche).

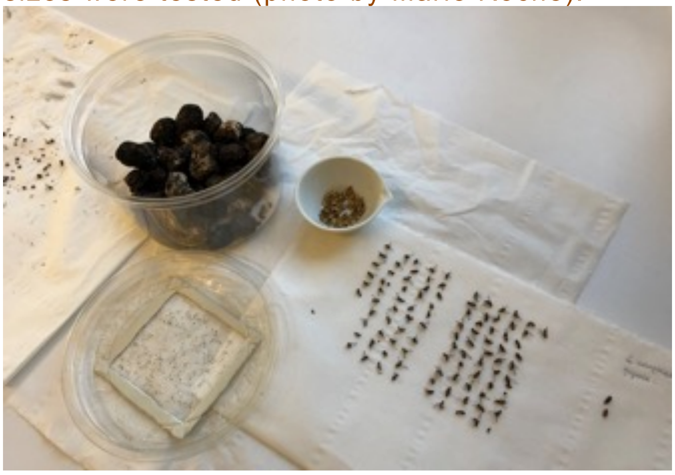

Figure 17. Collection of olive fruit flies after a mesh size test. Flies that managed to escape the container by going through the mesh and flies that remained trapped inside the container were counted, as well as the total number of pupae found in the box. Native parasitoids that were present inside and outside the box were also counted (photo by Gaylord Desurmont).

Both of the project components above and some additional side studies have been ongoing and extended after Dr. Manoukis' departure late September by EBCL scientists and technical staff. He will return to France in future summers to continue collaborative research with EBCL.

Development of the sterile insect technique (SIT) for biological control of disease-vector *Phlebotomus* sand flies in Greece (sabbatical research conducted at EBCL, Greece)

by Seth C. Britch and Alexandra Chaskopoulou

As part of EBCL's sabbatical program, Dr. Seth Britch, a USDA-ARS Research Scientist from the Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, conducted a collaborative research project during summer 2019 in Thessaloniki, Greece. Through this sabbatical research, Dr. Britch and EBCL built essential foundations for an unprecedented control effort using the sterile insect technique against sand fly vectors of disease. Phlebotomine sand flies are important vectors of pathogens harmful to humans such as *Leishmania* species parasites which cause leishmaniasis in at least 80 countries, with more than 1.5 million new cases and 20,000 deaths per year. Traditional adult and larval integrated vector management (IVM) techniques targeting sand flies have not been shown to be effective at reducing transmission to humans, and evidence is emerging that Phlebotomine sand flies may be evolving resistance to pesticides. The sterile insect technique (SIT) is a proven technology that leverages mating behavior in natural insect populations through mass release of sterilized males that may overwhelm female choice to outcompete fertile males, significantly reduce the number of viable eggs, and potentially crash the population. SIT applied to Phlebotomine sand fly population control could greatly enhance IVM strategies with a sustainable, low cost, and environmentally sound solution that does not contribute to the evolution of resistance.

The foundation is being prepared for an operational sand fly SIT control program over the three year sabbatical project by establishing a productive sand fly colony (years 1-3), optimizing male sand fly irradiation for sterility (year 2), and investigating the competitive ability of irradiated males in semi-field behavioral studies (year 3). The project is spread over three years in part to leverage natural sand fly population seasons each year for ongoing development of a field-collected colony of anthropophilic sand fly species such as *Phlebotomus perfiliewi* or *P. simici*. Initiation of a productive sand fly colony from field-collected specimens was the focus of summer 2019 work. This colony of local origin will be the eventual source of males for irradiation and field release against wild conspecifics. In parallel, we initiated a backup colony of *Phlebotomus papatasi* from specimens supplied by the Walter Reed Army Institute of Research (WRAIR), Silver Spring, Maryland, that could supply large numbers of male sand flies for baseline irradiation and behavioral studies through years 2-3.

Colony initiation from field-collected sand flies is classically difficult and required frequent, repeated collections coupled with intensive processing of specimens to isolate blood fed and potentially gravid females, induce oviposition, and transfer eggs to larval substrates. These efforts in summer 2019 yielded our first lab-reared adults, validating the process and generating valuable insight for optimizing and expanding the process in summer 2020, to include novel approaches to handling field caught individuals and calibrating the artificial blood-feeding protocol to local species. EBCL and Dr. Britch's development of a backup colony derived from WRAIR cultures similarly generated data to adjust rearing conditions attuned to local climate to increase colony productivity. Looking forward, establishment of a strong local-origin sand fly colony and maintenance of the WRAIR colony will continue. The team is on track to develop techniques to separate immature male sand flies and conduct irradiation of males in summer 2020.

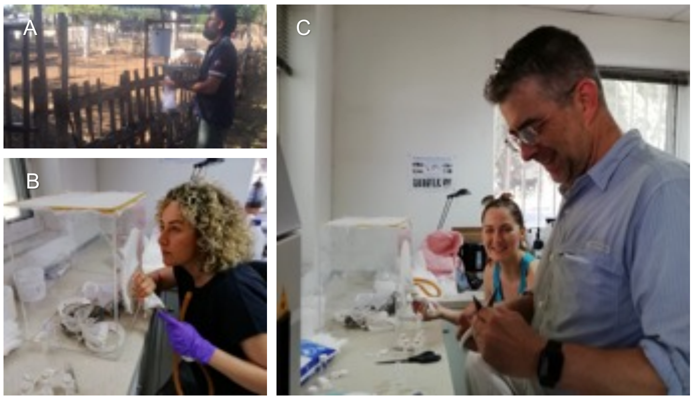

Figure 18. A) Michalis Miaoulis collecting sand flies in the field; B) Samiye Demir sorting blood fed females from the field; C) Alexandra Chaskopoulou and Seth Britch preparing vials.


Figure 19. (clockwise from top left) Non-blood-fed wild *Phlebotomus* females awaiting artificial blood feeding; artificial membrane-based blood feeding apparatus; adult blood fed *Phlebotomus* female from Litsas Farm (Thessaloniki, Greece); and late instar *Phlebotomus papatasi* from Walter Reed strain (backup colony). Photos by Seth Britch, Samiye Demir, Michalis Miaoulis.

Sino-American Biological Control Laboratory - Sino-ABCL

Determining the distribution and natural enemy complex of the Roseau Cane Scale in China

by Chenxi Liu

Roseau cane, *Phragmites australis*, is a critical species in the marshes of the Mississippi Delta. The roots protect riverbanks from erosion, mitigate sedimentation, provide important habitat for waterfowl and fish, and protect the interior from storm surges. However, in recent years, large areas of *Phragmites* cane have been dying in the lower Mississippi River Delta. These diebacks coincide with heavy populations of the invasive Roseau cane scale (RCS), *Nipponaclerda biwakoensis* (Hemiptera: Aclerdidae), which live on the stems of the reed and remove sap from the plant, leading to plant weakening and death. The dieback of Roseau cane is of significant environmental and economic concern.

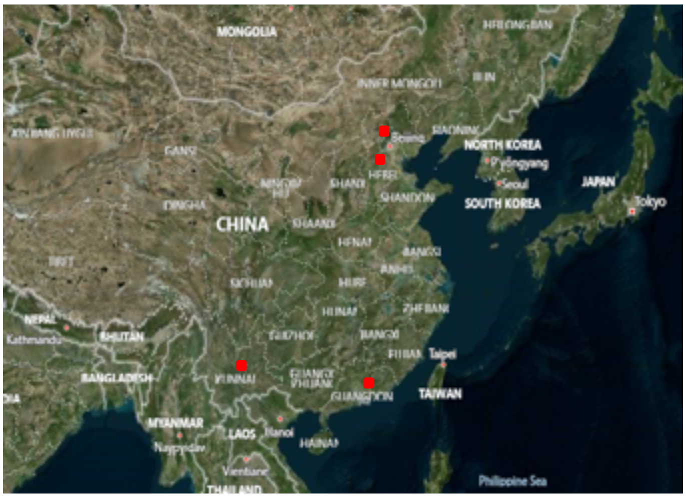

Figure 20. *Nipponaclerda biwakoensis* study sites in China (red).

Prior research has shown that natural enemies play an important role in suppressing populations of RCS in their native range. RCS is native to Asia and is known from reed wetlands. In the native range RCS is reported to have 3 to 6 generations per year. In China, the first-generation nymphs are observed feeding on new shoots in May and later settle under the new leaf sheaths of the reed stems. According to a standardized protocol for sampling and collection, research scientists from the Sino-American Biological Control Laboratory together with Chinese local collaborators completed three collections in Beijing, Hebei, Guangdong and Yunnan (Fig. 20) over the season – one collection in July, one in August, and one in September. Great progress has been made in the first year of this project, in which we aimed to determine the distribution and natural enemies of Roseau Cane Scale (*Nipponaclerda biwakoensis*) in China. The sites where phragmite stands grew along the river were chosen for survey and collections (Fig. 21). In the first year, survey was conducted according to the standardized protocol for sampling host plants, scale density, and the associated parasitoid community across the four provincial sites (Fig. 22).

Figure 21. Selecting the sites where phragmite stands grow along the river.

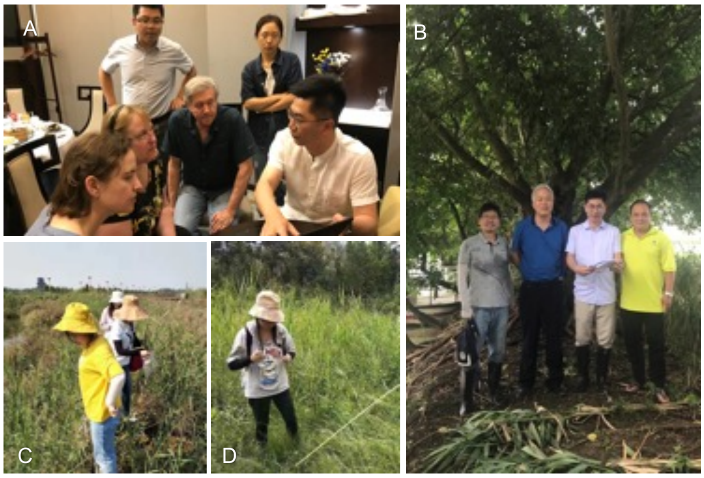

Figure 22. (A) Kim Hoelmer, Juli Gould, Hannah Broadley, Chenxi Liu and local collaborators discuss field survey plans. (B, C, & D) Sino-ABCL Research scientists and local collaborators conduct field survey and sampling.

The primary mission of the Sino-ABCL is to search for, identify, and evaluate potential natural enemies of pest insects, weeds, and plant diseases that affect Chinese and the 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. 22). 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 over 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.

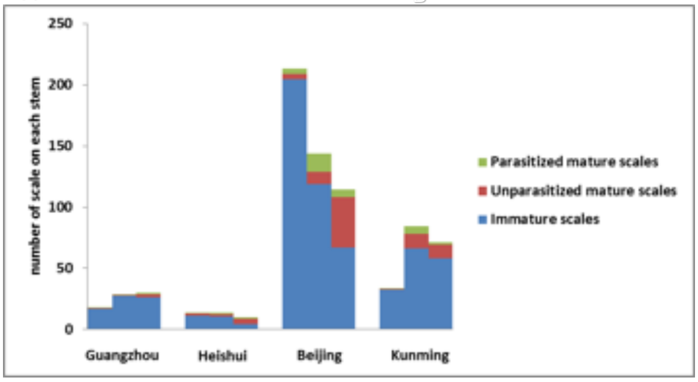

Figure 23. Investigation of scale and parasitism in the four survey sites in China.

Figure 24. *Nipponaclerda biwakoensis* -male.

Figure 25. *Nipponaclerda biwakoensis* gravid female.

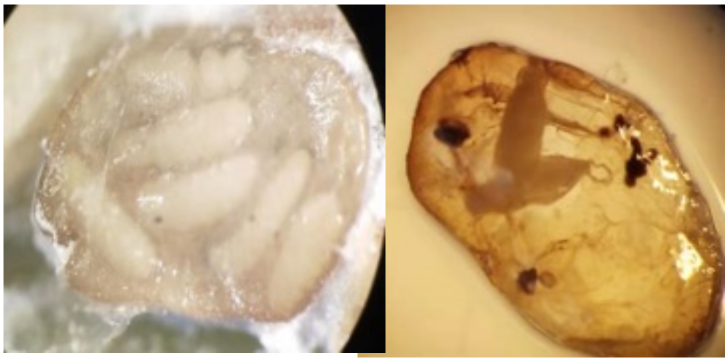

Figure 26. Parasitized scale.

In the first collection in China, an average of 66.67 percent of stems that were sampled had scale and the stands had an average of 24 live stems and 2 dead stems in the 0.25 m^2 quadrant by analyzing the survey data from the above four sites. Each infested stem had, on average, 66.33 scales that were immature, 3.32 scales that were mature. In the second collection in China, an average of 76.67 percent of the stems that were sampled had scale and the stands had an average of 19 live stems and 3 dead stems in the 0.25 m^2 quadrant. Each infested stem had 55.64 immature scales, 6.28 unparasitized scales, and 5.43 parasitized scales. Compared with the results of the first collection, there were fewer immature scales and more mature scales. In the third collection in China, the results showed that an average of 68.33 percent of stems that were sampled had scale and the stands had an average of 18 live stems and 3 dead stems in the 0.25 m^2 quadrant. As the reed withered, the populations of the scales on the reeds declined. Each infested stem had, on average, 56.44 scales and most were immature. Of these scales, 14.79 scales were unparasitized scales, 2.70 parasitized scales. The number of immature scales, unparasitized mature scales, and parasitized mature scales on each stem in the four sites in China was shown in Figure 23. Phragmite scale at different developmental stages (Figs. 24 & 25) and parasitized scale (Fig. 26) were found across the collections. Some holes on the reeds indicated where parasitoids emerged (Fig. 27). Emergence of the parasitoids is still ongoing for these collected samples, and up to the present four species of parasitoids have been identified in the survey sites in China (Fig. 28).

Figure 27. Holes on phragmite stands for wasp emergence.

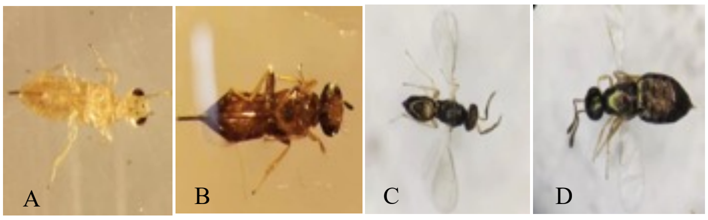

Figure 28. Parasitoids identified in the collections in China. A. *Astymachus japonicus* B. *Boucekiella depressa* C. *Aprostocetus sp.* D. *Platencyrtus parkeri.*

Recent Publications by ABCL

Elsayed, A.K., Wheeler, G.S., Purcell, M., Dyer, K., Zhang, J. & Tokuda, M. 2019. A new *Schizomyia* species (Diptera: Cecidomyiidae) inducing flower bud galls on Chinese tallow tree *Triadica sebifera* in its native range. Applied Entomology and Zoology 54 (4) 429-436.

McCulloch, G.A., Purcell, M.F., Harms, N.E., Grodowitz, M.J., Zhang, J., Sun-Hee, H. & Walter, G.H. 2019. Molecular screening of herbivorous flies collected from *Hydrilla verticillata* across China and Korea, setting up hypotheses for further exploratory surveys and tests. Biological Control 138 https://doi.org/10.1016/j.biocontrol.2019.104051.

Purcell, M., Harms, N., Grodowitz, M., Zhang, J., Ding, J., Wheeler, G., Zonneveld R. & Desmier de Chenon, R. 2019. Exploration for candidate biological control agents of the submerged aquatic weed *Hydrilla verticillata*, in Asia and Australia 1996. BioControl 64 (3) 233-247.

McCulloch, G.A., Makinson, J.R., Zonneveld, R., Purcell, M.F., Brookes, D.R., Gurdasani, K., Lake, E.C., Raghu, S. & Walter, G.H. 2019. Scrutinizing biological control survey data from the native range, the phylogeny and *Lygodium* fern host associations of Musotiminae moths. Biological Control 134 123-129.

Recent Publications by EBCL

Benante, J.P., James, F., Lawrence, K., Fansiri, T., Pongsiri, A., Ponlawat, A., Chaskopoulou, A. 2018. A comparative study of mosquito (Diptera: Culicidae) and sand fly (Diptera: Psychodidae: Phlebotominae) sampling using dry ice and chemically generated carbon dioxide from three different prototype CO2 generators. Journal of Economic Entomology. 112:494-498. https://doi.org/10.1093/jee/toy319.

Smith, L. 2018. Influence of host plant on wind dispersal by an eriophyid mite, *Aceria salsola*. In: Hinz, H.L., et al. (eds.), XV International Symposium on Biological Control of Weeds, Engelberg, Switzerland. 27-31 August 2018, p. 135.

Weyl, P., Cristofaro, M., Smith, L., Schaffner, U., Vidovic, B., Petanovic, R., Marini, F., Stutz, S. 2019. Eriophyid mites and weed biological control: does every silver lining have a cloud? XV International Symposium on Biological Control of Weeds, Engelberg, Switzerland. 27-31 August 2018, pp. 9-11.

Winkler, D.E., Bon, M., Cristofaro, M., Sforza, R., Marini, F., Auge, M., Kashefi, J., Smith, L. 2019. From genomic analysis of the rapid colonization of the exotic Sahara mustard (*Brassica tournefortii* Gouan 1773) in the United States to the search of its natural enemies. XV International Symposium on Biological Control of Weeds, Engelberg, Switzerland. 27-31 August 2018, p. 38.

Ben Ghabrit, S., Bouhache, M., Birouk, A., Bon, M. 2019. Macromorphological variation of the invasive Silverleaf nightshade (*Solanum laeagnifolium* Cav.) and its relation to climate and altitude in Morocco. Revue Marocaine des Sciences Agronomiques et Vétérinaires. 7(2), 234-251.

Jourdan, M., Thomann, T., Kriticos, K., Bon, M., Sheppard, A., Baker, G. 2019. Sourcing effective biological control agents of conical snails, *Cochlicella acuta*, in Europe and north Africa for release in southern Australia. Biological Control. 134, 1-14. https://doi.org/10.1016/j.biocontrol.2019.03.020.

Cuny, M.A., La Forgia, D., Desurmont, G., Glauser, G., Benrey, B. 2019. Role of cyanogenic glycosides in the seeds of wild Lima bean, *Phaseolus lunatus*: Defense, nutrition or both? Planta. 1-12. https://doi.org/10.1007/s00425-019-03221-3.

Porretta, D., Fotakis, E.A., Mastrantonio, V., Chaskopoulou, A., Michaelakis, A., Kioulos, I., Weill, M., Urbanelli, S., Vontas, J., Bellini, R. 2019. Focal distribution of diflubenzuron resistance mutations in Culex pipiens mosquitoes from Northern Italy is associated with the history of insecticide applications. ACTA TROPICA. 193:106-112.

https://doi.org/10.1016/j.actatropica.2019.02.024.

Martel, G., Auge, M., Talamas, E., Roche, M., Smith, L., Sforza, R. 2019. First laboratory evaluation of *Gryon gonikopalense* (Hymenoptera: Scelionidae) as potential biological control agent of *Bagrada hilaris* (Hemiptera: Pentatomidae). Biological Control. 135(48-56). https://doi.org/10.1016/j.biocontrol.2019.04.014.

Kerdellant, E., Thomann, T., Vitou, J., Sheppard, A., Guisto, C., Simonot, O., Sforza, R. 2019. The double life cycle of *Lepidapion argentatum*, a potential candidate for the biological control of French Broom. Biocontrol Science and Technology. 10.1080/09583157.2019.1597334.

Williams III, L.H., Pointurier, O., Deschodt, P. 2019. Affect of food provisioning on survival and reproductive success of the olive fruit fly parasitoid, *Psyttalia lounsburyi*, in the field. Arthropod-Plant Interactions. https://doi.org/10.1007/s11829-019-09684-1.

Fotakis, E.A., Giantsis, I.A., Avgerinou, A., Kourtidis, S., Agathaggelidou, E., Kapoula, C., Dadakou, G., Vontas, J., Chaskopoulou, A. 2019. Identification of Leishmania species in naturally infected sand flies from refugee camps, Greece. Emerging Infectious Diseases. 25(2):361-364. https://dx.doi.org/10.3201/eid2502.181359.

Bitume, E.V., Moran, P.J., Sforza, R. 2019. Impact in quarantine of the galling weevil *Lepidapion argentatum* on shoot growth of French broom (*Genista monspessulana*), an invasive weed in the western U.S. Biocontrol Science and Technology. 29(7):615-625. doi: 10.1080/09583157.2019.1573417

Desurmont, G.A., Kerdellant, E., Pfingstl, T., Kreiter, S., Auger, P., Tixier, M. 2019. Mites associated with egg masses of the viburnum leaf beetle *Pyrrhalta viburni* (Paykull) on Viburnum tinus L. Acarologia. 59(1):57-72. https://doi.org/10.24349/acarologia/20194311.

Sabbatini Peverieri G, Talamas E, Bon MC, Marianelli L, Bernardinelli I, Malossini G, Benvenuto L, Roversi PF, Hoelmer K (2018) Two Asian egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: *Trissolcus mitsukurii* (Ashmead) and *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae). Journal of Hymenoptera Research 67: 37-53. https://doi.org/10.3897/jhr.67.30883

Jaffuel, G., Puza, V., Hug, A., Meuli, R., Nermut, J., Turlings, T., Desurmont, G., Campos-Herrera, R. 2018. Molecular detection and quantification of slug parasitic nematodes from the soil and their hosts. Journal of Invertebrate Pathology. 160:18-25. https://doi.org/10.1016/j.jip.2018.11.005.

Giantsis, I.A., Chaskopoulou, A. 2018. Broadening the tools for recognizing sand fly breeding habitats: A novel molecular approach for the detection of Phlebotomine larval DNA in soil substrates. ACTA TROPICA. 190:123-128. https://doi.org/10.1016/j.actatropica.2018.11.008.

Stahl, J., Tortorici, F., Pontini, M., Bon, M., Hoelmer, K.A., Marazzi, C., Tavella, L., Haye, T. 2019. First discovery of adventive populations of *Trissolcus japonicus* (Ashmead) in Europe. Journal of Pest Science. 92:371-379. https://doi.org/10.1007/s10340-018-1061