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OBCL Newsletter

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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.
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

European Biological Control Laboratory - EBCL

EBCL during COVID-19

by Dawn Gundersen-Rindal

What a difference one year makes. Last year during the first week of April, 2019, the European Biological Control Laboratory (EBCL) celebrated its 100th anniversary with centennial events held in Thessaloniki, Greece and Montpellier, France. This year in early March, 2020, facing worldwide pandemic, EBCL staff in France and Greece went into confinement to prevent contagion and spread of the coronavirus COVID-19.

EBCL necessarily postponed two events we had anticipated hosting in Montpellier: a Bi-Annual Quadripartite Biological Control Meeting and a Toothpick Project® Biocontrol Workshop, both scheduled for late March, 2020. EBCL's dedicated technical staff coordinated work on a rotating and staggered schedule so that insects and plants reared for research or release were able to be maintained under strict quarantine conditions.

European countries took necessary precautions and made sacrifices to slow the spread of and mitigate the impact of COVID-19. After greater than two months of telework, on June 8th in Greece and June 22nd in France, EBCL staff without underlying health risk issues were allowed to return to work and to resume limited local travel. EBCL adopted new health and safety protocols requiring mask wearing, sanitation, and isolation of employees to prevent spread of infection. Starting on July 1st, 2020, EBCL scientists will be permitted to travel for exploration and collection purposes within several countries of the European Union with several restrictions. Travel outside of the European Union will be unlikely until

there is worldwide resolution to the COVID-19 crisis, which we hope may be sometime in 2021.

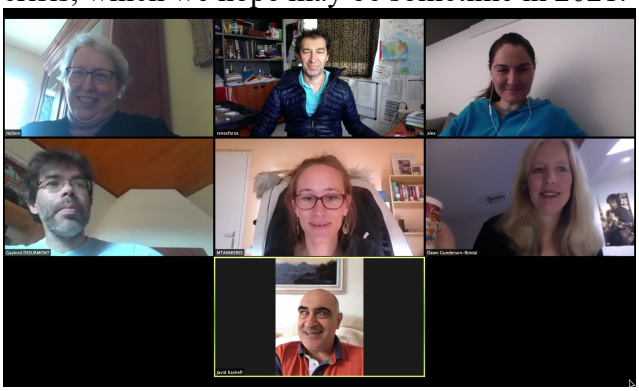

Figure 1. European Biological Control Lab scientists meeting virtually during confinement.

The olive psyllid, *Euphyllura olivina* (Costa) (Hemiptera, Psylloidea, Liviidae)

by Marie-Claude Bon, Marie Roche, Fatiha Guermache, Ludovic Manaargadoo and Mélanie Tannières

The olive psyllid, *Euphyllura olivina* (Costa) (Hemiptera, Psylloidea, Liviidae), (Fig. 2 and 3) naturally occurs throughout the Mediterranean Basin where it attacks the flower blossoms and soft growing tissue of olive trees, causing significant fruit yield reduction by up to 60%. First reported on olives in Orange County, in California, in 2007, the olive psyllid is nowadays present on the California coast between Monterey and San Diego. Although the olive psyllid is not currently considered an economic pest in California because it has not yet reached the olive production region of California's Central Valley, a classical biological control approach is already undertaken in California jointly by the California Department of Food & Agriculture (CDFA), USDA-ARS-Albany, Berkeley and Riverside Universities in California. The major primary parasitoid found in foreign collections in the native range and possible biological control agent is *Psyllaephagus euphyllurae* (Masi) (Hymenoptera: Encyrtidae)(https://doi.org/10.3390/insects11030146). Marie Roche contributed with Charlie Pickett from CDFA to collecting olive psyllids and parasitoids in Spain, identifying and shipping materials to California. As for species identification or confirmation of pests and natural enemies, DNA barcoding has recently become an important and routine practice in many EBCL projects, olive psyllid populations collected in Spain were barcoded and compared primarily with a French colony of the olive psyllid reared by Arnaud Blanchet (EBCL) and a Californian population collected by Charlie Pickett. Although preliminary, the barcode results evidenced for the first time that the olive psyllid collected in Spain is made of two distinct maternal lineages, but one comprising the Californian population, the EBCL colony and another population in Italy (Fig. 4). As potentially each lineage might interact differently with *P. euphyllurae*, the distribution of the "Californian" lineage, and potentially the identification of new lineages throughout this region must be further investigated, as this information will guide foreign exploration efforts based on more comprehensive understanding of evolutionary areas of origin and the evolution of parasitoid specificity to olive psyllid.

Figure 2. Olive psyllid adult.

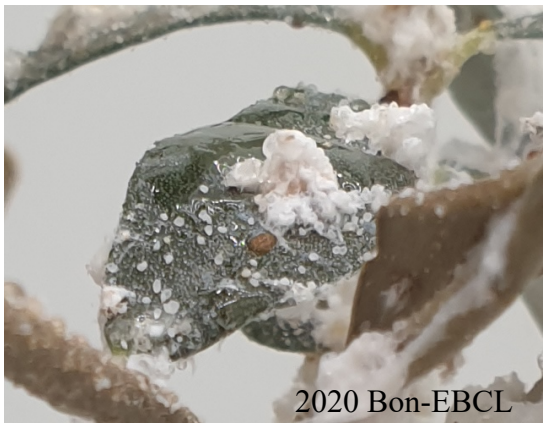

Figure 3. Olive psyllid nymphs and adults produce a white, waxy secretion which can cause premature flower drop during heavy infestations.

Figure 4. Phylogenetic relatedness between few olive psyllid populations including the Californian ones and congeners.

Parasitoid Biocontrol Agents of Olive Fruit Fly:

Part 1: Effects of artificial rearing conditions on parasitoid effectiveness

by Gaylord Desurmont, Mélanie Tannières, Marie-Claude Bon, Nicholas Minoukis, and Xingeng Wang

The olive fruit *Bactrocera oleae* (Diptera: Tephritidae) has been the main pest of olives since ancient times and has now spread worldwide, impacting the major areas of production of table olives and olive oil. Native to parts of Africa and Asia, and naturally present in the Mediterranean basin, this pest was reported in California in 1998, where it spread rapidly and became a serious threat to the olive industry (Daane and Johnson, Biological Control; 2010). Classical biological control has rapidly become a major focus of research for sustainable management of this pest, leading to the evaluation of several parasitoids from Africa and Asia (Sime, Daane et al. Bulletin Entomol. Research 2007, Daane, Sime et al. 2008, Wang, Johnson et al. BioControl; 2011). Two of these parasitoids, *Psyttalia lounsburyi* and *Psyttalia humilis* (Hymenoptera: Braconidae) have been introduced to California where *P. lounsburyi* successfully established (Daane, Wang et al. Biological Control, 2015). A third parasitoid, *Psyttalia ponerophaga*, native to Pakistan, has elicited interest as a possible additional biological control agent (Sime et al., 2007). It is a larval-pupal endoparasitoid that attacks larvae of its main host *B. oleae* within olives. Rearing of this parasitoid started in 2010 at EBCL with specimens initially collected in Pakistan and is still ongoing. A special rearing protocol was developed and optimized for *Psyttalia* parasitoids (Chardonnet, Blanchet et al., J. Applied Entomol; 2019). In this setup, parasitoids are reared on an alternative host, larvae of the Mediterranean fruit fly *Ceratitis capitata*. Larvae of *C. capitata* are presented to parasitoids on a polystyrene sphere coated with a thin layer of food and covered with Parafilm®. To stimulate oviposition, olives are attached to the sides of the polystyrene ball. From 2010 to late 2019, there were a total of 76 generations of *P. ponerophaga* reared using this method (Fig. 5).

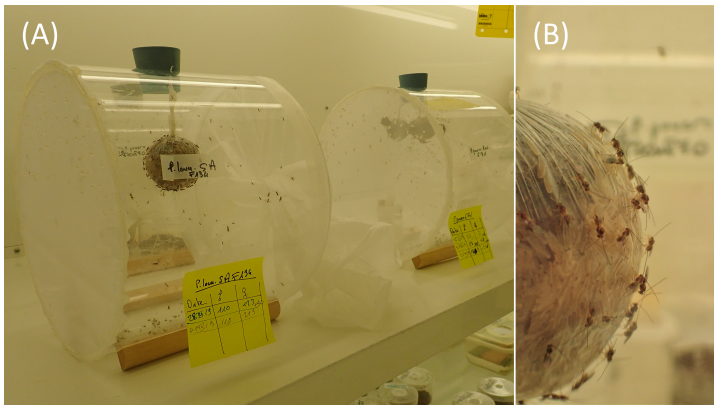

Figure 5. (A) Rearing method of *Psyttalia* parasitoids at EBCL. The ball inside the cage contains *C. capitata* larvae covered by a parafilm layer. (B) Close-up of parasitoids foraging on the parafilm layer.

The rearing method developed at EBCL has several key advantages: the alternative host *C. capitata* can be reared on artificial diet all year long, and the artificial support can expose large numbers of larvae at once to parasitoids, which is ideal for mass production. However, rearing a parasitoid on an alternative host and on an artificial support brings up the question of whether or not these rearing conditions impact characteristics of the parasitoid such as its effectiveness at parasitizing its main host *B. oleae* and its host specificity (Canale and Benelli, J. Pest Science; 2012). Prolonged rearing under artificial conditions may select for ecological and behavioral traits detrimental for the value of the parasitoid as a biological control agent (Bautista and Harris, Entomol Experimentalis et Applicata; 1997). A study was designed in 2019 at EBCL to test the hypothesis that EBCL's *P. ponerophaga* laboratory colony differs both in host specificity and effectiveness at parasitizing *B. oleae* compared with wild populations of *P. ponerophaga*. To test this hypothesis, freshly field-collected *P. ponerophaga* individuals obtained from Pakistan were compared with EBCL's lab-reared *P. ponerophaga* colony in tests of host specificity and parasitism effectiveness. Host specificity tests were conducted with two non-target species present in North America: the black cherry fly *Rhagoletis fausta*, and the Cape-ivy gall fly *Parafreutreta regalis* (Diptera: Tephritidae) under choice and non-choice conditions. For the parasitism effectiveness tests, field-collected parasitoids and lab-reared parasitoids were presented commercial olives infested with 10-day old *B. oleae* larvae for 48h, and parasitism rates were calculated based on the number of flies and parasitoids emerging from the olives. Field-collected parasitoids

were then separated in two groups: one reared on *B. oleae*-infested olives for three generations, and another reared on *C. capitata* using EBCL's protocol for three generations. Parasitism effectiveness was measured and compared to EBCL's lab colony at each generation. All bioassays were conducted in EBCL's quarantine facility under temperature-controlled conditions (22 ± 2 °C, 16L: 8D, 30-40% RH) (Fig. 6).

Figure 6. Illustration of the bioassays. Left: rectangular containers used for host specificity choice-tests; right: cylindrical containers used for host specificity non-choice tests and parasitism effectiveness tests.

Complete results of the host specificity tests will be known at the end of *Rhagoletis fausta*'s obligate diapause period (summer 2020). Results of the parasitism effectiveness showed that there was no overall effect of rearing on parasitism: the lab-reared colony of *P. ponerophaga* (Population "R") was as effective at attacking *B. oleae* inside olives as the field-collected parasitoids reared on olives for three generations (Population "O") and field-collected parasitoids reared on *C. capitata* for three generations (Population "CC"). In other words, there was no short-term or long-term negative consequences of EBCL's rearing protocol on the ability of *P. ponerophaga* to attack its main host inside olives. It is important to mention that the population maintained on olives quickly declined (both in numbers and in parasitism effectiveness) after one generation whereas the population maintained on *C. capitata* remained healthy. The difficulty of keeping fresh olives through the year certainly impacted the quality of the *B. oleae* larvae exposed to parasitoids and consequently affected parasitoid fitness in the population "O".

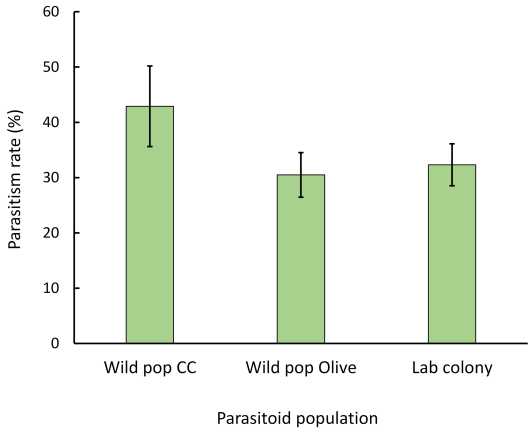

Figure 7. Effects of rearing conditions on parasitism rate of *P. ponerophaga*. Wild pop CC: field-collected individuals reared on *C. capitata* for 3 generations. Wild pop olive: field-collected individuals reared on olives for 3 generations. Lab colony: individuals reared on *C. capitata* for 76 generations.

The results of the effectiveness of parasitism tests plead in favor of using EBCL's rearing protocol to maintain a continuous *P. ponerophaga* rearing (Fig. 7). Several aspects of the ecology of *P. ponerophaga* that may play a role in its realized value in the field as a biological control agent, such as its dispersal ability and long-distance foraging behavior, were not investigated during this study and could potentially be the focus of future studies.

Part 2: Persistence of two Wolbachia variants in parasitoids following their release and establishment in California

by Mélanie Tannières and Marie-Claude Bon

Laboratory colonies [Kenya origin (K) and South Africa origin (SA)] of one biological control agent of the olive fruit fly (*Psyttalia lounsburyi* (Hymenoptera: Braconidae)), have been mass-reared in the EBCL quarantine facility as described in (1) and shipped to our co-operators in California [primarily California Dept. of Food and Agriculture (CDFA)] for over a decade. From 2005 to 2015, more than 60,000 individuals of *P. lounsburyi* have been released in different counties by our cooperators in California who confirmed the widespread establishment of the biocontrol agent in coastal California. Genotyping of the populations established in California prior 2015 evidenced that they are predominantly of SA origin while they represented only 3.9% of the total number of female parasitoids released. It also showed that the SA mostly established in central coast of California and

K established along the southern coast although individuals from SA origin were also recovered at these locations (Fig. 8).

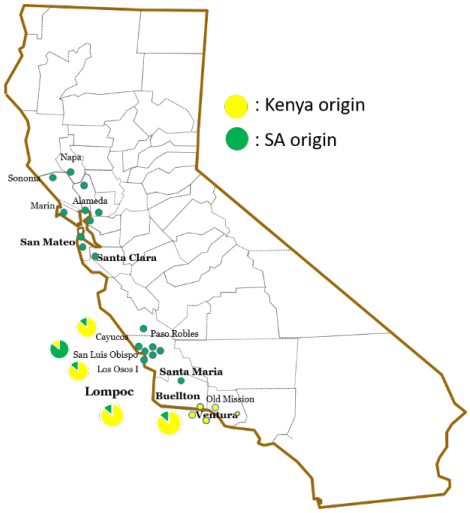

Figure 8. Origin of *Psyttalia lounsburyi* populations recovered in California

For decades, the effects on host reproduction of α-proteobacteria *Wolbachia* have drawn attention and been increasingly investigated, particularly in parasitoids. At the onset of the biocontrol program, in 2005, *Wolbachia* infection status was assessed for both K and SA quarantine colonies, revealing the presence of two genetically different *Wolbachia* variants (type I and II). In K, a double infection (type I and II) was observed in 96% of individuals, only 4% being mono-infected with type I. In SA, a single infection by type II was detected in 50% of the tested individuals, with others being uninfected.

In 2018, the Wolbachia infection status in K and SA quarantine colonies was tested revealing the following pattern: a stable double infection in K, and the spread of type II in SA with more than 95% of parasitoids being infected (Fig. 9). Interestingly, the same evolution of pattern of *Wolbachia* infection status was observed in recovered Californian populations. Results suggested that *Wolbachia* infection status was not dramatically affected by 1) new environment into which populations were released and/or 2) host switching (from medfly to olive fruit fly). Results tended to support the idea of *Wolbachia* providing a fitness advantage to its host. However, different *Wolbachia* variants can confer diverse benefit/cost ratios and we hypothesized that infection by type I or coinfection of type I and II might have played a role in limiting the establishment of K in California. To explain the better establishment of SA in California, factors such as differences in life history traits or the microbiome which could play a crucial role on fitness and adaptability cannot be excluded. Thus, microbiomes associated with these biocontrol agents are being investigated via 16S rRNA-based Next Generation Sequencing methods.

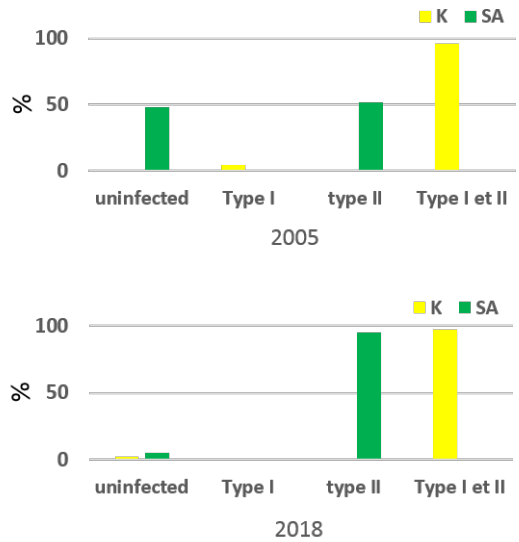

Figure 9. Evolution of *Wolbachia* infection status of the two quarantine colonies

New exciting collaborations at EBCL Thessaloniki, Greece

by Alexandra Chaskoploulou

Two new collaborations have recently been initiated at EBCL, Thessaloniki, Greece :

(1) New EBCL – American Farm School Master thesis project. The first joint MSc research project between EBCL and the American Farm School, Thessaloniki, Greece, was initiated in 2020. Androniki Christaki, a graduate student at Perottis College of the American Farm School, will be working under the supervision of Drs. Kiki Zinoviadou (AFS) and Alexandra Chaskopoulou (EBCL) on examining and quantifying the nutritional aspects of mosquitoes (protein, lipid, and mineral content) in various species and developmental stages to explore the application of mosquitoes as feed in the field of agriculture. Considering the growing population in the world and the increasing demand for livestock production, edible insects have been gaining ground as animal feed. Research has shown that edible insects provide the necessary amounts of proteins, fatty acids, amino acids and micronutrients to feed livestock (or even humans). Commonly used insects as animal feed include grasshoppers, crickets, cockroaches, termites, scales, beetles, flies, fleas, bees, wasps and

ants. Some mosquito species have also been used as an alternative food source in aquaculture because of their rich protein content, high availability, and high population densities. However, it is still not a common practice to use mosquitoes as feed and there is almost no empirical evidence on the nutritional benefits of mosquitoes due concerns relating to pathogen transmission. Androniki will address these gaps by assessing the nutritional value and microbiological risk of mosquito species that are produced in high numbers in irrigated rice fields.

(2) EBCL & the VectorNet Consortium. **Vector**Net, a consortium of experts in medical and veterinary entomology, is a joint initiative of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), which started in May 2014, and is now in its second iteration (2019 - 2023). **Vector**Net provides scientific support to ECDC and EFSA for their assessments on vectors and vector-borne disease risk, prevention and control. In December 2019, EBCL entered into an agreement with the **Vector**Net consortium to perform a literature review on vector control practices and strategies against West Nile virus (WNV) vectors. West Nile virus infection represents a serious burden to human and animal health worldwide because of the capacity of the virus to disperse fast and adapt to a large variety of environments causing unforeseen and large epidemics. In the absence of effective vaccination, vector control remains the primary line of defense in preventing and containing WNV outbreaks. Under the umbrella of **Vector**Net a comprehensive technical report on vector control strategies for WNV was produced by Alexandra Chaskopoulou (EBCL) in collaboration with **Vector**Net's coordinators Drs. Marietta Braks and Wim van Bortel and under the technical guidance of ECDC's medical entomologist Dr. Olivier Briet. The report collated information on WNV vector management experiences across the world under specific operational contexts and identified gaps in knowledge and capacities relating to effective WNV vector management practices.

Australian Biological Control Laboratory - ABCL

ABCL COVID-19 Response

by Matthew Purcell

With the increase in cases of COVID-19 in Australia in March 2020, ABCL staff, in conjunction with the CSIRO biological control of weeds team co-located at our laboratory in Brisbane, implemented a two-team plan to ensure the security of valuable laboratory and quarantine insect cultures and plant stocks from Australia and overseas. Each team was separated spatially and temporarily to avoid a scenario where all biological control staff members could be instructed to isolate at home all at the same time due to the possibility of cross contamination. Thankfully, infections rates in Australia have been extremely low and no infections have been recorded on site which is usually attended by almost 1000 State and Federal Employees. During the lockdown period from March through May, insect colonies were successfully maintained, and some progress could be made with laboratory research on assessing biological control agents. No new research, field surveys or travel were permitted by CSIRO during this period to reduce staffing on site. As restrictions ease our staff presence is increasing and local field surveys within the state of Queensland permitted. Our collaborators in South Korea, China and India where also in lockdown with restrictions currently continuing for field surveys in China and India. As restrictions ease in these countries, we will rely heavily on their cooperation to conduct field surveys in our absence given we are unlikely to be permitted to travel internationally at least until 2021. We will steadily increase of field research across Australia as restrictions ease though extreme care is required near indigenous communities susceptible to infection by COVID19. A return to work, four stage plan is being implemented for all CSIRO research staff with completion expected in September 2020.

ABCL Data Management Strategies

by Jeff Makinson

Every biocontrol project will involve multiple collections at numerous locations and will ultimately record many thousands of insect, plant and pathogen specimens with details of their interactions. That's without even scratching the

surface of host-specificity, life history and the myriad of other lab-generated data. But unlimited data (surprisingly!) doesn't equate to unlimited happiness, unless its generation and interrogation work in your favor.

At ABCL we have over 30 years of data (a lot of it hand-written) in many historical formats from terrestrial, aquatic and pest biocontrol projects in 14 countries taken at around 2000 unique field sites. The focus of our data management has been on field-collected data as this is arguably the source of the best information on plant-insect interactions and the area where time-efficiency is the most crucial. We created a single multi-user relational database to capture all field-collected data from all projects in a uniform manner, whilst allowing for the unique requirements of sometimes vastly different project needs. All information on locality, plant phenology, environmental parameters, host plants, insect specimens and their species interactions are searchable to provide data relevant to our research goals (Fig. 10).

Recently we have tried to eliminate much of the double-handling of data as is practicable. None of us has time, when back in the lab, to re-enter field data into the database. To this end we developed a phone/tablet app (Fig. 11) that works offline (ideal for remote fieldwork and work in foreign countries) and captures all the same field data with the addition of taking photos, recording geospatial information and linking to mapping apps. Collection data can then be uploaded to Google Sheets when Wi-Fi or mobile data is available and, from there, integrated into the database with a minimum of repeated effort. Hardcopy data will always have a role in our work, since you can never control when and where an observation will prove important, but our goal is to minimize the amount of double data entry through a tailored digital strategy that will enhance our data management and improve our ability to ask and answer relevant questions of the data we generate.

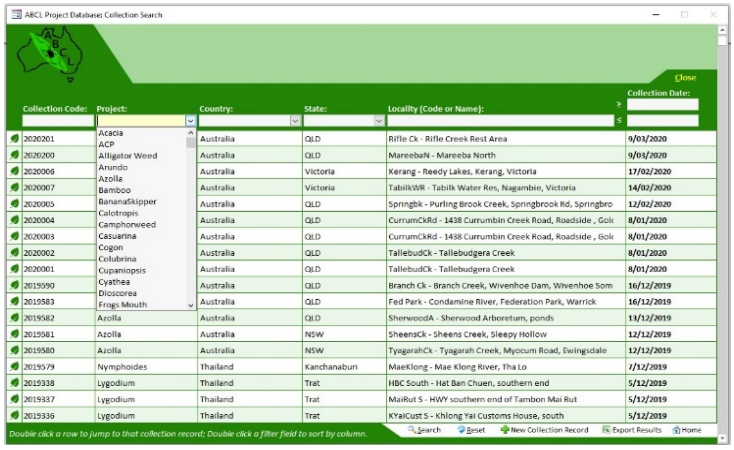

Figure 10. Screen grab of one of the forms within the database

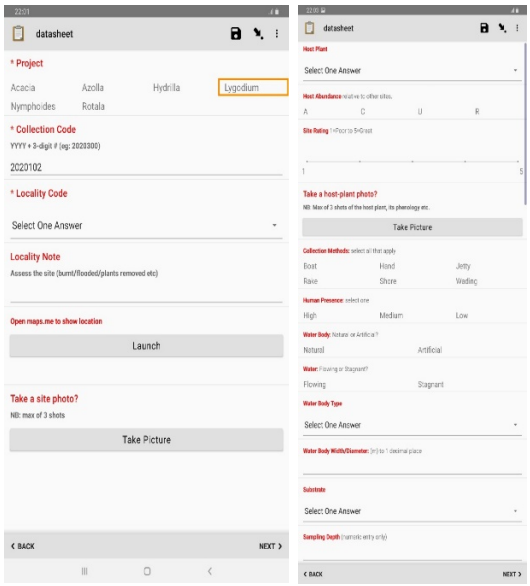

Figure 11. Screen grab of the mobile app for field data

Foundation for the Study of Invasive Species - FuEDEI

Biocontrol Agent for Cactus Moth

By FuEdei authors & Willie Cabrera Walsh

The unintended arrival of *Cactoblastis cactorum* to Florida and its expansion in North America represent a threat to *Opuntia*-based agriculture and natural ecosystems in USA and Mexico. *Apanteles opuntiarum* (Fig. 12) is an endoparasitoid that attacks *C. cactorum* and has potentiality as biocontrol agent due to its specificity, wide distribution and occurrence. Laboratory rearing methods utilizing excised cladodes for *C. cactorum* as host larvae of *A. opuntiarum* were developed, but need a continuous cactus supply and risk

microorganisms compromising the rearing. For parasitoids, host associated olfactory cues are the most important signals leading to host location, including sex pheromones, odor of host metabolic sub products like feces, and chemicals emitted by the attacked plant. Because little attention has been paid to behavioral differences of parasitoids in presence of hosts reared on artificial diet, the objectives of the present work were to determine the effect of the meridic diet for *C. cactorum* on parasitoid ability and behavior, and determine if prior experience influences the response of *A. opuntiarum* to hosts. Parasitism rates and sex ratio were evaluated using cladodes or meridic diet as food source for larvae (Fig. 13). Also, behavioral experiments evaluated the effect of prior experience (larvae or frass from larvae fed on cactus or diet) on host searching, encounter, and attack on host larvae. We found that parasitism behavior of *A. opuntiarum* was negatively affected by the use of meridic diet to feed host larvae. The most influential factor that elicited excitatory behavioral responses was the type of substrate used, not the prior experience. However, a slight effect was observed due to the previous encounter with host frass, indicating a relevant stimulus for host location and acceptance. We recommend for laboratory mass rearing procedures of *A. opuntiarum* adding frass from cactus fed larvae and a piece of cactus during parasitoid mating to maximize parasitism rates and percentage of female offspring.

Figure 12. *Apanteles opuntiarum* attacking first instar larvae of *Cactoblastis cactorum* in a prickly pear plantation.

Figure 13. Larvae of *Cactoblastis cactorum* reared on cactus (left) or artificial diet (right).

Species of *Heteroperreyia* (Hymenoptera: Pergidae) feeding on Brazilian peppertree, *Schinus* spp. (Anacardiaceae), including a new species

by Andrés F. Sánchez-Restrepo and Fernando Mc Kay

The Brazilian peppertree (*Schinus terebinthifolia* Raddi, Anacardiaceae) is a Neotropical species whose native range extends along the Atlantic coast of Brazil from Paraiba south to Rio Grande do Sul states, west to northeastern Argentina and adjacent Paraguay and Uruguay. This species has been introduced to many countries (e.g., Australia, South Africa, USA) as an ornamental and is now recognized as one of the worst weeds worldwide.

Biological control programs for *S. terebinthifolia* began in Hawaii in the 1950's and 1960's, and continued in Florida during the 1980's and 1990's. More recently, surveys in the native range of *S. terebinthifolia* came up with many new species of potential biological control agents. Among these, the leaf-feeding thrips *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae), which has been recently released in the USA. Another potentially host-specific species is the defoliating sawfly *Heteroperreyia hubrichi* Malaise. Native to Argentina, Brazil, Uruguay, and Paraguay (Schmidt and Smith 2006), *H. hubrichi* has been found feeding on the leaves of *S. terebinthifolia*.

While conducting surveys of plant use under natural conditions in the native range of *S. terebinthifolia* in southern Brazil in March 2018, a new species of *Heteroperreyia* was discovered. The new species was described by Dave Smith (USDA-ARS SEL) as *Heteroperreyia kava* based on morphological and genetic analyses (Smith et al. 2019 https://doi.org/10.4289/0013-8797.121.4.704) (Fig. 14). "Káva" is wasp in Guarani, the language

of the indigenous people of the type locality. Since sawfly larvae often store peptides that may be toxic to wildlife that feeds on them, the larvae of *H. kava* could pose a threat to native and domesticated fauna in the invaded range. Consequently, specificity testing, tests will also have to evaluate the toxicity factor before release in the field.

Figure 14. *Heteroperreyia kava* larvae and lateral view of head.

Field surveys in Argentina and Paraguay in search of natural enemies of the invasive weed *Passiflora foetida*

by Mariano Maestro and Willie Cabrera Walsh

Researchers from FuEDEI have conducted field surveys in Northern Argentina and Paraguay, looking for natural enemies of the stinking passionflower, *Passiflora foetida*. This plant is native to Central and South America, ranging from Mexico and the West Indies to Central Argentina, and is an invasive weed in several countries in the Pacific region, Australia, and South East Asia. The surveys comprised 41 sampling sites (Fig. 15), where the presence of *P. foetida* was assessed in order to map its local distribution. Insects feeding on *P. foetida*, and plant tissue with eggs or signs of disease were collected and conveyed to FuEDEI for identification and rearing.

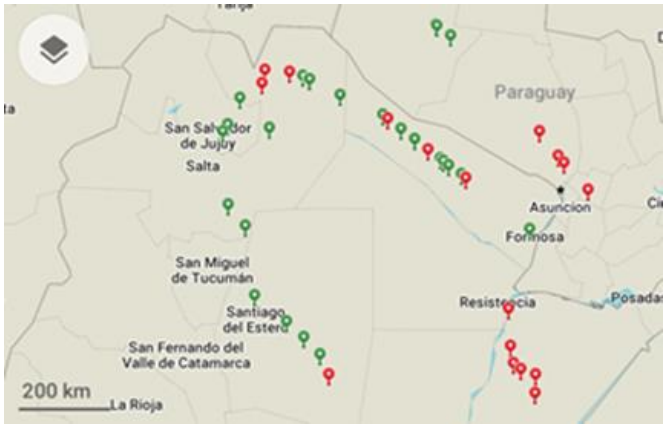

Figure 15. Locations visited during the surveys. Those where *P. foetida* was found are represented in green.

The following natural enemies were found:

1. Unidentified disease possibly viral: Symptoms included stunted growth, yellowing veins, and abnormal, lumpy development of leaves (Fig 16). Whole diseased plants were collected and planted at FuEDEI, where attempts were made to transmit the disease to healthy plants by sap contact and grafting, with yet inconclusive results.

Figure 16. Abnormal development on leaves of *P. foetida*.

2. Hemiptera:
   i. Miridae: A species of mirid was found feeding on *P. foetida* leaves (Fig. 17). Where the insects fed, the leaves exhibited discoloured marks characteristic of sap-sucking damage (Fig. 18). The mirids where collected and identified as *Engytatus* sp. (Bryocorinae: Dicyphini). A colony was established in FuEDEI for further studies.
   ii. Berytidae: These were conspicuously present on *P. foetida* plants in one particular location (S 23° 43' 48" W 64° 37' 12"). The damage caused to the plant was not evident. A colony has recently been established at FuEDEI, and the species is yet to be identified.
   iii. Stink bugs: Clusters of green, round stink bug eggs were found in the underside of leaves (Fig. 19). They are currently being reared in FuEDEI to establish their association with the host plant.

Figure 17. Myrid identified as *Engytatus* sp.

Figure 18. Damage caused by sap-sucking insects on leaves of *P. foetida*.

Figure 19. Unidentified stinkbugs hatching from eggs laid on underleaf of *P. foetida*.

Coleoptera: Eggs, larvae, and adults of a species of Chrysomelid awaiting taxonomical classification were commonly found in the field. The eggs where present in clusters in the underside of leaves, while the larvae fed on leaf and shoot tissue (Fig. 20). The damage caused by the adults to the plant, if any, was not evident on the field. Larvae and eggs were collected to establish a colony in FuEDEI for further studies.

Figure 20. Chrysomelid larvae feeding on *P. foetida* in the field.

3. Lepidoptera:
   i. Larvae of the gulf fritillary (*Agraulis vanillae*) (Nymphalidae: Heliconiinae) were commonly found on the field, feeding on leaves. Specimens from Southern Formosa (S 26° 19' 12" W 58° 0' 0") were collected and a colony was established in FuEDEI, in order to study if any differences, such as host races, exist between the different populations.
   ii. Larvae of another lepidoptera, awaiting taxonomical classification, were found on the field feeding on immature fruits of *P. foetida* (Fig. 21). The larvae bore holes on the fruit tegument and feed on the immature seeds, and pupate inside the emptied fruits. When presented with immature fruits of *P. caerulea*, there was only superficial damage to the outer layers of the fruit, although the larvae fed on the seeds when the fruit was presented cut in half instead of whole. No damage leaves or other tissues was observed.

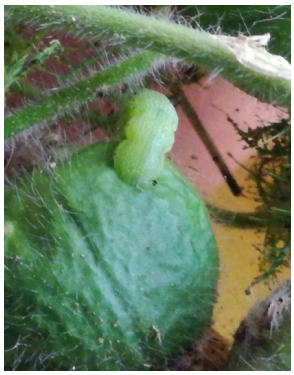

Figure 21. Larva of unidentified lepidoptera boring into an immature fruit of *P. foetida*.

These species are currently being studied in FuEDEI in order to ascertain their taxonomical identity, the details of their life cycle, and the nature of their association to *P. foetida*, which will hopefully be of use to determine their potential as biocontrol agents.

New norms for natural resource-access and benefit sharing in Argentina

by Luis Calcaterra and Willie Cabrera Walsh

Argentina's Ministry of the environment (SGAyDS) held an informative meeting for administrators and scientists last November in Buenos Aires, in order to present and explain the new law adapted to the Nagoya Protocol (No. 410/2019) (Fig. 22). This law aims at protecting diversity, sustainability in the use of natural resources, and ensure fair benefit sharing of said resources. Allegedly, Argentina is one of the first countries to have adapted completely to the Nagoya Protocol in its legal system.

According to the authorities of the SGAyDS, the spirit of the law aims to stimulate research, unify criteria across federal and provincial authorities, and see that the provinces reap a fair share of the benefits of research in their territories. It –allegedly- should also simplify the procedures to obtain access permits for non-commercial research, and establish clear and workable procedures for commercial developments and patentable research as well.

Other than this, obtaining permits in Argentina continues to be a five-tier procedure involving 1. Obtaining collection permits from the provinces or Natl. Parks; 2, 3. Transport and informed consent authorizations from the same; 4. Export permits from the SGAyDS; and 5. Final export permits from the Animal and Plant Health Inspection Service (SENASA). Human, agricultural and other resources included in the ITPGRFA, are not included in the new law. No reference to waiting timeframes was included, so time will tell how "workable" these laws are for international cooperation.

Figure 22. Argentina's Ministry of the environment (SGAyDS) meeting held November 2019 in Buenos Aires.

Sino-American Biological Control Laboratory - Sino-ABCL

Identification of toxins in a predatory bug, *Arma chinensis*

by Chenxi Liu

Pentatomidae is one of the largest Heteroptera families, comprising about 10% of the species estimated in the suborder. *Arma chinensis* is a predaceous bug that preys upon a large variety of species and can effectively suppress agricultural and forest pests in the orders Lepidoptera and Coleoptera. Venoms of Pentatomidae predators are known to possess long-term, non-lethal paralytic effects on their prey. Latrotoxins were firstly found in *Latrodectus* spider venom, which affect the nervous system of prey. To date, the venom has been found to contain five insecticidal toxins, termed α, β γ, δ and ε-latroinsectotoxins. There is also a vertebrate-specific neurotoxin, α-latrotoxin, and one toxin affecting crustaceans, α-latrocrustatoxin. Based on the current *A. chinensis* genome data, Sino-ABCL explored the characterization and expression profiles of toxin genes in this predatory bug.

To identify potential toxin genes, toxin-like and venom auxiliary ploypeptides from *A. chinensis*, all protein sequences were compared using BLASTp to an exclusive database for toxins and venom proteins in the UniProt Animal Toxin Annotation Program, restricting the E-value to 1E-5. The resulting putative toxin genes were considered positive if the presence of the toxin motif or family domain (partial or complete) could be confirmed in their predicted sequence. This validation step relied on resulting putative toxin genes compared with Pfam domain according to the Pfam annotation for the uniport toxin database.

Based on the *A. chinensis* transcriptome data, we identified toxin gene transcripts in different developmental stages and proceeded with k-meas clustering analysis to ascertain toxin gene expression across the *A. chinensis* life cycle. In addition, we dissected *A. chinensis* adult and collected salivary glands for transcriptome sequencing. Identified *A. chinensis* toxin genes were aligned with the salivary gland transcriptome and their expression profile, distribution on chromosomes were developed.

One hundred and eighty-eight latrotoxin genes (Pfam ID: PF12796.6), including α-latrotoxin, α-latrocrustatoxin and δ-latroinsectotoxin, accounted

for the highest proportion equaling 37.08% of total identified *A. chinensis* toxin genes. By clustering analysis, we found that 67 latrotoxin genes were tandemly-arrayed on six chromosomes, forming 26 gene clusters (Fig. 23), suggesting that most *A. chinensis* latrotoxin genes evolved by gene duplication. The largest number of clusters was on Chromosome 5 (Chr 5), including 34 latrotoxin genes. We found α-latrocrustatoxin in the *A. chinensis* genome, which maybe a possible explanation for why *A. chinensis* can prey on some coleopteran insects in addition to lepidopterans.

Based on the *A. chinensis* developmental transcriptome, we identified 505 toxin transcripts expressed across nymphal and adult stages and conducted k-meas clustering analysis (Fig. 24). We found 82 up-regulated toxin genes from the 2nd instar nymph to adult stages as compared with 1st instar nymph stage. This provided molecular evidence for *A. chinensis* predatory behavior suggesting 1st instar did not prey until development into the 2nd instar. Based on the *A. chinensis* adult salivary gland transcriptome, 59 latrotoxin genes were identified, indicating these latrotoxins play important roles in predatory behavior.

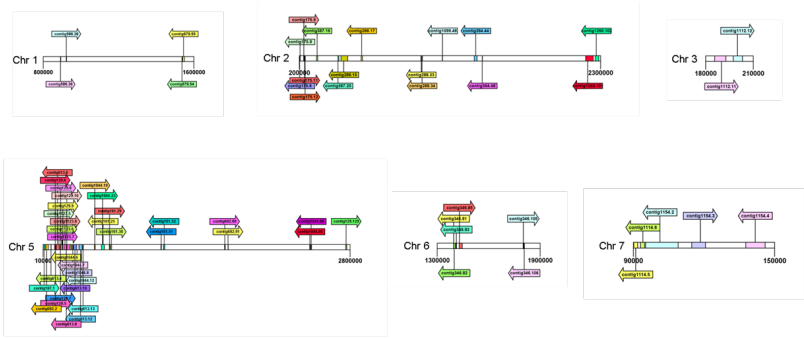

Figure 23. locations of latrotoxin genes and clusters in *A. chinensis* chromosomes. Sixty-seven latrotoxin genes were tandem-arrayed on six chromosomes, forming 26 gene clusters. All chromosome representations are drawn to scale.

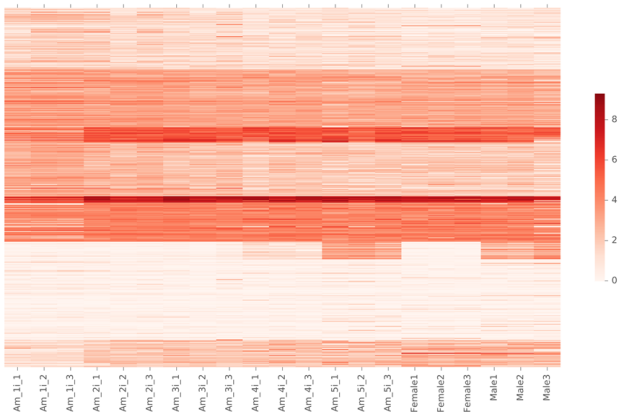

Figure 24. Clustering analysis of toxin genes across *A. chinensis* nymphal and adult stages.

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