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. This has been a long term relationship originating in 1985. 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. It has a permanent staff of 1 American and 7 foreign scientists, 9 technicians and 5 administration/support. Contact: Lincoln Smith, Link.Smith@ars.usda.gov

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

The Sino-American Biocontrol Laboratory (SABL) is based in Beijing, China. Contact: Liu Chenxi, liuchenxi@caas.cn

Foundation for the Study of Invasive Species - FuEDEI

Cactus moth parasitoid Apanteles opuntiarum: Effect of prior experience on parasitization behavior

by Laura Varone, Ana Faltlhauser and Guillermo Logarzo

Apanteles opuntiarum (Hymenoptera: Braconidae), is a larval parasitoid that attacks the cactus moth, Cactoblastis cactorum (Lepidoptera: Pyralidae) (Fig. 1), an invasive pest of prickly pear Opuntia ficus-indica and other native Opuntia species in North America. Learning is known to affect host searching and recognition in some parasitoids. We performed no-choice behavioral experiments aimed to optimize the quality of laboratory-reared parasitoids. We conducted experiments with A. opuntiarum to evaluate the effect of prior female experience on host searching, encounter and attack behaviors. Naíve females were exposed individually to one type of prior experience condition for 24 hours. These conditions consisted in exposing the females to four prior conditions, plus a control: 1) larvae of C. cactorum fed with cactus, 2) larvae of C. cactorum fed with artificial diet, 3) frass from larvae feeding on cactus, 4) frass from larvae feeding on diet, and 5) only honey and water. Every treatment included honey and water as well. We recorded the time until the female arrived on the substrate (larva or frass), and the time spent “grooming”, probing or ovipositing, and exploring.

Figure 1. Female parasitoid Apanteles opuntiarum stinging a Cactoblastis cactorum larva.
We found that prior experience in *A. opuntiarum* did not influence the speed in finding the stimulus (larvae or feces of *C. cactorum*), or parasitizing larvae. However, females that previously experienced cactus frass stung a higher proportion of larvae fed on cactus (Fig. 2). Also, the larvae of *C. cactorum* fed with *Opuntia* were significantly more attractive to *A. opuntiarum* than larvae fed on an artificial diet. Thus, we do not recommend the use of artificial diet for mass rearing purposes of *A. opuntiarum*.

**Figure 2.** Proportion of *Cactoblastis cactorum* larvae stung by *Apanteles opuntiarum* under different combination of previous experience of the female and stimulus offered.

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**Waterhyacinth, *Eichhornia crassipes*, studies in Argentina 2015-2016**

by Alejandro Sosa, Marina Oleiro, Guillermo Cabrera Walsh, M. Cristina Hernández, Fernando McKay, and Mariel Guala

Water hyacinth, *Eichhornia crassipes* is a floating weed native to South America, but distributed around the tropics, subtropics and some temperate areas of the world since the XIX\(^{th}\) century, where it clogs waterways, irrigation canals, rivers and lakes in temperate, subtropical and tropical regions. It is considered one of the worst aquatic weed in the world.

Biological control was first used against this weed in 1974 in a reservoir in north-western Argentina, Dique Los Sauces, a dam located in La Rioja Province in a semi-arid landscape in western Argentina (Fig. 3). The lake of 120 hectares was invaded by water hyacinth, where it covered more than 80 % of the water surface. In April 1974 the weevil *Neochetina bruchi* (Coleoptera: Curculionidae) was released with the purpose of controlling the plant, making this event the first attempt to manage this plant using biological control. Currently, the weevils *Neochetina* spp. and others arthropods, are widely used in several countries, with notorious biocontrol successes such as Lake Victoria (Africa), Papua New Guinea, and Eastern Cape Province. In Argentina a recent invasion in a small lake south of Buenos Aires, El Ojo Lake, San Vicente, Province of Buenos Aires (Fig. 4), motivated returning to Dique Los Sauces after 40 years to evaluate the situation there, with the purpose of comparing plant cover and weevil density through time in both water bodies. Monitoring these evolving systems may help understand and improve the action of new agents as well as those already in use.

**Figure 3.** Dique Los Sauces, 1974 and 2017, before and after 40 years of BC of WH utilizing *Neochetina bruchi*.

**Figure 4.** Visual history of waterhyacinth invasion in El Ojo lake during the summers of 2009 to 2016: a) 2009, b) 2012, c) 2013, d) 2014, e) 2015, f) 2016.
Monitoring waterhyacinth in the Los Sauces reservoir indicates that after 42 years the control of the plant is almost absolute, with one slight resurgence in the late 1990’s. Today the plant is only located in a small section of the lake, where the few plants remaining (less than one hectare) host large weevil populations, working as a refuge for the insects (Fig. 5).

Releases were conducted at El Ojo during the summer of 2014-15 with the aim of establishing and/or increasing the number of herbivore insects for the biological control of waterhyacinth. We used the weevils *Neochetina bruchi* and *N. eichhorniae* and the planthopper *Taosa longula*. The weevils are widely distributed in South America. Their natural southern distribution boundary is the lower delta of the Paraná River. The planthopper is naturally distributed in Northern Argentina and other more tropical areas in South America. In order to ensure the establishment of the inoculum and their effect on the plants, the releases were carried out in floating cages covered with white polyester gauze sleeves. Five cages per species were used, releasing 139 adults of *N. eichhorniae* and 40 adults of *N. bruchi* and 40 nymphs of *T. longula* per cage. The purpose was to obtain a high density of insects inside each cage so as to release a large initial population, and maximize the chances of successful mating and establishment so as to minimize Allee effects.

Two types of controls were used to measure the insect’s impact: a) 5 cages with plants with herbivorous insects already present in the lagoon; b) 5 other cages with insecticide-treated plants. To evaluate the success of the establishment and the actual effect of these insects in the lake, five plants were randomly extracted from the cages 4 to 6 weeks after insect release to measure the following parameters: fresh weight of the five plants, number of reproductive structures of the plants (flower peduncles and number of leaf IV), maximum diameter of leaf petiole IV, width and length of leaf IV, leaf area, number of leaves with adult feeding marks of *Neochetina* sp., number of *Neochetina* sp. larvae, number of *Neochetina* sp. adults, number of *Neochetina* sp. larvae, number of eggs, number of leaves with *Megamelus scutellaris* feeding / oviposition markings (already present in the lake), number of leaves with *Neochetina* sp. larval activity, marks of *Thrypticus* sp. (also present in the lake).

The historical growth of the waterhyacinth population in the El Ojo lake was analyzed using aerial images (Google Earth) (Fig. 4). The invasion became clearly detectable, with a noticeable increase in area, in 2012. At this point the open water was dominant, except for coastal reed mats. By April 2013 about 9.3% of the lake’s surface was covered. In January 2014, the area covered was three times greater than the previous year (32.9% of the lake). In the last available image of February 2015, ~60% of the lake was covered. The rapid growth in coverage can be assimilated to a logistic growth model of the type \( r = \ln\left(\frac{N(t2)}{N(t1)}\right)/(t2 - t1) \) (GLM, \( P < 0.0054 \)) with a growth rate of 0.0021 ha/day, equal to 21 m²/day. In addition, this surface growth corresponds to a theoretical logistic growth curve proposed for this plant (Wilson et al. 2005) with an initial dry weight of 35 kg (about 46 plants of 0.750 kg of wet weight), growth rate \( r = 0.015 \) kg.days⁻¹ in a body of water with about 2% of dissolved nitrogen, of a temperate climate, in an insect-free situation.

At El Ojo Lake only the establishment of the weevils was confirmed. The cage experiment showed that insects could control the plant, reducing biomass and density compared to control treatments (Fig. 6). Both the weevils produced enough damage to reduce the biomass from about 90 kg/m² to 60 kg/m² and density from 60 to 40 plants (ramets)/m². The two control treatments were significantly different from each other. The one without insecticide also showed fewer plants and less biomass, which could be explained by the presence of other insects in the lake, the mining fly *Thrypticus* sp. and the planthopper *M. scutellaris*.
Figure 6. Growth of water hyacinth in the different caged treatments in the El Ojo lake.

Two years after releasing the insects, plant density and plant biomass diminished significantly. Even though the lake is still highly infested due to the percentage of the lake surface covered by plant (about 80%), plants were much lighter and lose from each other (less ramification, allowing for more mat dispersal), coinciding with a weevil population increase (Fig. 7).

Figure 7. Evolution of water hyacinth weight and weevil populations in El Ojo lake.

Sino-American Biological Control Laboratory (SABCL)

The agreement for the establishment of SABCL in the Institute of Biological Control, CAAS, was signed in November 1988. It was the first long-term cooperation project on agricultural science and technology between China and USA, and it is an important platform for scientists in IPP to exchange and cooperate with USA. In the past two decades SABCL has carried out more than 10 research projects including studies, surveys and collection of agents for invasive insects: Asian Longhorn Beetle, soybean aphid, wheat stem sawfly, emerald ash borer, brown marmorated stink bug and spotted wing drosophila, and invasive weeds: saltcedar and leafy spurge.

Figure 8. Staff at SABCL.

Mass rearing Arma chinensis
by Liu Chenxi

Arma chinensis (Hemiptera: Pentatomidae) is a predaceous bug that is native to China, Korea and Mongolia. A. chinensis can effectively suppress a wide range of agricultural and forest pests, including lepidopteran, coleopteran, hymenopteran and hemipteran species. The Colorado potato beetle (CPB), one of the most important exotic invasive pests of potatoes and tomatoes in Asia, has developed resistance to most of the insecticides used for its control, and has the potential of developing resistance to Bacillus thuringiensis (Bt) toxins. Preliminary tests indicate A. chinensis is able to control CPB in field applications.

SABCL has been developing methods to mass rear A. chinensis for inundative biological control (Fig. 9). After several years of studies on metabonomics, nutrigenomics and artificial diet development on A. chinensis, we developed a meridic diet for continuous rearing of A. chinensis. Alternatively, we also designed a series of facilities for mass rearing armyworm, and the larvae and pupae were used as prey for mass rearing A. chinensis. Based on that, we established a mass-rearing center in Zunyi, China in 2014. Now, this beneficial predator has been applied in greenhouse and other crops for pest biological control in Tianji and Guizhou, China.
Casuarina or Australian pine is a serious invasive weed of coastal areas in the United States especially southern Florida. This weed is a complex of three species, C. equisetifolia, C. glauca, and C. cunninghamiana and their hybrids. Although these trees were planted as valued ornamentals, wind breaks and for erosion control, since their introduction they have also threatened fragile coastal habitats which support many valued Florida species, including the federally threatened American crocodile and five species of sea turtles.

Over many years ABCL has been conducting native range surveys for potential biological control agents for Australian pine. A complex of insects feed on Casuarina stems, leaves, fruit, and seeds. A major factor that limits the reproductive output of the Casuarina spp. in Australia is the damage caused by this complex of herbivores. The Torymidae gall wasp, Bootanelleus orientalis, has been collected widely in Australia and reared from the cones of six Casuarina species (Fig. 10). Field populations on C. equisetifolia are being monitored at field sites near Brisbane to elucidate seasonal trends. Laboratory testing has focused on whether immatures require live fruit to complete development, which appears likely. Dissections of field collected fruit indicates that mature seeds are preferred. This insect is likely to be specific and could be useful in controlling the reproductive capacity of Casuarina spp. in Florida and will avoid conflicts of interest relating to unsightly damage to ornamental trees.

Of particular interest is a gall inducing wasp Selitrichodes utilis (Hymenoptera: Eulophidae) (Fig. 11). Under laboratory conditions potted C. glauca plants were heavily galled by S. utilis and severely damaged causing the death of some individuals. In host range testing, C. glauca was the preferred host though galls also developed on C. cunninghamiana and C. equisetifolia. No galls were observed on two Allocasuarina species tested. Further exploration will be conducted in Australia to search for S. equisetifolia; a species which attacks introduced C. equisetifolia in Guam. Its native range is unknown but given the damage it has inflicted on C. equisetifolia in Guam, it could be an excellent biological control agent.
Other insects of interest under evaluation include; the leaf binding moth, *Cryptophasa irrorata* (Lepidoptera: Xyloryctinae), the defoliating moth species *Calathusa maritima* (Lepidoptera: Noctuidae) and *Zauclophora pelodes* (Lepidoptera: Xyloryctinae), the sap-sucking psyllid *Casuarinicola australis* (inset left) and Cecidomyiidae flies that gall the foliage and damage seeds.

Conflicts of interest exist in the introduction of biological control agents for Australian pine given its value as an ornamental and its “suggested” use as a wind break around citrus orchards to prevent the spread of citrus canker. Given its shallow root system, the tree has caused significant property damage in Florida due to trees toppling over during hurricanes so its ornamental value has been tempered. Though suggested as a wind break many years ago, it has yet to be utilised by the citrus industry for that purpose.

**Exploration for Biological Control Agents of Invasive Aquatic Plants**

by Matt Purcell, Bradley Brown and Phil Tipping

ABCL has been exploring for biological control agents of the submerged aquatic weed hydrilla, *Hydrilla verticillata* since the 1980’s. Biological control agents have been introduced into the US but their impact on hydrilla has been Limited. More agents are needed, particularly insects adapted to monocious hydrilla which is expanding northwards in the US and threatens the Great Lakes. Exploration by ABCL continues to focus in the Republic of Korea, the origin of US monocious hydrilla, and also in mainland China were the greatest genetic diversity of hydrilla occurs.

Other introduced aquatic plants native to Australasia are becoming problematic in the southern states of the US. Round leaved tooth cup, *Rotala rotundifolia* (Fig. 12), is becoming a serious weed in ponds and irrigation channels in south Florida and has the potential to be a more serious weed than hydrilla. Like hydrilla, stem fragments can become new plants which has significant implications for disturbance through mechanical control. Unlike hydrilla, the plants also form viable seeds. Initial surveys began recently in the native range in China and a defoliating moth has already been observed in Hong Kong.

Crested floating heart, *Nymphoides cristata*, is a water lily spreading in Florida where it is now found in at least 7 counties and also occurs on Lake Okeechobee and in the Big Cypress Preserve (Fig. 13). This aquatic plant also occurs further north into South Carolina where it has significant coverage on Lake Marion. *Nymphoides cristata* is also native to Asia and surveys for biocontrol agents are planned for 2017.

Feathered mosquitofern, *Azolla pinnata* is problematic in northern Florida, particularly during the winter. This floating fern spreads rapidly over water surfaces through vegetative reproduction forming dense mats which often turn a distinctive red colour. Worldwide analysis of *A. pinnata* plants by Dr Paul Madeira (USDA ARS IPRL, Fort Lauderdale) determined that the genetic match for the US genotype of *A. pinnata* in the native range was collected near Brisbane, Australia. A *Bagous* sp. weevil which severely damages this fern has been collected from this region and colonised for testing against native US *Azolla* species including *A. filiculoides* and *A. caroliniana* (Fig. 14).
European Biological Control Laboratory - EBCL

EBCL scientist elected president of the European Society of Vector Ecology
by Lincoln Smith and Alexandra Chaskopoulou

Alexandra Chaskopoulou, was elected in February 2017 as the new president of the European branch of the Society for Vector Ecology (SOVE). SOVE is an international organization that promotes research on suppression of nuisance organisms and disease vectors through environmental management, biological control, public education, and appropriate chemical control technology. Alexandra will serve for 3 years and will organize the society’s bi-annual conferences.

Host age suitability for two egg parasitoids of bagrada bug
by Matthew Augé, Guillaume Martel, René F.H. Sforza and Lincoln Smith

In order to improve methods for maintaining laboratory colonies and to better understand optimal conditions for host specificity testing, we conducted experiments to measure the ability of parasitoids to attack host eggs of different ages. We studied two species of egg parasitoids originating from Pakistan: *Trissolcus hyalinipennis* and *Gryon* sp. (Platygastridae) in the EBCL quarantine laboratory in France. We exposed female parasitoids (20 replicates for *Gryon* sp. and 16 for *T. hyalinipennis*) to 6 eggs for 1 h at 22°C. Host eggs were collected daily and held for different durations to provide eggs of different ages, from 0-1 to 3-4 days old. In addition, some 0-1-day-old eggs were frozen at -80°C. Only one age class of eggs was exposed to parasitoids at a time (no-choice). We observed that both *Gryon* sp. and *T. hyalinipennis* could parasitize live host eggs, regardless of their age up to 4-days-old (Fig. 15). However, frozen eggs were much less suitable. The results indicate that both parasitoid species can attack host eggs up to 4 days old, and possibly older. However, frozen eggs generally are not suitable under our conditions. This means that the use of frozen eggs for sentinel eggs to survey for parasitoids in the field would probably not detect either of these parasitoid species.

Mosquitoes and Resistance: A global Challenge
by Alexandra Chaskopoulou

Control of mosquito borne diseases is being seriously challenged by the ongoing development of insecticide resistance by mosquitoes. Scientists from EBCL Greece are involved in several studies in collaboration with the Agricultural University of Athens and the Department of Biology in Crete attempting to elucidate the mechanisms of resistance of vectors to insecticides. One study was
recently published at PLOS Neglected Tropical Diseases and was focused on the Asian tiger mosquito, *Aedes albopictus*, which is a highly invasive species that transmits arbovirus diseases, including chikungunya, dengue and Zika. Resistance of this mosquito to the organophosphate larvicide temephos was recently associated with the up-regulation, through gene amplification, of two carboxylesterases; *CCEae3a* and *CCEae6a*. This study looked at the worldwide distribution and origin of the amplified esterases, which is of great value for designing and implementing efficient vector control programs. Individuals with amplification of both esterases were found in Greece and Florida (U.S.A), representing a single amplification event that spread between the two countries, highlighting the importance of passive transportation of disease vectors carrying resistance mechanisms. In addition, individuals with amplification of *CCEae3a* but not *CCEae6a*, representing a second and independent amplification event, were found in Florida. The worldwide haplotype diversity observed for *CCEae3a* is consistent with the highly invasive nature of the *Aedes albopictus*. The passive movement of mosquitoes by modern transportation enables the rapid dispersal of new resistant biotypes globally, which is likely to accelerate the decline in efficacy of existing insecticides. This further increases the need for development of novel vector control tools that will rely not just on chemical interventions but will also use biorational/biological control technologies. For more information see: [http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005533](http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005533)

**Does a root-galling weevil affect the microbial community of hoary cress?**

by Mélanie Tannières

Hoary cress, *Lepidium draba* (Brassicaceae), is a deep-rooted perennial mustard introduced to the U.S. during the late 19th century. It invades crops, rangelands and riparian lands, and is a noxious weed in 14 states. The gall-forming weevil *Ceutorhynchus assimilis* has been evaluated as a prospective biocontrol agent for several years. Root galls induced by *C. assimilis* in the native range were observed to be infected by the ubiquitous soilborne fungus *Rhizoctonia* sp., raising the question whether there is an important interaction between the fungus, insect and host plant. A field experiment with four conditions (1] Control, 2] Weevil, 3] *Rhizoctonia solani*, 4] Weevil + *R. solani*) and 13 to 17 replicates was conducted at EBCL. Plant mortality rate was observed to be higher although not statistically significant in the condition where weevil and the pathogen are combined (21.8%) than in the treatment with the weevil alone (13.8%), suggesting a potential synergy between the weevil and the pathogen.

In order to study the change in microbial community in response to the introduction of the biocontrol agent, bacterial communities from the different experimental conditions were analyzed by sequencing the 16S rRNA gene. This showed a non-significant modification of the structure and the diversity of bacterial populations in presence of the weevil and/or *Rhizoctonia solani* (Fig. 16). The replicates within a condition do not cluster together indicating a higher variability among replicates than among conditions.

**EBCL collaboration with the European Center for Disease Prevention & Control (ECDC) on West Nile virus control**

by Alexandra Chaskopoulou

The European Center for Disease Prevention and Control (ECDC) has launched a Vector Control Analysis (VeCA) project to develop a tool to appraise and compare different vector control strategies against West Nile Fever. West Nile virus (WNV) represents a serious burden to human and animal health because of its capacity to cause unforeseen and large epidemics. Understanding the interaction of ecological factors that affect WNV transmission is crucial for preventing or decreasing the impact of future epidemics. The VeCA project aims to increase our knowledge on WNV vector ecology and control. This project also aims at optimizing resources allocation and improving cost-effectiveness of vector control against West Nile virus. EBCL Greece is partnering with ECDC in studies to evaluate the effectiveness of commonly used vector control techniques against WNV vectors. For more information access article on Parasites & Vectors at [https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-016-1736-6](https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-016-1736-6)
Figure 16: Mean relative abundance heat map of the 100 most abundant microbial OTUs associated with *Lepidium draba* based on the sequence of the 16S rRNA gene. Each column represents one sample; each row represents one OTU. Conditions: yellow = control; blue = weevil; violet = *Rhizoctonia*; green = weevil + *Rhizoctonia*. Samples and OTUs were clustered using Euclidian distances and complete linkage. Scale bar represents normalized abundances from low (blue) to high (red).

The assessment of fungal community using NGS technology will allow us to study the response of bacterial and fungal populations to the introduction of one biocontrol agent. This approach will be developed in the context of the evaluation of a biocontrol agent with experiments conducted under natural conditions. A better understanding of tri-trophic interactions between an invasive plant, a biocontrol agent and the microbial communities should give new clues in fight against invasive species.
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