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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: Michael Grodowitz, michael.grodowitz@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

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

Australian Biological Control Laboratory - ABCL

Aquatic Weed Biological Control and COVID

Matthew Purcell, Nathan Harms and Bradley Brown

Much of the exploration for biological control of aquatic weeds conducted by ABCL has been performed in the Asian native range of several invasive US weed species. With the onset of the COVID pandemic, travel to the Asian region from Australia has been paused for the foreseeable future due to government restrictions and availability of flights to Asia. ABCL has been relying on its collaborators to conduct surveys, though they themselves have had restrictions on their movements and ability to operate effectively.

Recent research on the biological control of the submerged weed hydrilla, *Hydrilla verticillata* has focused on finding the matching genotypes of the U.S. introductions of this invasive weed in the native range and identifying adapted herbivores. ABCL has evaluated a Korean leaf-mining *Hydrellia* sp. (Ephydridae) fly from monoecious hydrilla in quarantine in Brisbane, Australia. The genotype of this fly was only found on the monoecious hydrilla in South Korea matching the U.S. introduction. Unfortunately, the fly has been rejected as a biological control agent as its host range was too broad and included a *Potamogeton* species and a close hydrilla relative, *Elodea canadensis*, native to North America. Other agents have been identified in China and Korea that could be evaluated for biological control of monoecious and dioecious hydrilla in the U.S., including insect herbivores found on a new hydrilla genotype recently discovered in the Connecticut River. However, with the difficulty in accessing these insects due to COVID travel restrictions, in the short term our research will pivot to Australia looking at potential hydrilla herbivores in the cooler regions of Australia matching climatically with the cooler regions of the US where hydrilla is or has the potential to be a problem (Fig. 1). There are fewer COVID restrictions for travelling throughout Australia. Surveys are planned specifically for the border regions between the states of New South Wales and Victoria

close to the southern latitude equivalent of the Connecticut River.

ABCL has also been working closely with

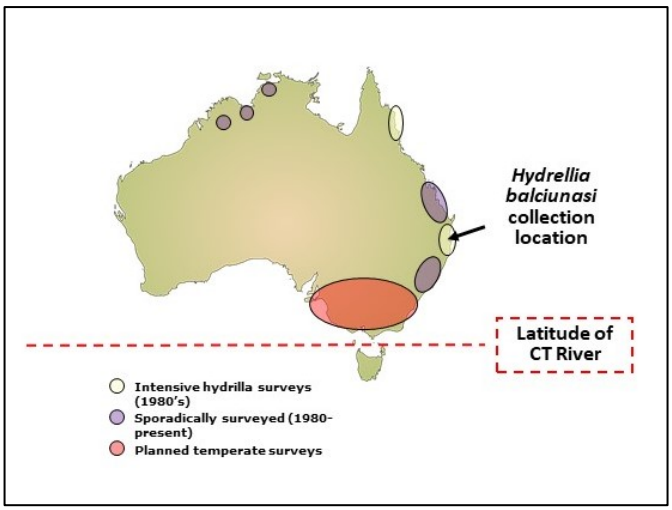

Figure 1. Planned survey regions for of hydrilla, Hydrilla verticillata, in temperate regions of Australia.

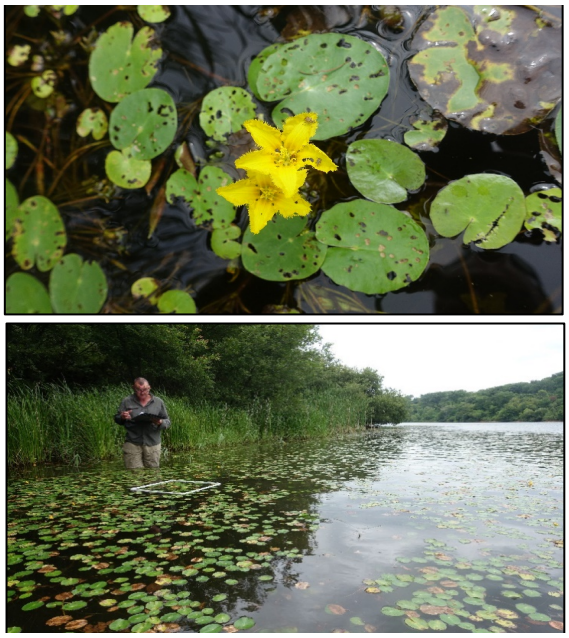

Figure 2. Yellow floating heart, Nymphoides peltata damaged by Bagous weevils in China (a). Demography surveys of yellow floating heart, N. peltata in South Korea (b).

collaborators in China, South Korea and India on exploration for biological control of crested and yellow and floating hearts, *Nymphoides cristata* and *N. peltata* respectively. Surveys of *N. peltata* had been conducted in South Korea in 2018 and 2019. In 2020, field research has been delayed due to COVID though our collaborators at Hankyong University have recently begun exploratory field surveys and demography studies (Fig. 2a) which will be continued up until the onset of winter. A leaf-mining *Hydrellia* fly has been discovered damaging the leaf laminas and petioles. Additionally, in China a *Bagous* weevils was very common on *N. peltata* from Shenyang to Harbin north of Beijing (Fig 2b.). The adults attacked flowers while larvae damaged fruit below the waterline. Surveys in India are yet to start with restrictions on movements due to the pandemic. Scientists in Thailand and Japan have agreed to collect dried leaf specimens for DNA analysis to determine the origin of US floating heart introductions. Early analysis indicates a European origin for the U.S. introduction of *N. peltata,* but few herbivores have been detected in that region.

Trichilogaster bud gall wasp: a priority biocontrol agent for earleaf acacia

Christine Goosem and Ryan Zonneveld

Earleaf acacia (*Acacia auriculiformis*) is an evergreen tree native to Australia, Papua New Guinea and eastern Indonesia that has developed into a damaging environmental weed in Florida (Fig. 4). The invasive tree threatens pinelands, scrub and hardwood hammocks, and has been listed as a Category I invasive weed by the Florida Exotic Pest Plant Council (FLEPPC) since 1993.

Figure 4. Demography surveys of yellow floating heart, N. peltata in South Korea.

From 2017, the ABCL has conducted regular native range surveys in Australia to identify and prioritize a list of insect herbivores showing the greatest potential as biocontrol agents for earleaf acacia (Fig. 5). One of the most promising agents is a bud galling wasp, *Trichilogaster* sp. nov. (Hymenoptera: Pteromalidae: Ormocerinae) which was found year-round at sites in northern Queensland (Fig. 6).

Figure 5. Ryan Zonneveld (ABCL) conducting native range surveys of earleaf acacia.

Gall-inducing insects are known for their host-specificity to plants. Galling diverts plant resources away from branch and flower development and into gall formation, which provides food and shelter to the developing wasp larva.

Two

Figure 6. Trichilogaster gall wasp on earleaf acacia. Larva developing inside gall (left), adult female (right).

congeners, *T. acaciaelongifoliae* and *T. signiventris*, have proven to be extremely successful biocontrol agents in South Africa against *A. longifolia* and *A. pycnantha*. Both agents significantly reduce the reproductive output of their host Acacias and within two generations, seed production had been reduced by >95% on heavily infested trees.

The ABCL has been refining methods for rearing the earleaf acacia bud gall wasp. The wasp induces substantial single and multilocular galls (each chamber 5-20mm in diameter) at leaf bases and developing vegetative and reproductive buds and is likely to be highly damaging (Fig. 7). Extensive galling of young, infested seedlings can cause mortality.

Figure 7. Multilocular galls at leaf bases and vegetative buds.

A colony was started at the ABCL in October 2018, by exposing potted Queensland and Florida plants to *Trichilogaster* adults emerging from field-collected galls. Three months later, both plant genotypes were heavily galled. Emerging adults were monitored for adult oviposition behavior, longevity and egg-adult development on fresh plants (Fig. 8). Adults that emerged from the galls were parthenogenetic females that immediately began depositing viable eggs at new buds.

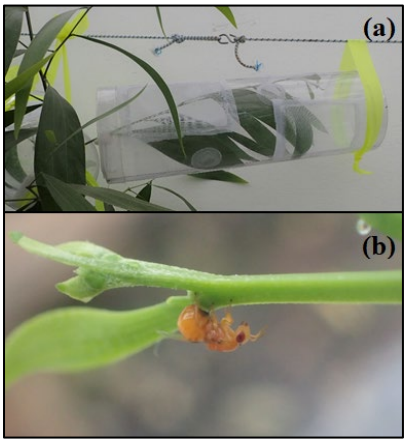

Figure 8. a) Gall wasp rearing cylinder b) Oviposition behavior at bud.

Fast-forward two years, and the ABCL has a whole glasshouse full of galled earleaf acacia plants, with several gall wasp adults emerging daily. We have experimented with plants of different ages, sizes and those that have been cut right back and are re-shooting. All plant-types including seedlings (≤10cm), large 4-year-old mature plants and re-shooting plants have been readily galled (Fig. 9). Immature galls become visible about one-month post-oviposition and the first adults emerge about 2-3 months post-oviposition. Adult longevity is very short; on average around 2 days.

Figure 9. Bud galls on an earleaf acacia seedling.

Preliminary host-range screening results in Australia are indicating that this gall wasp will likely be an Acacia specialist and only develop through on earleaf acacia and its closest relatives. Ten plant species have been screened and galls have only been induced on one non target, *Acacia mangium*, an Australian native and very close relative which is known to hybridize with earleaf acacia naturally in the field.

The extensive galling observed in the glasshouses as well as the historical success of *Trichilogaster* gall formers as biological control agents offers great promise for the specificity and efficacy of this novel species on earleaf acacia. It could be particularly impactful on young seedlings as well as mature, seed producing and/or reshooting earleaf acacia trees due to the energy-intensity of galling.

ABCL Publications

Harms, N. E., D. A. Williams and M. F. Purcell (2020).The role of overseas genetic surveys to potentially accelerate biological control development for a new *Hydrilla verticillata* introduction in the USA. Biocontrol: 10. https://doi.org/10.1007/s10526-020-10050-x .

Minteer, C. R., M. C. Smith, P. Madeira, C. Goosem, R. Zonneveld, J. Makinson, G. S. Wheeler and M. Purcell (2020). Is biological control for earleaf acacia (*Acacia auriculiformis*) feasible in the United States? Biocontrol Science and Technology https://doi.org/10.1080/09583157.2020.1833305 .

Smith, M. C., S. A. Wright, B. Brown, M. Purcell, P. D. Pratt, P. Clark and J. A. Lollis (2020). Fundamental host range of *Lophodiplosis indentata* (Diptera:

Cecidomyiidae), the last proposed biological control agent for *Melaleuca quinquenervia* (Myrtaceae) in Florida. Biocontrol Science and Technology 30(10): 1073-1082. https://doi.org/10.1080/09583157.2020.1787345 .
Wineriter-Wright, S. A., M. C. Smith, M. A. Metz, J. R. Makinson, B. T. Brown, M. F. Purcell, K. L. Barr and P. D. Pratt (2020). The biology of *Casmara subagronoma* (Lepidoptera: Oecophoridae), a stem-boring moth of *Rhodomyrtus tomentosa* (Myrtaceae): descriptions of the previously unknown adult female and immature stages, and its potential as a biological control candidate. 11(10): 653; https://doi.org/10.3390/insects11100653 .

Sino-American Biological Control Laboratory – Sino - ABCL

Salivary Gland Secretome Analysis Reveals A Novel Mechanism of Horizontal Gene Transfer in the Brown Marmorated Stink Bug

Chenxi Liu

Herbivory is a highly sophisticated feeding behavior that requires abilities of plant defense suppression, phytochemical detoxification, and plant macromolecule digestion. For plant-sucking insects, salivary glands (SGs) play important roles in herbivory by secreting and injecting proteins into plant tissues to facilitate feeding. Little is known on how insects evolved secretory SG proteins for such specialized functions. In this study, we aimed to investigate the composition and evolution of the secretory SG proteins in the brown marmorated stink bug, *Halyomorpha halys*, and whether these proteins empower the insect to become a successful invader worldwide feeding across a wide host range.

We re-sequenced its SG transcriptome and predicted hundreds of secretory proteins with the largest categories involved in metabolic and cellular processes, linking them to diet digestion and host manipulation. Interestingly, a group of secretory SG phospholipase C (PLC) proteins exhibited high sequence similarity to bacterial homologs (Fig. 1). We performed a high-resolution phylogenetic analysis using all Gammaproteobacteria PLC sequences retrieved from the NCBI NR database to further trace the donor species of these HGT-related PLC genes (Fig. 2). As expected, the 10 Hhal-PLC proteins formed a separated subclade, which, surprisingly, were clustered with another subclade consisting of sequences mainly from a bacterial genus, Xenorhabdus, with strong support (posterior probability = 1). Interestingly, the non-Xenorhabdus PLCs in this clade formed their own subclade, showing closest phylogenetic relationship with those (Xe_vie-PLC2 and Xe_vie-PLC3) of Xenorhabdus vietnamensis, suggesting

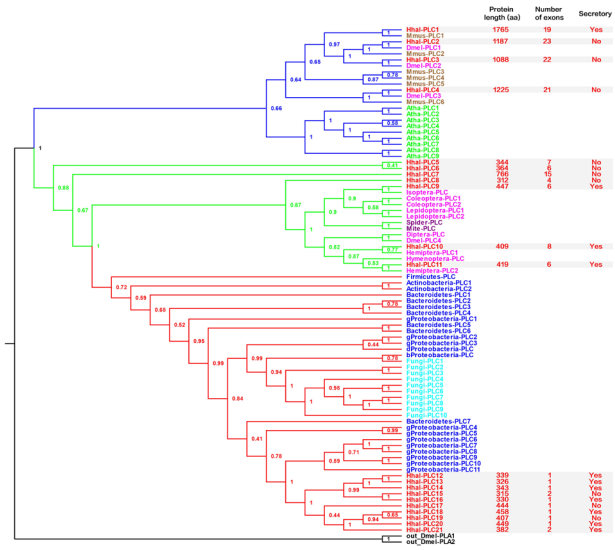

Figure 1. Phylogeny of PLC proteins derived from H. halys and a variety of kingdoms of organisms. The eukaryote, arthropod, and bacteria PLC clades are in blue, green, and red, respectively. Clade posterior probabilities are indicated at nodes. Taxa ar are color coded per organism lineage with the outgroup PLA proteins in black, H. halyes (Hhal) in red, Mus musculus (Mmus) in brown, Arabidopsis thaliana (Atha) in green, Drosophila melanogaster (Dmel) and other non-Hhal insect species (shown in Order names) in pink, arachnids in purple, bacteria in blue, and fungi in cyan. Bacteria species are shown in their phylum names except Proteobacteria which are shown in class names, i.e., bProteobacteria = Betaproteobacteria, dProteobacteria = Deltaproteobacteria, and gProteobacteria = Gammaproteobacteria.

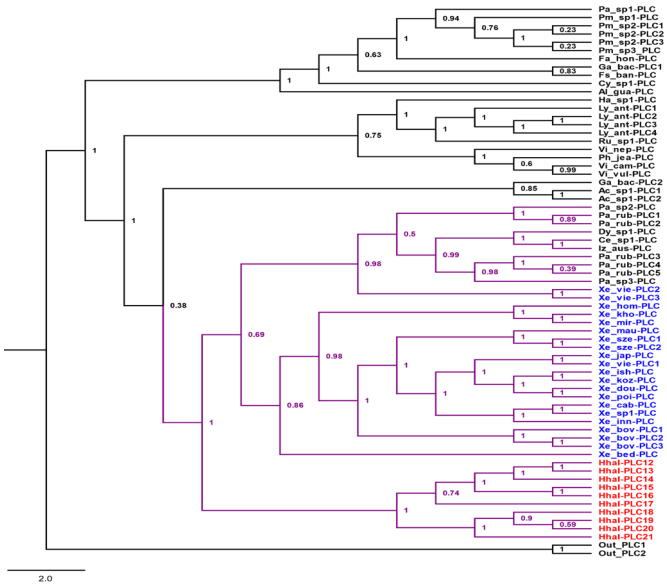

Figure 2. Phylogeny of the HGT-derived H. halys PLC proteins and Gammaproteobacteria PLCs. The tree is rooted on two Bacteroidetes PLC proteins. Clade posterior probabilities are indicated at nodes. The clade comprising H. halys (Hhal) PLCs (in red) an nd their most closely related bacterial PLCs, particularly Xenorhabdus (Xe) are highlighted in purple. First two letters of the Gammaproteobacteria taxa denote the genera, and three characters following an underscore symbol denote the species.

possible horizontal gene transfers occurring between these species, likely starting with a transfer from X. vietnamensis to one of these non-Xenorhabdus species (Figure 2). Nevertheless, the Hhal-PLCs were most closely related to the homologs of several *Xenorhabdus* species, indicating that the genes encoding these proteins, including the ones in the insect genome and those of *Xenorhabdus* species, shared a common ancestor. Thus, their genes were demonstrated to be acquired by *H. halys* through the mechanism of horizontal gene transfer (HGT) from bacteria, specifically, from *Xenorhabdus*, a genus of Gammaproteobacteria living in symbiosis with insect-parasitizing soil nematodes. These indicated that the bacterium-to-insect HGT was mediated by nematodes, a mechanism involved with the tripartite symbiotic and parasitic interactions. Typically, the nematode carries its symbiont in a specialized region of the intestine, termed the receptacle, until the nematode invades the insect hosts, penetrates their hemocoels, and releases *Xenorhabdus* spp. into the insect hemolymph. Once in the insect the bacteria reproduce and secrete a complex of virulent factors in coordination with nematodes to suppress insect immunity and kill the insect host. The bacteria also offer essential nutrients to nematodes by degrading the insect cadaver and meanwhile produce a diversity of antimicrobial molecules to prevent the growth of antagonistic microorganisms. This specialized lifecycle of *Xenorhabdus* spp., which involves pathogenic and mutualistic interactions with the different invertebrates hosts, the insects and nematodes, respectively, provides an opportunity for bacterium-to-insect horizontal gene transfer because the bacteria are released into an open circulatory system, potentially bringing bacterial DNA close to an insect host germ cell, and thus allowing DNA integration and transmission to the next generation if the host survives the attack. qRT-

PCR analysis demonstrated that Hhal-PLC12, -PLC13, and -PLC16 were expressed at significant higher level in salivary gland than other tissues tested, including head,

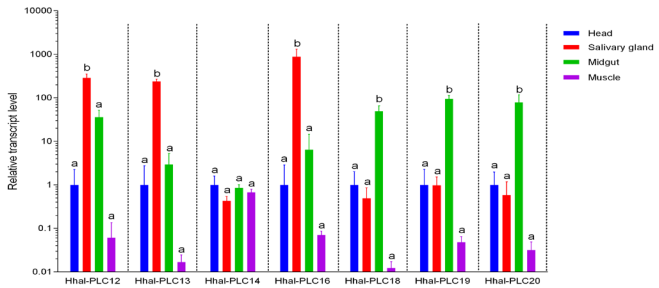

Figure 3. Quantitative reverse transcription PCR analysis of seven horizontally transferred PLC genes in the head, salivary gland, midgut and muscle of *H. halys*. Data shown are mean relative expression ± SE. Different letters on error bars indicate significant difference (*P*-value < 0.05).

midgut and muscle (Fig. 3). In contrast, expression of Hhal-PLC18, -19, and -20 was significantly upregulated in midgut compared to other tissues. However, Hhal-PLC14 was similarly expressed in all these four tissues (Figure 3). The diverse expression profiles and distinct secretory features in the HGT Clade of Hhal-PLCs indicated the genes have been evolved towards functional diversification following their horizontal gene transfer and expansion in the insect genome. Thus, this study revealed that an herbivore can facilitate its feeding through gaining genes from an endosymbiont of its parasite in a tripartite relay of host manipulation.

Foundation for the Study of Invasive Species - FuEDEI

Little Fire Ant

Luis Calcaterra

The little fire ant (*Wasmannia auropunctata*) is a major agricultural problem in Hawaii, and it is spreading in the continental U.S. The shift from a sexual reproductive system in native populations to clonal-dominant populations in human-modified habitats (introduced and native)

Figure 1. Small sexual colony (top) and clonal supercolony with large number of queens (bottom).

(Fig. 1) has played a key role in the successful invasive capability of this and other invasive ant species. This shift results in supercolonies with a reduction in genetic variability and, consequently, a loss of intra-colonial aggression. However, a loss of natural enemies likely also contributes to the success of this ant in introduced areas.

Historically, prospecting for classical biological control agents has been conducted by collecting ant colonies, followed by close examination for the presence of pathogens, parasites and predators in either the field or laboratory. Since there are no known macroscopic effective natural enemies of *W. auropunctata* that can be used in a biological control program, the search for microorganisms, such as viruses, with next-generation sequencing (genomic tools) is being applied. Ants and bees have shown to host several dozen viruses, some of them responsible for colony decline. This is the case of the red imported fire ant (RIFA), *S. invicta*, and likely the LFA. Surveys were conducted in central eastern and northeastern Argentina to collect *W. auropunctata* samples. Samples from 39 colonies were collected, preserved and sent to Dr. Steven Valles in the CMAVE, ARS-USDA. Metagenomics and next generation sequencing are being employed to discover natural enemies (virus) that may be used as biological control agents against this pest ant. Preliminary results indicate the presence of a lot of copies of viral sequences that could be replicating in the LFA.

Improving the Success and Perception of Biological Control Through Community Engagement and Awareness

Alejandro Sosa, Laura Varone, Ana Faltlhauser, Fernado Mc Kay and Guillermo Cabrera Walsh

How can we improve the success of any management strategy? Both prevention and success ultimately rely on public awareness and perception. In countries like Argentina this is necessary not only for raising/increasing the acceptance and visibility of biological control, but also to show the importance of international collaboration to implement biological control programs.

Since 2015, FuEDEI has dedicated efforts to raise awareness of the threat posed by invasive plants and arthropods, and the prospects for implementing biological control programs among the scientific and educational community, policymakers/administrators, and the general public. In order to achieve this, we have organized biocontrol courses, workshops, online surveys and several talks. Also, we have been providing two undergraduate classes (biological control and invasive species) for General Ecology, and one postgraduate course "Biological Control of Weeds and Pests", both at the University of Buenos Aires, the most prestigious science college in Argentina, and one of the top three in Latin America. The course includes not only lecturers from FuEDEI, but other argentine institutes (INTA and CONICET), and international guest lecturers like Rodrigo Díaz (Louisiana State University). With these activities we involve every year approximately 100 undergrad and 35 postgraduate students who would otherwise have no contact with the discipline of biological control.

Another main activity this year was the organization of the first online workshop for primary and secondary school teachers on Environment and Biodiversity training. Held at the *Universidad Nacional de Hurlingham* we had more than 150 participants. In this two-day workshop we also worked closely with international lecturers: Kim Weaver (Centre for Biological Control, Rhodes University South Africa) and Iris Stiers (*Vrije Universiteit*, Belgium) sharing their experiences on community engagement and citizen science in their countries.

We hope these activities will help to increase the acceptance and visibility of biological control among the argentine scientific and education community, as well as across society as a whole.

Cabomba

Marina Oleiro and Mariel Guala

Fanwort, *Cabomba caroliniana*, is a common plant in streams and lakes in clear and deep waters in Argentina. It has become invasive in Australia, southeast Asia, China, India, and parts of Europe and North America. During the last year, our efforts have been

Figure 1. H. natans pupa on cabomba tip

devoted to improve rearing methods in laboratory of the aquatic weevil *Hydrotimetes natans* (Fig. 1), the only specific herbivore found on this plant to date. Field observations and research by colleagues at the CSIRO indicate this weevil has very demanding rearing conditions.

During the pandemic we were able to maintain the colony in small tanks (10 liter and 40 liter tanks) in controlled conditions, but the rearing efficiency was irregular during the year due to variations in climatic conditions and the difficulty to obtain fresh plant material because of the Covid-19 restrictions. So, in July 2020 we initiated rearing using two 10 liter tanks with 20 adults each to evaluate the rearing efficiency under more strictly controlled temperature, pH and dissolved gases. After three months we obtained 69 new adults from 40 initial adults, and the adults are alive and keep laying eggs. It was discovered that *Hydrotimetes* did better in tanks where plant material was changed frequently (i.e., twice a week, Table 1).

	1	2	3	4
# Adults still alive	9	16	10	14
# New adults	25	44	-	-

Table 1. Adults obtained from the rearing tanks under controlled conditions.

Rearing was scaled up to 100-liter tanks with water heaters and CO_2 diffusors in order to begin the mass rearing of the weevil under less controlled conditions (Fig. 2). Each tank was filled with four pots of planted cabomba and we released 30 marked adults in each tank.

Figure 2. "Cabomba glasshouse": on the left small tanks for rearing under controlled conditions. On the right, the 100-liter tanks connected to pH sensors and CO_2 diffusors

Although the larger tanks produced comparatively fewer adults than the smaller, more tightly controlled tanks, the possibility of obtaining many new adults in big tanks and under less restricted conditions is a promising result (Table 2). Another set of four tanks have been set up to try to improve the mass rearing method.

	A	B	C	D	**Total**
OLD	16	11	15	5	**47**
NEW	10	17	15	3	**45**
Total	**26**	**28**	**30**	**8**	**92**

Table 2. Adults recovered from the mass rearing tanks.

Peanut smut, (*Thecaphora frezii*)

Mariano Maestro and Willie Cabrera Walsh

Thecaphora frezii (Basidiomycota) is a soil-borne pathogenic fungus, and the causal agent for peanut smut. This is currently the most damaging and prevalent disease of peanut farming in Argentina. It was first reported in the country in 1995, and by 2011-2012 it was present throughout the peanut-producing region.

Following an initial infection of the plant pegs during the budding stage, the fungus develops inside the peanut pods, with damage ranging from abnormal development of the seeds, with small sori on them, to their complete replacement by teliospores which are later released in the soil. Because there are no symptoms on the aerial part of the plant, it is difficult to assess the incidence and severity of the disease until the harvest. This exacerbates the economic losses, because the farmers will often give their fields all the usual agricultural inputs without knowing what their profit margins will be.

At present, management strategies of the disease include crop rotation (mostly with maize), deep ploughing to bury the teliospores deeper into the ground, and treatment of the seeds with fungicides to reduce initial inoculum. These strategies, however, have drawbacks of their own. Development of disease-resistant cultivars is under way, but yet without definitive results.

Argentina does not have a relevant local market for this crop, but it is one of the world's leading exporters of high-quality confectionery grade peanuts, with a share of 14.9% of the total market, valued in $445.64M in 2019. Although the usual safety measures are observed to prevent the disease from spreading into other peanut-producing regions through the export of infected seeds, experience teaches us that it pays to be prepared. Researchers from FuEDEI are working in cooperation with Dr. Rebecca S. Bennett, at the USDA-ARS- Wheat, Peanut, and Other Field Crops Research Unit, OK, to identify the different pathotypes of *T. frezii* and their

distribution in the peanut-producing region in Argentina, with a view to increasing our knowledge of the variability of the disease, and consequently developing a suitable management strategy.

We are currently processing infected samples from the main peanut-producing area in central Argentina to obtain monosporic cultures, *in vivo* and *in vitro*, from the different areas, with the aim of obtaining enough pure inoculum to compare them genetically.

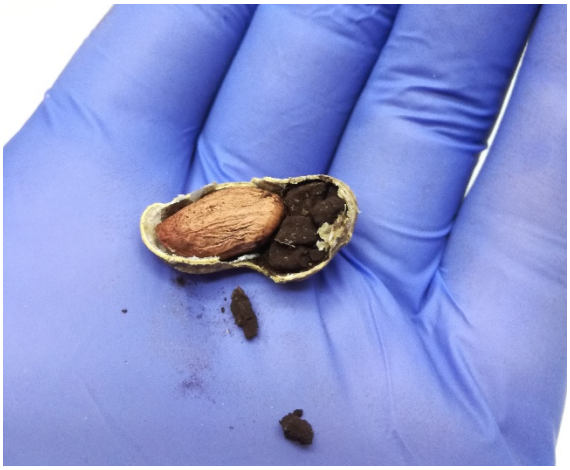

Figure 4. Open peanut showing abundant smut teliospores where the seed should be.

Untangling *Lysathia flavipes*, a confusing flea beetle associated with water primrose (*Ludwigia* spp.)

Ana Faltlhauser, Alejandro Sosa and M. Cristina Hernández

Water primrose (*Ludwigia grandiflora* ssp. *hexapetala*, Onagraceae) is an aquatic plant that can form dense mats that can overrun the banks of deep, fast-moving water bodies, and completely cover lakes and canals. Its invasion can alter water flow, bank structure, displace native plants, and affect water quality and biodiversity. It is native from South America but has invaded the U.S. and many countries in Europe. One of the natural enemies described many years ago in Argentina is *Lysathia flavipes* (Boheman). Both the larvae and adults of this flea beetle are defoliators, and they inflict heavy damage to water primrose. However, this species has also been recorded feeding on parrot's-feather, *Myriophyllum aquaticum*, and was therefore ruled out as a candidate to control *Ludwigia* in the 1980s due to its wide host range.

Recently, morphological observations suggest that more than one insect species might have been described. If this were corroborated, a potential specific control agent for *Ludwigia* could result from this research. To further investigate this, we are initiating studies to describe the morphology, host associations, and genetics of different *Lysathia* populations from *Ludwigia* and *Myriophyllum* in Argentina. The molecular approach, called species delimitation analysis, is based on coalescent simulations to test hypotheses about species limits. It can incorporate data from multiple loci and does not require species to have diverged to the point of being reciprocally monophyletic. The morphological description is based on samples from the different hosts and localities, and it will provide evidence if more than one entity is present.

Also included in this study is *Lysathia* sp. a successful biocontrol agent released in South Africa for parrot's-feather that hasn't been described yet, and that shows similar morphological traits to *L. flavipes*. The combination of molecular tools, detailed morphological comparisons, and host associations will help clarify and provide more information on this complex group of Neotropical flea beetles.

European Biological Control Laboratory - EBCL

From international collecting trips to a deeper understanding of local parasitoid assemblages: how Covid-19 shaped the viburnum leaf beetle project at EBCL in 2020

Gaylord Desurmont and Elven Kerdellant

The viburnum leaf beetle biological control project started at EBCL as early as 2016 but international collecting efforts for natural enemies of this invasive pest in North America did not start until 2019. At that date, a large sampling effort was done in Western Europe and egg masses of the beetle were

collected from 11 countries (Fig. 1). This effort had promising results and high numbers of parasitoids emerged from the eggs collected. Parasitism rate was as high as 25% in some locations. The same sites were supposed to be resampled in 2020, this time looking for larvae and/or adults of viburnum leaf beetle, and collection efforts were planned to be extended to Nordic countries and further east. This exciting program was cut short by the Covid-19 pandemic. All international travels were banned at EBCL during the first wave of the pandemic, which coincided with the period of egg hatch and larval development of the viburnum leaf beetle. Fortunately, this insect is also present in France and several sites had been previously identified in the vicinity of the EBCL laboratory in the Montpellier area with infested *Viburnum tinus* shrubs. Thus, since large international collecting trips were impossible, local prospection efforts were intensified. The focus of the work also changed. Instead of looking at the broad assemblage of natural enemies of the insect, we investigated two more precise research questions: 1) do the egg parasitoids of the viburnum leaf beetle also parasitize eggs of the closely related and locally common elm leaf beetle? And 2) what abiotic factors are correlated with the presence and abundance of the beetle in the area? Between May and September 2020, a total of 251 sites within 50 km distance of Montpellier were investigated for the presence of the viburnum leaf beetle, and 35 for the presence of elm leaf beetle. All local collecting trips were done following the sanitary rules and regulations in place in France at the time of the trips and all the sanitary protocols and recommendations internal to EBCL at the time.

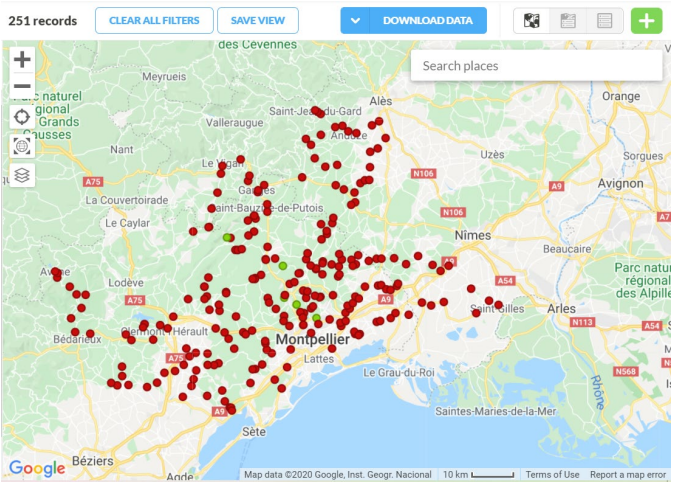

Figure1. Sites investigated for the presence of the viburnum leaf beetle between May and September 2020.

These local trips were a success at different levels. Firstly, we discovered nearby sites where the viburnum leaf beetle was outbreaking, which is not very common for this species in its native range. Interestingly, these outbreaking sites were often nearby sites where the insect was totally absent on its host plant. Populations of the viburnum leaf beetle had been observed to be highly fluctuant in the south of France but these observations were purely anecdotal. Having gathered information on more than two hundred sites in the Montpellier area will be highly valuable to investigate the factors that are associated with the abundance of this beetle in this part of its native range and understand the circumstances under which these outbreaks occur in the field.

Secondly, these local trips also led to a better understanding of the local natural enemies of the viburnum leaf beetle and the elm leaf beetle (Fig 2).

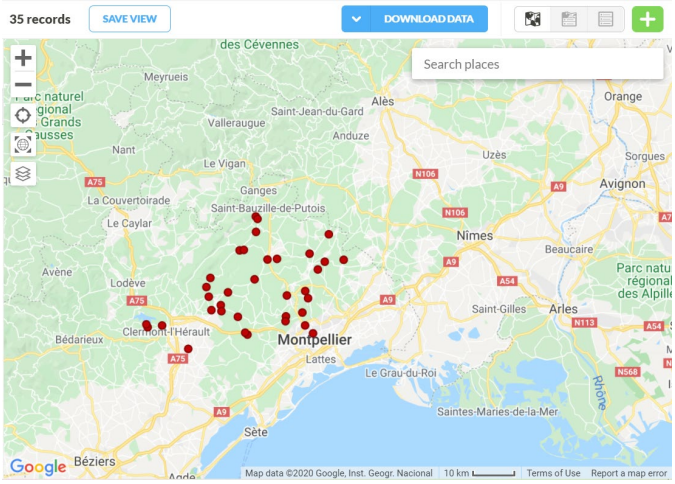

Figure 2. Sites investigated for the presence of the elm leaf beetle between May and September 2020.

A total of 1064 parasitoids emerged from the elm leaf beetle egg masses collected and we were able to determine that there is indeed some level of overlap between the egg parasitoid assemblages of the two beetle species. The large number of parasitoids collected meant that we had enough individuals to test some interesting hypotheses regarding the genetic divergence and variation in some morphological traits between individuals of these parasitoids depending on the host they emerged from. These ongoing analyses will help evaluating the host specificity of viburnum leaf beetle egg parasitoids and their ecological role and impact in local food webs, with direct implications for classical biological control perspectives.

In conclusion, the challenge posed by Covid19 led a brutal stop for international collection

efforts for this project but was also an opportunity to have a deeper look at our local field resources and led to unexpected exciting new discoveries regarding the viburnum leaf beetle and its parasitoids. International trips may resume in 2021 if the sanitary situation improves in Europe. Otherwise, there is no shortage of "local questions" to investigate. Even though classical biological control programs often do require extensive amounts of long-distance travels, being stationed within the native range of an invasive pest means some amount of field work can still be conducted at times when travels are banned or strictly limited.

Celebrating the International Education Week: 16-20 November 2020

Alexandra Chaskopoulou

International Education Week (IEW) is an opportunity to celebrate the benefits of international education and exchange worldwide. This year EBCL-Greece in collaboration with American Farm School and Perrotis College celebrated the IEW by creating a thread of short videos telling stories of how faculty and researchers contributed to international education (Figure 1). Alexandra Chaskopoulou, research scientist at EBCL-Greece and affiliate faculty of Perrotis College, explained how her laboratory in Thessaloniki has hosted more than 50 students during the last 4 years, from across the world and across all levels of education. Students have been very excited for the opportunity to participate in research projects that improve their knowledge and technical skills but most importantly they value the opportunity to work in an international environment and build meaningful friendships with peers from across the world.

Top, Left: Leighanne Penna, Associate Dean of Student Services, American Farm School
Top, Right: Kiki Zinoviadou, Professor, Perrotis College
Bottom, Left: Helen Yarenis & Eleni Kantyltzoglou, Study Abroad Coordinators, American Farm School
Bottom, Right: Alexandra Chaskopoulou, Research Scientist, USDA-ARS EBCL

USDA-ARS has always been committed to educate students in agricultural research, and research internships are an effective method to reach out to the student community and attract them to agricultural sciences. EBCL laboratories with their two locations in France and Greece present unique opportunities for both US and Europe-based students to participate in international research programs. Through International research students improve their understanding and appreciation of science but also expand their understanding of the world. Every year EBCL researchers work closely with universities from across the world to support undergraduate and graduate research programs of common interest. Happy International Education Week to All!

West Nile Virus Research Updates for 2020 Mosquito Season

Alexandra Chaskopoulou

While the number one priority of public health authorities globally is to decrease the spread of COVID-19, protection of the public from vector-borne diseases remains an essential service, and public health authorities across the world are maximizing their efforts to prevent any interruptions in vector surveillance and control operations. EBCL-Greece in collaboration with Greek departments of public health and vector control agencies continued to survey for West Nile virus (WNV) during the 2020 mosquito season. EBCL scientists in Greece

have been working in improving a WNV early warning surveillance system specifically targeting the threatening WNV lineage 2 strains. The system relies on detecting viral-RNA in field-collected mosquitoes and screening of sentinel-chickens for WNV specific antibodies. During 2020 the system was successfully implemented and provided information on WNV mosquito circulation and enzootic transmission two months prior to human cases allowing for targeted and proactive vector control interventions.

The EFSA's (European Food Safety Authority) and ECDC's (European Center for Disease Prevention and Control) comprehensive technical report on vector control strategies for WNV (authored by Alexandra Chaskopoulou -EBCL, Marietta Braks, Wim van Bortel) has been published this November and is available online at: https://www.ecdc.europa.eu/en/publications-data/vector-control-practices-and-strategies-against-west-nile-virus. The report collated information on WNV vector management practices across the world under specific operational contexts and identified gaps in knowledge and capacities relating to effective WNV vector management practices.

Understanding the estivation behavior of invasive snails to develop new pest management strategies

Gaylord Desurmont, Priscillia Hanache, Thierry Thomann, Valérie Caron

Background

Snails are paradoxical organisms: even though they have evolved from marine ancestors and typically prefer dark and humid environmental conditions due to their soft body prone to desiccation, many species have adapted to hot and dry climates. Some of these species have been accidentally introduced to other areas of the world and have become invasive: this is the case of *Theba pisana*, *Cochlicella acuta*, *Cochlicella barbara*, and *Cernuella virgata*, which are all native to the Mediterranean basin and are considered serious invasive pests in Australia. Despite having a generally negligible impact on plants, they can represent a major threat for cereals due to their shells being harvested and mixed with grains, causing post-harvest grain contamination.

Left: the four snail species tested in the study (top left: Theba pisana; bottom left: Cernuella virgata; top right: Cochlicella Barbara; bottom right: Cochlicella acuta). Right: example of aggregation of snails on a post during summer in Australia. Ph *Left: the four snail species tested in the study (top left: Theba pisana; bottom left: Cernuella virgata; top right: Cochlicella Barbara; bottom right: Cochlicella acuta). Right: example of aggregation of snails on a post during summer in Australia.* *Photos (left): Priscillia Hanache.*

In order to survive under arid and semi-arid conditions, snails typically observe a prolonged period of rest during the warmest months of the year, a behavior called aestivation (or estivation). When daily temperatures start rising above a certain threshold, snails of the four species abovementioned climb on vertical supports such as stems and poles to estivate, typically in groups that can gather large numbers of individuals, a phenomenon referred to as "clustering". It is generally accepted that the main benefit of climbing on vertical supports is to escape the heat of the ground, and this benefit is more pronounced in groups of snails than in individual snails. Even though the aestivation behavior of snails is well known, its modalities remain vastly underexplored. It is for example unknown whether snails estivate in groups as a result of an active aggregation behavior or simply because they compete for the same estivation spots. A study was conducted in 2019 as a collaborative effort between the European Biological Control Laboratory (EBCL) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Montpellier, France, in order to improve our understanding of the estivation behavior of *T. pisana*, *C. acuta*, *C. barbara*, and *C. virgata*. The main objective of the study was to investigate snail preferences for different estivation supports and to determine whether snails use visual or chemical cues to locate estivation sites. The study was conducted with hopes of developing new management tools against these species. Many management strategies have been implemented against invasive snails in Australia, including a large classical biological control program using parasitic flies, but none of them have been completely successful.

The experiments of this study were conducted by Priscillia Hanache, a Master of Sciences student in Ethology and Ecology from University Jean Monnet (Saint Etienne, France) under the supervision of Thierry Thomann (CSIRO), Valérie Caron (CSIRO), and Gaylord Desurmont (EBCL).

Material and methods

All snails from the four species tested were collected in the Montpellier area. Experiments were conducted under both laboratory and field conditions, testing the following hypotheses: a) snails can discriminate between vertical supports of different heights and thicknesses based on visual cues, b) snail choices are affected by the presence of congeners or snails of another species on vertical supports, c) snail choices are affected by the presence of mucus of congeners or of another species. In order to test these hypotheses, laboratory and field choice-tests were designed. In the laboratory, innovative test arenas were designed. Briefly, a test arena was a circle limited by white cardboard. Black supports (pieces of black paper) were placed side by side against the white background and individual snails were released in the center of the arena, facing the two supports. Snails were left in the arena for 10 minutes and snails that did not make a choice (i.e. climbed on a support or touched the white background) were considered as "no-choice". To test hypothesis b), the supports were replaced by black poles: one of the poles had ten snails of the same species or ten snails of another species, and the other pole remained snail-free. Individual snails were released in the center of the arena facing the two poles. To test hypothesis c), snails were again placed in the center of the arena facing two poles. One of the poles had a mucus trail of the same snail species or another snail species leading to it, and the other pole was mucus-free. In the field, hypothesis a) was the only one tested, and the experimental setup was different. Field test plots were quadrats with nine vertical poles in each quadrat. Poles within each quadrat were varying either in diameter (three diameters tested, three poles per diameter) or in height (three heights tested, three poles per height). A hundred snails of the same species were released in the middle of each quadrat and were recollected 24 hours later. The number of snails that had climbed on each pole during the experimental period was counted.

Most of the experiments were conducted at two different time points during the year: once in the spring (March-May), at a time of the year when snails are still active, and once in summer (July) during the natural estivation period of the four snail species tested.

Left: experimental setup for support thickness choice-test under laboratory conditions. Right: experimental setup for support thickness choice-test under field conditions. Photos: Priscillia Hanache.

Results

Although the results of the study are currently being analyzed and will be the subject of a publication in a peer-reviewed scientific journal, we can already conclude that the study was highly successful. In the laboratory snails of the four species were perfectly able to orient toward the different vertical supports in the test arenas and were highly responsive. This is promising for future studies and shows that the behavioral ecology of these four snail species can be thoroughly studied under laboratory conditions. Moreover, snails showed marked preferences for supports with certain characteristics of diameter and height, which allows us to conclude that they do discriminate among vertical supports based on visual cues. However, the study remained unconclusive regarding the role of chemical cues in snail orientation. Chemical analyses of the air contained in snail rearing boxes revealed that snails were actually quite odorless: only trace amounts of volatile organic compounds were detected. In the field, the quadrat choice-test design worked very well. Snails climbed eagerly on the supports offered to them, often forming clusters that gathered up to dozens of snails. The 24h experimental period was thus sufficient to elicit the "climbing and clustering" behavior associated with estivation. Results of the field choice-tests matched nicely the results obtained in the laboratory in terms of snail preferences.

Conclusions

Snails have successfully colonized many arid and semi-arid environments, and the estivation behavior is a critical ecological adaptation that contributes to their survival under such harsh abiotic conditions. It is therefore not surprising that snails are selective when it comes to choosing estivation sites. Our study sheds light on some of their

preferences for vertical supports and are highly promising but many aspects remain to be explored. We tested supports varying in height and diameter, but other factors could play a role such as the color, shape or material used for the supports. It also remains to be elucidated whether snails manage to recognize their congeners chemically and adjust their estivation preferences depending on the presence of other snails. In terms of application for pest management in areas where these snails are invasive, the fact the snails prefer vertical supports showing certain characteristics of height and diameter means that attractive vertical supports could theoretically be developed. Such "bait" supports could be planted inside or in the vicinity of cereal fields in order to divert the snails from climbing onto cereal stems. However, such an approach would require that snails are attracted from a distance to the "bait" supports, and it remains to be elucidated whether or not this is possible in the field. Additional field tests under realistic conditions are needed to further explore the potential of exploiting the estivation behavior of invasive snails for management purposes.

Current Status of Cooperative Research with BBCA - Biotechnology and Biological Control Agency
Rome, Italy

Massimo Cristofaro

The Biotechnology and Biological Control Agency (BBCA), located near Rome, Italy is a small non-profit Foundation, born in 2000. The staff includes 2 Research Entomologists, 1 Biologist, 2 technicians, 2 Ph.D. students and one administrator. BBCA is working very closely with USDA ARS-EBCL, France in several projects on the biological control of weeds and insect pests; in addition, BBCA has research agreements with other USDA-ARS laboratories, located in USA and few other projects with other research institutes. Below is an updated list of ongoing projects on weeds and insect pests.

WEEDS	PARTNERS	ARTHROPOD PESTS	PARTNERS
Yellow star thistle	USDA-ARS - EBCL	Bagrada	EBCL
Russian thistle	USDA-ARS - EBCL	BMSB	FEM-EBCL-USDA
Scotch thistle	USDA-ARS - EBCL	Cattle fever tick	EBCL- UniNaples
Medusa-head	USDA-ARS - EBCL	Medfly	FEM
Ventenata	EBCL	Drosophila suzukii	UniRome1-FEM
Cheatgrass	USDA-ARS	Red palm weevil	INRA (France)
Giant reed	USDA-ARS		
Guinea grass	USDA-ARS		
Tree of heaven	EBCL		
Ice plants (3 species)	USDA-ARS		
Leafy spurge	USDA-ARS		
Rush skeletonweed	MSU - BLM		
Russian olive	MSU - CABI		
Perennial pepperweed	CABI - BLM		
Houndstongue	UniIdaho - USDA		
Saltcedar	CSU - South Africa		
Russian knapweed	CABI		
Dyer's woad	CABI		

The 2020 was a peculiar year worldwide, because of the COVID-19 pandemic. In particular, Italy was facing a very difficult period at the beginning of the spring (from mid-March until the end of May): the pandemic in that period was extremely severe, and we were in full lockdown mode, completely isolated from the rest of the world, and confined in our own houses. For this reason, BBCA lost the possibility to carry out most of the research activities (and all the foreign travels) at the

Figures 1 - 3. From left to right - laboratory bioassay on giant reed, BBCA facilities; open field test for the tree of heaven mite at the BBCA facilities, Rome, Italy; ice plants near Trapani, Sicily.

beginning of the spring. In the middle of March, we decided to organize the research activities (particularly the ones connected with field travels) in a "modular way", according to 4 possible different scenarios.

i) In the worst case (full lockdown), I was considering the possibility to work on projects that can be managed within the laboratory space and the town where we are living: this was the case, during April and May, of two weed projects (giant reed, *Arundo donax*, and tree of heaven, *Ailanthus altissima*) and one insect pest (BMSB, brown marmorated stink bug, *Halyomorpha halys*), plus the open field test carried out in Plovdiv (Central Bulgaria) by our Bulgarian cooperators (only the maintenance of the field plot during the year 2020), for a total of 5 targets.

ii) If the sanitary situation improved by mid spring and we were allowed to travel and perform field work in the entire Italian territory (this was suitable since the beginning of June), it would be possible to improve our field activities, including 2 other important weeds (cheatgrass and medusahead), plus the first surveys a collections for a new target weed complex (ice-plants) and some work on the biocontrol of Bagrada bug, for a total of 9 targets (7 weeds and 2 insect pests).

iii) The best (realistic) scenario (travels allowed within Europe), was reached just at the beginning of July. We were able to perform some field surveys in Greece, too late for insect collections (on cheatgrass), but still in time at least to collect seeds of our target weed.

iv) Realistically, I did not believe it would be possible for us to carry out surveys outsi de Europe during 2020: however, at the beginning of October, we were able to perform a nine-day trip in Central Kenya, where we collected a suitable number of eriophyid mites on Guineagrass and send them to our cooperator John Goolsby, USDA ARS TX.

In conclusion, our previsions were correct, and, at the end, we were very fortunate: MAY. during the month of May we performed a successful laboratory bioassay focused on the selection of a nematode-free colony of the leafminer midge *Lasioptera donacis* of

Figures 4 - 6. From left to right - the pentatomid pest Bagrada hilaris; the blooming ice plant Carpobrotus edulis in Sicily; cheatgrass in Central Greece.

giant reed (**Fig. 1**); and we carried out the second open field host range test with an eriophyid mite (*Aculus mosoniensis*) associated to the **tree of heaven** (**Fig. 2**), since both target weeds and candidate agents are very common in Rome area; JUNE. during the month of June it was possible to travel in Sicily (**Fig. 3**) (we made collections on **medusa-head**, **cheatgrass, ice-plants** and **Russian thistle)** and we started to do some research activities in laboratory with **BMSB**; JULY. on July, we started some field work together with EBCL on **Bagrada** in Sicily (**Fig. 4**), where we also made several leaf samplings on **ice plants** (**Fig. 5**), at the end of the month we performed a field trip in Greece where we sampled cheatgrass and ice plants (**Fig. 6**); AUGUST. in August we close the open field test on the **tree of heaven** and collect 2 weevil species on **saltcedar** (**Fig. 7**); OCTOBER. finally, at the beginning of October we performed a successful field trip in Nanyuki area (Central Kenya), where we collected an important population of a new species of eriophyid mite (*Abacarus* sp.), associated to **Guinea grass** (**Fig. 8**): the mites have been sent to the USDA-ARS quarantine facilities in Texas. NOVEMBER. we described the morphology of the seed galling wasp *Tetramesa amica* on medusa-head.

In particular with **Bagrada** and the **tree of heaven**, we were able to set up a joint research program with EBCL: the work with the pentatomid-bug pest is still ongoing, with weekly shipment of sterile eggs to the EBCL, while the open field test of the tree of heaven has been closed and we are processing the results. Regarding the other target weeds and pests, the lockdown interfered with the possibility to perform observations and to carry out bioassays on the life cycle of the target species and their selected biocontrol agents. Regarding **YST** and **Scotch thistle**, BBCA organized -in cooperation with 2 scientists of the University of Plovdiv- an open field test at the beginning of November 2019 in a field plot in the campus of Plovdiv University (Central Bulgaria) (**Fig. 9**): everything went well, test plants were growing, but the COVID-19 pandemic in Italy and Bulgaria did not allow to collect the 2 weevil species (*Larinus latus* and *L. filiformis*) and release them in the field plot during the late spring and the early summer of 2020. We remained in touch with our Bulgarian cooperators in order to have the field plot in place for 2 years, with the purpose to carry out -hopefully- the multiple open field host range experiment during the spring and early summer of 2021.

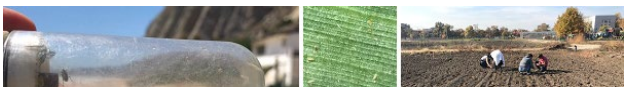

Figure 7 - 9. From left to right - Coniatus weevils on saltcedar; Abacarus mites on Guinea grass near Nanyuki, Kenya; field plot in Plovdiv, central Bulgaria (November 2019).

PUBLICATIONS.

De Biase, L. Smith, **M. Cristofaro, 2019.** Three prospective agents instead of one*? Cryptic diversity of the biological control agent* Psylliodes chalcomera. Biological Control. DOI: 10.1016/j.biocontrol.2019.103998

G. F. Sutton, K. Canavan, M.D. Day, A. den Breeyen, J.A. Goolsby, **M. Cristofaro**, A. McConnachie, I.D. Paterson, **2019.** *Grasses as suitable targets for classic weed biological control.* BioControl, https://doi.org/10.1007/s10526-019-09968-8

P. Weyl, G. A. Asadi, **M. Cristofaro**, B. Vidovic, R. Petanovic, **F. Marini** & U. Schaffner, **2019**. *The host range and impact of* Aceria angustifoliae *(Eriophyidae), a potential biological control agent against Russian olive* Elaeagnus angustifoliae *(Elaeagnaceae) in North America*. Biocontrol Science and Technology, https://doi.org/10.1080/09583157.2019.1675144

D.M. Suckling, M.C. Levy, **G. Roselli**, V. Mazzoni, C. Ioriatti, M Deromedi, **M. Cristofaro** & G. Anfora, **2019**. *Live traps for adult brown marmorated stink bugs*. Insects 10, 376; DOI: 10.3390/insects10110376

D.M. Suckling, **M. Cristofaro, G. Roselli**, M.C. Levy, A. Cemmi, V. Mazzoni, L.D. Stringer, V. Zeni, C. Ioriatti & G. Anfora, **2019**. The competitive mating of irradiated brown marmorated stink bugs, Halyomorpha halys, for the sterile insect technique. Insects, 10, 411, doi:10.3390/insects10110411

M. Cristofaro, G. Roselli, **F. Marini**, E. de Lillo, R.U. Petanovic, B. Vidovic, M. Augé & B.G. Rector, **2020.** *Open field evaluation of* Aculodes altamurgensis, *a recently described eriophyid species associated with medusahead (*Taeniatherum caput-medusae*)*. Biocontrol Science and Technology, DOI: 10.1080/09583157.2019.1711021

V. Di Ilio, **M. Cristofaro, 2020**. *Polyphenolic extracts from olive mill wastewater as a source of biopesticides and their effects on the life cycle of the Mediterranean*

fruit fly Ceratitis capitata *(Diptera, Tephritidae)*. International Journal of Tropic Insect Sciences, https://doi.org/10.1007/s42690-020-00224-6

F. Freda, M. Masi, J. Kashefi, **M. Cristofaro,** S. Musmeci & A. Evidente, **2020**. *Acaricidal activity of the plant sesquiterpenoids α-costic acid and inuloxin A against the cattle ectoparasitic tick,* Rhipicephalus (Boophilus) annulatus. International Journal of Acarology, https://doi.org/10.1080/01647954.2020.1805002

I. Pervukhina-Smith, R.F.H. Sforza, **M. Cristofaro,** J. Smith, S.J. Novak, **2020.** *Genetic analysis of invasive populations of* Ventenata dubia (Poaceae): *an assessment of propagule pressure and pattern of range expansion in the Western United States*. Biol. Invasion, 22: 3575-3592, https://doi.org/10.1007/s10530-020-02341-2

Upfront Research to Control Bagrada Bug

R. F. H. Sforza and G. Martel

Since 2015, EBCL is conducting research on Biocontrol of the severe pest bagrada. In less than a decade, *Bagrada hilaris* Burmeister (Hemiptera, Pentatomidae) (=bagrada) has become a major threat to crucifer crops in the Americas inducing yield losses as high as 25 % in Arizona. Originated from Asia and Africa, bagrada was first discovered in California in 2008, and rapidly spread to other U.S. states including Arizona, and New Mexico. Bagrada is absent from the continental Europe. Currently in the quarantine of EBCL, permanent rearings of the bug originated from California and South Africa are routinely maintained, plus a population of a promising egg parasitoid.

Bagrada, also a pest in its native range, and what about natural enemies?:

Wherever bagrada is present in the native range of Asia and Africa, it is considered a pest on cole crops. Since 2018, Kenya and South Africa, as parts of the native range, were surveyed by EBCL. Bagrada was found on broccoli, cabbage and rooibos plants, as well as on wild brassicas. In the frame of the biocontrol program, we always searched for native parasitoids. In Kenya, few egg parasitoids were collected from exposed sentinel eggs and are still under investigation for their identity. In South Africa, a Master student, Susana Das Neves, from the University of Stellenbosch is working on local populations of bagrada in rooibos tea fields. The purpose of her work is to expose sentinel bagrada eggs at different heights both aboveground (0 to 40cm) and belowground in the field to catch native egg parasitoids and also to install Malaise traps in highly infested tea fields. The tea area is located near Clanwilliam in the Western Cape Region. Together with Susana, we visited a citrus orchard on the farm (Fig. 1) and found a large population of bagrada on wild *Brassica* sp. in between the citrus tree rows. All bagrada stages were present, mated couples

Figure 1. Field work in South Africa to collect bagrada in Citrus orchard (RFH Sforza)

were numerous and located in the cracks at the crown of wild plants. Few adults were buried in the sand (4-5 cm). *Brassica* plants were severely damaged (punctures). The impact of bagrada on rooibos plants occurs when wild crucifiers are dried out, and bagrada colonies moved on plants with green foliage later in the summer. We collected nearly 500 individuals by beating Brassica plants directly into the bugdorm cage. Bagrada infestation on wild *Brassica* is very localized, sometimes on 1 or 3 big Brassicas, with no bagrada found on surrounded plants at few meters distance.

In 2020, an additional survey was conducted on the island of Pantelleria (Italy) to observe the impact of bagrada on caper plants. Bagrada was introduced there since the 1980s, most likely from Africa. Sentinel eggs were also exposed during the course of the summer with no evidence of parasitism so far (Fig. 2).

Figure 2. Field work on Pantelleria island with Guillaume Martel installing Petri dishes with sand to collect bagrada eggs naturally oviposited (RFH Sforza)

Catch me if you can?:

Since 2018, EBCL is hosting a PhD student, Guillaume Martel to work specifically on bagrada and its egg parasitoid, *Gryon gonikopalense* Sharma (Hymenoptera, Scelionidae). Various approaches were investigated like the life history traits of the parasitoid (Martel et al., 2019). Beyond its reproductive capacities and its high longevity, its major advantage is its ability to detect and parasitize buried eggs of bagrada (Martel & Sforza 2019). Pentatomid stinkbugs usually deposit clustered eggs on their host plants. As an exception, the phytophagous pest *Bagrada* buries single eggs in the ground. We then aimed to evaluate the capacity of *G. gonikopalense* to respond to the original bagrada ovipositional behavior. Our results showed that *G. gonikopalense* exhibits a well-adapted foraging behavior observed by tracing and successfully parasitizing 2mm-deep buried bagrada eggs, which was further supported by video recordings. Four conditions of egg detectability and foraging duration were tested (Figure 3). After 2

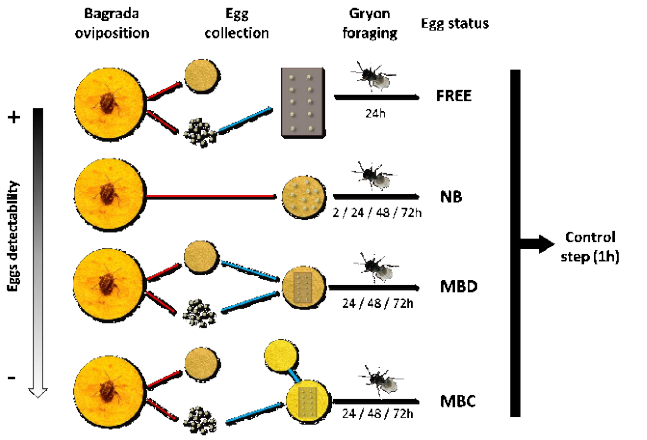

Figure 3. Experimental design for assessing the foraging ability of Gryon gonikopalense (Hymenoptera, Scelionidae) on eggs of Bagrada hilaris (Hemiptera: Pentatomidae). Experiments were conducted with non-buried eggs (FREE), naturally buried eggs (NB) and manual buried eggs with either clean (MBC) or disturbed (MBD) sand. Eggs from MBD and MBC were grouped on a cardboard before burial. Coloured arrows represent different steps: red for egg and substrate collection, blue for preparation of egg series and black for parasitoid exposure (test followed by control).

hours, 71% of tested parasitoids succeeded, and after 72 hrs 100% of them succeeded (Figure 3 and 4). Our data suggested that host egg detection was based on chemical cues since parasitism success was reduced by up to 20% when eggs were manually buried under a clean substrate (without chemicals) or a substrate previously used for oviposition but disturbed. Evidently, *G. gonikopalense* can overcome both the egg detection and physical barrier constituted by the sand to parasitize the pest, though fitness limitations must be considered.

Where to go now?:

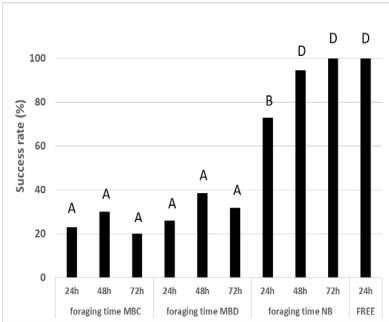

Figure 4. Parasitism success rate of Gryon gonikopalense exposed to Bagrada hilaris non-buried eggs (FREE), naturally buried eggs (NB), and manually buried eggs with clean (MBC) or disturbed (MBD) sand. Foraging times were 24, 48 or 72 hours for categories NB, M BC, MBD and 24 hours for FREE. Values associated with the same letter were not statistically different (P > 0.05).

Exploration is highly needed, because a combination of well adapted egg parasitoids to bagrada oviposition behavior will help controlling the pest in *Gryon gonikopalense* was collected from Pakistan, but the genus Gryon is diversified and co-evolved species with bagrada are likely to be present in Africa.

In the laboratory, we already started to work on the egg foraging by *G. gonikopalense*, especially on its capacity to detect and parasitize buried eggs. In addition, we looked at the role of the host plant on the distribution pattern of bagrada eggs and the response of *G. gonikopalense* to these different patterns if any. All this ongoing research at EBCL aims to provide at short term a biocontrol response to the bagrada invasion in the U.S.

References:

Martel, G., Augé, M., Talamas, E., Roche, M., Smith, L., & Sforza, R.F.H. 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

Martel, G., & Sforza, R.F.H. 2020 Catch me if you can: novel foraging behavior of an egg parasitoid, Gryon gonikopalense, against the stinkbug pest, *Bagrada hilaris*. Journal of Pest Science (submitted).