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In This Issue

Introduction

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

The **Australian Biological Control Laboratory (ABCL)** is based in Brisbane, Australia. The facility is run through a Specific Cooperative Agreement between USDA-ARS and Australia's Federal research body, CSIRO.
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The **European Biological Control Laboratory (EBCL)** is based in Montpellier, France, and has a satellite laboratory in Thessaloniki, Greece. Contact: Lincoln Smith, Link.Smith@ars.usda.gov / www.ars-ebcl.org

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

The **Sino-American Biocontrol Laboratory (SinoABL)** is based in Beijing, China.
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Australian Biological Control Laboratory - ABCL

Potential expanded facilities for ABCL

by Matt Purcell

ABCL is collocated with the CSIRO tropical invasive plants team in Brisbane. While ABCL conducts exploration in Australia and Asia for biological control agents which are invasive in North America, the CSIRO tropical weeds team conducts biological control research on weeds which are invasive in Australia, including African boxthorn, parkinsonia, sow thistle, fleabane, Brazilian pepper and tropical soda apple.

Over several years the funding for these Australian-focused weeds has increased significantly leading to an expansion of the team and the need for facilities with a greater capacity. At the same time, ABCL has increased its portfolio of weed targets to include new terrestrial targets such as ear leaf Acacia, *Acacia auriculiformis*, and aquatic targets such as crested floating heart, *Nymphoides crista*. This expansion by both teams has put great stress on the current infrastructure at the EcoSciences Precinct in Brisbane. This facility is shared between multiple CSIRO Business Units as well as science departments from the state of Queensland. Being a multistorey building with the main plant and insects culturing facilities (including quarantine) located on the roof, there is little room for expansion (Fig. 1). ABCL in conjunction with the CSIRO tropical weeds team is seeking to lease plant growing facilities off site at Redlands, approximately 22 km east of the current facility in Brisbane.

Figure 1. Quarantine, plant growth and insect rearing facilities are predominantly in a restricted space on the roof of a multistorey building at the Ecosciences Precinct in which ABCL is located.

Extensive glasshouse space (both evaporative cooled and air-conditioned) is available for leasing from the Queensland State Government (Fig. 2). Although having two sites will be a logistical challenge, this is the best way to address current research needs, and going forward it provides a mechanism to address the significant fluctuations in research funding.

Figure 2. Air-conditioned and evaporatively cooled glasshouses are available for lease by ABCL/CSIRO at Redlands, 22 km east of ABCL.

The ABCL host organisation, CSIRO, is seeking to provide funding for the leasing of glasshouse space at Redlands, a dedicated vehicle to facilitate the logistics of moving stock between locations, and extra labour to address requirements across two sites.

Biological control of ear leaf Acacia

by Christine Goosem, Jeff Makinson, Matt Purcell, Muhammad Nawaz, Paul Madeira and Ryan Zonneveld

Exploratory surveys to find biological control agents of ear-leaf Acacia, *Acacia auriculiformis*, have been underway for several years. Initial surveys were conducted over tropical north Queensland where this tree species is native. Subsequently, molecular analysis of plant tissue samples was undertaken by Dr. Paul Madeira at the USDA Invasive Plant Research Laboratory in Fort Lauderdale, Florida. These plant tissue samples were collected by ABCL staff from the native range of *A. auriculiformis* at field sites in Queensland and the Northern Territory. Samples were also taken from seedlings grown at ABCL from seed obtained from the Australian Tree Seed Center, operated by CSIRO in Canberra. These seeds had been collected and deposited at the Center from not only multiple sites in the Australian distribution of this tree, but also from Papua New Guinea and Indonesia. The results indicated that the origin of the US introductions appears to be from the Northern Territory. In 2018, exploratory surveys for biological control agents in Australia have now refocused on this region.

One of the potential biological control agents discovered thus far is a Chrysomelidae beetle, *Calomela intermerata*, whose adults and larvae both feed on the young foliage of *A. auriculiformis* (Fig. 3).

Figure 3. The leaf-feeding Chrysomelidae beetle, *Calomela intermerata*, a potential biological control agent of ear leaf Acacia, *Acacia auriculiformis.*

This beetle has been collected in both the Northern Territory and in Queensland but genetic characterisation determined a 10% difference (C01) between populations. However, in the laboratory adults readily interbreed and produce viable offspring. In July surveys of *A. auriculiformis* will be conducted from Queensland across to the Northern Territory and *C. intermerata* beetles will be collected in the transitional zones between regions and subsequently genetically characterised. The biology and life history of both populations is being compared in laboratory studies and their performance on *A. auriculiformis* sourced from Queensland, the Northern territory, Papua New Guinea and Indonesia is to be tested.

Calomela intermerata is one of many exciting potential agents discovered thus far. Others include gall-forming *Trichilogaster* wasps (Fig. 4), seed-feeding *Melanterius* weevils and leaf tying Gracillariidae moths, *Macrobathra leucozancla* and *Leptozestis* spp.

Figure 4. *Trichilogaster* galls on *Acacia auriculiformis* collected from North Queensland.

Foundation for the Study of Invasive Species - FuEDEI

Biological control of Water Primrose

by Ana Faltlhauser, M. Cristina Hernández

Two species of water primrose, *Ludwigia grandiflora* subsp. *hexapetala* and *L. peploides* subsp. *montevidensis* are invasive weeds in USA that originated from South America. The genus *Ludwigia* is highly diverse, and species are grouped by 'sections' based on their similarities. The two species invading the USA are in the Jussiaea Section, and are characterized by their fast growth and the ability to reproduce both sexually and vegetatively. These two species are also invasive in France, England, Belgium, Italy and the Netherlands.

The thrips *Liothrips ludwigi*, found on both *Ludwigia* species in the field, is being studied as a biocontrol agent candidate at FuEDEI (Fig. 5). It has very promising features, such as shoot tip feeding and a narrow host range. In host specificity tests, *L. ludwigi* was highly specific to *L. g.* subsp. *hexapetala* and *L. p.* subsp. *montevidensis*.

Figure 5. *Liothrips ludwigi*, adults and nymphs, a prospective biological control agent of water primrose.

We are studying the comparative performance of the thrips on both species of invasive *Ludwigia* through multiple generation crossings, in order to evaluate if any maternal effects could be carried over in our laboratory cultures. Such effects could affect the performance of a population eventually released in the field. The research was performed by the newest staff addition to the *Ludwigia* project, biologist Ana Faltlhauser. Ana obtained a scholarship from the National Scientific and Technical Research Council (CONICET) to do her PhD at FuEDEI from April 2018 to April 2023.

Ana's research will concentrate on the search for and identification of prospective biological control agents of weedy water primrose species, as well as ecology and phenology of the target plants (Fig. 6). This research also aims to develop an augmentative biological control program in rice fields of Argentina, where the plants are native (Fig. 7).

Figure 6. Studying diversity of *Ludwigia* species in the Paraná River delta, Argentina.

Figure 7. Rice field in Corrientes, Argentina, infested with *Ludwigia bonariensis* (Macrocarpon sect.).

Collaborative projects at FuEDEI

by Guillermo Cabrera Walsh

Although FuEDEI's main program is associated to the USDA, ever since the 1990s, while it was still an ARS overseas laboratory, it has undertaken several projects with other foreign institutions, notably the CSIRO, ARC-PPRI, and CABI. Recently too, we have accepted PhD and post-doc students from the CONICET (National Scientific and Technical Research Council of Argentina). Most of these projects were of interest to the USDA as well, and in the particular case of our CONICET grantees, their projects were creatively woven into our ARS projects in such a way as to cater for Argentine interests, while enhancing FuEDEI's research capacities. Following are brief accounts of some of our external research programs.

Floating pennywort (*Hydrocotyle ranunculoides*) (CABI).

This floating, rooted aquatic Araliaceae is becoming a weed of concern in Europe and western Australia. Its thick floating growth produces water fouling, and hinders water use, flood control and hydroelectric generation. The weevil *Listronotus elongatus* is a specific and damaging natural enemy that is being considered for release in the UK (Fig. 8). FuEDEI is currently working on obtaining permits to export the weevil to England, and performing cold tolerance tests in order to assess if this weevil, which inhabits the mild temperate lower delta region, could adapt to the British winters.

Figure 8. Damage by the weevil *Listronotus elongatus* on floating pennywort; Top: adult on leaves, Bottom: larva and pupa in the stolon.

Parkinsonia (*Parkinsonia aculeata*) (CSIRO).

This spiny tree in the Fabaceae was introduced in Australia as an ornamental and hedge tree. It is widespread through Western Australia, the Northern Territory and Queensland, covering some

8,000 km^2, but has the potential to invade a much larger area. Three Argentine agents developed at FuEDEI have been released to date: the seed beetle *Penthobruchus germaini*, and the defoliating caterpillars *Eueupithecia cisplatensis* and *E. vollonoides* (Geometridae) (Fig. 9). The three agents have established, and so far their impact is variable.

Figure 9. The defoliating caterpillar (*Eueupithecia vollonoides*) larva on a Parkinsonia leaf that was released in Australia.

Bellyache bush (*Jatropha gossypifolia*) (Queensland Department of Agriculture and Fisheries).

This perennial in the spurge family is considered a noxious weed in Australia and Puerto Rico. Widespread surveys in Paraguay and Bolivia have resulted in the discovery of several yet unidentified herbivores. The most promising ones so far seem to be the midges in the genus *Prodiplosis* that produce dramatic damage in the plant tips (Fig. 10).

Figure 10. Tip galls produced by the midge *Prodiplosis* sp. on Bellyache bush in Bolivia.

Leaf-cutter ants (LCA) (FuEDEI - CONICET).

LCA are one of the most important pests in the Neotropics, from Argentina to southern US (Texas and Louisiana). The CONICET PhD student, Nadia Jimenez, is doing her postgraduate project at FuEDEI under the guidance of FuEDEI scientist, Luis Calcaterra. They have focused on *Acromyrmex lundii* and *A. ambiguus*, the two main species of LCA affecting willow and poplar plantations in the lower Parana Delta (Fig. 11).

Results so far indicate that ants can cause between 55 and 90% losses in timber volume of willows and poplars in the first three years, depending on the tree variety. *A. lundii* is considered the most damaging of the two ant species, owing to its wider climatic tolerance and higher ability to defoliate. Control measures should probably concentrate on this species, although their cryptic nests, compared to the conspicuous *A. ambiguus* domes, make it difficult to locate.

Figure 11. A worker of the leaf-cutter ant, *Acromyrmex lundii*, carrying a piece of willow leaf.

Cabomba (*Cabomba caroliniana*) (CSIRO).

Cabomba is a submerged plant native to southern South America and, allegedly, south-eastern US. It is considered an important weed in Australia, India, China and Southeast Asia. It is also a weed of growing concern in Europe, northern US, and Canada. As with most aquatic weeds, it poses problems related to water management and use, as well as biodiversity, in that it tends to displace native plants, and the fauna associated to them.

Cabomba has been sampled and inspected for herbivores and pathogens in Argentina, Brazil, Uruguay, Paraguay, Bolivia, and several states in the Southeast US. To date only one specific herbivore has been discovered, the weevil *Hydrotimetes natans*, which appears to be endemic to northeastern Argentina and southeast Paraguay (Fig. 12).

Attempts to rear this weevil in controlled conditions in Australia and Argentina have so far failed. FuEDEI and CSIRO are currently evaluating chemical and physical variables that may influence weevil reproduction, and attempting new ways to rear the weevil in captivity.

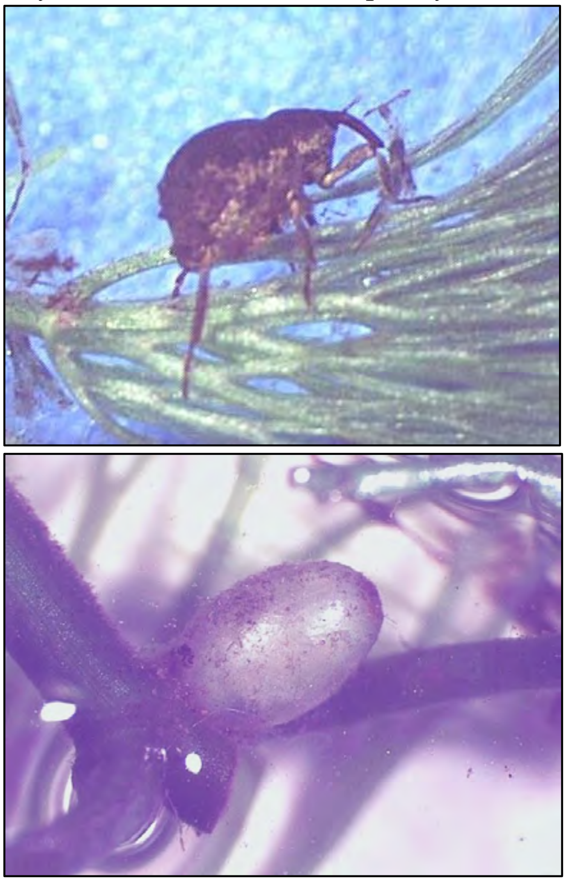

Figure 12. The weevil *Hydrotimetes natans*, a prospective biological control agent of cabomba; Top: adult on plant; Bottom: pupal case attached to stem.

Sino-American Biocontrol Laboratory (SinoABL)

Biological control of citrus psyllid in China

by Chenxi Liu

Citrus greening disease (huanglongbing, HLB) is considered the world's most destructive citrus disease. The Asian citrus psyllid (ACP), Diaphorina citri, is the most important pest of citrus worldwide because it serves as a vector of the citrus HLB pathogen, Candidatus Liberibacter spp. The psyllid infests most of the citrus-producing areas in the world, including Guangdong, Guangxi and Jiangxi and other several provinces in the southern China. New investigation by Ministry of Agriculture of China showed the distribution of the psyllid is expanding northwards in China (Fig. 13).

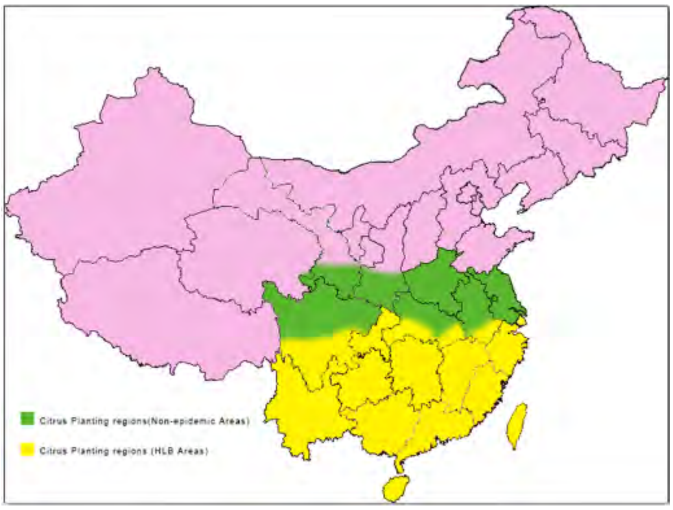

Figure 13. Presence of huanglongbing (HLB) in China; yellow = infested, green = non-epidemic area.

Biological control has been the key strategy for sustainable management of citrus psyllid due to the increasing resistance of the pest to insecticides and the negative effects of pesticides. We investigated the biodiversity and rates of predation and parasitism by natural enemies of the psyllid, including predators and parasitoids, by using Malaise traps (Fig. 14) and collecting psyllid nymphs at four commercial orchards where insecticides were not applied, in Ganzhou city, Jiangxi province (Fig. 15).

Figure 14. Malaise trap to monitor natural enemies of Asian citrus psyllid in a citrus orchard at Jiangxi, China.

Figure 15. Collection of citrus psyllids to monitor parasitism.

Although Asian citrus psyllid (ACP) is known to feed on up to 27 species within seven genera of Rutaceae in China, *Murraya exotica* has been shown to be a good host. We used both *M. exotica* and citrus as host plants for rearing ACP in our laboratory (Figs. 16 & 17).

The parasitoid, *Tamarixia radiata* (Hymenoptera: Eulophidae), was reared from mummies of fifth instar citrus psyllid nymphs collected in citrus orchards in Jiangxi province in April 2018. Interest in *T. radiata* for biological control of psyllid has grown in response to continued spread of HLB, and the evident searching and colonization capabilities of the parasitoid.

Figure 16. *Murraya exotica* used as host plant to rear citrus psyllid in China.

Figure 17. Citrus used as host plant to rear citrus psyllid in China.

The male and female of *T. radiata* are similar in color and body structure, except for antennae and a somewhat darker abdomen in the male (Fig. 18). The female antenna has 8 segments, both funicle and club with 3 segments covered with fine, short setae. The male antenna is more slender, and 9-segmented.

Future studies will focus on mass rearing *T. radiata* for the release in citrus orchards. Another objective is the evaluation the biodiversity and predation rate of ladybird beetles and lace wings by using DNA barcoding to assess their potential for controlling the psyllid populations.

Figure 18. Adult parasitoid of the Asian citrus psyllid (*Tamarixia radiata*); Top: female, Bottom: male.

European Biological Control Laboratory - EBCL

Genetic diversity and possible origin of venenata grass

by Stephen J. Novak, René Sforza and Massimo Cristofaro

Ventenata (also known as wiregrass or North Africa wire-grass; *Ventenata dubia*), is a recently introduced invasive annual grass in the western USA that is replacing native perennial grasses and forbs. It has minimal forage value for livestock or wildlife, and its shallow root system makes soils prone to erosion. It is spreading rapidly and is difficult to control. In order to help discover prospective biological control agents, it is important to understand the genetic diversity of the target weed and its probable region of origin. Genetic analysis using enzyme electrophoresis (allozymes) was conducted on 51 populations in the USA and 38 in Europe.

The results indicate the presence of multiple genotypes in the USA, many of which match samples from eastern Europe (Fig 19). So far, no prospective arthropod biological control agents have been found, but exploration is continuing.

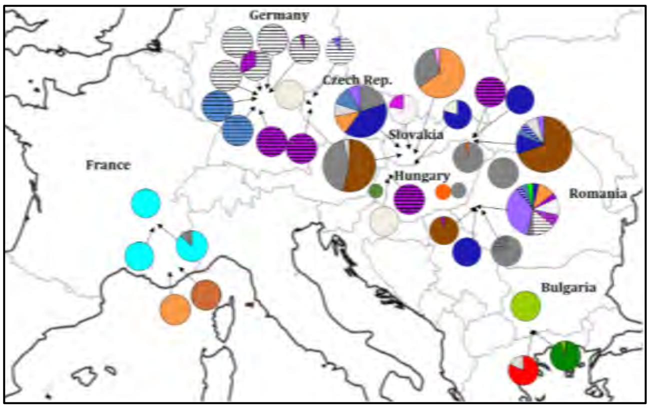

Figure 19. Geographic distribution of multilocus genotypes of 38 populations of ventenata grass in Europe (each color is a different genotype). Dark blue matches plants found in 8 states (CA, ID, MT, NV, OR, UT, WA, WY); orange matches some samples in OR; red mat.

Ecology of giant reed shoot tip fly

by Gaylord Desurmont

Giant reed (*Arundo donax*) is an invasive weed of riparian habitats in the southern half of the United States. Native to Asia, the species is believed to have been initially introduced into North America from the Mediterranean region, and two biological control agents have been released in the USA to control its populations: the Arundo wasp, *Tetramesa romana*, and the Arundo scale, *Rhizaspidiotus donacis*. The leaf sheath miner, *Lasioptera donacis*, has also been approved for release. The chloropid fly *Cryptonevra* sp. (Diptera: Chloropidae) has been suggested as a possible additional biocontrol agent, but its taxonomy and biology are uncertain. The fly is thought to attack giant reed shoots when they are young (< 75 cm tall), and larvae develop in the decaying shoots. Attacked shoots turn yellow and quickly die and dry out (Fig. 20).

Figure 20. Dead *Arundo donax* shoot possibly infested by *Cryptonevra* sp. Young shoots growing in the field sites are tagged, and the ones dying are brought back to the laboratory to be dissected to check for presence of *Cryptonevra* sp.

To provide insight into the ecology and field biology of *Cryptonevra* sp., a study was initiated in 2017 at EBCL and is currently ongoing. This study involves repeated observations at 10 field sites located in the south of France. Young shoots are monitored, and shoots showing signs of possible *Cryptonevra* infestation are cut and dissected in the lab to check for the presence of live fly larvae and pupae. Sticky traps are deployed in all sites to evaluate the abundance of *Cryptonevra* sp. adults. Finally, manipulative experiments are conducted under laboratory conditions at EBCL to understand the mechanisms of *Cryptonevra* sp. attacks on *A. donax* shoots and the effects of water stress on *A. donax* vulnerability to *Cryptonevra* sp.

Although the study is still ongoing, the first results are promising: *Cryptonevra* sp. adults were found at every field site and show a clear seasonal abundance pattern, peaking in mid summer (Fig. 21), and they were absent during winter (November-February). Dissections of shoots showing symptoms of *Cryptonevra* sp. infestation resulted in the collection of over 2000 fly larvae and pupae, which need to be identified by either genetic analyses or morphological examination to determine which are *Cryptonevra* sp.

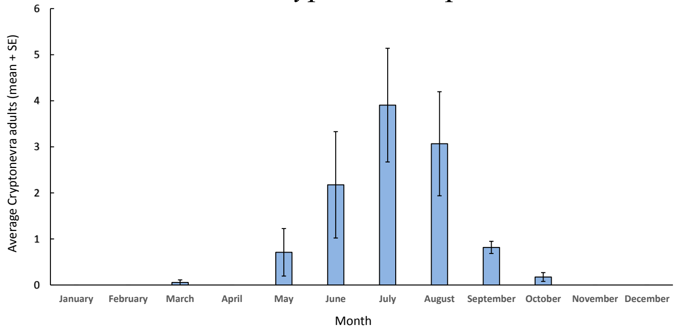

Figure 21. Pattern of seasonal abundance of adult *Cryptonevra* sp. at 7 field sites in the south of France.

Shipping longhorn beetles to cooperators in Austria, Canada, China and USA

by L. Smith and N. Ramualde

This spring EBCL sent larvae of Asian and citrus longhorned beetles (*Anoplophora glabripennis*, *A. chinensis*) to cooperators at the Department of Forest Protection in Vienna, Austria to train dogs to detect infested trees. The surveillance program has been so successful that there was a shortage of insects and infested wood which are needed to train dogs.

EBCL also sent insects to cooperators in Canada and the USA to establish colonies in quarantine laboratories at the USDA-ARS in Newark, DE, the US Forest Service in Cambden, CT, and the Canadian Forest Service in Sault Ste. Marie, Ontario.

Preserved specimens were sent to the Main Inspectorate of Plant Health and Seed Inspection at Toruń, Poland for reference to identify insects by plant health inspectors, and to China for population genetics studies by Dr. Xiao-Yi Wang at the Chinese Academy of Forestry, Beijing.

Recent Publications by EBCL

Daane, K.M., Middleton, M.C., **Sforza, R.F.H.**, Kamps-Hughes, N., Watson, G.W., Almeida, R.P.P., Correa, M., Downie, D. and Walton, V. 2018. Determining the geographic origin of invasive populations of the mealybug *Planococcus ficus* based on molecular genetic analysis. PLoS ONE 13(3): e0193852.

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Schaffner, U., **L. Smith**, M. Cristofaro. 2018. A review of open-field host-range testing to evaluate non-target use by herbivorous biological control candidates. BioControl 63(3): 405-416. doi.org/10.1007/s10526-018-9875-7

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