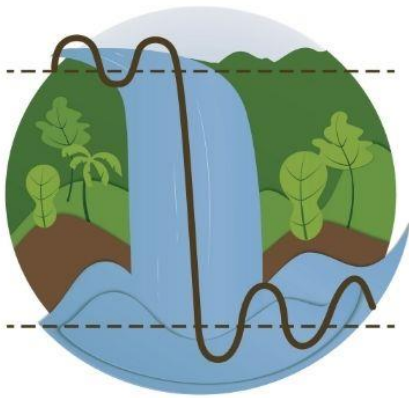


# PROCEEDINGS



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BIOLOGICAL CONTROL  
OF WEEDS**  
2023. IGUAZÚ, ARGENTINA

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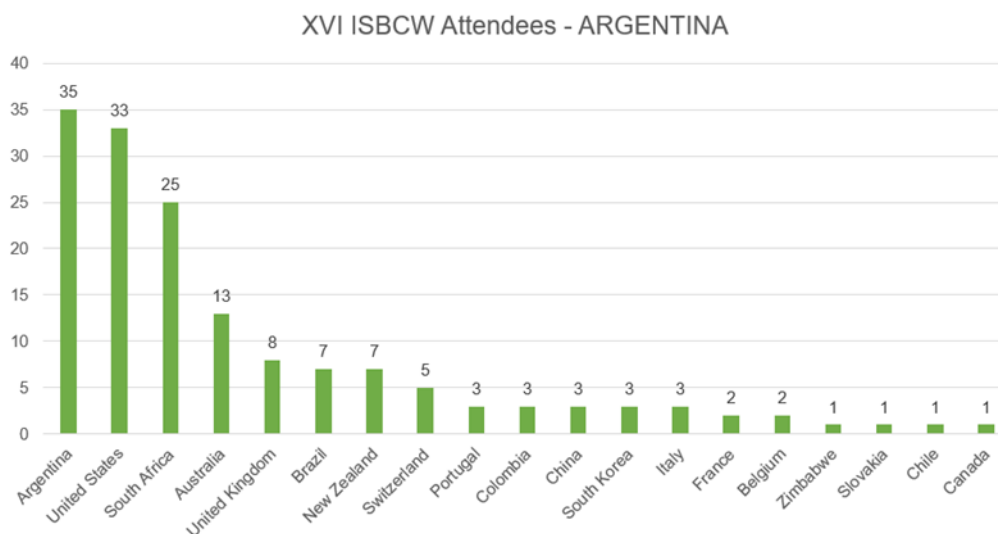


## PREFACE

### Symposium synopsis

The XVI International Symposium on Biological Control of Weeds was held in Puerto Iguazú, Misiones, Argentina in May 2023. This was the first time the meeting took place in South America. The closeness of the venue to the Iguazú falls and the largest remnants of the Atlantic forest, a UNESCO World Heritage Site, added an extra perk to the meeting. We visited the falls during the mid-symposium tour on a wonderful cool sunny day, which allowed everyone to enjoy the scenery at its very best. Again, at the symposium's banquet, the view of the rainbow over the falls from the privileged vantage point of the gardens of the Gran Meliá Iguazú Hotel, is a memory we will keep forever close to our hearts.

The XVI ISBCW was organised in 11 scientific sessions, with 75 oral presentations and 100 posters by 156 participants from 19 countries and 64 institutions from all over the world. Given the post pandemic global economy and sanitary travel restrictions, these numbers are quite satisfactory. We had the highest percentage of female attendees ever (45%) while the percentage of first timers remained quite similar to previous meetings. The countries with the highest representation were, in order, Argentina, the United States of America and South Africa.



The meeting took place amidst a very relaxed and friendly atmosphere that stimulated the free exchange of ideas and fruitful discussions during both oral and poster sessions. Keynote speakers did a wonderful job at laying the foundations of each session, most speakers kept to their allotted time, making the job of moderators very easy. All in all, the scientific programme was completed as planned. Social gatherings were animated and good-spirited, with members of different delegations getting to know each other or meeting again in person after a long time. It was obvious that everyone was happy to be together again after the isolation imposed by the pandemic.

We are very proud of having hosted this edition of the ISBCW in Argentina and sincerely hope everyone enjoyed it as much as we did. We would like to thank all attendees for

coming, and for their contributions, as well as our sponsors, who helped us keep the very high-quality standards set by previous meetings. We will keep our shared time in Iguazú as one of our most cherished memories.

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### **Local organisation**

Amerian Portal del Iguazú Hotel, Iguazú events and conventions centre  
Cuenca del Plata  
María Laura Romero Mirci

## **Symposium Production**

Cabrera Walsh, Anderson, Mc Kay, Sosa, Hernández and Faltlhauser managed general organisation. Faltlhauser was in charge of the general coordination and communication in social networks. Diluvio Comunicación and Hernández developed the logo. Diluvio Comunicación and Faltlhauser developed the web site. Hernández and Sosa handled abstracts. The scientific committee reviewed all abstracts and voted the awards. Hernández and Anderson did the technical editing and digital composition of the Book of Abstracts and Proceedings. Faltlhauser and Oleiro were in charge of the selection of gifts. Peard and Seal were in charge of the economic administration.

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Special thanks are due to the moderators for managing their sessions and to all members of the scientific committee for reviewing submitted abstracts and selecting oral presentations. We warmly acknowledge Camila Amarilla from American hotel and Conventions Centre, Yazu Utzugi from Cuenca del Plata, and María Laura Romero Mirci for an impeccable assistance. Thanks to Emanuel Grassi, from Instituto Misionero de Biodiversidad, for local contacts. We are also very grateful to all the people that helped in publicising the event and shared their ideas and advice to help in the organisation. Finally, we are greatly indebted to all attendees from all over the world who trusted us and contributed with their presence, good work and high spirits to make this meeting a huge success.

## **Symposium sponsors**

The following Institutions contributed to the realization of the XVI ISBCW by funding or supporting student participation:

- United State Department of Agriculture – Agricultural Research Service, USDA-ARS.
- Fundación para el Estudio de Especies Invasivas, FuEDEI, Argentina.
- Commonwealth Scientific and Industrial Research Organisation, CSIRO, Australia.
- Agencia Nacional de Promoción Científica de la Investigación, el Desarrollo Tecnológico y la Innovación, Argentina.
- Ministerio de Ciencia Tecnología e Innovación- MinCTI, Argentina.
- Manaaki Whenua - Landcare Research, New Zealand.
- Centre for Biological Control- CBC, South Africa.
- Department of Primary Industries, New South Wales, Australia.
- Centre for Agricultural Bioscience International- CABI, UK and CH.
- Grupo AVINEA, Argentina.
- International Organisation for Biological Control – Global IOBC, supported the participation of 10 young researchers.

## **Venue of the Next ISBCW**

The XVII ISBCW will take place in Paihia, Bay of Islands, New Zealand's North Island in 2026 and will be organised by Manaaki Whenua – Landcare Research.

## TARGET AND AGENT SELECTION

Moderator: Marion Seier (CABI, UK)

Oral presentation

### Weeds of missed opportunity: a plant pathologist's perspective

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Biological control of weeds with plant pathogens was inaugurated in the 1970s and plant pathologists have explored both classical (C) and biopesticide (B) approaches since then. The choices made of target weeds and the biocontrol agents to tackle them has had far reaching consequences for each project, as well as for the history of the discipline as a whole. In retrospect, it seems puzzling that weeds of minor importance such as Northern jointvetch (*Aeschynomene virginica*), milkvine *Morrenia odorata*) and round-leaved mallow (*Malva pusilla*) were chosen as targets by the pioneers of B – leading to technical successes but commercial failures. Each of those examples appears to have been motivated not by the individual relevance of the target weed, but rather by the pathogen effect as observed in the field. The commercial failures of COLLEGO, DEVINE and BIOMAL, initiated by “pathogens of opportunity”, have discouraged further advances – and industry involvement – in bioherbicide development. But now, after a 50-year hiatus, interest is on the rise. Nevertheless, the issue of objective target prioritization remains mostly a pending issue for B. Conversely, C with pathogens had started on the right foot in the 1970s, with the introduction of a rust fungus from Europe to control skeleton weed in Australia. The outstanding success of this pioneering project was a reference point for the potential of fungi (and, in particular, the *Pucciniales*) as C agents of invasive alien weeds. Other successes with rust fungi followed. These were initiated upon request and were based on “weeds of opportunity”. Target selection in C now often involves prioritization protocols and decision trees. Nevertheless, lack of funding for promising initiatives, or long delays, still plague the field of C. Several intrepid scientists, often working under challenging scenarios, have dedicated years of work to push the discipline ahead and to raise its profile. Here, some illustrative examples of C and B projects, involving the use of fungi, which have stalled or been consigned to history, have been selected for discussion: namely, C targets – *Eichhornia crassipes*, *Ligustrum robustum*, *Miconia calvescens*, *Mitracarpus hirtus*, *Passiflora foetida*, *Psidium cattleianum* and *Rottboellia cochinchinensis*; B targets – *Cirsium arvense*, *Cyperus rotundus* and *Euphorbia heterophylla*. Valuable information has been generated along the way by the scientists involved in these “failed” projects. The loss of this legacy – typically, hidden in unpublished reports – together with anecdotal information and personal insights, is unfortunate. Such information is of great value and represents half-opened “windows of opportunity” for the future generations of weed biocontrollers. We need to preserve this material heritage and make it publicly available for the common good.

### **The South African biological control target selection system**

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Biological control is an effective and sustainable method for management of invasive alien plants (IAPs), and has been implemented on 68 of the 367 plants that are listed under South Africa's National Environmental Management: Biodiversity Act (NEM:BA) regulations. With limited funding available, it can realistically only be considered for a subset of the remaining alien plants for which biological control has not yet been implemented. To address this, the Biological Control Target Selection (BCTS) system was developed to create an objective, transparent and easily usable method to prioritise the IAPs that are most suited and in need of biological control. The BCTS system builds on twelve previously developed prioritisation systems and encompasses thirteen attributes that are combined to present the highest predictive powers to rank targets. The BCTS system was applied to untargeted NEM:BA species, alien grasses and those present on the Prince Edward Islands. The tool has successfully prioritised IAPs that represent good investments for biocontrol in each of these groups.

## **My weed is better than yours: How to rank the environmental weeds in Europe for biocontrol purposes?**

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Globally, classical biological control has been applied for over 100 years in North America, Australasia and South Africa, but has as yet not been widely used against invasive alien weeds in Europe. Only five active programmes exist there so far. Despite this slow start, the interest is growing rapidly. In this paper, we applied an existing framework to develop a ranked list of environmental weeds named in the EU Regulation on Invasive Species (the “Union list”) for biocontrol. We used the scoring system developed by Paynter et al. (2009). This system is based on 1) existing knowledge of the weed impacts (the *Impact* score), 2) the amount of effort needed to deliver a classical biological control programme targeting them (the *Effort* score) and 3) the feasibility and likelihood of success of a classical control approach (the *Biocontrol feasibility* score). A final total ranking score was then obtained for each species by applying the formula: *Impact* score x *Biocontrol feasibility* score x 1 / *Effort* score. All species included in the Union list, a total of 40 weed species from 25 different families, were ranked from lowest to highest based on their final total ranking score value. To characterise a prioritised group, we used two different approaches. First, we used the final total ranking scores where the sequence of species was visually divided into groups of low, medium and high potential. Then a cluster analysis, a method to group similar observations into a number of clusters based on the observed values of the different variables (*Impact*, *Effort* and *Biocontrol feasibility*) for each weed, was applied. From this, we identified 16 environmental weeds in Europe for which classical biological control has relatively high potential. The top three weed species are *Pontederia crassipes* (Pontederiaceae), *Pistia stratiotes* (Araceae) and *Acacia saligna* (Fabaceae). Existing knowledge that can underpin any future investments in such activities against some of these weeds will be discussed. How the results of this analysis could guide European governments, decision makers and other stakeholders in managing these key environmental weeds in the context of classical biological control will also be discussed.

## Developing biological control for novel weed biocontrol targets in Vanuatu. Google to the rescue?

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*Solanum torvum* (Solanales: Solanaceae), *Urena lobata* (Malvales: Malvaceae), *Senna tora* and *S. obtusifolia* (Fabales: Fabaceae) have become major introduced pasture weeds in Vanuatu and a biocontrol program to mitigate their impacts commenced in 2018, funded by the New Zealand Ministry of Foreign Affairs and Trade. It has long been recognized that surveys for candidate weed biocontrol agents should focus on the center of origin of the weed species. However, there was considerable uncertainty regarding the native ranges of both *S. torvum* and *U. lobata*. For example, the Kew Gardens ‘Plants of the World Online’ website considers *S. torvum* to be native to the Neotropical region, from Mexico and the Caribbean region, through Central America, and northern South America to eastern Brazil. Other authorities state that it is native to the Antilles from where it has subsequently been spread. Similarly, “Plants of the World Online” indicates that *U. lobata* is native throughout much of the tropics, including Central and South America, the Caribbean, Africa, and Asia west of the Wallace Line. Some authors have suggested that Africa is the true native range, and others Asia. We searched the published literature and online databases for host records of arthropods and plant pathogens associated with these plants to identify regions with the highest diversity of potentially host-specific natural enemies and coupled this with very simple climate matching (using Köppen climate classification). This approach identified that the Antilles and SE Asia were the most promising places to survey *S. torvum* and *U. lobata*, respectively. Subsequent genetic matching indicated that populations of the two species in these respective regions are a good match to invasive populations in Vanuatu, validating our choice of survey locations. Promising candidate agents for both weed species, identified by the literature searches, were prioritized using a simple initial ‘triage’ scoring system. Specificity testing has been completed for the top-ranking candidate agents for both weed species, indicating that they are both adequately host-specific for release in Vanuatu as well as demonstrably damaging in the native range. These case studies indicate that there is enough information online to enable internet sleuthing to greatly assist with the survey stage of biocontrol programs against novel weed biocontrol targets.

**Prospects for the biological control of South African fireweed (*Senecio madagascariensis*) in invaded countries - field surveys and DNA barcoding elucidate the host ranges of candidate agents**

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Native to southern Africa, *Senecio madagascariensis* Poir. (Asteraceae) has invaded several countries that include Australia, the USA (Hawaii), Japan, Argentina and Brazil, with severe infestations in Australia and Hawaii. Given its negative impacts on livestock farming and the environment, biological control is an attractive option. Surveys in South Africa have identified several promising insect agents that include root feeders, stem borers and capitulum feeders. Host-specificity requirements are stringent for Australia, which harbours some 87 native *Senecio* species, as well as Argentina (260 species), but less so for Hawaii, which harbours no native species in the tribe Senecioneae. Native-range surveys on non-target *Senecio* species were carried out over several years in South Africa to elucidate the field host range of the most promising candidates. Adult and larval specimens collected across the range of *Senecio* species were subjected to DNA barcoding to differentiate between species and link them with their host plants. The COI gene phylogenies indicated that neither the root-feeding nor the capitulum-boring candidates were restricted to *S. madagascariensis* and are thus unsuitable for countries with a diverse native *Senecio* flora (e.g. Australia and Argentina). In contrast, stem-boring candidates, notably the weevil *Gasteroclisus tricostalis* (Thunberg), displayed more restricted host ranges. While, stem-boring agents represent the best prospects for Australia, all prioritized agents may be suitable for Hawaii, due to the more distantly related native flora. Native-range studies such as this are useful in eliminating unsuitable candidates at an early stage, thus precluding investment in time-consuming laboratory studies in quarantine. Furthermore, DNA barcoding has highlighted potentially host-specific agents that should be prioritized for further host-range testing.



**The blackberry cane-boring sawfly – what does a barcoding survey tell us about its host specificity?**

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European blackberry taxa (*Rubus fruticosus* L. agg.) are a major threat to natural and agricultural ecosystems in Australia. Biocontrol using the leaf rust fungus, *Phragmidium violaceum* (Uredinales) has achieved some advances in the suppression of susceptible *Rubus* genotypes, but the rust is not effective in low rainfall or moisture-stressed habitats. New agents for blackberry are still required and research should concentrate on natural enemies attacking blackberry crowns and primocanes (first year canes) because they are likely to have greater impacts on infestation characteristics. Larvae of the cane-boring sawfly, *Phylloecus faunus* Newman (= *Hartigia albomaculata*) (Hymenoptera: Cephidae) tunnel within the primocanes causing them to weaken and break thereby reducing daughter plant production. Initial host-specificity testing conducted during the 1970s indicated that some Rosaceae species might be at risk of attack, although it was suspected that the lab-based trials might have overestimated the true host range of this insect. Using DNA barcoding to rapidly identify larval specimens, we conducted a field survey in Mediterranean Europe to further our understanding of the field host range of *P. faunus* as a first step to assessing its potential as a candidate agent for the biological control of European blackberry in Australia. All specimens of *P. faunus* were collected exclusively from *Rubus fruticosus* and no evidence of the sawfly was found in *Rosa canina* – a species for which larval development was observed in host specificity testing in the 1970s – plants growing nearby. Instead, a different sawfly species, *Cladardis elongatula* (Klug) was found within *R. canina* canes. This study gathered supporting evidence that the ecological host range of the blackberry sawfly might be more restricted than initial studies suggest. Further investigation to assess its safety for introduction into Australia should be considered.

**Biological control of *Navua sedge (Cyperus aromaticus)*: challenges due to genetic differences in the target weed**

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*Navua sedge (Cyperus aromaticus)*, a native of equatorial Africa, is an extremely aggressive perennial sedge affecting beef, dairy, and sugarcane industries in the wet tropical regions of northern Queensland, Australia. *Navua sedge* is also a major weed of crops and pastures in Fiji and other South Pacific Islands. Biological control is the most cost effective and long-term management approach for this weed. Native range surveys in Kenya, Tanzania and Nigeria have identified the smut fungus *Cintractia kyllingae* that infects flower heads and seeds and the rust fungus *Uredo kyllingae-erectae* that infects leaves and stems as prospective biological control agents. Strains of the smut fungus from Tanzania and Nigeria readily infected Australian *Navua sedge* plants; however, rust strains from Nigeria and Tanzania did not infect them. To target native range surveys and to find strains of *U. kyllingae-erectae* compatible with the Australian *Navua sedge*, we investigated the population genetics of *Navua sedge* in the introduced and the native range using genotyping by sequencing. *Navua sedge* samples were collected in Queensland, Australia in the introduced range and from Kenya, Tanzania including Zanzibar, and Nigeria in the native range. Also included in the study were Herbarium samples of *Navua sedge* from its native range, specifically: Congo, Comoro Islands, Ethiopia, Equatorial Guinea, Gabon, Ghana, Kenya, Madagascar, Malawi, Mauritius, Nigeria, Reunion Island, Seychelles, Sudan, Tanzania including Zanzibar, Uganda, and Zambia. The study identified two genetically distinct populations in Queensland which are genetically distinct from populations in eastern and western African countries but are closely aligned with populations from Madagascar and the Comoro Islands in the Indian Ocean, off the coast of southern Africa. As the Madagascan population of *Navua sedge* is the one which is predominant in Australia, survey work was redirected to Madagascar to assess the occurrence and collect strains of *U. kyllingae-erectae* on *Navua sedge*. Future survey work will focus on the Comoro Islands to collect additional strains of *U. kyllingae-erectae* on *Navua sedge*. Host specificity tests for the rust will commence at CABI, UK, if the Australian populations of *Navua sedge* are proven to be susceptible to rust strains from one or both countries.

**Seeking better versions of the same agent *Aphalara itadori* – the knotweed story continues**

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Japanese knotweed, *Reynoutria japonica*, Giant knotweed, *Reynoutria sachalinensis* and the hybrid, *Reynoutria x bohemica* have been problematic invasive plant species in Europe and North America since their introduction from Japan in the 19<sup>th</sup> Century. *Aphalara itadori* Shinji (Hemiptera: Psyllidae), a sap-sucking psyllid specific to these knotweed species, collected from Mt. Aso, Kyushu Island, Japan (Kyushu line), has been released into the United Kingdom (UK) and subsequently into North America, as a classical biological control agent after approval by Department for Environment Food and Rural Affairs in 2010. Reproduction of the Kyushu line has been observed on *R. japonica* at several release sites, with some overwintering; however, long-term establishment has proven challenging. The application of CLIMEX modelling led to a further survey in 2019 which focused on collecting psyllids from further north on Honshu Island, Japan, with higher Composite Match Index values to the UK. Psyllids were collected from Murakami, Niigata Prefecture, northern region of Japan (Murakami line) where extensive and severe leaf curling damage attributable to psyllid nymphs was observed, quite unlike anything seen with the original Kyushu psyllids. The Murakami psyllid was confirmed as *A. itadori* based on both molecular analysis and morphological characteristics. A molecular screen for disease contamination in the Murakami line found that it was not infected with any of *Candidatus Liberibacter* spp. nor *Candidatus Phytoplasma* spp. which other psyllid species have been known to vector, indicating that the curling damage was likely to be caused by the physical interaction of the psyllids. Host-specificity testing of the psyllid using the most important nine selected non-target plant species showed the host-specificity of both the Murakami and Kyushu lines are very similar. The results also suggest that the Murakami line performs better on *R. x bohemica* than on *R. japonica*. Based on those results, releases of the Murakami line were granted in the Netherlands in 2020, and in the UK and Canada in 2021. The Murakami line has been released onto *R. japonica* and *R. x bohemica* populations in the UK and showed more curling damage on *R. x bohemica* than on *R. japonica* in the field. In this presentation, our research with the new Murakami psyllids on knotweed species is reported.

**Re-initiating classical weed biological control in Zimbabwe - where to start?**

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In Zimbabwe, various ecosystems are increasingly becoming fragmented due to anthropogenic activities and alien plant invasions. A recent review revealed that management of invasive alien weeds in the country is limited. To date, nine biological control agents have been intentionally released against five weeds since the 1960s. These releases were mainly on floating aquatic macrophytes and only one terrestrial invader, *Lantana camara* L. No new agents have been released since 2009 although 10 biological control agents released in neighbouring South Africa have spread naturally into the country, substantially suppressing some problematic weeds. Using the Biological Control Target Selection (BCTS) system, 19 invasive alien plants that could be successfully managed through classical weed biological control were identified, and for 12 of these, this could be achieved at minimal cost, as agents are available within the region. Amongst the priority weeds is *Acacia mearnsii* De Wild. which is invading montane grasslands particularly in eastern Zimbabwe, compounding conservation efforts in the endemic rich biome. Pre-release surveys to quantify seed banks, impacts of invasion on native vegetation diversity and ascertaining the presence/absence of known biological control agents were undertaken at three sites in Nyanga National Park, Zimbabwe. Results showed that *A. mearnsii* is rapidly spreading via seeds, threatening natural vegetation and current mechanically based management initiatives are not yielding the desired results. In addition, the two known agents, *Dasinuera rubiformis* Kolesik (Diptera: Cecidomyiidae) a flower bud galling agent and the seed feeding weevil, *Melanterius maculatus* Lea (Coleoptera: Curculionidae) were not found in Zimbabwe. Considering the overwhelming success of *A. mearnsii* biological control in South Africa, biological control should be considered as a management option. One limiting factor for *D. rubiformis* establishment in South Africa has been climate. Therefore, climate matching has been used to ensure the climate in Zimbabwe is suitable for the species. This step marks the first substantive action towards implementing recommendations made by weed experts towards the sustainable management of weeds in the country.

**Field results of Europe's first mite biocontrol agent released against the aquatic weed, *Crassula helmsii* (Saxifragales: Crassulaceae)**

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The gall-forming mite, *Aculus crassulae* (Acari: Eriophyidae) was approved for release in Great Britain (GB) against the aquatic weed, *Crassula helmsii* (Saxifragales: Crassulaceae) in 2018, as the fourth officially sanctioned biological control agent in Europe. *Crassula helmsii*, a mat-forming weed which was introduced to GB in the 1900s as an oxygenating pond plant is now widespread and dominating still waterbodies across GB. Pre-release quarantine testing demonstrated that *A. crassulae* was highly host-specific, and could survive and develop under GB conditions. With a degree day requirement of 175 degree days for complete development, there is potential to complete four generations in some parts of the country. The mites have now been released at 19 sites across England and Wales and now that the specific habitat requirements of the mites are understood, early signs of establishment have been observed at sites in southern parts of GB. As a highly adaptable weed, *C. helmsii* can grow in different forms depending on water depth, and water levels increase considerably during the winter months. This is in contrast with *A. crassulae* which requires low and consistent water levels for winter survival. Some *C. helmsii*-infested waterbodies are therefore considered unsuitable for mite establishment and pre-release site assessments are necessary to ensure the appropriate plant types are available for *A. crassulae*. The effects of global climate change mean that patterns of precipitation and drought are less predictable, with increased intensity and frequency, affecting the ability to choose the best sites. Despite these limitations, the mites have shown the ability to spread and establish at natural sites and the first field observations will be discussed.

**Managing *Prosopis*: an update on biological control efforts made in South Africa**

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Mesquite trees (*Prosopis* spp.) are known to be one of South Africa's most widespread and damaging genera of invasive plants. Despite biological control offering the only viable cost-effective solution to managing *Prosopis* invasions in the long-term, until recently the programme has been limited to the use of seed-feeding agents, namely the bruchids *Algarobius prosopis* (Coleoptera: Chrysomelidae) and *Neltumius arizonensis* (Coleoptera: Chrysomelidae), given the perceived benefits offered by the trees. Although *Prosopis* trees may offer benefits in terms of shade, fuelwood, fodder and timber, research has highlighted the overwhelmingly negative socio-economic and ecological impacts exerted by dense *Prosopis* stands across much of their invasive range in South Africa. Substantial expenditure on chemical, manual and mechanical controls have offered limited success and failed to halt the spread and densification of *Prosopis*, prompting renewed endeavors toward improving biocontrol impacts. The most recent of these biocontrol efforts have included the assessment and release of two new agents, namely the podlet weevil *Coelocephalopion gandolfoi* (Coleoptera: Brentidae) and the leaf-tying moth *Evippe* sp. #1 (Gelechiidae: Lepidoptera), both of which were released in South Africa during 2021. Although the podlet weevil maintains the potential to be highly damaging, severely reducing the reproductive output of *Prosopis* given its feeding and development on immature pods / seeds prior to dispersal, its establishment has yet to be confirmed. Whereas the leaf-tying moth has established at a single release site to date, however, bioclimatic assessments highlight that the moth is likely to be climatically constrained, being most effective predominantly in the hottest arid regions of the country. In addition, native range surveys have also been undertaken, in collaboration with research groups in both the United States (New Mexico State University) and Argentina (FuEDEI), to identify new and damaging potential candidate agents. These renewed biocontrol efforts against *Prosopis* remain promising, aiming to drastically reduce the spread and impacts caused by these trees in the near future. Overall, this research advocates strongly for the continuation of biocontrol efforts against *Prosopis* species in South Africa and discusses the anticipated constraints as well as potential avenues for future research.

**Prospects for the biological control of crystalline ice-plant, *Cryophytum crystallinum***

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The crystalline ice-plant, *Cryophytum crystallinum* (L.) N.E.Br. (Aizoaceae), (also known as *Mesembryanthemum crystallinum* L.) is indigenous in South Africa and Namibia, but has become a serious environmental weed in California (USA) and Mexico. It is also present in arid coastal habitats in the Mediterranean, where its status as an indigenous plant is disputed. In California and Mexico, *C. crystallinum* forms dense monocultures to the detriment of indigenous biodiversity and salinizes the soil. It is an ecosystem engineer, driving biodiversity loss, and disrupting ecosystem functioning in island habitats containing many endemic species. Genetic matching to determine the origin of the invasive populations has indicated that the south-west of South Africa is the likely origin of the invasive populations, but since samples from the Canary Islands were closely matched to those from California, it is possible that it was introduced to North America from there. There are two promising candidates for biological control that have been detected in South Africa, a stem-mining weevil, *Lixus carinerostris* Boheman, and a root-girdling weevil, *Calodemas prolixus* Faust. Both have been recorded only on the target weed or congeners. Field collections confirmed that *L. carinerostris* is found on *C. crystallinum* in coastal habitats in the area of South Africa known to have a Mediterranean climate similar to that of California. Preliminary host specificity testing suggests that it can feed and reproduce on a congener, *Crystallinum guerichianum* (Pax) Schwantes which is common in both inland and coastal locations in southwestern South Africa, but not on other plants, including a South African representative of the only two native genera of the Aizoaceae that are native to the continental U.S.A. The prospects for biological control of *C. crystallinum* are promising.

**The search for safety: The case of the French broom weevil**

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Prior to release of a biocontrol agent against an invasive weed in the introduced range, several steps need to be verified to guarantee safety for the new environment. The French broom weevil, *Lepidapion argentatum* (Coleoptera, Brentidae), attacking French broom (*Genista monspessulana*; Fabaceae) in its Mediterranean native range, is one good example of how different approaches can help to clarify its safety and sanitary status. We first verified its taxonomic status as data from literature did not match with the observations that we made, such as a dual oviposition behaviour. Weevil females lay eggs both in French broom stems and fresh seedpods, a behaviour that could have belonged to two different weevil species according to the literature. Integrative taxonomy was used to potentially delineate cryptic species among individuals emerging from pods and stems. Both phylogeny using a multi-locus approach and a morphological examination concurred with the hypothesis of one single species. Secondly, we measured its potential adverse impact on 36 non-target plant species including 11 species in the lupine group, closely related to the broom group. In no-choice tests only two lupines (*Lupinus arboreus* and *Lupinus chamissonis*) showed presence of stem galls and larvae, but with a minimal impact compared to the controls. Additionally, no impact was observed either on lupine seedpods in no-choice tests or on lupine stems in choice tests, but on target brooms. Finally, field sampling of larval body remains present in seedpods and galls, and emerged adults was performed to check their health status from the source population to make sure they do not carry plant pathogens or entomopathogenic microbes. Using microbial isolation and DNA barcoding methods, eight ubiquitous saprophytic fungal genera were identified, and no entomopathogenic fungi or bacteria were evidenced. The multi-level approach presented here gives to our knowledge the confidence we could put into this potential biological control agent. We ascertained the genetic and microbiological status of the source population that could be used for a future introduction into either the USA or Australia, or wherever French broom is a threat to the environment. In addition, although the fundamental host range revealed some non-target impacts, the ecological host range assessed through choice tests showed zero impact on lupines. As a conclusion, our data are very encouraging for arguing that *Lepidapion argentatum* could be an efficient and safe candidate for the control of the invasive French broom.



**Biological control using *Cyrtobagous salviniae* as a potential strategy for managing the invasion of *Salvinia molesta* in Madagascar**

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Madagascar has 14,000 species of vascular plants, but 101 species are recognized as invasive, of which 17 species are aquatic. *Salvinia molesta* is one of the aquatic species that has become problematic both environmentally and socio-economically. Between August 2015 and June 2020, a study was conducted to assess the distribution of this weed across the island, and its socio-economic and biological impacts, to develop a control strategy. *S. molesta* was first recorded by farmers around 1980. Currently the weed is present in 14 regions of Madagascar, across four bioclimates (humid, sub-humid, dry and sub-arid), ranging from 0 to 1579m asl where it colonizes lentic ecosystems, especially rice paddies. In these ecosystems, *S. molesta* is associated with other the invasive species *Pontederia crassipes*, *Pistia stratiotes* and *Azolla filiculoides*. In the presence of *S. molesta*, fish and freshwater shrimp production decreased by 82% and rice yield by 30%, despite requiring an additional expense of US\$ 1,107/ha for control. To gauge biological impacts of the species on biodiversity, a manipulated outdoor trial was conducted to determine the level and nature of competition of *S. molesta* against the indigenous close relative *Salvinia hastata*, using an additive series density model. This trial showed that *S. molesta* outcompeted *S. hastata* eight-fold. These results illustrate the magnitude of the *S. molesta* invasion both socio-economically and biologically. However, no action plan has been established to manage the species. Some sporadic manual control measures have been implemented, but this has no effect due to the high speed of propagation of the species. Thus, biological control using *Cyrtobagous salviniae* could provide a long-term strategy for reactive management and control when taking into account the current wide distribution of the species across the island.

### **A good match is hard to find – the case of classical biological control of *Rubus niveus* in Galápagos**

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Introduced in the late 1960s for agricultural purposes, *Rubus niveus* (Rosaceae, subgenus *Idaeobatus*), commonly called Mysore raspberry or ‘mora’ in Spanish, is considered to be the most problematic invasive plant species in Galápagos. Native to the temperate regions of the Himalayas, this invasive weed is present in the wetter parts of the five larger islands of the archipelago. Its current total cover is estimated to be more than 5,000 hectares and it is rapidly spreading. *Rubus niveus* can grow up to four meters in height and the dense spiny thickets not only have a detrimental effect on farmland but also have a destructive impact on native biodiversity; preventing regeneration of the unique endemic daisy tree *Scalesia pedunculata* by shading out its seedlings. Current methods of control rely on herbicides and manual labour, but these are expensive and unsustainable. Thus, it is accepted that classical biological control (CBC) needs to be an essential part of the integrated management strategy for this invasive species. As there are no native members of the Rosaceae, and only one commercially-important introduced *Rubus* species present in Galápagos, *R. niveus* makes an ideal target for CBC. Research into the potential for CBC of *R. niveus* commenced in 2014 with the focus on fungal pathogens. A wealth of rust fungi, particularly species belonging to the genus *Phragmidium*, has been recorded on *R. niveus* in its native Asian range. A member of this fungal genus, *Phragmidium violaceum*, has already been a successful CBC agent of invasive *Rubus* species in Chile and Australasia. However, the taxonomy of these rusts is complex and it cannot be ruled out that some of the listed species are synonymous. Furthermore, it is likely that there have been misidentifications of the *Rubus* host as this genus is considered to be one of the most challenging taxonomic groups due to hybridization, polyploidy and apomixis. Additionally, phenotypic plasticity can make correct identification of *Rubus* species difficult.

**Summary of biological control of *Arundo donax* in North America (Poales: Poaceae)**

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*Arundo donax* L., a.k.a. giant reed, or carrizo cane is a large perennial grass native from Mediterranean Europe, northern Africa, the Middle East and Asia that was introduced to North America. However, giant reed has become an invasive weed in the Rio Grande Basin of Texas and Mexico, in California, as well as South America, South Africa, Australia, and New Zealand. Giant reed forms dense thickets along rivers, canals and reservoirs, removing water that is needed for agriculture in regions where rainfall is scarce, promotes wildfires, displaces native plants and animals, hinders access and visibility for law enforcement, and facilitates the invasion of cattle fever ticks along the international border with Mexico. USDA-ARS developed the world's first biological control program targeting an invasive grass, focusing on *A. donax*. Two insects, a shoot tip-galling wasp (*Tetramesa romana*) and a rhizome- and shoot-feeding armored scale (*Rhizaspidotus donacis*) were introduced to the Binational Lower Rio Grande Basin in 2009 and 2011, respectively. Over 1 million arundo wasps were released through a mass-rearing program, and they have dispersed established throughout the Rio Grande Basin. By the sixth year after release, the arundo wasp had reduced live biomass by 32% along the Rio Grande. This reduction translates to a water savings valued at \$4.4 million USD per year. The arundo armored scale, now established at many sites in the Lower Rio Grande Basin, does not disperse rapidly, but in release plots, in combination with the arundo wasp, has reduced live biomass by 55%. The arundo wasp and armored scale have been released and are established at many sites in northern California. A third agent, the arundo leafminer *Lasioptera donacis*, was released in Texas in 2016, but did not establish. Additional releases are planned in California where climatic conditions may be more favorable. Methods for successful management of this invasive weed include the integration of mechanical to biological control strategies. Stands are topped at 1m using a tractor with a side mounted boom with attached sickle cutter bar, followed by attack of arundo wasps on subsequent abundant lateral shoots. Mechanical topping with a tractor mounted sidebar mower, is effective in reducing live biomass. Over 700 km of the Rio Grande have been topped over multiple years accelerating declines in biomass. Biological control integrated with mechanical topping is likely to be the most critical tool used to reduce the economic and environmental damage caused by *A. donax*.

***Asteromyia modesta* (Diptera: Cecidomyiidae) a potential biological control agent of *Conyza* spp. (Asteraceae)**

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*Asteromyia modesta* is a gall forming midge associated with Asteraceae. The species has been recorded inducing galls on the leaf tissue of five species from the genus *Conyza* Less., in Brazil. Considering the potential of gall-inducing insects as weed biological control agents, no-choice and choice host-specificity tests were performed on 140 plant species (from 59 families and including 123 genera), primarily from the Asteraceae family (61 genera and 81 species) with *Conyza sumatrensis* (Retz.) E. Walker as the control. The experiment was carried out in entomological cages using plants propagated from seeds. Insects were obtained from the colonies maintained at the Forest Monitoring and Protection Laboratory of the Regional University of Blumenau. Due to the concurrent performance of the tests and the short life cycle of *A. modesta*, the plants were tested separately in groups of seven species, every 30 days. For multiple choice tests, each cage contained one seedling of each non-target plant species to be tested, one seedling of *C. sumatrensis* (control) to which 50 unsexed and newly emerged adults of *A. modesta* were added, totaling six replications for each plant species. In no-choice tests, the same number of replicates were used, however, 20 newly emerged and unsexed adults of *A. modesta*, were released in PVC pipes containing only one plant species. After the period of each test, the plants were evaluated for the total number of leaves, leaves with galls and total number of galls per plant species. A total of 1,920 plants were evaluated and 25,200 adults of *A. modesta* were used during 600 days of experimentation. In no choice tests only, the non-target plant *Letostelma maximus* supported development of galls, with an average of 20,3 galls per plant compared to an average 111 for on *C. sumatrensis*. It should be noted that *L. maximum* is a representative of the same subtribe (*Conyzinae* Horan.) of the genus *Conyza* Less., which includes four more genera, all with distributions restricted to the American continent. Results suggest *A. modesta* has potential as a biological control agent, but additional work is recommended to determine if it is a member of a cryptic species complex, requiring additional biological and molecular investigations.

**Bionomics, immature morphology and host range of *Podomachla apicalis* (Lepidoptera: Erebidae), a potential biological control agent of *Senecio madagascariensis* (Asteraceae)**

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The Madagascar fireweed, *Senecio madagascariensis* Poiret (Asteraceae), is a serious poisonous pasture plant killing livestock. Away from its natural enemies in the native range, fireweed is a significant weed of agricultural grasslands which has become naturalized over a wide range of the world including Afrotropical region, Australia (New South Wales, Queensland), Hawaii (Hawaii, Maui islands), South Carolina, the Neotropics (Argentina, Brazil, Colombia, Mexico, Uruguay, Venezuela), and has recently been reported as a noxious weed in Japan (Kyushu, Seto, Tohoku). Biological control is being considered in Australia and Hawaii as a long-term solution to managing this invasive plant. Surveys for biological control agents in the native regions in South Africa, Eswatini, and Madagascar identified several herbivorous insects that can be used for biological control. Hawaii was the first nation to release the Madagascar fireweed moth, *Secusio extensa* (Butler), (Lepidoptera: Erebidae), in 2013 that has become well established contributing to biocontrol efforts. A second defoliator moth, *Podomachla apicalis* (Walker), (Lepidoptera: Erebidae), native to South Africa and Tanzania was introduced as a suitable biocontrol agent. It was tested in quarantine but not released. Characteristics of immature stages and adults, reproductive biology, and host range of *P. apicalis* are described for the first time from a colony originated from KwaZulu Natal, South Africa and maintained under laboratory condition at the Hawaii Department of Agriculture Insect Containment Facility. Host-specificity tests were conducted to determine its potential for field release in Hawaii. Host-range testing of 26 Asteraceae species showed that *P. apicalis* appeared to prefer members of the tribe Senecioneae as permissible hosts (56 – 96% adult eclosion), (*Crassocephalum crepidioides*, *Emilia fosbergii*, *Erichtites hieracifolia*, *Senecio vulgaris*, and *Senecio madagascariensis*). Besides Senecioneae, no-choice specificity assessments showed that larvae produced partial defoliation and completed development to adult stage on other plants of Asteraceae members of the tribes Arctotideae (*Gazania* sp.), Calenduleae (*Calendula officinalis*), Helenieae (*Flaveria trinervia*), and Heliantheae (*Bidens pilosa*) as inferior hosts (2.0 - 2.8% adult eclosion). No feeding was observed on other 11 non-Asteraceae host plants. Although *P. apicalis* was highly fecund when reared on the target host (mean potential fecundity  $174.5 \pm 18.7$  eggs/female) and a short life span of  $30.3 \pm 0.4$  days, laboratory no-choice tests indicated that the moth had a wide host range that included other Asteraceae species. Further testing was terminated, the study contributes towards a better understanding of the characteristics, bionomics, and host preferences of this African tiger moth. Hawaii is planning additional agent selection activities in Africa for other potential agents.

### **Impacts of *Asteromyia modesta* (Diptera: Cecidomyiidae) on natural populations of *Conyza* spp. in southern Brazil**

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Galls are anatomical deformations in stems, leaves, fruits and flowers, caused by insects that consume plant tissue or use it to complete their biological cycle. The anomalous growth of tissues alters the format, structure and physical processes, preventing or hindering the normal plant development. *Asteromyia modesta* is a gall inducer selected as a potential control agent for *Conyza* spp., and thus, a study was carried out to quantify the leaf area damaged by this insect on *Conyza sumatrensis* var. *leiotheca*; *C. sumatrensis* var. *sumatrensis*; *C. canadensis*; *C. glandulitecta*, and *C. bonariensis* naturally present in southern Brazil. Thirty plants with the presence of galls of each species were randomly collected in the field and taken to the Regional University of Blumenau for measurement of total height, number of leaves, number of leaves with galls, and total number of galls per plant. Subsequently, the leaves containing galls were cleaned with damp paper and scanned. A total area of leaf blade and an area containing galls were measured using Image-Pro Plus software (precision of 0.01 mm). All variables were tested for homoscedasticity (Levene), normality (Shapiro-Wilk), variance (Kruskal-Wallis), and difference (Mann-Whitney). The average values from total height, leaves with galls and total number of galls were statistically higher in the species *C. glandulitecta*, *C. sumatrensis* var. *sumatrensis*, *C. sumatrensis* var. *leiotheca* compared to *C. canadensis* and *C. bonariensis*. The smallest number of leaves with galls and total number of galls was registered in *C. bonariensis* and *C. canadensis*. However, the ratio of the total number of leaves to the total number of leaves with galls was higher in *C. bonariensis* (3.0%) compared to the others, *C. sumatrensis* var. *leiotheca* (2.4%), *C. glandulitecta* (1.8%), *C. canadensis* (1.4%) and *C. sumatrensis* var. *sumatrensis* (1.3%). The total leaf area compromised by the development of galls for each species was also obtained and will be presented. Preliminary results suggest a greater number of galls in some species, but the impact ratio is greater for smaller individuals. However, other natural factors can be could affect damage levels, such as the gall inducer preference for a given species, host developmental stage, and parasitoid density.

**Some aspects of reproductive biology of *Lixus caudiger* (Curculionidae: Lixinae), a potential agent for the biological control of *Conyza* spp. (Asteraceae)**

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The genus *Conyza* (Asteraceae), native to the Americas, is considered an exotic invader in several locations and is included in the international database of weeds resistant to herbicides. *Lixus caudiger* (Curculionidae: Lixinae) is of interest for use as a biological control agent for *Conyza* spp. in Australia, but no specificity studies with *L. caudiger* have been initiated so far. In order to verify the relationship between the life stages of *L. caudiger* and the host plants, individuals of *Lixus caudiger* and *Conyza* spp. were analyzed between the years 2020 and 2022, in the region of Blumenau, Santa Catarina, Brazil, performing field collections and establishing a colony in a greenhouse. The study allowed observations into preferences for oviposition sites, characteristics of eggs, larvae and pupae, determination of larval instars, sex ratio, host preferences, as well as population fluctuation. A new methodology for breeding *L. caudiger* was developed, involving the insertion of eggs and larvae in a fissure in the live host plant. Results suggest there was low significance in the relationship between height and diameter of the host plant for oviposition, with most eggs ( $1.47\text{mm} \pm 0.05$  in length and  $0.94\text{mm} \pm 0.07$  in diameter, 2-4 per oviposition) found in the upper 1/3 of the host plant, and larvae found in the central region. 225 larvae were analyzed, and five larval instars were estimated. The sex ratio was 0.52, with males having longer time between the first instar and the adult phase. A large population peak was identified between August and November, and a smaller peak between May and June. The new methodology showed positive results, with a significant increase in individual survival. Information on pupae and the influence of temperature were also evaluated. It is concluded that *Lixus caudiger* has a great impact on the host plant, directly interfering with the stem cambium in the larval and pupal stages, and its reproductive biology is related to the seasonality of the host plant, indicating a potential as biological control agent for *Conyza* spp. if approved in the host range tests.

**Collaborative efforts to manage the cryptic invader *Limnobium laevigatum* in southern African water-ways**

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While the South American fresh water invader *Limnobium laevigatum* has been present in African water ways since the early 2000's, no management efforts have been implemented against the weed. Often unnoticed due to its similarity to *Pontederia crassipes* (water hyacinth), *L. laevigatum* has spread rapidly, forming large, dense monospecific mats. Climate matching suggests large regions of Africa, including Botswana, Kenya, Malawi, South Africa, Tanzania, Zambia and Zimbabwe would be climatically suitable for invasion. Due to the high number of wind and water dispersed seeds, small seedlings and quick downstream spread, further rapid invasion is expected - causing severe socio-economic problems especially for rural livelihoods that depend on natural resources as well as negatively impacting native biodiversity (especially in hotspots, Okavango and Bangwelu swamps). Considering the overwhelming success of the biological control of aquatic species on the African continent, and an already known potential agent for the species, biological control should be considered as a management option. To this end, a consortium has been formed including researchers from Zimbabwe and South Africa (CBC), Argentina (FuEDEI) and Switzerland (CABI) to develop an effective biological control programme. *Listronotus cinnamomeus* has already been identified as potentially host specific and prioritized as a candidate agent. A population of the weevil has already been located close to the FuEDEI laboratory in Argentina. Additional host specificity studies will commence in 2023. It is hoped that the collaborative effort will result in the successful implementation of a biological control program with the aim of reducing the current and potential negative impacts of this aquatic invader.



**Natural enemies of *Rubus anglocandicans* (Rosales: Rosaceae) target-specific surveys for the biocontrol of blackberry in Australia**

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*Rubus anglocandicans* A. Newton is the most widespread taxon of the *Rubus fruticosus* aggregate in Australia, however it has never been specifically surveyed for potential biocontrol agents. This is because previous surveys concentrated on *Rubus* spp. in continental Europe, whereas *R. anglocandicans* is native to the UK. It wasn't until 2003 that the taxonomy of Australian taxa was revised, confirming that the predominant blackberry taxa occurring Australia was *R. anglocandicans*, and not *R. procerus* as previously thought. Subsequently, we undertook surveys for natural enemies on *R. anglocandicans* from early summer to mid-autumn in 2021. A total of 56 invertebrate and three pathogen species were collected from 22 sites around central England where *R. anglocandicans* is widely distributed. Disappointingly, the majority of the insect species recorded were either polyphagous or were vagrants on the plant not directly associated with the target, with only a few species being regarded as host-specific at the *Rubus fruticosus* aggregate level. These were the galling wasp, *Lasioptera rubi* (Schrank) (Diptera: Cecidomyiidae) and the bramble-feeding moth, *Thyatira batis* (Linnaeus) (Lepidoptera: Drepanidae). One explanation for the depauperate presence of specialist natural enemies could be that the evolutionary timeframe in which *R. anglocandicans* has been in existence has been most likely relatively short. In addition, *R. anglocandicans* occupies only a small natural range. Possibly, the combination of these two factors may have so far restricted the development of host specific antagonists in the UK. A possible exemption to the lack of host specific invertebrates at the species level may still be found within the eriophyid mites. Generally, eriophyid mites have the potential to be highly host specific and can become effective biological control agents.

Our results show that there are some genetic differences between *R. anglocandicans* from Australia compared with the plants sampled in the UK. A better understanding of the genetic differences between the Australian and European populations of *R. anglocandicans* may support the acquisition of more efficient or host-specific natural enemies by narrowing down the area of origin of the invasive population in Europe.

**Biological control of parrot's feather, *Myriophyllum aquaticum* in temperate biomes**

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Parrot's feather, *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae), is a popular ornamental plant that has become problematic worldwide, due to its ability to spread easily and turn into an invasive species. Originally from the Amazon basin, parrot's feather has now spread worldwide and is considered invasive in several temperate regions such as western North America, Europe and South Africa. In the invaded range, parrot's feather forms dense impenetrable mats which affect stream flow, interfere with irrigation, recreational use and increase the risk of flooding. In addition, it degrades water and habitat quality resulting in reduced native species richness. Recently, biological control programmes targeting parrot's feather in western North America have been initiated. There are three potential biological control agents worth further investigation. The leaf-feeding flea beetle (*Lysathia* sp.) and the stem-mining weevil (*Listronotus marginicollis*) are both native to South America and have been developed for South Africa; the former has been released. The third, a North American native weevil (*Phytobius vestitus*), was recently identified damaging parrot's feather in Louisiana. Yet, to be able to use these insects as biological control agents against parrot's feather, several requirements should be met, such as their ability to perform and survive in a different climate from their original one, as well as to establish any potential to spread onto native non-target plant species. Using *Lysathia* sp. as model system, host-specificity tests are now underway for closely related species with parrot's feather as controls. In addition, life-history traits of the insect developing on different plant species, and at different temperatures will be measured. Preliminary results suggest that the more complex the habitat is, the more *Lysathia* sp. tends to be specific to parrot's feather, suggesting a narrow ecological host range. With this work we hope to highlight the natural enemy suitability for a novel ecological niche, and try to answer the question of what should be prioritized for testing, safety or efficiency?

### Northern temperate weeds programme in South Africa

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The high elevation grasslands of South Africa are important systems for water security, as they provide nearly half of all the country's water run-off and are a key biodiversity hotspot with high plant endemism. The biome is also an invaluable resource, supporting livestock central to the livelihoods and economies of commercial, small-scale, communal farming/agriculture. These grasslands are under severe threat from invasive alien plants, particularly species deriving from the cooler Northern Temperate regions of the Globe. Unfortunately, in South Africa, there has been greater focus on invasive alien plant management in riparian areas than on the invaders of mountainous catchments. Therefore in 2017, a programme was supported by the National Government's: Department of Forestry, Fisheries and the Environment, Working for Water Programme to try to bring the benefits of biological control to the mountain grasslands of South Africa. Thus far, research has focused on biological control feasibility and ecological and socio-economic impact studies on several northern temperate weeds. This has allowed the programme to prioritise species for further consideration. Over the next five years, the programme will prioritise studies on five weed species for pre-release studies (*Ailanthus altissima*, *Cotoneaster pannosus*, *Genista monspessulana*, *Robinia pseudoacacia*, and *Pyracantha angustifolia*). While a further two species (*Gleditsia triacanthos* and *Hypericum perforatum*) have been targeted for post-release evaluation studies and mass rearing as they already have biocontrol agents on them but may benefit from additional releases.

**Potential impact of the stem-boring weevil *Gasteroclisus tricostalis* on the invasive fireweed (*Senecio madagascariensis*)**

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The stem-boring weevil *Gasteroclisus tricostalis* (Thunberg) (Curculionidae) is under investigation as a biological control agent for *Senecio madagascariensis* Poiret (Asteraceae) in Australia and Hawaii. Besides mandatory host-specificity testing, pre-release studies on the weevil's impact can be a useful predictor of its efficacy when released in the invaded range. In this study, we investigated the response (i.e., growth, floral production, and biomass accumulation) of mature flowering *S. madagascariensis* plants to varying loads of *G. tricostalis* larvae in their stems. Although there were negative relationships between all plant response variables and larval loads, these were only significant in the production of side branches (shoots) and floral components (capitula). Regression analyses estimated that six and 12 larvae per mature plant could prevent the production of new capitula and new shoots, respectively. A significant negative relationship between the percentage of eggs that resulted in larval recoveries and the number of eggs deposited per plant may suggest aggressive interactions between the larvae. Additional studies have indicated that *G. tricostalis* has a more dramatic impact on young pre-flowering plants, often causing mortality. Since high floral production is a major driver of invasions by *S. madagascariensis*, substantial reductions in flowering by *G. tricostalis* should make a significant contribution to the weed's management.

**Antagonistic fungi: could they be a limiting factor to fungal weed biocontrol success?**

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Classical biological control, or biocontrol, of invasive weeds involves the intentional introduction of exotic, usually co-evolved plant pathogens and insects, for the permanent establishment and long-term control of a target plant. Agent establishment, effectiveness, and safety are the critical elements for a successful weed biocontrol programme. However, establishment and effectiveness of agents on the invasive plant often vary throughout the invaded range with about two-thirds of weed biocontrol agents failing to suppress their target weed. There are many documented reasons why weed biocontrol agents do not establish or are ineffective when they do, and the presence and accumulation of natural enemies in the invaded range is one of them. Endophyte-enriched, invasive weeds and those forming mutualistic associations with indigenous, native endophytes could explain the lack of consistency of some classical biological control introductions. However, another variable could be factored into the mix: mycoparasitism, where one fungus parasitises another, the natural enemy of the plant's natural enemy. Mycoparasitic interactions are common in nature, form part of the microbiota of plants, and are considered significant contributors to fungus-fungus antagonism. Mycoparasites kill plant pathogens, protect the plant from abiotic and biotic stressors, and reduce disease incidence and severity at the plant population level. Their exploitation as biocontrol agents in agriculture is well documented. However, mycoparasites may potentially affect classical fungal biocontrol agents of invasive weed species. We discuss the successes and failures of fungal agent programmes, with a focus on the potential antagonistic role of fungal endophytes and mycoparasites.

**The smut *Cintractia kyllingae* and the rust *Uredo kyllingae-erectae*: two potential classical biological control agents for *Cyperus aromaticus* in Australia**

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*Cyperus aromaticus* (Cyperaceae), also known as Navua sedge, is a perennial, rhizomatous sedge native to equatorial Africa and countries in the western Indian Ocean. An introduced noxious weed in southeast Asia and a number of South Pacific Islands, the sedge has also become an aggressive invader in Australia, following its introduction to the wet tropical regions of northern Queensland in the 1970s. Navua sedge not only causes negative impacts on the biodiversity of native grasslands, but also affects the beef and dairy industries and crop farming. The current management of the weed based on chemical and mechanical means is expensive and unsustainable, and gives only short-term control. Therefore, *C. aromaticus* has been approved as a target for biological control in Australia, which, if successful, would offer a cost-effective and long-term management option. A biological control programme commenced in 2017 and native-range field surveys in Kenya, Tanzania and Nigeria showed the smut fungus *Cintractia kyllingae* and the rust fungus *Uredo kyllingae-erectae* to severely infect inflorescences and leaves/stems of Navua sedge, respectively. Several strains of *C. kyllingae* were exported from Nigeria and Tanzania to CABI-UK, and sporidial cultures of the pathogen were established, both *in planta* and *in vitro*. Teliospores and sporidia of Nigerian and Tanzanian smut strains proved to be infective to Australian Navua sedge plants. Inoculation studies also showed that younger flower heads of the sedge are more susceptible to the pathogen, with more smutted seeds developing, compared to older flower heads. A Tanzanian smut strain, showing highest virulence towards Australian sedge plants, has been selected for further research. In contrast, none of the assessed strains of the rust *U. kyllingae-erectae* ex Nigeria and Tanzania infected the Australian sedge. Directed by molecular analyses of *C. aromaticus* herbarium material, additional survey work for the sedge was undertaken in Madagascar in 2022 to source rust strains compatible with Australian Navua sedge. Though not previously recorded from this country the rust was commonly present in Navua sedge populations in eastern and northern Madagascar; at some field sites, severe rust infection of leaves and stems of the sedge caused die-back of affected plants. Inoculations studies showed that rust strains ex Madagascar can infect and develop uredinial sporulation on the Australian Navua sedge. This poster reports on our research undertaken with the smut and rust as potential biological control agents for Navua sedge in Australia and outlines the next steps.

**Pre-release studies on *Robinia pseudoacacia* in South Africa**Chikowore Gerald<sup>1</sup>, Wolmarans Abigail.<sup>1</sup> & Martin Grant D.<sup>1,2</sup><sup>1</sup>Centre for Biological Control, Department of Entomology and Zoology, Rhodes University, Makhanda (Grahamstown), South Africa.<sup>2</sup>Afromontane Research Unit, Department of Zoology and Entomology, University of the Free State, Phuthaditjhaba, South Africa.

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Montane grasslands in South Africa are an important biodiversity hotspot with high plant endemism. However, weeds originating from temperate regions are rapidly spreading, threatening the integrity and functioning of this ecosystem. *Robinia pseudoacacia* (Fabaceae), a prolific invader from North America, is one of the species affecting high elevation grasslands particularly in eastern parts of the Free State Province, South Africa. Management of this plant through chemical and mechanical means is not effective due to its clonal spread. Thus, the Centre for Biological Control is currently considering candidate defoliating biological control agents to assist in the management of the species. In order to determine the benefits derived from the pending biological control agents, we measured the impact of the species on the grassland ecosystems. Using digital repeat photography, temperature and light loggers, we determined the phenology of *R. pseudoacacia*, and measured temperature and light intensity in invaded and adjacent uninvaded grassland. We further used pitfall traps and the line-point intercept method to determine arthropod and plant community assembly within the two habitats. Finally, we compared arthropod herbivory on the species between the native region, the United States of America, and invaded ranges of Europe and South Africa, to ensure released agents will have a significant impact. Results showed that *R. pseudoacacia* alters the biophysical components of grassland ecosystems and subsequently native plant and arthropod community assembly. Average temperatures and light intensity were at least 2°C and 1200 lumens/ft<sup>2</sup> lower respectively in habitats invaded by the weed than in open grasslands. These alterations in environmental conditions drive changes in plant and arthropod communities. Grass communities differed between invaded and uninvaded habitats by approximately 96% with the former dominated by alien nitrophilous species. The abundance of endemic grassland arthropods was lower in invaded habitats with Acridids totally excluded. Our results further showed that known pests of *R. pseudoacacia*, that can reach high densities in both the native range and in Europe, are absent in South Africa. These results justify the need to sustainably manage *R. pseudoacacia* and also provide essential baseline data for the future evaluation of the biological control programme. Considering the limited success achieved by current management efforts, biological control is expected to contribute significantly to the management of the species.

***Nassella trichotoma* (Poales: Poaceae) crown and root rot diseases in Argentina revisited: preliminary results**

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*Nassella trichotoma*, a grass species native to South America, has become a noxious weed in many regions of the world. In Australia and New Zealand, it has been targeted for classical biological control. Initial studies to identify potential fungal biocontrol agents started in Argentina in 1999 and ended three years later having discounted a rust and a smut fungus. Work on a crown rot associated with tussock die-back symptoms, and believed to be caused by a corticioid fungus at the time, was less detailed, and abandoned with inconclusive results. Almost 20 years later the problem is being revisited, with research focused on plants with die-back symptoms. New surveys have been conducted and diseased plants collected and studied in the laboratory. Surprisingly, five different corticioid fungi were found associated with the crowns and roots of declining tussocks. Several species of *Fusarium*, belonging to four different species complexes, were also isolated from crowns and/or roots of declining tussocks, while *Bipolaris* sp. was isolated from a seed of *N. trichotoma* recovered from a burial trial within a patch of diseased plants. Artificial inoculations with all the obtained isolates were carried out on healthy *N. trichotoma* plantlets. After many failed inoculation experiments, it was concluded that the corticioid fungi were not acting as pathogens but rather as secondary colonisers on senescing plant material. In contrast, all the plantlets inoculated with *Bipolaris* sp. and *Fusarium* spp. developed some degree of crown and root discoloration and rot, and the inoculated fungi were all re-isolated from affected tissues. Because these pathogens were also found associated with seeds recovered from the soil around diseased plants, healthy seeds were inoculated with *Bipolaris* sp. and one *Fusarium* isolate in a separate experiment, causing a great decrease in their germination compared with non-inoculated ones. Surviving seedlings were less vigorous than those grown from healthy seeds. Overall, although all the tested isolates (other than the corticioid ones) were shown to cause some level of disease, none of them produced the die-back symptoms observed in the field. It is now presumed those are caused by more than one of these pathogens acting together in an additive or synergistic manner. At least two of them have been shown to have an impact on plant recruitment processes. More work is needed to clarify the etiology of this disease in Argentina and to fully assess the suitability of the involved fungi as biocontrol agents.



***Passiflora foetida*: prospects for biological control**

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*Passiflora foetida* L. (stinking passionflower), native to Central and South America, is an invasive weed across the Asia-Pacific region, including Australia. Its impacts span environmental and agricultural contexts in Australia. As an invasive herbaceous vine, it is commonly found in riparian ecosystems, forest margins, coastal habitats and ruderal areas (e.g., road verges and disturbed habitats) across tropical and subtropical parts of northern Australia. The weed has significant impacts on native vegetation through the formation of dense mats that smother native plants, and by carrying fires into tree canopies. Its climbing/smothering habit similarly results in negative impacts on tree crops (e.g., sandalwood plantations) and post-mining restoration efforts. Currently, the management of this species is largely dependent on physical (e.g., hand-pulling) and chemical (e.g., herbicides) control tactics, but these methods are not cost-effective and sustainable at the spatiotemporal scale of the weed's infestation. As a result, efforts are underway to investigate biological control options for this weed. To date surveys in the native range (Argentina, Brazil and Colombia), guided by ecophysiological and population genetics studies of *P. foetida sensu lato*, have identified a range of pathogens and insects that are being studied for their prospects as candidate biological control agents. These prospective agents are being screened in the native range, through a combination of field observations and laboratory host-specificity tests, for their ability to develop on commercial passionfruit cultivars and other non-target species. Based on these native range studies, a cercosporoid fungal pathogen, and a leaf sucking mirid, *Engytatus passionarius* have been identified as having biocontrol potential and have been exported to Australia for further testing. Both species are currently progressing through host specificity testing. If they pose negligible risks to non-target species regulatory approval will be sought for release.

**The leaf-mining flea beetle *Epitrix* sp. is suitable for release as a biological control agent for *Cestrum* spp. (Solanaceae) in South Africa**

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Several *Cestrum* species, commonly known as ink berries, are declared weeds in South Africa. Two species (i.e. *Cestrum parqui* and *C. laevigatum*) have become highly invasive in the country, with *C. parqui* being predominantly found in inland provinces while *C. laevigatum* is highly abundant along the east coastal regions of the country. *Cestrum* species are generally toxic to animals including livestock, poultry and humans. A biological control programme of *Cestrum* spp. was resumed in South Africa in 2014, with surveys and search for potential agents initiated in the native region (South America). Among several natural enemies found in the native range was a leaf-mining flea beetle *Epitrix* sp. (Coleoptera: Chrysomelidae) which has since been introduced into quarantine in South Africa for host-specificity testing. Latest findings suggest that the beetle is adequately host-specific and highly damaging and therefore suitable for release against *Cestrum* spp. in South Africa. While *Epitrix* sp. is being described as a new species, an application to release the beetle is being prepared for submission to regulatory authorities. Because of the magnitude of *Cestrum* spp. invasion in South Africa, a mix of natural enemies will be required to mitigate their target plants' invasion in the country.

**Assessing potential new biocontrol agents for silverleaf nightshade *Solanum elaeagnifolium* (Solanales: Solanaceae)**

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Biological control has been proposed to reduce the extent and impacts of silverleaf nightshade *Solanum elaeagnifolium*; a Weed of National Significance in Australia. The leaf beetle *Leptinotarsa texana* was introduced to South Africa from the USA and is now an effective biological control of silverleaf nightshade in that country. However, Australian quarantine studies concluded *L. texana* is not suitable for introduction to Australia. Recent overseas surveys and experiments in the weed's native range have highlighted two new agents with potential for Australia: the lacebug *Gargaphia arizonica* from Texas, USA, and a mite *Aceria* sp. from Argentina. Research is now focussed on assessing the risk of these prospective agents to Australian *Solanum* spp. and to closely related crops such as potato and tomato.

**Results of international natural enemy surveys for biological control of yellow floating heart, *Nymphoides peltata* (Asterales: Menyanthaceae) in the US**

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Yellow floating heart, *Nymphoides peltata* is a widespread floating-leaved aquatic invasive plant in the US and biological control is being considered as a sustainable method of control. From 2018-2022, surveys to locate host-specific and damaging natural enemies were conducted in Europe and Asia as were studies to compare the plant demography between the introduced and native ranges. Yellow floating heart is genetically most-closely related to populations in Europe, but we found no promising agents in this region, except for two previously reported fungal pathogens. In Asia, 11 arthropod species were identified as potential biocontrol agents based on observed damage in situ and literature reports about host-specificity. Of particular interest are at least three species of *Bagous* weevils, one of which may be *Bagous charbenensi*, and a leaf-mining *Hydrellia* fly species, yet to be identified. In Korea and China, the generalist leaf-cutting moth *Elophila interruptalis* is common as were other leaf-cutting moths observed in domestic surveys in the US. A major discovery was the damaging fungal pathogen, *Septoria villarsiae* isolated from plants in a private pond in Maine- a first record in the Western hemisphere. The next steps for this program should include initial host-specificity and impact assessments of the fruit-feeding *Bagous* weevils from China and Korea and the leaf-mining *Hydrellia* sp. fly from South Korea.

**Natural enemies of the invasive plant *Harrisia martinii* (Caryophyllales: Cactaceae):  
Their use in biological control from the perspective of native and introduced areas**

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*Harrisia martinii* is a much branched, erect to climbing cactus with a native range from eastern Paraguay to northeast of Argentina. In Australia this species is invasive and can form dense infestations, choking out other pasture species and causing injuries to livestock. *Harrisia* cactus was introduced to Australia in the 1890's. Since then, it has been steadily increasing in density and distribution in Queensland and New South Wales. In Australia, integrated management strategies for this cactus include chemical and mechanical control, as well as biological control with agents that were imported from Argentina in the 1970s and 1990s. However, an adequate level of control has not been achieved. The aim of this research project is to further the knowledge of the interactions between *Harrisia martinii* and its natural enemies in the native range of Argentina, with a view to utilizing insects from Argentina as biological control agents in Australia. The research focuses on two biocontrol agent candidates: the fly *Dasiops bourquini* (Diptera: Loncheidae) and the beetle *Nealcidion cereicola* (Coleoptera: Cerambycidae). After localization and collection of the insects from the north of Argentina, laboratory cultures will be established for further observation. Field observations will also be carried out to understand the environmental requirements and mating habits to rear these species. Once the methodology has been established, a small collection from the laboratory cultures in Argentina will be exported to Australia. Methods and environmental conditions used in Argentina will be replicated at the Queensland Department of Agriculture and Fisheries (QDAF) quarantine laboratory facility in Brisbane to produce new cultures that will be used for host-specificity testing on significant and economically important species. This project is relevant because very little is known about the biology and ecology of *D. bourquini* and *N. cereicola*. This research will further the knowledge of these two native insect species in Argentina and establish a collaborative relationship with biological control researchers in Australia. If the insects are proven to be safe and are approved for field release in Australia, this work will be instrumental in helping to control one of Australia's worst invasive species.

**Potential of *Septoria araujiae* (Capnodiales: Mycosphaerellaceae) as a classical biocontrol agent for moth plant, *Araujia hortorum***

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*Araujia hortorum* (Apocynaceae) is native to Argentina where it is appreciated for its medicinal, nutritional, and ornamental value. In several countries where it has been introduced for the latter, it has become an invasive weed, and in the case of New Zealand it has been declared a target for classical biological control. The rust fungus *Puccinia araujiae* is, to date, the most studied of its fungal pathogens and shows very good prospects as a biocontrol agent. Nevertheless, little is known about the other pathogens that affect this plant in its native range. Studies are being conducted to build a body of knowledge on the fungal diseases affecting *A. hortorum* in Argentina. Surveys were carried out in the province of Buenos Aires in search of plants with symptoms of disease. In several populations across the province, plants were observed to be severely damaged by a foliar disease which was found to be associated to *Septoria araujiae* (Fungi, Ascomycota). Diseased plants exhibited extensive angular necrotic leaf spots, and in some cases, extensive necrotic areas on fruit surfaces. A detailed morphological description of the fungus was made, and its pathogenicity and specificity investigated, together with its penetration mode and the optimum incubation conditions for infection and disease to occur. To this end, artificial inoculations were performed on healthy plants of *A. hortorum* and nine other species within the Apocynaceae under controlled environmental conditions. All inoculated *A. hortorum* plants developed symptoms 14 days after inoculation at 20°C. The fungus was re-isolated from affected leaves, thus fulfilling Koch's postulates. Infection was shown to occur directly through the epidermis. Four other species belonging to the genera *Araujia* and *Oxypetalum* were found to be susceptible to the disease, indicating the fundamental host range of the pathogen is circumscribed to the subtribe Oxypetalinae. *Septoria araujiae* may be considered as a prospective biological control agent for *A. hortorum* due to its high virulence and its narrow host range. Furthermore, it was found to have quite a wider geographical distribution than *P. araujiae* in the province of Buenos Aires, indicating its ability to infect the host under a wider range of environmental conditions, and thus to impose a negative pressure on the host at locations where the rust may not. It is therefore suggested as an interesting potential complementary biocontrol agent.

**Measuring impact of an invasive tree, *Triadica sebifera*, on arthropod communities in Louisiana: Baseline data needed prior to biological control**

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The invasive tallow tree, *Triadica sebifera* (L.) Small, has modified many ecosystems in Southeastern USA by displacing native species and reducing biodiversity. Coastal prairie habitats and longleaf pine savannahs are two sensitive communities which are limited in distribution and are susceptible to tallow tree invasion. Even though tallow is highly valued by beekeepers, it can greatly reduce understory plant diversity with potential negative effects on native pollinators and ground-dwelling arthropods. Therefore, this study aims to establish long-term plots to quantify the effect of *T. sebifera* on pollinators and ground arthropods in managed pine forests in Louisiana. In November 2021, eight plots (2 ha each) were established within Marsh Bayou Wildlife Management Area located in Oakdale, Louisiana. Initial measurements included plant composition and DBH of all trees found in selected plots. The site is dominated by *Pinus taeda* (46%) but largely invaded by tallow trees (34%). Pre-treatment arthropod data was collected using pitfall traps and colored pan traps placed in five subplots per plot in April 2022 (160 traps total). Data on pollinators and vegetation are not presented here. A total number of 1,825 specimens were identified consisting of six taxa: Entognatha, Insecta, Chilopoda, Diplopoda, Malacostraca, and Arachnida. The most abundant groups were Collembola (46%), followed by Hymenoptera (19%) and Araneae (12%). Within insects, the most common families were Formicidae (46%), Carabidae (16%), and Zorotypidae (12%). In August 2022, four of eight plots were cleared using heavy equipment, mulched, and burned. Post-treatment arthropod data was collected in October 2022 (same as before). A bimonthly sampling of ground arthropods, pollinators, and understory vegetation will be continued from March to October 2023 and 2024. Long-term studies are critical to quantify changes in food webs during the management of invasive species in pine forests in Louisiana.

### **Update on *Solanum mauritianum* (Solanaceae) biocontrol in South Africa**

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*Solanum mauritianum*, native to subtropical South America, is invasive over large parts of South Africa. Two insect biological control agents, namely the lace bug, *Gargaphia decoris* and the flower-feeding weevil, *Anthonomus santacruzi*, were released in 1999 and 2008, respectively. Both agents have established in the field where they can be damaging but are mainly limited to low altitude sites. Prominent *S. mauritianum* invasions also occur in high altitude regions within South Africa (> 1000 m) that experience cold dry winters. Consequently, these agents have not established in these regions. A candidate biocontrol agent, the flower-feeding weevil *Anthonomus morticinus* was collected from temperate areas in Uruguay during 2020. These regions are climatically similar to the cooler high-altitude regions of South Africa. Laboratory thermal assessments indicate that *A. morticinus* is better adapted to lower temperatures than its congener, *A. santacruzi*. However, humidity trials indicate that its low humidity tolerance is not dissimilar to its congener. Host-range testing of *A. morticinus* shows it to have an equal or narrower host range relative to *A. santacruzi* on agricultural Solanaceae. Work to test *A. morticinus*' host range on native South African Solanaceae is ongoing and results thus far are promising.



### **The influence of climate on the establishment and efficacy of *Evippe* sp. #1 in South Africa**

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Several species of spiny leguminous trees within the genus *Prosopis* L. (Fabaceae) now occur as widespread invasive alien plants in South Africa, exerting severe negative socio-economic and ecological impacts. Given these impacts, South Africa recently released the leaf-tying moth *Evippe* sp. #1 (Gelechiidae: Lepidoptera) as a biological control agent against *Prosopis* in 2021. The widespread invasion of these *Prosopis* spp. across a vast and climatically diverse range of South Africa has led to concerns regarding the establishment and impact of the agent. Therefore, this study aimed to assess the constraints posed by climate to the potential establishment and efficacy of *Evippe* sp. #1 using both climatic matching, carried out using CLIMEX, and thermal-physiology assessments, namely the critical thermal and lethal temperatures. Climatic analyses revealed relatively high (71%) and moderate (66%) matches of South Africa to the native (Argentina) and introduced (Australia) ranges of *Evippe* sp. #1 respectively. In addition, thermal assessments of *Evippe* sp. #1, particularly of the 4<sup>th</sup> instar larvae, determined a  $CT_{min} = 0.9 \pm 0.3$  °C and  $LLT_{50} = -11.1 \pm 0.4$  °C, which suggest the moth may establish over a large range but is thermally best-suited to the warmest arid regions of South Africa. Overall, these assessments propose that the establishment and performance of *Evippe* sp. #1 populations are likely to be constrained by climate in parts of South Africa, particularly within the cooler semi-arid and temperate regions of the country. Promisingly, these climatic comparisons also suggest that *Evippe* sp. #1 may become established in the hottest parts of the Northern Cape province, which remains a major biological control target region for South Africa given it suffers the highest levels of *Prosopis* invasion and impact. Therefore, ongoing biological control efforts should focus releases primarily in the most climatically well-suited areas as to promote greater establishment of the moth. More broadly, this research highlights the importance of climatic considerations in biological control programmes.

**Biocontrol efforts against invasive *Tamarix* (Tamaricaceae) in South Africa:  
2014 - 2023**

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*Tamarix chinensis* and *T. ramosissima* native to Eurasia, are dominant invasive shrubs or trees in riparian ecosystems mainly located in the central to western half of South Africa. In 2014, a research programme was initiated at Wits University to explore the feasibility of using biological control against these species. However, *Tamarix usneoides*, which is native to South Africa has created additional challenges in finding host specific biocontrol agents. While the invasive *Tamarix* and the indigenous *T. usneoides* have slightly different distributions, they do co-occur. Consequently, the two invasive species and the indigenous *T. usneoides* have readily hybridized to form three additional hybrid genotypes. Abundance measurements of the invasive *Tamarix* in the Eastern and Western Cape Provinces show that the weed continues to spread. Hence, management interventions are required, of which biological control offers the best long-term prospects. The Wits' research has to date rejected the leaf-feeding beetle, *Diorhabda carinulata*, and the scale insect, *Trabutina mannipara*, as potential biological control agents of invasive *Tamarix* due to non-target feeding on native *T. usneoides*. As of early 2023, the leaf feeding weevil *Coniatus tamarisci* was also rejected due to non-target feeding on native *T. usneoides*. This high rejection rate is of concern because *T. usneoides* belongs to a clade that is distinct from *Tamarix chinensis* and *T. ramosissima*, yet candidate agents tested to date appear generalist across the *Tamarix* genus. Thus, more focused native range surveys are required to find suitable host specific agents.

**Host range studies on *Aceria* sp. (Acari: Eriophyidae), a biocontrol candidate of *Solanum elaeagnifolium* in its native range**

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*Solanum elaeagnifolium* Cav., silverleaf nightshade (Solanaceae), is a deep-rooted, summer-growing perennial herb, with an amphitropical native distribution (USA and Mexico, and the southern hemisphere, Argentina, Paraguay, Uruguay and Chile). In its invasive range in Australia, several options of management have been proposed, highlighting the necessity of biological control. The plant presents three clearly separated genetic lineages, two in South America with polyploid populations and a third with only diploids in North America and Australia. In Argentina the plant is present as diploid, tetraploid and hexaploid. Several natural enemies have been recorded in the weed native range, and proposed as potential biological control agents, one of them is *Aceria bicornis* Trotter (Acari: Eriophyidae). To search for this potential biocontrol agent, and to associate its occurrence with *S. elaeagnifolium* genetic variability, we conducted field trips in Argentina and preliminary field and laboratory host-range studies. During these trips, plants with mite-like damage were found at different localities. Moreover, we estimated mite field host range by checking other native *Solanum* for the presence of *Aceria* or other related mites and also were collected. In each site, plants and mites were collected for morphological identification and genetic analyses. For the mite identification, leaves with symptoms (galls) were collected and put in vials with ethanol (96%) and preserved for further morphological and molecular analysis. For rearing, *S. elaeagnifolium* plants with symptoms of *Aceria* were collected and taken to the laboratory. Leaves with galls were put in contact with asymptomatic plants of *S. elaeagnifolium* and 8 varieties of potato plant and eggplant in a non-choice design. Plants were potted and enclosed in fine-mesh cages for 2 months. Mites from field and laboratory tests were genetically analyzed using a fragment of the mitochondrial *COI* gene in order to identify mites found on *S. elaeagnifolium*, other native *Solanum* species and from host specificity tests without the need of morphological studies. The species found was *Aceria* sp, which was the same collected in each site. *Aceria* was collected only on the target weed in the field on only one plant lineage. In the laboratory host range study, it was found on the *S. elaeagnifolium* and on 2 plants of one variety of potato. The utilization of *Aceria* sp. as a potential agent against *S. elaeagnifolium* is still a challenge due to difficulties on mite identification and rearing.

**Classical biological control of the ice plant, *Carpobrotus edulis* (Caryophyllales: Aizoaceae)**

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The ice plant, *Carpobrotus edulis*, a mat forming, succulent perennial herb native to South Africa has been introduced to multiple continents as a horticultural species, but in numerous countries has escaped cultivation and become a problematic invasive. In the UK *C. edulis* is well established along coastlines to the south and west, forming large, dense mats to the exclusion of native plants, stabilising dunes, colonising cliffs and altering soil properties. *Carpobrotus edulis* is listed on Schedule 9 of the Wildlife and Countryside Act in England and Wales, meaning it is illegal to plant or otherwise cause the plant to grow in the wild, and populations are often targeted for control using manual and chemical approaches, however this can be challenging and expensive on sensitive and hard to access sites. The plant was flagged as a key concern in sensitive sites in the UK and CABI was commissioned to produce a biocontrol feasibility dossier for Natural England. *Carpobrotus edulis* was found to have various damaging natural enemies, particularly scale insects and a novel pathogen from its native range. One scale, *Pulvinariella mesembryanthemi*, caused significant damage to *C. edulis* when accidentally introduced to California in the 1970s. The plant had been introduced to the region over several decades as ground cover, for dune stabilisation and erosion control, but following its accidental introduction, the scale insect caused extensive damage to *C. edulis* to the point that researchers introduced several natural enemies to successfully control the scale, allowing the plant to recover with the result that *C. edulis* is now considered invasive in the region. *Pulvinariella mesembryanthemi* had been reported from the Isles of Scilly in the UK and anecdotally also from Cornwall. A survey was conducted in 2022 in these areas to identify natural enemies of *C. edulis*, particularly *P. mesembryanthemi*. The scale was located, although in low density, and brought into culture to assess its impacts in the lab and to identify parasitoids using it as a host. CLIMEX assessment is currently underway as it is suspected that *P. mesembryanthemi* may be climate limited in the UK in addition to being subject to predatory pressure. The next phase of the research will focus on native range surveys in South Africa to identify specific and damaging invertebrates and/or pathogens that could have potential for use as biocontrol agents against *C. edulis*, both in the UK and in other regions negatively affected by the plant such as the Mediterranean islands and coastal regions.

**Significant genetic structure in *Macrobathra* moths feeding on *Acacia auriculiformis*– implications for prioritising biological control agents**

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*Acacia auriculiformis* A. Cunn. ex Benth. is a native Australian tree that has now become a category 1 invasive weed in Florida, USA. Previous research has identified *Macrobathra* moths (Cosmopterigidae) as potential biological control agents for this weed, but little is known about the genetic diversity and structuring of these moths. In this study, we compared the genetic structure of four common *Macrobathra* spp. across the geographic distribution of *A. auriculiformis* and across regions where *A. auriculiformis* does not occur to assess whether any of these moth species may comprise unrecognised cryptic species. We found contrasting patterns of genetic structuring among the four moth species, indicating that host-specific cryptic species could be present in *Macrobathra arrectella* Walker and *Macrobathra diplochrysa* Lower. Further, we identified a deep genetic disjunction in both *M. arrectella* and *Macrobathra callipetala* Turner across the Gulf of Carpentaria, a pattern that is also found in *A. auriculiformis*. The geographic distribution and host plant associations of the distinct mitochondrial lineages of each of these moth species should be further evaluated with additional ecological sampling, and the species status of these lineages tested directly, using additional molecular screening and/or carefully designed cross-mating tests.

**Selection of potential agents for an invasive cactus weed, *Trichocereus spachianus***

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*Trichocereus spachianus* (Cactaceae) is an increasing concern as an invasive alien plant in South Africa. The invasion of this cactus species into rangelands is a major issue as it reduces grazing area for both indigenous wildlife and livestock. Biological control has been very successful in controlling several cactus species in South Africa and is considered a promising management strategy to reduce the impacts of *T. spachianus*. In particular, the use of a gall-forming mealybug, *Hypogeococcus* sp., has been successful in controlling the spread of invasive cacti that are closely related to *T. spachianus*. Although the indigenous distribution of *T. spachianus* is believed to be in Argentina, there are no confirmed records of the species in natural populations in the country. Surveys were conducted in 2019 in Argentina to locate wild *T. spachianus* populations and to search for potential agents, with particular focus on *Hypogeococcus*, but no *T. spachianus* populations were found outside of cultivation and managed gardens. The species was described from a potted plant in a botanical garden and all the records of the plant in the native distribution are now believed to be misidentifications. As it was not possible to collect potential agents from the target weed, several *Hypogeococcus* species and lineages were collected off other cactus species and imported into South Africa for further testing. Results indicated that all *Hypogeococcus* tested experienced low initial nymph survival, level of establishment and fecundity on *T. spachianus* compared to control cacti, so none were damaging enough to warrant further consideration as biological control agents. One further candidate biological control agent, *Dactylopius confertus*, a cochineal that is known to use *Trichocereus* species as a host, is being tested. *Dactylopius confertus* was accidentally released in Namibia where it utilises the cactus weed *Harrisia pomanensis*, a close relative of *T. spachianus*. Based on preliminary findings, *D. confertus* appears to be a more promising biological control agent than the previously evaluated *Hypogeococcus* as it has a greater suitability (based on duration of survival, development and fecundity) to *T. spachianus* than *H. pomanensis*. The successful use of cochineal in controlling other invasive cactus species, combined with the promising preliminary results, makes *D. confertus* a strong candidate for biological control of *T. spachianus* for South Africa.

**Native range exploration in the southwest United States reveals potential biological control agents for *Prosopis* (Fabaceae) for South Africa**

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Successful importation biological control of weeds is based on the collection and identification of appropriate host specific agents during native range surveys. Several species of mesquite, *Prosopis* spp., are considered weedy in arid and semi-arid regions worldwide, including in Australia and southern and eastern Africa. Despite current control efforts, *Prosopis* invasion in South Africa has expanded rapidly over the past 15 years. As a result, there is interest in increasing the supply of biological control agents against *Prosopis* species in South Africa. Over two field seasons, we conducted surveys of *Prosopis* and related trees, including *Vachellia*, *Mimosa* and *Senegalia* species across the southwest United States, including California, Arizona, New Mexico, and Texas. We used multiple collection methods, including visual surveys, black lighting, and beating of foliage. To prioritize host-range testing, we developed a matrix to rank potential biological control agents for *Prosopis*. We ranked insects according to their ability to damage host plants, host specificity, feeding guild, abundance, and prevalence. We collected and identified over 2000 individual insects. Coleoptera was the most well represented order among our specimens, with over 1000 individuals across 17 families. We also collected Lepidoptera representing eight families, as well as nine families of Hemiptera. Based on our field notes and published literature, we identified the insects with the most potential to damage or kill *Prosopis* species including *Hemileuca juno* Packard (Lepidoptera: Saturniidae), *Agrilus viridescens* Knull (Coleoptera: Buprestidae), *Hippomelas sphenicus* (LeConte) (Coleoptera: Buprestidae), and *Chrysobothris gemmata* LeConte (Coleoptera: Buprestidae). We will continue to survey for these and other host-specific insects and develop preliminary host range testing protocols.

**Biocontrol of Old Man's Beard, *Clematis vitalba* (Ranunculales: Ranunculaceae):  
fungi re-visited**

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Old man's beard (*Clematis vitalba*) is a climbing shrub of the family Ranunculaceae with a native range extending from Europe to Afghanistan. The species was introduced into New Zealand as an ornamental but due to its ability to grow and spread rapidly, it is now one of the country's most damaging invasive alien plants, impacting negatively on biodiversity. A biological control programme began in the 1980s which resulted in the approval of two insect agents, the sawfly *Monophadnus spinolae* and the agromyzid fly *Phytomyza vitalbae*. A fungal agent, '*Phoma clematidina*' was also released and establishment was confirmed at a number of sites throughout New Zealand. However, despite being virulent initially, the pathogen did not persist in the field. In 2020, a project to reassess the potential of fungal agents from the native range commenced. After completion of a literature review and a CLIMEX study to identify the climatically best-matched regions in the native range, field surveys for fungal pathogens were undertaken in the UK, Germany and Spain. We report on the results of these surveys and discuss the significance of recent molecular revisions of '*Phoma clematidina*'. The progress of pathogenicity assessments conducted with *C. vitalba* plants from New Zealand and other key species will also be presented and discussed.



## Targeting *Iris pseudacorus* (Iridaceae) for biological control in South Africa and beyond

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*Iris pseudacorus* L. (Iridaceae) is an emergent aquatic macrophyte originating from Europe, north Africa, and western Asia, and is an increasingly problematic invader in the southern hemisphere, including South Africa. By forming dense rhizomatic mats in the absence of natural enemies, *I. pseudacorus* outcompetes co-occurring indigenous biota, causing serious environmental and socio-economic challenges. *Iris pseudacorus* is a declared invader in South Africa, Argentina, New Zealand, the United States of America, and Canada, but little information is known regarding the species' invasive potential, particularly in the southern hemisphere, hindering the effectiveness of control efforts. This study addresses this knowledge gap in a South African context, providing valuable insight into the invasion autecology of *I. pseudacorus*, and options for biological control. For effective management and control of *I. pseudacorus* in South Africa and the global south, its distribution and invasive potential must be determined, and its population genetics understood. Through field surveys, *I. pseudacorus* infestations were confirmed in eight of the country's nine provinces. These surveys indicated that South African *I. pseudacorus* populations have enhanced their sexual reproductive output relative to native range populations, with ~ 83 % seed germination. Using inter-simple sequence repeats (ISSRs), high genetic diversity was observed within and between populations of *I. pseudacorus*, confirming the employment of sexual reproductive strategies, and providing evidence for gene-flow between and within populations. Moreover, a weak negative correlation was observed between geographic distance and genetic similarity, indicating a largely anthropogenic spread of *I. pseudacorus*. Because enhanced reproductive output in the invaded range is likely due to the absence of natural enemies, biological control is likely to render *I. pseudacorus* less capable of reproduction and spread. *Aphthona nonstriata* is one of three natural enemies of *I. pseudacorus* from its native range. Host-specificity testing demonstrated this species is specific to the genus *Iris*, which is not native to South Africa, and herbivory by *A. nonstriata* causes substantial damage to *I. pseudacorus* individuals, even when the beetle is present in low numbers. These results contribute to the development of appropriate adaptive and integrated management strategies to control *I. pseudacorus* invasions in South Africa, and should be implemented before South African *I. pseudacorus* infestations reach the severity observed elsewhere. Furthermore, *A. nonstriata* could be considered for release in New Zealand and Argentina where *Iris* is also non-native.

**Seasonal dynamics and damage potential of *Dacus persicus* (Diptera: Tephritidae) and *Paramecops farinosus* (Coleoptera: Curculionidae): two perspective agents for biological control of invasive *Calotropis procera* (Apocynaceae)**

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*Calotropis procera* (Apocynaceae), commonly known as calotrope or rubber plant, is a small tree native to the Indian subcontinent, the Middle East and northern Africa. Calotrope has become an invasive weed in Australia and the Pacific and parts of southeast Asia and south America. Seasonal dynamics and damage potential of two perspective biological control agents, a fruit fly, *Dacus persicus* Hendal (Diptera: Tephritidae) and a weevil *Paramecops farinosus* Schoenherr (Coleoptera: Curculionidae) on host *C. procera* were studied across 9 sites over two seasons in its native range in Punjab, Pakistan. The weevil starts infesting fruits in the early summer (April-May) while fruit fly in mid-summer (June) with first signs of fruit infestation by fruit fly appearing in mid to late summer (June-July). The population of *D. persicus* adults consistently increased with increasing minimum temperature up to 23°C before it started to decline in late autumn (Oct-Nov). Peak fruit infestation by weevil was observed in early – midsummer period (April – June) when on average 57% of fruits harvested were infested with weevil eggs or larvae. Weevil infestation gradually declines in late summer (July onwards), while fruit fly infestation increased slowly and peaked in late summer (August – Oct). In this season, the fruit fly and weevil infestation of fruit was 42% and 25%, respectively with about 33% fruit being healthy. A very small fruit proportion (< 1%) was infested by both fruit fly and weevil. The fruit availability declined significantly (c. 90%) in early winter (Nov-Dec) as compared to summer with no fruit was found on plants from January onwards until March. Across all sites and seasons, *D. persicus* seed damage (50% or more fruit<sup>-1</sup>) was 60% greater as compared to *P. farinosus*. Similarly, *D. persicus* reduced the fresh biomass of host fruits 33% and 46% greater than *P. farinosus* or healthy fruits, respectively. The season-long presence and high damage potential indicate that both agents effective and could be prioritised for biological control of *C. procera* in its introduced ranges.

**The leaf-feeding chrysomelid *Blaptea elguetai* (Coleoptera: Chrysomelidae), a potential biological control agent against Chilean flame creeper, *Tropaeolum speciosum***

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Native to Chile, Chilean flame creeper (*Tropaeolum speciosum*) has become highly invasive in New Zealand where it is a threat to native vegetation. The lack of herbivores on this vine in New Zealand suggested it would be useful to look for its natural enemies in its native range. During the spring of 2019, chrysomelid larvae were found feeding on leaves of Chilean flame creeper in General Lopez, Vilcun county, Chile. Later surveys revealed the presence of leaf-feeding adults that were identified Biocontrol efforts against invasive Tamarix (Tamaricaceae) in South Africa: 2014 - 2023 as belonging to the species *Blaptea elguetai*. These findings prompted additional exploration to learn about the beetle in the field and to collect adults for further studies in the laboratory. The beetle was the most common herbivore feeding on Chilean flame creeper in its native range. The dark blue and bronze-coloured beetles lay eggs in protected sites on their host and in the commonly co-occurring Chilean bamboo shrubs. In the laboratory, adults confined in Petri dishes lined with tissue paper, fed continuously on fresh Chilean flame creeper leaves, and laid eggs on leaves and on filter paper. Upon hatching, the larvae completed their development from eggs to adults within 50 to 60 days in two studies with parental populations from different sites. There were five larval instars. Surveys to determine the field host-range of the beetle were conducted on five of the most closely related native congeneric endemic *Tropaeolum* species in Chile. These species flower during late winter in central and northern Chile (600 to 2000 km from the distributional range of Chilean flame creeper). No leaf-feeding beetles were noticed on the congeneric species, suggesting that the host range of the beetle may be restricted to Chilean flame creeper in its native range, where there is a lack of geographical overlap with its congeneric species. These preliminary field and laboratory results led to the importation of the beetle into the Manaaki Whenua – Landcare Research containment facility at Lincoln, New Zealand, in November 2022, for further studies.

**Impact of the invasive American duckweed, *Lemna minuta* (Alismatales: Araceae), on freshwater ecosystems and potential for its biological control in Europe**

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Some floating aquatic plants are known to be highly invasive outside their native range, posing a serious threat to freshwater ecosystems. Dense mats of floating plants can reduce water quality and hinder navigation, fishing and recreational activities. Given their strong negative impact, classical biological control strategies have been developed and refined over the years to limit the damage of aquatic weeds including *Pistia stratiotes*, *Pontederia crassipes* and *Salvinia molesta*. However, one group of floating plants that have received little attention are duckweeds, tiny plants that grow in slow-moving or stagnant water bodies. Among them, *Lemna minuta*, native to North America, is rapidly spreading to many countries of Europe, where in recent years it has proven to be one of the non-native aquatic species with the highest invasive potential. The wide distribution of *L. minuta* is due to its high reproductive rate, which allows it to rapidly colonize extensive water bodies and form thick floating mats on the water surface, which reduce oxygen, light penetration and temperature in the water column. These conditions alter the biotic component of invaded ecosystems, leading to a decline in species richness and plant cover of the macrophyte communities, as well as an alteration in the specific composition of the animal communities. Due to the negative impacts associated with *L. minuta*, there is an urgent need to identify a biological control agent that can limit the weed's spread. Unfortunately, biological control of *L. minuta* has been unexplored to date, and information on its natural enemies is lacking. Recent preliminary surveys in its native range have found several insects associated with *L. minuta* that damage its fronds, including the shore-fly *Lemnaphila scotlandae* (Diptera: Ephydriidae) native to North America and the weevil *Tanysphyrus lemnae* (Coleoptera: Eirrhinidae) native to both North America and Europe; however, these insects have not proved to be sufficiently specific against *L. minuta* and further investigations are needed. In particular, it will be extremely important to enhance taxonomic studies on the entomofauna associated with this duckweed, combining morphological and genetic approaches, in order to clarify the complexity of co-evolutionary relationships in the geographic range of origin of this plant.

**Specificity test results of *Engytatus passionarius* (Hemiptera: Miridae), a recently discovered natural enemy of the stinking passionflower *Passiflora* (*Dysosmia*) *foetida* (Passifloraceae)**

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The stinking passionflower (*Passiflora foetida* L., Passifloraceae) is a species of creeping herbaceous vine native to the Americas, and an important invasive weed in some countries, including Australia. Several field surveys were conducted in its native range in Argentina and Paraguay to compile a list of its natural enemies, and pinpoint those that might be suitable candidates as biological control agents. Out of the natural enemies found, a new species of plant bug, *Engytatus passionarius* (Hemiptera: Miridae), discovered in northern Argentina, was the only species with no recorded alternative hosts. In this work we analyze the results of no-choice specificity tests performed with *E. passionarius* on 12 species of the genus *Passiflora* (including the commercial passion fruit, *P. edulis*). Both feeding and survival of *E. passionarius* were assessed. These results show that *E. passionarius* only found two species on the test list to be acceptable sources of food: *P. foetida* and *P. chrysophylla*. *Passiflora morifolia* received marginal feeding damage, but all *E. passionarius* confined with *P. morifolia* died after 15 days (on average), and could not reproduce, indicating *P. morifolia* is not a suitable host. On the other hand, *P. chrysophylla* shares many morphological traits with *P. foetida*, and is considered by some taxonomists to be a subspecies of the latter, which makes its acceptability to *E. passionarius* relative, or at least deserving of further testing. Moreover, neither *P. chrysophylla* nor *P. morifolia* exist in the areas invaded by *P. foetida*. These results make *E. passionarius* the most promising candidate for biological control of *P. foetida* available so far.

**Resuming evaluation of *Thrypticus* spp. (Diptera: Dolichopodidae) in their native range, as biocontrol agents of Water Hyacinth**

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The mining flies *Thrypticus truncatus* Bickel & Hernández and *Thrypticus sagittatus* Bickel & Hernández (Diptera: Dolichopodidae) were first evaluated as biological control agents for the water hyacinth *Pontederia crassipes* (Martius) Solms-Laubach (Pontederiaceae) 15 years ago, in Argentina. The project was discontinued for many years, until the end of 2021, when it was started up again. Biological aspects were studied, and the first part of the research was focused mainly on *T. truncatus* which was specific on water hyacinth. The larvae of both species feed on sap, using the microenvironment inside the aerenchyma to develop the immature stages. Both species are active and reproduce on water hyacinth from spring to the end of fall, and overwinter as larvae inside the mines in the petioles. We also found that the larval activity allows for the infection of the petiole tissues with about 33 fungi species. Because other aspects were not investigated, we set the following objectives: to obtain laboratory rearing methods under controlled conditions (at least for one species), to find ecological differences between the two species and complete the specificity tests at least for *T. truncatus*. We first surveyed sites with previous records of the presence of *Thrypticus* spp. until we found one with plants and insects in abundance, located at the Carabelas river, at the Paraná delta, Buenos Aires province (34°04'52''S; 58°48'80''W). We sampled it periodically from December 2021 to June 2022, and from November 2022 to April 2023. In each visit, we collected petioles with mines and, in the laboratory, we incubated the basal 30 cm where the mines are located, in 20 lt black buckets with a transparent flask attached to the lid (at 25 ± 2° C). Once the adults emerged, we separated the two species, sexed and counted the specimens using a cold plate to handle them. We presented the results as population dynamics of both *T. truncatus* and *T. sagittatus* during two consecutive summer season collections. We found that the two species increased their abundances at different times through the season. We also showed preliminary results from the first attempt to establish a laboratory culture of *T. truncatus* in green house conditions.

Moderator: Sara Montemayor (UNLP, AR)

Oral presentation

### **Rapid evolution of a plant invader in response to biological control and global warming**

**Keynote speaker:** Sun Yan<sup>1</sup>.

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Invasive alien plants together with their natural enemies from the native range used as biocontrol agents are ideal study system to address questions of whether and how fast organisms adapt to changing environments. Climate change is likely to impose further selection on invasive plant populations in interaction with the biocontrol process. We conducted an experimental evolution study to get insights into the evolvability to a biocontrol insect and global warming of the European plant invader, *Ambrosia artemisiifolia*. We tracked genomic and metabolomic changes across generations in field populations and assessed plant offspring phenotypes in a common environment. Using an integrated Bayesian model, we show that increased offspring biomass in response to warming arose through changes in the genetic composition of populations. In contrast, increased resistance to herbivory arose through a shift in plant metabolomic profiles without genetic changes, most likely by transgenerational induction of defences. Importantly, while increased resistance was costly at ambient temperatures, warming removed this constraint and favoured both vigorous and better defended plants under biocontrol. Climate warming may thus decrease biocontrol efficiency and promote *Ambrosia* invasion, with potentially serious economic and health consequences. Furthermore, I developed a novel population density distribution model that integrates by species' vital rates and defence strategies extricated from field observations and experiments. This model allows to more accurately predict the distribution and population density of a selected plant invader and its biological control agents on a global scale, as well as the ecological effects and cost savings of biological control management. These results provide a scientific foundation for green management interventions on invasive plants, and they add to the theory of rapid evolution in invasion science.

**The phenological consequences of photoperiodism for introduced biological control agents**

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In classical biological control, populations of agent species are moved across the globe to locations where the climate and seasonal regime differ (to varying degrees) from one to which they are adapted. Phenology and voltinism are often predicted for agents in the new range using simple degree-day models, but these neglect the fact that most multi-voltine insects (in temperate zones) use photoperiod as a primary cue for aligning their life cycles with the season duration. We incorporated photoperiodism into phenology models for several weed biocontrol insects and demonstrate how the combined responses to day length and temperature will interact in complex ways. Potentially consequential outcomes include: (1) emerging too early or too late relative to the availability of the host, (2) diapausing too early or too late relative to onset of fall, and (3) voltinism that is highly variable year to year (leading to booms and busts). Using spatialized daily climate data and latitude- and date-specific photoperiods, we can model and map the geographic patterns of predicted phenological mismatch across continents. From these results, we offer some generalized recommendations for improving success with future biological control introductions.



**Present and future climatic suitability of the aquatic invasive plant *Iris pseudacorus* (Iridaceae) and its candidate biocontrol agents**

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*Iris pseudacorus* is both a prized ornamental and an invasive aquatic plant that tends to grow dense monospecific stands, displacing the local vegetation and altering the hydrology of freshwater ecosystems. Originally from Europe, this species has historically invaded North America and Japan, and more recently spread through Argentina, South Africa and Australasia, where it is now a target for biological control. Field surveys within its native range have led to the selection of three candidate biocontrol agents: the flea beetle *Aphthona nonstriata* (Coleoptera: Chrysomelidae); the seed weevil *Mononychus punctumalbum* (Coleoptera: Curculionidae); and the sawfly *Rhadinoceraea micans* (Hymenoptera: Tenthredinidae). Prioritizing the best candidates for different regions constitutes a critical step, which could save significant time and resources. Accordingly, the objectives of this work were i) to predict *I. pseudacorus* invasions and range shifts in the context of climate change and ii) to identify the best suite of candidates for different invaded ranges. To do so, we modelled the present and future bioclimatic suitability of *I. pseudacorus* and its candidates using the software MaxEnt. Occurrence records for the four species were sourced from GBIF, cleaned and filtered to minimize the influence of sampling biases. The models were trained upon present climatic data using a custom selection of bioclimatic variables representative of the annual temperature and precipitation means, extremes and seasonality. Worldwide model projections were made both for present climate and for the projected time period 2040-2060. Model performance was considered high based on different evaluation metrics (i.e. area under curve, true presence ratio and Continuous Boyce index). Our results highlight a clear distinction between predictions for the Northern and Southern Hemispheres. In North America and eastern Asia, the area climatically suitable for *I. pseudacorus* is expected to increase and shift northwards. As for its biocontrol agents, very low suitability is predicted across these regions, further decreasing under future climatic conditions. On the other hand, climatically suitable areas for the plant in South America, southern Africa and Australasia are predicted, on average, to shrink in response to climate change. A decrease in climatic suitability is also expected for its candidate biocontrol agents which, however, would still maintain a significant range overlap with their host. These results can be used to prioritize areas most at risk of invasion and identify which combination of candidates could potentially provide the best level of control across different invaded ranges.

**The Target Score, an index that may be applied in present and future climatic scenarios, to recognize target regions for pests and biological control agents**

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The recognition of regions where the environmental conditions are highly suitable and where higher population densities are expected provides a key parameter for the management of pest species and for the implementation of biological control. In both cases, the impact depends directly on the availability of suitable areas and the overall population abundance. Different Ecological Niche Models (ENM) can be used to recognize such regions: Maxent models for regions where environmental conditions are highly suitable, and Minimum Volume Ellipsoids (MVE) for regions where higher population densities are expected. Maxent models assess the ecological requirements of species based on their known occurrences and aim to identify different regions or climatic scenarios that suit these requirements. Minimum Volume Ellipsoids models consider that species' fundamental niches can be operationalized as an MVE and that the highest population abundances are expected to occur in places with environments close to, or in the center of, the fundamental niche (Niche Centroid), where fitness is assumed to be highest. We propose to use an ensemble modeling strategy combining the information provided by ENM through the implementation of an index we have developed, the Target Score (TS), which allows the identification of regions where environmental conditions are highly suitable and where higher population densities are expected. The TS ranges between 0 and 1, values close to 1 correspond to areas where environmental conditions are highly suitable and where high population abundances are expected. Therefore, they represent regions where, for example, a pest species would be expected to be a major threat, or a biological control agent would be expected to be highly effective. As Maxent models and MVE can be projected to future climatic scenarios, the TS score can also be used to assess how climate change may affect pests and biological control agent populations.

### Effects of elevated CO<sub>2</sub> on *Megamelus scutellaris* (Hemiptera: Delphacidae) and its yeast-like endosymbionts (YLS)

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Two major threats to biodiversity are the rising levels of greenhouse gases causing climate change, and biological invasions. Invasive species have spread worldwide due to rising global temperatures and increased atmospheric CO<sub>2</sub> levels. Therefore, it is crucial to develop effective strategies that have minimal impact, provide early warnings, consider climate change scenarios, and plan future responses. Regarding weed biological control, it is necessary to know how these increased CO<sub>2</sub> levels would affect not only the target weed but also bio controllers that are being used in the present. Water hyacinth, *Pontederia crassipes*, is an invasive and aggressive aquatic plant around the world. It is native to the Cuenca del Plata and Amazon Basin in South America and can significantly alter invaded aquatic ecosystems. To control the plant, *Megamelus scutellaris*, a South American delphacid-planthopper, is mostly utilized as a biocontrol agent. It feeds and reproduces on *P. crassipes*. Its entire life cycle is dependent on this weed, and when population densities grow enough, they can cause severe leaf chlorosis and a reduction in plant vigor, eventually leading to increased senescence rates. Yeast-like endosymbionts (YLS) are microorganisms that live in *M. scutellaris* fat abdomen tissue, and are essential to their survival, as they provide nutritional functions such as the synthesis of essential amino acids and steroids, and nitrogen recycling. Quantity of YLS and *M. scutellaris* weight and size have a positive correlation. It is expected that endosymbionts from planthoppers such as *M. scutellaris*, will suffer some kind of consequence of elevated CO<sub>2</sub> conditions, and that consequence can have a direct impact on their hosts. To study that possible impact, specimens of *M. scutellaris* living on water hyacinth were raised for 3 months in two different CO<sub>2</sub> environments: current (cCO<sub>2</sub> - 400 ppm) and elevated (eCO<sub>2</sub> - 800 ppm). After that period, all insects were collected and analyzed to determine the age structure of the populations and sex ratio. Finally, 10 nymphs V and 10 adults were randomly selected from each population to be weighted and YLS counted under a binocular microscope. We found no significant differences in age structure, total individuals and sex ratio due to eCO<sub>2</sub>. However, insects raised under eCO<sub>2</sub> conditions showed a lower YLS/weight ratio than the ones raised under cCO<sub>2</sub>. We suggest that this should be further studied as it could mean that projected eCO<sub>2</sub> conditions could negatively affect fitness on control agents like *M. scutellaris*.

## TAXONOMY AND BIOLOGICAL CONTROL

Moderator: Iain D. Paterson (CBC, SA)

Oral presentation

### **Phylogenetic systematics and integrative taxonomy for the successful biocontrol of weeds**

**Keynote speaker:** Marvaldi Adriana E.

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Taxonomy and systematics are at the base of any biological research, providing the names and evolutionary context to access the biological information needed. The biological control of weeds (BCW), like many other areas, often suffers from the “taxonomic impediment” generated by incomplete taxonomic knowledge or lack of available taxonomic experts on the particular taxa involved (i.e., weed plants and their potential biocontrol agents). There is an estimation that about 80 % of all extant species remain unknown. That is particularly critical among hyperdiverse taxa (e.g., angiosperms, beetles) and highly biodiverse areas like the Neotropics. Moreover, most groups with described species require revision by specialists using modern taxonomic methods. Taxonomic experts in different plant and animal groups have the knowledge and experience to perform or supervise the accurate identification of the species involved in the study, or to recognize if they are non-native species, or if they are new to science and then in need of being described, named and classified. Their expertise can be crucial for identifying the challenging organisms often involved in BCW, like hybrids, parthenogenetic lineages, or cryptic species. Systematics can also contribute with phylogenetic information, i.e., hypotheses of evolutionary relationships between species and higher taxa, which can be most valuable for comparative purposes because of their predictive power. Molecular tools for confirming species identification are helpful and becoming common practice, but they can only illuminate, not replace, the taxonomic expertise required to identify based on morphological evidence. A fundamental practice is to deposit voucher specimens in scientific institutions to ensure the reproducibility of the research. Voucher specimens are essential to verify the reliability of the original identification. Besides their crucial importance, the possibility of linking research data or information to the source specimens is still not always easy or even possible. During this conference, I will refer to some case studies selected to illustrate the value of “good” taxonomy and systematics in BCW. I will emphasize the need for collaborative research between practitioners of BCW and systematic biologists.

**Comparing the host specificity of a South African weevil and North American planthopper: the challenge of host specificity that is “just right” for management of *Nymphaea mexicana* (Nymphaeaceae) and its hybrids in South Africa**

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Understanding the genetic structure of an alien invasive plant is important in assessing the host specificity of potential biocontrol agents and allows researchers to focus their surveys on plants that are genetically similar to invasive populations. For *Nymphaea mexicana* Zuccarini (Nymphaeaceae), an invasive waterlily in South Africa, understanding the genetic structure is especially important because of the existence of several hybrids in the invaded range. Biocontrol research for this species is still developing, and the challenge is to find an agent that is specific enough not to threaten native South African plants, but not so specific that it does not affect the hybrids as well as *N. mexicana*. Pre-release surveys in South Africa revealed that a native weevil, *Bagous longulus* Gyllenhal (Coleoptera: Curculionidae), has expanded its host range to include *N. mexicana*. Another potential agent, *Megamelus toddi* Beamer (Hemiptera: Delphacidae) was also imported from the native range of *N. mexicana* for host specificity tests in quarantine conditions. Hence, in this study, we conducted genetic work to determine the putative parents of the *Nymphaea* hybrids in South Africa, and compared the host specificity of the South African weevil and North American planthopper on the South African *Nymphaea nouchali* Burm. f., invasive *N. mexicana*, and a *N. mexicana* hybrid. The results from these studies allow us to improve our efforts at developing biocontrol for *N. mexicana* by relating host specificity to plant genetic structure and enables us to make informed decisions to take further steps to maximize the likelihood of success.

**The *Tetramesa* (Hymenoptera: Eurytomidae) as biological control agents of invasive African grasses: a molecular investigation**

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South Africa is a larger donor than receiver of invasive grasses, where approximately 15% of the country's native grass species have become naturalised elsewhere. However, the application of biological control to invasive grasses has been approached with trepidation in the past due to concerns of a lack of host-specific herbivores. Herbivorous wasps in the *Tetramesa* Walker genus (Eurytomidae) feed exclusively on grasses, and the success of the biocontrol agent, *Tetramesa romana* Walker, in the USA for the control of *Arundo donax* (Poaceae) has reignited interest in the search for other host-specific *Tetramesa* that could be used for the control of invasive grasses. Despite the high diversity of African grasses, only about 2% of the globally-described *Tetramesa* species are from Africa. Here, we present 197 COI DNA barcodes and 199 28S rRNA genetic sequences from wasps collected during field host-range surveys of 59 grass species in South Africa. These surveys were performed in search of possible biological control agents for multiple South African grasses that have become invasive in Australia (*Sporobolus pyramidalis* Beauv., *S. natalensis* Steud., *Eragrostis curvula* Nees, *Andropogon gayanus* Kunth) and the USA (*Megathyrsus maximus* Jacq.). Our species delimitation results support the existence of at least five native southern African *Tetramesa* species (with COI evidence of multiple intraspecific groups) that are new to science. Differentiation of the *Tetramesa* species collected was not possible due to their extreme morphological similarity and a lack of taxonomic knowledge for the group in Africa. As such, phylogenetic techniques and species delimitation analyses were applied to determine the field host-ranges of the novel *Tetramesa* taxa identified during our study. We have evidence of possible host-specific *Tetramesa* on *S. pyramidalis*, *S. africanus*, and *Hyparrhenia hirta* L. The wasps on *E. curvula* appear to be oligophagous, although still specialised to the *Eragrostis* genus. This would exclude the use of these *Tetramesa* species in regions with high diversities of indigenous *Eragrostis*. We have also uncovered a number of unresolved clades, which are likely linked to recent rapid divergences. This work has provided evidence of at least four potential *Tetramesa* taxa that hold promise for grass biological control, and has highlighted the importance of using extensive native-range survey data in conjunction with molecular work to guide agent prioritisation and the development of biological control programmes. Surveys such as this are also important contributors to species discovery, particularly in under-sampled regions such as Africa.

**Phytoplasmas, arthropods and *Ambrosia artemisiifolia* without pollen**

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Phytoplasmas (*Candidatus* genus *Phytoplasma*) are wall-less bacterial plant pathogens traditionally considered serious pests of crops worldwide. They are obligate symbionts of plants and arthropods and are usually transmitted to plants by arthropod vectors. Common ragweed (*Ambrosia artemisiifolia*, Asteraceae) is a North American plant that is currently invading Europe and has serious negative impacts on agriculture, biodiversity, and human health. We wanted to know the vector potential of arthropods in transmitting phytoplasmas. Field surveys were conducted in southern Slovakia in 2020-2021. Sweep nets and visual observations were conducted. Forty-seven true bugs (Hemiptera: Heteroptera), fifty-seven cicadas (Hemiptera: Auchenorrhyncha), and two eriophyoid mites (Acari: Eriophyoidea) were recorded and identified to species. Potential phytoplasma carriers or vectors included 4 true bugs and 18 cicadas. Molecular detection was performed to determine and identify phytoplasmas in plants. The phenotype of phytoplasma-infested plants was significantly altered. The deformed male inflorescences formed sterile seed-like structures instead of pollen, and female seeds were not formed at all on symptomatic plants. We hypothesize that the obligate parasite, a phytoplasma, along with the ability of arthropods to transmit vectors, may be responsible for the disruption of pollen production and overall sterility of plants. These exciting findings and the resulting ecological implications will be highlighted.

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**Eriophyid mites for the biological control of *Ailanthus altissima* (Sapindales: Simaroubaceae): how many species are available?**

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Eriophyid mites (Acari: Eriophyidae) are phytophagous arthropods known for being highly specific and impactful on their host plants, and therefore increasingly valued as biological control agents of weeds. Due to the extremely small size (*around 200 μm*), their correct morphological identification requires slide mounted specimens. Different mounting procedures may lead to poorly made illustrations, uncertainties over key morphological characters, and hence misidentifications. In addition, some species are characterized by an overwintering morph that is morphologically different to the spring–summer morph. Taken together, these aspects may induce an overestimation of the number of species. Integrative taxonomy, combining insights from various disciplines (phylogeography, comparative morphology, phylogeny, ecology, development, behavior, etc.), is now considered the most appropriate method to define eriophyid mite species. This is the case with the eriophyid species associated with *Ailanthus altissima* (Tree of heaven, Toh), a deciduous tree native to China and considered one of the most invasive species worldwide. Four eriophyid species were originally described: *Aculops ailanthi* Lin, Jin et Kuang, *Aculus taihangensis* (Hong et Xue), *Aculus altissimae* (Xue et Hong), and *Aculus mosoniensis* (Ripka), with the latter recently synonymized with *A. taihangensis*. *Aculops ailanthi* and *A. taihangensis* are the only two species found beyond the native range of *A. altissima*, in the US and Europe, respectively. Both species are considered promising candidate biological control agents of Toh. In particular, host range tests and impact assessments of *A. taihangensis* provided good indications of its specificity for *A. altissima* and effectiveness in reducing the fitness of new sprouts and seedlings. The morphological description of the four eriophyid species originally described was poor, and the possibility of further synonymies has been investigated. Recent insights from phylogenetic analyses based on ITS1 and mtCOI have indicated that *A. taihangensis* is also present at least in two US states (Colorado and Virginia). Further investigations based on a larger sample size in the US are currently ongoing to clarify if *A. taihangensis* is a junior synonym of *Ac. ailanthi*. A correct understanding of the identity of *A. taihangensis* and *Ac. ailanthi*, and as a result, of their distributions, is particularly important for the purpose of biological control of a globally invasive species such as Toh, especially due to the possibility that the same eriophyid mite species is associated with *A. altissima* in many of the countries where the plant is invasive.



**Two host-specific cryptic species on one weed- will disentangling their species status and ecology help improve biocontrol?**

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Two cryptic species of *Eueupithecia* moths, native to South America, are host-specific to *Parkinsonia aculeata* and have been introduced to Australia as biological control agents against this weed. A 4% CO1 divergence has been recorded across the two and slight disparities across them are evident in their internal genitalia. Hybridization in captivity raises questions of their species status. Do they, indeed, represent two species (as defined in population genetics terms), or is the variation recorded intraspecific? We know nothing of how the insects in each of these designated groupings interact spatially and temporally with one another across the native and introduced ranges. Therefore, we developed a mechanistic model of the mating system of each, based on behavioral observations, and assessed whether gene flow occurs between the two in the areas of their overlap, in both Argentina and Australia. Behavioral observations revealed overlap in the diel patterns of the mating behavior of *Eueupithecia cisplatensis* and *E. vollonoides*, and pheromone trapping in the field in Australia demonstrated that males are attracted to the pheromones of both types of females. Further, cross-mating laboratory tests of these two species demonstrated that hybridization does occur in the laboratory, but it is asymmetrical. Males of *E. cisplatensis* mate with females of *E. vollonoides*, but males of *E. vollonoides* do not mate with females of *E. cisplatensis*. Nevertheless, genotyping by sequencing techniques revealed no introgression across the two species in areas of sympatry in both the native and introduced ranges. From this, we anticipate that species boundaries between these two species will be maintained in Australia, regardless of them being released in the same areas. That is, these insects occur as two independent species gene pools in nature, and the unique qualities of each can be exploited to enhance biological control of *Parkinsonia*. This conclusion validates the efforts made in the early stages of this program to mass rear *E. cisplatensis* and *E. vollonoides* independently of one another and to match their releases in Australia to environments similar to those from which each came. This is likely to maximize their relative impacts as biocontrol agents across different environments in Australia. Periodic use of these molecular techniques during the monitoring stages of biocontrol programs also allows us to compare the pre and post release populations in the introduced range, and should provide substantial insight into the long-term establishment and movement of these biocontrol agents relative to one another.

**First report of *Cladosporium tenuissimum* causing leaf spots on sourgrass**Firmino André L.<sup>1</sup>, Rodrigues Jairla G.<sup>1</sup>, da Nóbrega Thaisa F.<sup>2</sup> & Vieira Bruno S.<sup>1</sup><sup>1</sup>Instituto de Ciências Agrárias, Universidade Federal de Uberlândia. Monte Carmelo, Minas Gerais, Brazil.<sup>2</sup>Ourofino Agrociência, Guatapar, So Paulo, Brazil.

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Sourgrass (*Digitaria insularis*) is a highly competitive weed, with a fast and aggressive development, considered a major problem in the Brazilian agricultural scenario. Certain biotypes are resistant to the herbicide Glyphosate and to inhibitors of acetyl coenzyme A carboxylase (ACCase). Thus, the search for alternative means of control becomes essential, with the biological method being an excellent option to the use of chemical herbicides. The objective of this work was to describe the morphology and prove the pathogenicity of the fungal isolate KDI 0118 on sourgrass plants showing leaf spot. The isolate was obtained from a collection of cultures of the sourgrass from the states of Minas Gerais, So Paulo and Gois. It was reactivated on potato-dextrose-agar (PDA) culture medium for 7 days, under a 12-hour photoperiod. Fungal colonies: 19.8–31.4 mm in diameter, flat with entire margins, olive green with a thin white border and dark green reverse, aerial mycelium dense, with a velutinate to flaky appearance. Microscopic observation showed a morphology compatible with the genus *Cladosporium*: pale brown hyphae, 0.70–1.79  $\mu\text{m}$  diam. Conidiophores macronematous, light brown, solitary, not constricted, 22.11–85.73  $\times$  0.83–2.0  $\mu\text{m}$ , 1–7 septate. Conidiogenous cells integrated, terminal or intercalary, cylindrical-oblong, geniculate, 2.21–18.47  $\times$  0.55–1.96  $\mu\text{m}$ . Thin-walled, pale brown, cylindrical ramoconidia with narrowed base, 3.17–6.91  $\times$  0.22–2.03  $\mu\text{m}$ , aseptate. Conidia acrogenous, formed in branched chains, ellipsoid, obovoid, subhyaline to pale brown, with distinct hila, 0.99–5.00  $\times$  0.97–2.76  $\mu\text{m}$ , aseptate. Using a taxonomic key, the isolate was identified temporarily as *C. tenuissimum*. Subsequently, the isolate was inoculated onto young sourgrass plants by placing mycelium discs on the leaves, in order to comply with Koch's postulates. All inoculated plants showed intense leaf spots and the isolate was recovered by indirect isolation from the symptomatic tissue, proving its pathogenicity. *Cladosporium tenuissimum* has previously been reported as a phytopathogen of crops such as rice, sugarcane, sorghum and maize. This is, to our knowledge, the first report of *C. tenuissimum* as a phytopathogen of sourgrass, a relevant fact for future studies evaluating the potential of this isolate as a biocontrol agent for this important weed.

**Evaluation of *Anthracocystis panici-leucophaei* potencial for the management of *Digitaria insularis***

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Sourgrass (*Digitaria insularis* (L.) Fedde (Poaceae)) is one of the most important weeds of annual and perennial crops in South America. It is a perennial C4 grass with a very high infestation potential because it is capable of reproducing by seeds and rhizomes, producing about 40,000 seeds/plant/year. An additional problem is that most populations of *D. insularis* that infest annual crops in South America are resistant to glyphosate herbicides. During a survey of phytopathogenic fungi associated with *D. insularis* in the Alto Paranaíba region (Minas Gerais state, Brazil), diseased plants were observed in the municipality of Monte Carmelo, showing severe drying of leaves and stems, drastic reductions in plant size and, in many cases, panicles and seeds were not present in infected plants. The panicles were covered by a mass of teliospores. The present work aimed to clarify the identity of the causal agent of this smut by molecular and morphological methods and to preliminarily evaluate the use of this pathogen as a biological control agent of *D. insularis*. Both phylogenetic analysis based on the ITS region, and morphological observations, revealed that the fungus belongs to the species *Anthracocystis panici-leucophaei* (Brefeld) McTaggart & R.G. Shivas. Inoculations with teliospores and sporidia of *A. panici-leucophaei* on *D. insularis* plants resulted in typical smut infections resulting in a significant reduction in target weed growth. The phenological stage of *D. insularis* influences the sensitivity to the fungus with newly emerged seedlings with one pair of leaves being most sensitive. The fungus systemically colonizes the plants and induces the formation of most of the panicles with galls containing teliospores of *A. panici-leucophaei*, considerably reducing seed production. The fungus reduces growth parameters of sourgrass plants up to 102 days after sowing such as: plant height, root length, stem diameter, dry mass weight of root, shoot dry mass weight, number of leaves per plant, number of tillers, number of inflorescences and number of tillers with smut. The BSV2 isolate of *A. panici-leucophaei* has potential as a biological control agent against sourgrass (*D. insularis*).

**Towards biological control of invasive North American *Rubus* species in South Africa: species identities, genetic diversity and origins**

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Various species of *Rubus* (Rosaceae) (brambles) from North America, Europe and Asia have become serious invaders in temperate, wetter regions of South Africa over the past century. Until recently, the complex taxonomy of the genus, which includes hybridization between alien and native species, together with potential conflict-of-interest with berry growers and consumers, have confounded prospects for biological control of alien *Rubus* species. However, a recent study indicates that the berry-growing industry in South Africa is very small and unlikely to be significantly harmed by biocontrol, and that local utilization of wild-growing fruit is negligible. By contrast, the negative effects of these invasions on biodiversity, forestry and pastoral agriculture are substantial. Several recent studies on taxonomy and phylogeny of *Rubus* species and hybrids in South Africa have also clarified which species and hybrids are present. Previous biocontrol projects targeting *Rubus* in South Africa were undertaken in the 1990s and early 2000s, with a focus on species of North American origin, but no suitable agents were found. It appears that North American brambles are still the most suitable targets, due to a relative lack of hybridization with native taxa and their highly invasive status, particularly in KwaZulu-Natal (KZN) province. Two taxa, members of *Rubus* section *Cuneifolii* and *Rubus* section *Arguti*, previously referred to, respectively, as the upright and the sprawling forms of *Rubus cuneifolius*, are the most abundant and widespread species in higher- and mid-elevations of KZN. A preliminary field survey in South Africa observed few natural enemies on these two invasive taxa, in contrast to those on sympatric native *Rubus*, indicating that the reverse may also be true, namely that natural enemies of *R. sect. Cuneifolii* and *R. sect. Arguti* in North America may be host specific. A molecular study using microsatellites is currently underway to determine genetic diversity of these two taxa in South Africa, and to match them with taxa in North America. A final stumbling block is our relatively poor understanding of the biosystematics of *Rubus* in eastern North America, which includes a large number of species. Two possible strategies to resolve this are: (1) to undertake molecular studies on a subset of the species within these two sections in North America, and (2) to expose South African trap-plants at selected sites in North America in order to attract and harvest natural enemies as candidates for importation into South African quarantine.

**A mycological exploration for potential biocontrol agents of sourgrass: *Bipolaris yamadae* causes leaf spots and foliage blight on *Digitaria insularis***

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Sourgrass (*Digitaria insularis* (L.) Fedde (Poaceae)), native to tropical and subtropical America, is regarded as one of the worst weeds in Brazilian agriculture. The intensive use of herbicides has resulted in the selection of *D. insularis* populations resistant to glyphosate and, more recently, to ACCase inhibitor herbicides. New management methods are urgently needed to tackle this weed. Biological control has never been attempted against *D. insularis*, but is a possible alternative. Surveys and preliminary assessments of the native mycobiota of *D. insularis* for potential biocontrol candidates is ongoing. We examined the taxonomy and pathogenicity of one isolate (KDI 0124) obtained from diseased sourgrass collected in the state of Minas Gerais, Brazil. Plants in the field showed leaves with necrotic lesions, light brown, irregular, elongated on the margins and apex, 5.5 - 10 x 0.4 - 0.7 cm. One pure culture was obtained from necrotic tissues of leaves. In PDA (potato dextrose agar), after seven days, the colonies measured 84 mm diam. These were flat, with an entire border, felted aerial mycelium, grayish, with sporulation. Morphology was typical for *Bipolaris/Curvularia*-complex: conidiophores 61.5–181 × 1.8–2.3 µm, macronematous, isolate or in small groups, simple, 5-8 septate, somewhat geniculate, with a swollen, darker brown basal cell; conidiogenous cells 5–21 × 2–3.5 µm, mono- or polytreptic, terminal or sympodial; conidia 17.5–38.5 × 5–8 µm, curved, sometimes straight, elliptical or obclavate, wider in the middle or just below, tapering towards the apices, pale to dark brown, 3–9 distoseptate, with a thickened external hilum. Chlamydo spores 3.5–8.5 × 3.5–5.5 µm, globose brown, terminal sometimes forming chains. This morphology places the *D. insularis* fungus in *Bipolaris yamadae* (Y. Nisik.) Shoemaker (Pleosporaceae). In order to confirm that identification DNA was extracted and selected regions were PCR-amplified. The ITS, EF1- $\alpha$  and GPDH genes were sequenced. Phylogenetic analysis using RAxML based on combined sequences data confirmed the morphological identification of the fungus as *B. yamadae*. Five young 20 days sourgrass plants were preliminarily inoculated with culture discs placed over the leaves in order to fulfill Koch's postulates. All inoculated leaves of *D. insularis* test-plants showed severe leaf spot lesions, similar to those observed in the field, and *B. yamadae* was re-isolated. *Bipolaris yamadae* is a species known only as a plant pathogen, reported on grasses. Until this finding the only known hosts were: *Panicum capillare*, *P. maximum*, *P. miliaceum* and *Saccharum officinarum*, and this is the first record of the pathogen from Brazil. Evaluations of its potential as a biocontrol agent for *D. insularis* are being conducted.

**Herbivorous insects associated with *Passiflora foetida* (Passifloraceae) in Colombia: Identification, damage and considerations on their usefulness for biological control**Clavijo-Giraldo Alejandra<sup>1</sup>, Salazar Héctor A. <sup>1</sup>, Sathyamurthy Raghu<sup>2</sup> & Uribe Sandra<sup>1</sup>.<sup>1</sup>Grupo de Investigación en Sistemática Molecular, Facultad de Ciencias, Universidad Nacional de Colombia. Medellín, Antioquia, Colombia.<sup>2</sup>CSIRO Health and Biosecurity. Brisbane, Queensland, Australia

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*Passiflora foetida* L. is a vine of the Passifloraceae family native to South and Central America that has been widely introduced in many tropical regions outside the native distribution where it is considered an invasive species that threatens native biodiversity. The methods widely used for its control (chemical and manual) are usually very expensive and have a low long-term impact. One of the main impediments to biological control is that very little is known about its biology and the ecological relationships it presents with organisms that can act as natural enemies in its native range. Research efforts carried out in Colombia in recent years seek to answer these questions, studying the possible antagonistic ecological relationships between phytophagous insects associated with the plant, including the evaluation of their specificity, to propose suitable biological control options. *Philonis inermis* Champion (Curculionidae: Cryptorhynchinae) and *Pronotacantha* sp. nov (Berytidae: Gampsocorinae) have been observed causing significant damage to *P. foetida* in Colombia, and even when the plant was in contact with or near other *Passiflora* species there was no damage to the non-target plants, indicative of the specificity of both insect species. *Philonis inermis* was described from specimens from Mexico, with later reports in other Central American countries and in Venezuela. Aspects about its life cycle, immature stages and host plants were unknown until now. The same is true for the genus *Pronotacantha*. In both cases, the taxonomic identification of the collected specimens was based on external morphological characters of the adults and on characters of the genital structure of the males. Molecular sequences of the mitochondrial gene *cox1* were also obtained, which were analyzed in conjunction with morphological data. Finally, experiments were carried out under laboratory conditions, to record and describe the immature stages and life cycle of both insects. Knowledge of how these insects damage the plant, new distribution records, field host specificity observations, and protocols for mass-rearing will be valuable if either insect are considered as potential agents. This study resulted in the first record of the genus and species of *P. inermis* in Colombia and the discovery of a new and undescribed species of *Pronotacantha*.

**Biology and specificity of phytophagous insects associated with *Conyza bonariensis* (Asteraceae)**

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*Conyza bonariensis* (L.) Cronquist (Asteraceae) is indigenous to temperate parts of South America and is invasive globally. An inventory of insects associated with the *Conyza bonariensis* (L.) Cronquist (Asteraceae) was carried out in order to identify candidate biological control agents. Surveys for insects and the characterization of damage from insects to the plant was carried out in eleven municipalities of the department of Antioquia, Colombia. Six insect species were recorded on the plant in three orders and four families (Tephritidae, Choreutidae, Pterophoridae and Miridae) which had not been reported as phytophagous of the plant in the country. The taxonomic identification of the species was made based on morphological and molecular characters and was subsequently verified with the help of specialists. The species found were: *Eutreta rhinophora*, Loew (Tephritidae), *Trupanea bonariensis*, Schrank (Tephritidae), *Caloreas cydrotia*, Meyrick (Choreutidae) and *Lioptilodes* sp Zimmerman (Pterophoridae), *Puto barberi*, Cockerell (Pseudococcidae) and *Proba vitiscuttis*, Stal (Hemiptera). Observations in lab of immature stages and damage on plants of *Conyza* and based on literature and previous works of natural history of phytophagous insects, it was specified the host range of these species, of which *T. bonariensis* is the most promising to considering it as potential agent for its use in future biological control programs of this weed. The life cycle of the species *T. bonariensis* was studied under laboratory conditions. The larval stage of fruit fly was found to develop in the galls present on the stems of the plants. The formation of galls affects and modifies the phenology and growth of the plants, producing ramifications and reducing their size. The duration of the cycle was 45 to 50 days: The egg lasted 5-8 days, larva 25-30 days with three instars pupa 8-13 days and adult  $16.7 \pm 5$  days. The importance of phytophagous insects as limiting factors for the development of *C. bonariensis* is highlighted. Therefore, the description of the life cycle, behavioral observation, hosts and parasitoids of the fruit fly *T. bonariensis* plays a strategic role as it is the first record of this species for Colombia.

**First detection of the broad-nosed knapweed seed head weevil, *Bangasternus fausti* (Coleoptera: Curculionidae) in Canada**

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The broad-nosed knapweed seed head weevil, *Bangasternus fausti* (Reitter, 1890), is a palearctic species that has been introduced to the western United States for biological control of invasive diffuse and spotted knapweed (*Centaurea diffusa* Lam. and *C. stoebe* ssp. *micranthos* (Gugler) Hayek: Asteraceae) (Smith & Mayer 2005; Winston et al. 2012). It was established in both Oregon and Montana after releases began in 1990 (Lang et al. 2000; Smith & Mayer 2005), and it has since been released in or redistributed to Washington, Idaho, and areas of the southwestern USA (Winston et al. 2014). *Bangasternus orientalis* (Capiomont, 1873), a palearctic congeneric of *B. fausti*, has also become established in the western USA following biocontrol releases on invasive yellow starthistle (*Centaurea solstitialis* L.) (Winston et al. 2014). Neither species of *Bangasternus* are known to occur in Canada (Bousquet et al. 2013), nor have they been approved for biocontrol releases in Canada (Winston et al. 2014). However, there are now several dozen observations of *Bangasternus* spp. in the southern interior of British Columbia, Canada reported via iNaturalist.org, suggesting that one or both of these species have moved northward from the USA. Here, we surveyed the southern interior of British Columbia for *Bangasternus* species. Using a combination of insect taxonomy – specifically diagnostic male genitalic morphology, molecular diagnostics - cytochrome *c* oxidase subunit I (COI) DNA barcoding, and host-plant association, we provide evidence that the broad-nosed knapweed seed head weevil, *Bangasternus fausti* is now established in southern British Columbia, Canada. We discuss its potential role in providing suppression of diffuse knapweed and its possible interactions with the existing seed head feeding biocontrol agent community.



**Specificity (Redemption?) of a potential agent, falsely accused of being polyphagous, to control *Jatropha gossypifolia* (Euphorbiaceae) in Australia**

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*Jatropha gossypifolia* (Euphorbiaceae), a shrub native to tropical America, is a major and expanding weed in dry tropical Australia. During surveys in Paraguay and Bolivia a leaf-feeding cecidomyiid was discovered that was identified as the crop pest *Prodiplosis longifila*, a bud midge that is an important pest of Solanaceae, asparagus, citrus, and other crops in the Neotropic. However, in Paraguay there were no reports of *P. longifila* damage on any crop species, even when growing near *Jatropha* plants severely damaged by the midge. Suspecting the existence of a cryptic species, or other different, specialised clade, the first approach was to run host specificity tests on 13 recorded *P. longifila* hosts. We obtained larval feeding damage and adult emergence only in *J. gossypifolia* replicates. Our recent (2022) morphological and molecular studies revealed that the midge was, in fact, a new species, named *P. hirsuta*. Based on the host specificity tests, morphological and molecular results, a new set of more typical no-choice trials were performed on six different species within the Euphorbiaceae family (including cassava). Only two species in the genus *Jatropha* (other than *J. gossypifolia*) were attacked. As there are no native *Jatropha* species in Australia, *P. hirsuta* may be considered a promising candidate. The results obtained in these experiments highlight the importance of confirming the identity and host range of potential agents (and target species), to ensure suitable agents are not discarded unnecessarily.

***Lysathia* sp. nov. and *Lysathia flavipes*: Molecular and morphological unraveling of biocontrol agents for *Myriophyllum aquaticum* and *Ludwigia grandiflora* subsp. *hexapetala***

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Water primrose (*Ludwigia grandiflora* ssp. *hexapetala*; Onagraceae) and parrot's feather (*Myriophyllum aquaticum*; Haloragaceae) are aquatic plants native from South America and coexist in their natural environment growing in shallow waters of the centre and north of Argentina. Both species are aggressive weeds that have invaded several countries. The flea beetle *Lysathia flavipes* (Coleoptera, Chrysomelidae) was found in the 80's causing heavy damage to water primrose and moderate damage to parrot's feather. In the 90's specimens of *Lysathia* sp. were imported from Rio de Janeiro province, Brazil, to South Africa where it was studied and released as a biocontrol agent for parrot's feather. These specimens were identified as an undescribed species and the lack of specialists on this complex Neotropical genus has hindered its description to date. On the other hand, extensive surveys on *Ludwigia* spp. in Argentina revealed frequent and abundant damage produced by *L. flavipes*. However, preliminary morphological studies showed variations in body coloration and female genitalia suggesting the presence of more than one species. To clarify this complex taxonomic situation with both species and to confirm if *L. flavipes* utilises both hosts, morphological and molecular studies were carried out. DNA was extracted from 48 individuals of *L. flavipes* (Argentina and Uruguay), *L. sp.* (South Africa) and *L. ludoviciana* (United States of America). The COI (~772-bp) region was amplified and sequenced in both directions from each sample with primers C1J2195 and TL2N3014. A phylogenetic tree (maximum likelihood and Bayesian methods) and haplotype network were obtained. Results showed that the *Lysathia* sp. utilised in South Africa, clearly separates from the samples collected in Argentina, except for two specimens of the north-east of Argentina, representing perhaps the southernmost distribution of the *Lysathia* sp. The remaining specimens, *L. flavipes sensu lato*, showed variability but not related to the host plants. There are three specimens collected as *L. flavipes* that are probably different/new species. *Lysathia flavipes s. lat.*, also separates from specimens of *L. ludoviciana* except for three samples that were more similar to the latter than to *L. flavipes*. To conclude, after morphological and molecular research, the *Lysathia* sp. utilised as biocontrol agent against *M. aquaticum* is a *species nova* that will soon be published with a full description and will be named after Dr. Carina Cilliers, from South Africa. After DNA results, specimens of *L. flavipes s. lat.* are also being described morphologically to re-describe the species.

**The *Hypogeococcus pungens* species complex: an update on the current status of its members in their native and non-native ranges**

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The *Harrisia* cactus mealybug, *Hypogeococcus pungens* Granara de Willink, 1981 (Hemiptera: Pseudococcidae), was considered to be a single species native to South America that fed on several plant species in the Cactaceae, Amaranthaceae, and Portulacaceae. However, recent studies indicate that it is actually a species complex that includes the true *H. pungens*. This complex is composed of at least five putative species, each one specialized on different host plants. One of the species in the complex is an undescribed *Hypogeococcus* sp. that was introduced in the 1970s in Australia, and later South Africa, for biological control of weedy cacti. The biological control agent released was collected from *Harrisia* spp. and *Cleistocactus baumannii* (Cactaceae) in Formosa Province, Argentina, and identified as *Hypogeococcus festerianus* Lizer & Trelles, 1942. This is a valid species that is restricted to Cactaceae, but is not part of the *H. pungens* complex. However, some years later, when M. C. Granara de Willink described *H. pungens* from material collected on Amaranthaceae from Argentina, the species introduced to Australia and South Africa reidentified, again incorrectly, as *H. pungens*. Two other South American *Hypogeococcus* species have spread to North America and the Caribbean, and one of them is a severe pest of native cacti in Puerto Rico. Biology, ecological interactions (host plant-mealybug/mealybug-natural enemy), and the current status of the members of the *H. pungens* species complex are discussed. Correct identification of the target species is key in classical biological control programs in order to identify natural enemies specific to the weed/pest in its native range. Furthermore, in new association biological control programs, it enables the selection of biological control agents that are closely related to the invasive alien plant or the insect pest.

**Rapid and inexpensive MALDI-TOF MS comparison for characterizing different populations of biological control agents and target weeds, and optimizing their matching**

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MALDI-TOF MS is a powerful and versatile technique that employs the MALDI soft ionization process to prepare large proteins intact in the gas phase that carry predominantly a single positive charge. When such charged proteins in the gas phase are accelerated by means of an electrical field along a tube held at high vacuum, their times-of-flight are proportional to the square root of their mass-over-charge ratios. Using this simple relationship, a mass spectrum can readily be generated for the protein components in a particular biological sample. Mass spectra of a subset of the expressed proteome (most commonly the highly-expressed acid-soluble proteins, including many ribosomal proteins) are often employed for the characterization and identification of biological samples. Much of the development of MALDI-TOF MS for the identification and/or characterization of biological samples has been shaped by human clinical microbiology, and a key driver has been the diagnosis of bacterial and yeast infections. In order to extend the range of this technique beyond microbiology, we have developed a highly-simplified and inexpensive method for MALDI-TOF MS sample preparation. This method lyses cells by immersion (or maceration, for plant or insect material) in aqueous acetonitrile containing trifluoroacetic acid (TFA) to selectively extract acid-soluble proteins, peptides, and other similarly-soluble basic cellular components. In this method, lysis and extraction are carried out in the presence of near-saturated and inexpensive-grade MALDI matrix. The resulting matrix-saturated lysate, which contains extracted acid-solubilized components, can then simply be dried down directly onto the MALDI-TOF MS sample plate and analyzed. Primarily to facilitate field work in Developing Countries, we have also developed methods for inexpensive and robust storage and shipping of plant (and/or insect) samples for MALDI-TOF MS analysis using immobilization by thorough drying on filter paper. In this presentation, we will show examples of rapid and inexpensive spectral comparison between different populations of target weeds and we will discuss optimizing the matching between control agents and their targets. In this context, principal-component analysis and closest-relatedness diagrams derived from spectral-comparison data can discriminate between the closely-related *Impatiens* spp. *Impatiens noli-tangere*, *Impatiens parviflora*, *Impatiens scabrida*, *Impatiens balsamina*, and two regional biotypes of the invasive weed *Impatiens glandulifera*.; and comparisons between blind-test spectra and reference-sample spectra are able to discriminate between four UK regional biotypes of *I. glandulifera* that differ in their susceptibility to the biological control agent Himalayan balsam rust (*Puccinia komarovii* var. *glanduliferae*) using both mature leaf material and seeds.

Moderator: Guillermo Cabrera Walsh (FuEDEI, AR)

Oral presentation

**National strategy on invasive alien species and biological control in Argentina, prospects and challenges**

**Keynote speaker:** Zalba Sergio M.<sup>1</sup>

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The Ministry of Environment and Sustainable Development of Argentina approved the first National Strategy on Invasive Alien Species (IAS) in May 2022, after ten years of extensive work involving hundreds of people from public and private institutions around the country. The document includes an updated diagnosis of the problem and guidelines for the prevention, containment, early detection, control and eradication of IAS. Objective 5 of the strategy highlights the need for innovative proposals aimed at developing, testing and adjusting management tools, including biological control. In turn; strategic axis 2 proposes “promoting initiatives for the use of biological control, especially in cases of widely distributed IAS, previously evaluating any associated impacts”. To assess the current situation with respect to these objectives, I reviewed research projects focused on the subject at the national level. For a total of 4,916 researchers in the areas of Agricultural; Environmental; Biology; and Earth, Water and Atmospheric Sciences, 79 included the word "biological control" in the description of their specialty or in the title of their research topic, 84 indicated they work with biological invasions, but only three combined both fields. Additionally, I explored the perception of people related to research and management of the environment and biological diversity, about the importance and limitations of BC for IAS management in natural areas (NA). A survey was sent to 1,730 persons, including all the participants of the national strategy. I received 133 responses, 22 from direct decision-makers in the public administration, 34 from technical advisors in those institutions, 19 from NGOs, 68 from academics, 22 from park rangers, and 22 from education and communication professionals. While 95 responded they would recommend/authorize/enforce BC of IAS in NA, including nature reserves, as long as it was an appropriate tool for the case, 27 said they would do so only "as a last option" and 11 would not do so at all, with a slightly higher rejection from the academic sector compared to those working in the public administration. Forty-two percent of respondents overestimated the number of IAS originally introduced for BC, however when asked about specific cases, only five species (less than 0.7% of the national list of IAS) were mentioned. These results highlight the need to promote research/management projects aimed at the control of IAS in AN, disseminate the potential and advantages of BC for the management of IAS, and publicize the procedures and safety of modern BC.

### **Biological control of weeds in Argentina. Present status and hope for the future**

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Argentina hosted pioneering classical weed biological control projects that were carried out by state institutions between the 1970s and 1990s, at a time when the practice was quite young in South America. A few releases of exotic agents against thistles and skeleton weed were carried out during those years, resulting in the successful establishment of most of them, but with little effectiveness in the control of the population densities of their target weeds, and poor post liberation studies. Despite the early development of these initiatives, the discipline did not prosper. Meager financial support and a prevailing tendency towards the use of chemical herbicides as the main tool in weed control, soon ended the initial interest in these more environmentally friendly enterprises. Notwithstanding, Argentina continued, and continues, to provide biological control agents for the control of weeds in many other parts of the world.

Although public concern about invasives and chemical-dependent agricultural practices has increased over time, it has not led to a greater acceptance of classical biological control of weeds. There appears to be a general unawareness of the potential of this practice amongst policy makers, and the public in general, whose perception is more oriented towards its potential risks than to the benefits derived from it. No regulations regarding declaring weeds as targets for classical biological control are in force in Argentina. Despite this, a biological control project against invasive *Tamarix* spp., proposed and carried forth by the authors' laboratories was recently funded by the Argentine Ministry of Science and Technology (MinCyT), indicating that the situation may be changing, and there could be some hope for the relaunch of this practice.

In addition, the possibility of exploiting resident fungal pathogens as bioherbicides, or natural enemies as part of integrated weed management programmes, is currently being explored for several significant weeds (*Sorghum halepense*; *Convolvulus arvensis*, *Amaranthus* spp., *Conyza bonariensis*, *Dipsacus fullonum*). These projects are (or were recently) funded by different public institutions such as the National Agricultural Technology Institute (INTA), Universidad Nacional del Sur (UNS), and the MinCyT. Manipulating natural enemies that are already present in the environment is generally perceived as less risky than importing exotic ones, and may help increase the acceptance of this control strategy in the near future.

Prospects are brighter today than a few years ago. We still need to work hard on publicising the benefits of weed biocontrol to gain more support.

**An overview of the Brazilian National Strategy for invasive exotic species -  
a proposal under construction**

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Invasive exotic species (IES) are species that are found outside their natural distribution area and their introduction/dispersion threatens local biodiversity. The introduction, establishment and reproduction of an IES characterizes what is known as a biological invasion, which will lead to structural changes in the ecology and balance of the invaded environment. Due to the importance of the topic, the Brazilian Ministry of the Environment - MMA, through the CONABIO Resolution of May 07, 2018, approved the National Strategy for Invasive Exotic Species, a document that provides for the planning of actions for a period of 12 years (2018 - 2030) that will guide actions to prevent the introduction, manage or control established populations and eradicate new IES infestations in Brazil. The strategy foresees an interdisciplinary approach, involving the various sectors that are affected by biological invasions, including federal, state and municipal governments, the private sector and civil society, and annual monitoring meetings. The national strategy implementation plan has 6 main components, with specific objectives and actions: C1 – Legislation, intersectoral articulation and international cooperation. C2 – Prevention, early detection and rapid response. C3 – Eradication, control and mitigation of impacts. C4 – Scientific research. C5 – Technical training. and C6 – Communication. Within the scope of objective 2.2 “developing and implementing criteria for analyzing the risk of biological invasion”, action 2.2.1 was implemented, through which risk assessment procedures and protocols were developed to support the registration of non-classical biological control agents. Furthermore, the biological control of weeds is action 3.1.13 of component 3, with the objective of “developing, implementing and monitoring measures for eradication, control and mitigation of impacts of invasive alien species and biological invasions”. Three groups were set up with members of the Ministry of the Environment, Ibama, Embrapa and Universities to develop this action, which aims to formulate a base text on biological control of weeds to be shared with the other members of the national strategy, clarifying the fundamentals and the importance of this technique to achieve the proposed objectives of controlling weeds that cause biological invasion in Brazil. This group also discusses the application of protocols for the introduction and release of natural enemies in Brazil and specific legislation that can ensure the application of biological control of weeds. The main components, their specific objectives and all the actions related to the biological control of weeds will be presented.

**Perspectives and challenges for implementing biological control in weed management in subtropical wetlands of Argentina**

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Urban wetlands of Argentina are particularly relevant because they provide water storage areas during flood events, reducing the area that experience catastrophic impacts. However, due to their morphometry and function, they are particularly vulnerable to the proliferation of aquatic weeds, which negatively impact biodiversity and ecosystem services. Although there are previous experiences in Argentina where classical biological control was applied in water bodies of temperate and arid regions, there are none in subtropical wetlands. This work aims to assess the most adequate target weeds and the agents better suited for a biocontrol initiative in subtropical urban wetlands. We also analyse the relevance of the structure and performance of these wetlands a weed biocontrol initiative. Preliminary monitoring in 12 urban wetlands allowed us to identify three target aquatic weeds showing potential for management with biological control: *Pistia stratiotes*, *Pontederia crassipes*, and *Victoria cruziana*. Current management of these weeds is restricted to mechanical methods, but it is expensive and does not provide a desirable level of control. In terms of abundance and impact, the most viable biological control agents could be *Neohydronomus affinis* and *Lepidolphax pistiae* on *P. stratiotes*, and *Neochetina bruchi*, *Neochetina eichhorniae* and *Cornops aquaticum* in *P. crassipes*. There is no previous experience in implementing biological control in *V. cruziana*, hence new biocontrol candidates should be evaluated. Almost all wetlands have highly variable areas and are fairly shallow (less than 4m deep), with a low frequency of frost during the year. They tend to have insect assemblages dominated by herbivores, with few detritivores, high plant productivity, weed invasion alternating with algal bloom episodes, polymictic circulation pattern of water, and eutrophic conditions. A multidisciplinary approach integrating examination of all these characteristics could improve the implementation of biological control and wetland recovery in the subtropics. Our results also suggest that urban wetlands can be interesting systems to implement and promote weed biological control in Argentina due to their high visibility and use, their key role in mitigating flooding in the cities and the rising trend in the urban planning for preserving and managing these systems in a more sustainable way. More taxonomic and ecological studies on biocontrol agents are recommended, integrating aspects related to water quality. The engagement of policymakers, citizens and stakeholders interested in preserving wetlands could substantially contribute to implementing effective biological control of aquatic weeds in subtropical urban wetlands of Argentina.



**Towards inaugurating classical biological weed control in Brazil: the rust *Maravalia cryptostegiae* vs. *Cryptostegia madagascariensis* s.l., the hope and the challenges**

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Rubber vine (*Cryptostegia madagascariensis* s.l.; Apocynaceae) – known as unha-do-diabo (devil's claw) in Brazil – has been the target of a classical biocontrol (CBC) programme since 2018, funded jointly by the Government of Ceará, the Syndicate of Carnaúba Wax Refiners of Ceará and the American multinational S.C. Johnson & Son. This invasive Madagascan vine is threatening the unique, semi-arid Caatinga ecosystem of Ceará and most of north-east Brazil; including natural stands of the iconic Carnaúba palm (*Copernicia prunifera*), source of the commercially-important carnauba wax. The Brazilian project is piggy-backing on the success of the Australian CBC programme controlling the congeneric Madagascan species *Cryptostegia grandiflora* with the rust fungus *Maravalia cryptostegiae* in northern Queensland. Associated with both *Cryptostegia* species in their native ranges, the rust was prioritized for assessment as a CBC agent for rubber vine in Brazil. Field surveys, covering the native range of *C. madagascariensis*, sourced numerous rust strains. Including some already held in the liquid nitrogen culture collection at CABI, a total of 19 rust strains were screened for their compatibility with and virulence to populations of the plant biotype invasive in Brazil. These studies were complemented by molecular analyses of *C. madagascariensis* populations from north-east Brazil, Kenya and Madagascar, in order to: verify the species identity; establish the origin of the Brazilian introduction; and assess the genetic variability of the Brazilian populations. The host-specificity of the best-matched rust strain has been assessed against a test-plant list representative of Brazilian-native apocynaceous genera and species, as well as locally-important non-target species from other plant genera (total of 48 non-target species). In parallel, field studies were conducted at three sites in Ceará over three years to establish the size and density of the *C. madagascariensis* populations present and collate data on plant performance, such as flowering, fruiting and seed set. Thus, future impacts of the rust can be assessed against an established baseline, were it to be approved for release as a CBC agent. In order to pave the regulatory way for introduction of the rust, simultaneous declarations of an environmental and phytosanitary emergency, due to the rubber vine invasions, are currently being pursued in the State of Ceará. This presentation gives an overview of the research completed, thus far, and discusses the suitability of the rust for introduction into Brazil as what would be the first exotic weed biocontrol agent implemented in this country.

**Economic loss of ecosystem services caused by two invasive macrophytes of wetland ecosystems in Argentina**

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Invasive alien plants are responsible not only for the loss of biodiversity, but also for causing direct and indirect damage to productive activities. *Hedychium coronarium* J. Koenig (Zingiberales: Zingiberaceae) and *Iris pseudacorus* L. (Iridaceae) are currently invading different biomes of Argentina. This work estimates the economic loss in ecosystem services due to *H. coronarium* and *I. pseudacorus* in Argentina, using benefit transfer methodology for the current distribution of these species. Potential distribution of both species was estimated performing a correlative ecological niche model using a maximum entropy method. Then, we estimated the area currently invaded by *H. coronarium* and *I. pseudacorus* in Argentina using the percentage of occupation of each species reported for Iguazú National Park and Punta Lara Natural Reserve respectively. Using a benefit transfer methodology and value coefficients weighted according to expert opinions, we determine the impact degree for different ecosystem services (freshwater supply, regulation of water flows, nutrient cycling, pollination, biological control, habitat refugia, erosion prevention, nursery service, genetic diversity, recreation and cognitive information) in four biomes (coastal systems, inland wetlands, freshwater lakes and rivers, and tropical forest) where these invasive plants are present. The impact of both species on ecosystem services was considered “negligible”, when impact degree was lower than 20%; “low” when up to 30%; and “moderate” when it reached 50%. Our results indicate a current estimated loss of ecosystem services value that ranges from 44.3 to 217 million USD/year for *H. coronarium*, and 530.9 to 4,037.97 million USD/year for *I. pseudacorus* using an ecological niche suitability above 0.8 and 0.6, respectively. Our results shed light on the magnitude of the potential economic impact of invasive plants on ecosystem services. We recommend that economic losses in ecosystem service values due to invasive plants be estimated more often and incorporated into the decision-making process to prioritize weed management strategies, including biological weed control.

**Exploring biological control of field bindweed, *Convolvulus arvensis*  
(Convolvulaceae), in Argentina: progress so far**

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Field bindweed, *Convolvulus arvensis* L., is a persistent, perennial invasive species in Argentina. Despite extensive research on its control, it continues to be one of the most serious perennial weed infesting farmland worldwide. *C. arvensis* occurs across a wide range of settings such as agricultural fields, pastures, lawns, roadsides, and other disturbed areas. Its ability to invade and persist in a variety of habitats due to its long-lived seeds, adventitious buds on its extensive root system, and carbohydrate reserves in its roots, make management more challenging. Mechanical disturbance can exacerbate the problem by spreading vegetative propagules and repeated use of herbicides can lead to the appearance of resistant biotypes. In 2019, a recently formed research team began a project to explore the feasibility of incorporating conservative biological control to an integrated management approach, focusing on organisms associated with the target weed in its adventive range. Surveys were conducted to search for phytophagous insects and fungal pathogens on field bindweed populations in the southwest of the province of Buenos Aires. Three beetle species of Chrysomelidae: Cassidinae and one of Lepidoptera were found feeding on the foliage at different sites. The occurrence of *Botanochara angulata* and the lepidopteran was negligible, therefore only the other two species, *Chelymorpha varians* and *Paraselenis* cf. *saltaensis*, were selected considering the high specificity to host plants, extensive feeding, and prolonged reproductive periods characteristic of many Cassidinae. Very recently, in January 2023, a fourth species of Cassidinae was collected. As a first step, the biology of these insects is being studied. Rearing in the lab has been achieved with variable degrees of success. Three fungal pathogens were also found causing conspicuous damage to foliage at several sites: a powdery mildew and the leaf spot fungi *Septoria convolvuli* and *Stagonospora calystegiae*. The last two were selected for further trials: pathogenicity was confirmed for both and specificity testing is partially completed with inconclusive results. Field observations and results of artificial inoculation tests indicate that, of the two pathogens, *St. calystegiae* is capable of inflicting the greatest level of damage to individual plants. Another fungus, *Colletotrichum* sp., was recently isolated from other leaf spots. Its identity and pathogenicity is under study. The diversity of natural enemies found to date is interesting and justifies further research into their suitability as biological control agents.

***Tamarix ramosissima* wood canker and branch death in Argentina: assessment of the role of associated fungi and possible implications for classical biocontrol**

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Saltcedars (*Tamarix* spp.), are among the invasive alien shrubs and trees present in Argentina causing major ecological and economic problems. As such, they have been selected as one of the pilot programmes of the National Strategy on Invasive Alien Species. A biological control (BC) project against *Tamarix* spp. was initiated in 2017, funded by the Ministry of Science, Technology and Productive Innovation with the main objective of assessing the feasibility of introducing the defoliating and stem damaging beetle *Diorhabda sublineata*, a BC agent successfully released against *Tamarix* spp. in the USA. It is common practice for these projects, to record the organisms associated with the target in its adventive range, to identify any that may either interfere with the BC agents intended for release, or that may themselves have potential as such. To this end, surveys were conducted to search for fungal pathogens in *Tamarix* populations in Argentina. *T. ramosissima* trees showing discoloration and death of branches were found at two sites located around 700 km apart. Several fungi were identified associated with these symptoms, among which *Cytospora unilocularis* and *Neomicrophaeropsis tamaricicola*, appeared as the most likely causal agents. Both were isolated on artificial culture media to provide inoculum for pathogenicity tests. Branches of six-month-old *T. ramosissima* potted plants were inoculated with *C. unilocularis* by either placing a drop of a conidial suspension in water into *ad-hoc* produced wounds or by spraying undamaged ones. Inoculation with *N. tamaricicola* was performed only on wounded branches. Control plants were treated with water alone. Wounded plants inoculated with *C. unilocularis* developed cankers, branch discoloration and death of branches after two months. Pycnidia in association with the symptoms were also observed on one of the plants. The fungus was re-isolated from the cankers allowing for the completion of Koch's postulates. To our knowledge, this is the first report of *C. unilocularis* causing disease on *T. ramosissima*. In this ongoing experiment, incipient symptoms have developed so far on the plants inoculated with *N. tamaricicola*. Both fungi have recently been described from the plant's native range, suggesting they must have arrived in Argentina together with their host. Because *C. unilocularis* seems to infect only through wounds, the effect of this pathogen would be enhanced should *D. sublineata* eventually be introduced, thus contributing to a better control of populations where the pathogen is already present.

**New reports of potential biological control agents for *Convolvulus arvensis* (Convolvulaceae) in Argentina: *Paraselenis* cf. *saltaensis* sp. nov. and *Poecilaspidella proxima* (Coleoptera: Chrysomelidae)**

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Surveys were conducted to search for insects attacking field bindweed, *Convolvulus arvensis* L. (Convolvulaceae), populations in the southwest of the province of Buenos Aires during the period 2019-2022. Three species of Coleoptera: Chrysomelidae: Cassidinae were found at different sites in the Bahía Blanca area: *Botanochara angulata*, *Chelymorpha varians*, and *Paraselenis* cf. *saltaensis* sp. nov. In January 2023, a fourth species of Cassidinae, *Poecilaspidella proxima*, was discovered at another location near Carmen de Patagones. Characteristics of Cassidinae beetles such as high specificity to host plants, extensive feeding, and prolonged reproductive periods make them good candidates as biological control agents. There are no studies of *Paraselenis* cf. *saltaensis* sp. nov., and *Poecilaspidella proxima* has not been cited for Argentina to date. Both native phytophagous species have been found feeding and thriving on field bindweed, a cosmopolitan introduced weed known for its potential to reduce crop yields. *P.* cf. *saltaensis* sp. nov. has been found on the same host species and also on another introduced species, *Convolvulus sepium* L. At this time, as a first step the biology of these species is being studied. Using *C. arvensis* as the host plant, *C. varians* was successfully reared in the lab for several generations. *P.* cf. *saltaensis* sp. nov. has been kept in the lab and multiple viable ovipositions have been recovered from the colony on field bindweed. However, the F1 adults did not lay eggs during 2022 in the lab. About 15 days after being collected, the adults of *P. proxima* from the field have been laying viable eggs and larvae are thriving. The identification of *P.* cf. *saltaensis* sp. nov. is ongoing as is the study of the biology of both species. Damage caused to *C. arvensis* and host specificity testing will be carried out once the biology of these species in the southwest province of Buenos Aires area is understood.

### **Yellow flag iris invasion in South America: is biological control needed?**

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*Iris pseudacorus* L. is an invasive alien species, introduced from Europe to many other countries in the world, being classified as highly invasive in many of them. This species not only causes significant ecological damage in natural environments, diminishing native flora and structurally modifying ecosystems, but can also invade urban and rural areas, generating large economic losses. Currently, there is an international alliance between countries from the native and introduced range aimed at testing the feasibility of applying classical biological control against this weed with the candidate *Aphthona nonstriata*, native to the Old World. Yellow flag iris has been cataloged as invasive with mandatory control in Argentina and Uruguay, and recorded as naturalized in Chile. The first record is from 92 years ago in Argentina, and recent studies showed that in this country it is present throughout a diversity of habitats, ecoregions, water conditions, and latitudes. Furthermore, studies of potential distribution predict that the invasion can continue to expand in the territory. In Uruguay, numerous coastal sites are invaded, including protected areas, and there are currently plans to control and research this plant. Despite the importance of the invasion, no bio-controllers or other management strategies have yet been assessed. We assessed the extent of the problem using citizen science, field exploration, literature reviews, and interviews with park rangers. Citizen science revealed a much higher level of invasion than previously recorded in Argentina, indicating the need to apply control. Different methods of mechanical removal and substitution are being applied in protected and priority areas of Argentina and Uruguay, and although they resulted effective for small infestations, they demand periodic effort, and are not feasible for large invasions. Although herbivorous species have been detected feeding on the vegetative and reproductive parts of the plant, our results suggest an absence of species with biocontrol potential for *I. pseudacorus* in Argentina. This information emphasizes the need to continue testing the feasibility of applying classical biocontrol with candidates from the native range of the plant that demonstrate specificity for *I. pseudacorus*

**Double impact of a weed biocontrol agent in Hawaii: *Secusio extensa* (Lepidoptera: Erebidae) releases for fireweed, *Senecio madagascariensis*, outspreads naturally to Cape ivy, *Delairea odorata***

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Fireweed and Cape ivy are invasive noxious weed in Hawaii that are native to South Africa. Fireweed, which is toxic to livestock, invaded the islands of Maui and Hawaii in the 1980s, and now infests an estimated 850,000 acres statewide. Cape ivy, a perennial vine that overgrows native Hawaiian forests, is also found in most coastal watersheds in Australia, California, Chile, New Zealand, and Oregon, where it has become a significant threat to riparian habitats. It smothers native vegetation, and its leaves contain toxins for birds and other wildlife. *Secusio extensa* (Butler) is a Madagascan tiger moth investigated in Hawaii for biological control of fireweed. After extensive host range studies that included 88 endemic and naturalized species (71 of which were Asteraceae), it was found to reproduce on six different species of the tribe Senecioneae that are weedy. The Senecioneae contain no native species in Hawaii, and its release was approved in 2013 for biocontrol of fireweed. Fortuitous non-target impact on Cape ivy was predicted because *S. extensa* developed equally well on this plant under laboratory conditions. Shortly after its release, *S. extensa* was found to spread naturally onto Cape ivy, causing a dramatic buildup of the moth population that spread over to fireweed and Cape ivy sites. A field trial at Puu Huluhulu native tree sanctuary showed a dramatic reduction of vines in less than a year after the release of 14925 adults and larvae during March 2015. Photos before and after the release were documented to show its impact. Only plants in the Senecioneae have been attacked, and the insect is spreading naturally to other islands, including Kahoolawe and Oahu. Predators and egg parasitoids have not been recorded to date, but recently the larval gregarious parasitoid, *Lespesia archippivora* (Riley) (Diptera: Tachinidae), was reported from Maui Island. Fireweed is a declared noxious weed in Argentina, Brazil, Colombia, Uruguay, and Venezuela. The prospect of biocontrol of fireweed using *S. extensa* is excellent. This update can be useful for future introductions of *S. extensa* to areas with fireweed or Cape ivy problems in South America

### **Classical biological control on *Tamarix* spp in Argentina: a plan for its implementation**

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*Tamarix* spp. (Tamaricaceae) are native to Asia and the Mediterranean region. They are used as ornamental plants, windbreaks, shade trees, dune stabilization, and erosion control. In USA, Mexico, Australia, South Africa, and Argentina they have become invasive weeds. They are capable of invading riparian and wetlands systems in arid and semi-arid inland and coastal areas. These species negatively impact on water courses and wetlands by consuming abundant water and salinizing the upper soil layers. In Argentina, there are four species of *Tamarix*: *T. ramosissima* Ledeb., *T. chinensis* Lour., *T. gallica* L., and *T. parviflora* DC. *T. ramosissima* is the most frequent and invasive species with an active propagation process (spontaneous recruitment and dispersal). *Tamarix* spp. are one of the seven pilot projects of the National Strategy for Invasive Alien Species in Argentina, which seeks to generate sustainable and consensual management strategies. However, the management of species that have been introduced intentionally and are valued can generate controversy and conflicts of interest. Therefore, a comprehensive analysis of ecological and social aspects is necessary to reach effective and lasting solutions. Due to its taxonomic isolation (no Tamaricaceae native to America), wide distribution, and invasiveness in natural areas, *Tamarix* spp. constitutes an optimal target for classical biological control in Argentina. *Diorhabda* spp. (Coleoptera: Chrysomelidae) is the first choice as biocontrol agent given its success in the USA. However, another specific insect of *Tamarix* spp., *Opsius stactogalus* (Hemiptera: Cicadellidae), is already present in Argentina, presumably arrived originally with the plant, but it does not produce significant damage on its host plant. Yet, *O. stactogalus* might be used in augmentative biocontrol strategies together with a classical biocontrol approach using *Diorhabda* spp.

Finally, we seek to evaluate the interaction of *T. ramosissima* with native and exotic arthropods, and study the socio-ecological perception of the weed in order to assess the support and challenges that the different control strategies for this weed could have. The ultimate goal is to lay the foundations to incorporate biological control as part of an integrated *Tamarix* management program to be applied in the country.



Moderator: Iris Stiers (VUB, BE)

Oral presentation

**Harnessing evolutionary principles to improve weed and insect biological control**

**Keynote speaker:** Szücs Marianna<sup>1</sup>.

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Biological control programs provide unique opportunities for rapid evolutionary processes to play out as agents are introduced into new habitats where they experience novel selection pressures. However, during the collection, rearing and quarantine stages agents will experience fluctuating and/or low population sizes, which can lead to reduced genetic diversity, and can compromise their evolutionary potential. We are just beginning to understand how rapid evolution may impact establishment success, adaptive ability, population growth and ultimately the effectiveness of biocontrol agents. In my lab we approach invasive species problems and new biocontrol releases from an evolutionary perspective. I will present examples of this for two weed and one insect biocontrol systems. For biological control of swallow-wort vines (*Vincetoxicum* spp.), we are releasing a defoliating moth, *Hypena opulenta*. This moth spent over a decade in quarantine before our laboratory colony was founded with 19 females in 2018. To alleviate potential inbreeding and increase chances of adaptation to the local climate we crossed our *H. opulenta* colony with individuals collected from a field-established population in Canada. Experiments indicate that the outbred colony created by these crosses has higher fitness than either the pure Canadian population or our laboratory colony. We are designing our field releases to test how the genetic background of the different *H. opulenta* populations may impact establishment success. For biological control of knotweeds (*Fallopia* spp.), we are facing a unique situation in Michigan. Of the two host races of the psyllid biocontrol agent, *Aphalara itadori*, the Kyushu (southern) host race that is adapted to develop on Bohemian and Japanese knotweeds appears to be a poor climate match for lower Michigan where these knotweed species are prevalent. The Hokkaido (northern) strain of *A. itadori* that should be a better climate match, on the other hand, has poor developmental success on the target knotweeds. We are exploring how hybridization between the two host races of *A. itadori* alters preference and performance on different knotweed species and whether hybrids may be best suited for releases in lower Michigan. For the invasive spotted wing drosophila, we investigated whether we could use experimental evolution to increase the virulence of native parasitoids on this novel host. We found that within three generations of selection we could increase the developmental success of two pupal parasitoids by 88% and 259%. These studies demonstrate different ways to harness evolutionary principles to improve biocontrol outcomes.

**Context-specificity in establishment, disease severity and impacts of a foliar pathogen on its host weed wandering trad, *Tradescantia fluminensis*, across broad climate and spatial gradients**

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It is well demonstrated that biological control agents (BCAs) can drive reductions in target weed abundance. However, the likelihood of BCA establishing, spreading, and impacting on host weed populations can vary significantly across a weed's introduced range, often in relation to broad ecological and climatic gradients. Wandering trad (*Tradescantia fluminensis*) is an invasive herbaceous weed that smothers groundcover vegetation in forest ecosystems across eastern Australia and New Zealand. Its invasion is strongly associated with reduced native vegetation diversity and altered community compositions. In eastern Australia, we released an approved BCA, the leaf-smut fungus, *Kordyana brasiliensis*, in 2020 on dense wandering trad infestations at 50 fixed monitoring plots (dimensions: 2 m × 5 m) located at 14 sites over a broad temperate-subtropical forest gradient (i.e., across a ~ 1,000 km span of latitude). At each plot, we quantified the abundance of wandering trad prior to BCA release, to provide a benchmark against which the development and impact of the agent could be evaluated over time. Microclimate (humidity, temperature) data were recorded in each plot using Hygrochron iButtons®, and annual rainfall and regional temperate data for each site were obtained from the Australian climate SILO model. Disease incidence (% weed stems infected), severity (% leaf surface area infected) and wandering trad abundance (% foliage cover) were recorded at 6- and 18-months intervals following the release. After 6-months, disease incidence increased significantly from ~ 60 % to 90 % stems over a daily maximum temperature range of 20 °C to 33 °C, and disease severity doubled from ~10 % to 20 % over that same temperature range. Disease severity (but not incidence) also increased exponentially with increasing relative humidity. Over that same period, wandering trad cover declined on average across all plots by ~10 %, but the magnitude of the decline did not vary significantly across the latitudinal gradient. In contrast, at 18-months after release, the magnitude of decline in wandering trad cover was greatest in warm, humid climates closest to the equator (~50-60 %), with only moderate declines under relatively cool, dry conditions in the southern region (~20 %). These results indicate that infection and impacts of the fungus on its host weed are slower during early stages of establishment under cool, dry conditions, and promoted under warm-hot and moist conditions consistent with a sub-tropical climate. Our data highlight the importance of climate context in predicting BCA establishment and impacts at broad spatial scales.

**Identifying the most effective cochineal species to control *Opuntia elata* and *Opuntia megapotamica* in South Africa**

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*Opuntia elata* and *Opuntia megapotamica*, two South American Cactaceae, are problematic invasive alien plants in South Africa. As is the case for many invasive cacti, biological control is the most effective management solution. Biological control of *Opuntia* species usually relies on cochineal insects as biological control agents. Cochineal species and lineages have variable impacts on different species of *Opuntia*, so although several *Opuntia* species may be suitable hosts for a particular lineage of cochineal, only one or two *Opuntia* species will be damaged sufficiently to warrant the use of the specific cochineal for biological control. It is therefore essential that the impacts of all the available cochineal species and lineages for a new target weed are compared to select the most effective agent. There are six cochineal species/lineages that are currently used for the control of *Opuntia* species and close relatives in South Africa. Both *O. elata* and *O. megapotamica* fall within the host ranges of these cochineals, but none are currently used for biological control for either target species. In addition, a new lineage of cochineal has been imported from the native distribution of the weed (Argentina) and is currently under evaluation in quarantine. This study compared the impacts of the six South African species/lineages of cochineals on both target plants. *Opuntia elata* was susceptible to the cochineal *Dactylopius austrinus*, which is used for the control of *O. aurantiaca* in South Africa. *Opuntia megapotamica* was susceptible to *D. opuntiae*, used for the control of *O. ficus-indica* and *O. stricta*, as well as *D. austrinus*. The new cochineal collected in the native distribution of both target plants is however more damaging than any of the cochineals already used for control of other *Opuntia* species in South Africa. The new cochineal will therefore be subjected to host specificity testing and if suitably specific and damaging will be released. This study suggests that cochineals from close relatives (old associations) are likely to be more damaging than those from more distant relatives (new associations) for new cactus biocontrol targets in the future.

**Explaining variable success of biological control of invasive spotted knapweed,  
*Centaurea stoebe* ssp. *micranthos* (Asteraceae), in British Columbia, Canada**

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Recent reviews have identified that variable control of widespread invaders is a common pattern. One such species is spotted knapweed (*Centaurea stoebe* ssp. *micranthos*), a widespread and problematic invader of rangelands worldwide, including in southern British Columbia (BC), Canada and much of the North American Northwest. Together with diffuse knapweed (*C. diffusa*), spotted knapweed has been the subject of a North American biocontrol programme since the 1970s, resulting in the release of 13 insect agents. Despite effective biocontrol of diffuse knapweed in BC, and widespread reductions in the density and fecundity of spotted knapweed across the province, pockets of abundant spotted knapweed remain with considerable costs to rangeland tenure holders. We found, on average, a long term decline in spotted knapweed density under biological control. However, control is highly variable among study sites. Here we report on ongoing work exploring potential mechanisms for this variability. We identified spotted knapweed life history traits, short-growing season sites as refugia from a key control agent species, and diverse land use practices as key predictors of knapweed success. Taken together, the longer term successful control of spotted knapweed in BC will require an integration of biological control efforts and revised land use to reduce this species' abundance to non-problematic levels.

***Lygodium microphyllum* (Schizaeales: Lygodiaceae) genotypes are associated with resistance to galling by *Floracarus perrepa* (Prostigmata: Eriophyidae) mites – consequences for native range exploration and provenance**

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The Old World climbing fern, *Lygodium microphyllum*, has been a weed in Florida since the 1960s. The *Floracarus perrepa* galling mites from Australia were released in 2008 to control the fern. The establishment and impact of the mites varied considerably across sites and across plants in experimental tests, with mites inducing galling on the leaves of some plants and causing browning of the leaf tips on others. Mites induced galling on 41% of plants during initial greenhouse research. During field releases across 63 plots, galls were observed in 10%, tip-browning damage in 44%, and neither galls or tip-browning damage in the remaining 46%. A genetic basis to fern susceptibility to mite galling was hypothesised. We used restriction-site associated DNA sequencing (RADSeq) to investigate whether the genetic structure of Florida *L. microphyllum* is associated with susceptibility and resistance to mite galling. Leaf material was collected from 13 sites across Florida and RADseq data was obtained for 190 samples. *Floracarus perrepa* galls were recorded as present or absent at each site based on current and past field measurements and observations. Analysis of *L. microphyllum* population genetic structure indicated that two genetically distinct populations were present, though with a considerable number of admixed individuals. The presence or absence of leaf galls was associated with these two genetically defined populations. Overall, we interpret these results to mean that two closely related *L. microphyllum* lineages invaded Florida. Admixture between them since then has decoupled the mechanism of gall resistance and susceptibility from the pattern of population genetic differentiation. Further, an ongoing analysis of global *L. microphyllum* samples will determine the biogeographic history and provenance of Florida *L. microphyllum* and investigate how individuals that established Florida *L. microphyllum* populations differ genetically from contemporary individuals. These results refocus the direction of the *L. microphyllum* biological control program and invite a mechanistic approach to understanding weed provenance and insect associations.

**Addressing climatic mismatches in biological control: *Alternanthera philoxeroides* in the USA as a case study**Knight Ian<sup>1</sup>, Harms Nathan<sup>2</sup>, Pratt Paul<sup>3</sup> & Reddy Angelica<sup>3</sup>.<sup>1</sup>US Army Engineer Research and Development Center, Vicksburg, MS 39180<sup>2</sup>US Army Engineer Research and Development Center, Lewisville, TX 75057<sup>3</sup>USDA ARS, Invasive Species and Pollinator Health Research Unit, Albany, CA 94710

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The southeastern United States is host to some of the world's worst tropical and subtropical aquatic weed species. Throughout the 20<sup>th</sup> century, many were the target of successful biocontrol programs. Recently, some weed species have expanded into more-temperate regions, but their biocontrol agents fail to establish or build up populations to provide adequate control in those areas. To address this lack of control, foreign exploration for climatically suited species or phenotypes has been the traditional solution. However, domestic surveys of introduced locally adapted populations may provide a lower cost approach that avoids regulatory burdens of evaluating new foreign biocontrol agents. To assist selection of agents for these areas and improve control, cold-tolerance must be measured and compared between candidates. However, there exist a range of assays and metrics, each differing in their implication and ecological significance. We discuss the approaches taken in the USA to address the issue of climate mismatch between the aquatic weed alligatorweed, *Alternanthera philoxeroides*, and its primary biocontrol agent, *Agasicles hygrophila*. A holistic approach to measuring cold-tolerance of foreign and domestic *A. hygrophila* populations was taken and comparisons were made between and among them. Although populations identified through exploration in the native range yielded substantially more cold-tolerant populations than those already in the USA (1.98 and 1.95°C lower critical thermal minima and chill coma temperatures, respective, 35% greater survival at 15°C, and 15.34% faster development at 15°C), they are still pending approval for release and may require years of additional waiting. Moderate variation in cold-tolerance among domestic *A. hygrophila* populations was identified by a latitudinal cline- southern populations were less cold tolerant than northern populations as measured by critical thermal minima ( $\Delta 0.88^\circ\text{C}$ ). Our results suggest local adaptation may have occurred for some populations of *A. hygrophila*, and provide insight into how long-term redistribution efforts in the southeastern USA may inhibit the local adaptation of populations. Advantages and disadvantages of foreign and domestic exploration, the value of various cold-tolerance metrics, preliminary evaluation of other *A. philoxeroides* agents, and our efforts to establish more cold tolerant agents in higher latitude locations are discussed.

**Population genetic structure of *Megamelus scutellaris* (Hemiptera: Delphacidae) and its implications for the biological control of water hyacinth (*Pontederia crassipes*)**

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*Megamelus scutellaris* Berg is a monophagous delphacid planthopper used as a biological control agent of water hyacinth (*Pontederia crassipes*) in Argentina, South Africa and the United States. In its native range, *M. scutellaris* is distributed across South America, from the Amazon basin to the Del Plata basin. Variations across insects' distribution range can promote plasticity or local adaptations and genetic differentiation among populations. In this regard, individuals from populations from Paraguay and Argentina have been introduced in the U.S. and differences in their performance have been reported across seasonal temperatures. These observed phenotypic variations, and the varying levels of success of *M. scutellaris* in establishing under different conditions, reinforce the need of studying this biological control agent from a genetic framework. In this work, a combination of genome-wide SNP data and mtDNA were used to evaluate mitochondrial and nuclear diversity and phylogeographic patterns along *M. scutellaris* known native distribution in Argentina. A total of eleven haplotypes were found, forming two separate haplogroups. The first one corresponded to northern populations of *M. scutellaris*, located in the biogeographic provinces of the Chaco and the Parana Forest, while the remaining haplotypes were found in southern populations, located in the Pampean province. Preliminary genomic analyses, based on a 5,928 SNP matrix, included DAPC and estimation of individual ancestry coefficients. Both these analyses suggested a similar pattern of spatial genomic variation, showing three separate lineages: two of them corresponding with the Northern mitochondrial haplogroup and the other matching the Southern haplogroup. This combined evidence indicates that the exported *M. scutellaris* populations, from Cordillera (PY) and Buenos Aires (AR), most likely belong to two different lineages. In the following steps of this project, the generated nuclear genomic data will be used to further understand the underlying historical and current climatic, environmental and geographical factors shaping the patterns of genetic variation among *M. scutellaris* native populations. This information can be useful in the development of biological control projects, taking such variations into consideration before the release of this agent into new areas or in areas where it has previously failed to establish.

**Investigating the enemy release hypothesis: a case study of *Lagarosiphon major* in New Zealand**

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Globalisation has played a very important role in connecting people and, therefore, ecosystems. One of the negative consequences of this development is the spread of invasive species outside of their natural range. Scientific studies have shown how devastating the effects of invasive species can be on both the environment and economies. There are many explanations for why invasive alien plants are likely to be more successful outside their native range. One such explanation is offered by the Enemy Release Hypothesis (ERH), which states that plants automatically become superior competitors outside of their natural range due to release from top-down stressors (herbivory, parasites, and diseases) that is evident in the absence of their natural enemies. The underlying assumption of the ERH is that natural enemies are important regulators of plant species populations, and that the pressures from these natural enemies are felt more readily by the native species compared to alien plants, and also that in the absence of such pressures, exotic plants are able to redirect more resources towards growth and reproduction while also successfully keeping the accumulated biomass. Classical biological control has previously been cited as evidence for the enemy release hypothesis. Thus, the aim of this study was to investigate the role of ERH on the invasiveness of *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in New Zealand. This was achieved by measuring and comparing several plant parameters (e.g., biomass, percentage cover, and macrophyte diversity) between South Africa (native range) and New Zealand (invaded area). Results show higher biomass, denser stands of *L. major*, and overall reduced plant diversity in invaded sites compared to the native range. In summary, these results can be interpreted as evidence for the ERH, and as such making the *L. major* invasion in New Zealand ideal for biological control. Two promising agents (*Polypedilum tuburcinatum*, and *Hydrellia lagarosiphon*) are currently in their last stages of consideration and testing.



**Origin and genetic variability of the invasive species *Stellaria media* (Caryophyllaceae) through morphological traits and molecular markers**

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*Stellaria media* (“Capipi”, “chickweed”) is a species native to Europe, West Asia and North Africa that behaves as a weed in barley, wheat, rye, soybean and sunflower crops. This species accumulates nitrates at potentially toxic levels for animals and is highly resistant to different herbicides. Moreover, it has several mechanisms that determine its invasive character, such as: high tolerance to water stress, high production of flowers and seeds per plant with high germination power and different seed dispersal mechanisms. The objectives of this work are to: (1) evaluate the variation in morphological traits of *S. media* in Argentina and (2) estimate the origin of its populations in our country from a phylogeographic analysis with nuclear sequences, ITS1 and ITS2. The observation of vegetative and reproductive structures was carried out from living material, fixed in with formaldehyde-aceticacid-ethanol (FAA) and/or herbarium specimens, using a stereoscopic microscopy and an ANOVA was performed to estimate the morphological variability. For the phylogeographic analysis, ITS sequences were obtained by PCR from four populations from the NEA (Northeastern) and NOA (Northwestern) regions and Buenos Aires Province, and those available in Genbank belonging to Canada (four populations), China (three populations), Czech Republic (four populations), England (four populations), Germany (two populations), South Korea (three populations) and United States of America (two populations). The sequences were aligned and analysed by maximum parsimony in MEGA X, to root the tree a *Spergula arvensis* ITS sequence was used. The ANOVA shows the presence of significant differences between study areas in plant height, degree of pubescence, leaf area (length x width of lamina), fruit size (length x width of capsule) and number of seeds. The phylogeographic analyses indicate that the Argentinian populations of *Stellaria media* present multiple origins, probably from North America, England or Eastern Europe. This would also indicate that the invasive process could have been the result of several introduction events, which is consistent with the morphological variability observed.

**Intraspecific variation in cold tolerance of the adventive parrot's feather weevil,  
*Phytobius vestitus*, in the USA**

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Cold tolerance of beneficial insects used for weed biological control may dictate where and under what conditions the insects should be expected to provide value as a management tool. Spatial variation in cold tolerance may occur due to evolutionary adaptations, and could inform exploration and prioritization of agent genotypes. Parrot's feather, *Myriophyllum aquaticum*, is a South American aquatic plant that is weedy in several locations around the globe. In the southeastern USA, where *M. aquaticum* has been introduced, a native weevil (*Phytobius vestitus*) has been identified that was historically associated with *M. heterophyllum* but damages *M. aquaticum* to the degree that infestations are rarely an economic problem there. As a first step in determining the potential value of this species outside the warm subtropical region of the southern USA, we conducted a series of experiments to investigate cold tolerance of *P. vestitus* from two geographically separate populations. Specifically, we examined populations of *P. vestitus* collected in Honey Creek, Oklahoma, USA (34.58°N latitude; USDA Plant hardiness zone 7a: -17.8 to -15°C winter minimum temperature), and Blind River, Louisiana, USA (30.09°N latitude; USDA Plant hardiness zone 9a: -6.7 to -3.9°C winter minimum temperature). We evaluated super cooling point (SCP), and the effect of rapid cold hardening (RCH) on critical thermal minimum (CTmin) and chill coma induction temperature. We then compared values to those previously reported for a successful agent of *M. aquaticum* in other countries. We found no differences in SCP between populations, but populations differed in the detection of RCH, and Oklahoma weevils were more cold tolerant, overall. There was only slight evidence for RCH in Louisiana weevils- CTmin was reduced by 35% when cooled slowly but chill coma was not affected by RCH. In contrast, RCH depressed CTmin by 83% and chill coma by 164% for the Oklahoma population. Both populations exhibited mean CTmin values below those previously reported for *Lysathia* sp., an agent that provided extensive control in South Africa. Our preliminary findings suggest that geographic variation in thermal tolerances of candidate agents may be used to locate better-adapted populations for use in similar climates around the world. In the case of *M. aquaticum* however, it may not be necessary to search beyond those populations sampled here, at least for climates similar to those where control occurred in South Africa.

**From where, how and how many times? History of the invasion of *Senecio madagascariensis* (Asteraceae) in South America**

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*Senecio madagascariensis* commonly named fireweed, is a southern Africa native species, recognized as invasive in Australia, south-east Asia, South America and the Hawaiian Islands. This species occurs in roadsides, fallows, wastelands and anthropized areas. An individual of *S. madagascariensis* can produce up to 18.000 seeds that are dispersed by wind. Unfortunately, these plants contain pyrrolizidine alkaloids that cause cumulative chronic liver damage and fatality, especially in monogastric livestock. Phylogeographic and population genetic analyses can provide important insights into the dynamics of biological invasions by elucidating geographical source(s), dispersal route(s), spread and the roles of evolutionary processes in invasion success. Thus, in order to have a more comprehensive understanding of the invasion of *S. madagascariensis* in southern South America, we aimed a) to clarify the origin of introduced genotypes and b) determine the genetic diversity and the structure of the invasive populations. To achieve our goals, we used a genetic characterization with internal transcribed spacer (ITS) from 57 populations belonging to Argentina and Brazil and 26 others populations available in Genbank belonging to different parts of the world of both native and invasive range. We analyzed the haplotype and nucleotide diversity and an AMOVA among collection sites. The evolutionary relationships among the sequences were performed, sequences of *S. inaequidens* from GenBank were used as outgroup. In addition, we used 8 microsatellite markers in 342 individuals for the 57 populations through which the genetic diversity was estimated using the parameters: number of alleles, allelic richness, expected and observed heterozygosity, deviations of the genotype frequencies Hardy-Weinberg equilibrium and the inbreeding coefficient ( $F_{IS}$ ). Moreover, the relationships between geographic and genetic ( $F_{ST}$ ) distances among populations were tested at the structure and scale of geographic differentiation among populations. The phylogeographic results indicate that the Argentinian populations are originating from South Africa, while the Brazilian ones represent diverse provenances. These results become more relevant with the evidence from population genetic studies from which Argentinian populations are represented by only one deme, whereas the populations that are present in Brazil are split into 2 demes represented by populations that occurred in the center of the distribution area and the other deme is represented in the coastal area of the country.

**Development of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on different geographic populations of *Parthenium hysterophorus* (Asteraceae)**

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*Parthenium hysterophorus* L. (Asteraceae) commonly known as parthenium weed, is a highly invasive herbaceous weed in more than 50 countries around the world. Parthenium weed has been reported to negatively affect crop and pasture production, biodiversity, and human and animal health. Here, we investigated the development of a specialized biocontrol agent, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) across a range of populations of parthenium weed, collected from its native (Argentina, Brazil, Mexico) and introduced (Australia, China, Pakistan) regions. First instar larvae were applied to fully grown leaves (one larva/leaf) of selected parthenium weed populations, inside a leaf clip cage and were studied for their development. The mean ( $\pm$ SE) larval developmental period (1<sup>st</sup> to 4<sup>th</sup> instar) was longer on native ( $12.8 \pm 0.8$  days) versus introduced populations ( $10.5 \pm 0.16$  days). Larval defoliation, survival and time to adult emergence were all higher for larvae on introduced than native populations. Similar trends were observed for larval weight, pupation period and the number of adults emerged. The mean ( $\pm$ SE) larval consumption of host leaf material was also greater in introduced ( $0.25 \pm 0.02$  g) than native populations ( $0.09 \pm 0.04$  g). These results support the theory of evolution of 'increased competitive ability' which suggests that invasive species, when introduced to new regions, may undergo rapid evolutionary changes in their morpho- and physio-chemical make-ups, to allocate more resources towards their growth rather than defense. These results would be insightful for the management of parthenium weed using classical biocontrol agents in introduced ranges.

## IMPLEMENTATION OF ACCESS AND BENEFIT-SHARING MEASURES: CONSEQUENCES FOR CLASSICAL BIOLOGICAL CONTROL OF WEEDS

Moderator: Fernando Mc Kay (FuEDEI, AR)

Oral presentation

### **Classical weed biological control and the Nagoya Protocol – it's time to put it to the test**

**Keynote speaker:** Hill Martin P.<sup>1</sup>

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The Convention on Biological Diversity (CBD) and the Nagoya Protocol (NP) establish the international legal framework for access and benefit sharing (ABS). The NP was initiated in October 2014 and has been ratified by many countries including those active in classical weed biological control. Whilst understanding the need for access and benefit sharing of biodiversity, the Nagoya Protocol was met with concern by the biological control community as many practitioners were skeptical of how efficiently it might be implemented in signatory states, and feared that it would significantly slow down the process of obtaining the necessary permissions to survey for, collect and export potential biological control agents (Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT)). The International Organization for Biological Control (IOBC) Global established a Commission on Access and Benefit Sharing in 2008 that has resulted in a selection of papers including a special issue of *BioControl* (2023) that essentially sets out a series of best practices for implementing the Nagoya Protocol in classical biological control. In kind benefit sharing through support of laboratories, student support, infrastructure sharing of agents has historically been a philosophy of the international weed biological control community. The weed biological control community has prided itself in practicing a public good science. However, given the new regulatory environment, is it time to test the system through financial compensation for biological control agents? The intentions of the NP are noble as it seeks to reward countries for protecting their biodiversity. However, the unintended consequences of the NP could be that only resource rich countries will be able to afford to practice classical biological control and it will be unaffordable to resource poor countries.

**Meeting access and benefit sharing requirements: a practical review of CABI's access to genetic resources for weed biological control**

Weyl Philip S.R.<sup>1</sup>, Djeddour Djamila H.<sup>2</sup>, Hinz Harriet L.<sup>1</sup>, Seier Marion K.<sup>2</sup>, Shaw Richard H.<sup>2</sup>, Smith David<sup>2</sup> & Cabrera Walsh Guillermo<sup>3</sup>.

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CABI is a not-for-profit international organization with the mission of improving people's lives worldwide by solving problems in agriculture and the environment, while engendering trust, facilitating science and ensuring that benefits are shared. CABI is in a unique position in that it both uses biological control agents and often acts as an intermediary between provider and user countries. CABI took a position to share benefits whether the provider country is party to the Nagoya Protocol or not. For transparency, CABI has also developed a publicly available access and benefit sharing (ABS) policy and ABS best practices built around the principles of the Nagoya protocol. CABI's ABS best practices aim to facilitate compliance with national legislation on ABS in the countries in which it works and in those that provide genetic resources for CABI's use. In Switzerland, CABI's ABS best practice is officially recognized in a public register with the benefit of reducing the risk of non-compliance while at the same time building trust with the provider and user countries. CABI UK, Egham summarized it's work on 116 projects involving genetic resources and although the majority were out of scope of UK regulation, monetary and/or non-monetary benefits have been shared with provider countries. Currently CABI, as all who access and use biological control agents, is following the global debate on how Digital Sequence Information (DSI) associated with the organisms worked with will be treated in respect to the implementation of the Nagoya Protocol. CABI evaluates how DSI impacts on biocontrol research and delivery and illustrates how important a good outcome of these discussions will be to allow biocontrol research to continue. In this presentation, using relevant examples, we will highlight how CABI deals with the requirements of the Nagoya Protocol and access and benefit sharing compliance with regards to the implementation of weed biological control research.

**Importing the rust fungus *Uromyces pencanus* as a biocontrol agent for Chilean needle grass, *Nassella neesiana*, into New Zealand – a decade long journey**

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Chilean needle grass [CNG] (*Nassella neesiana* (Trin. & Rupr.) Barkworth), mostly a pasture weed in New Zealand, outcompetes and displaces pasture species, and can cause major damage to stock. Native to Argentina, CNG is widespread in the New Zealand regions of Hawke's Bay and Marlborough, with smaller sites in northern Canterbury and Auckland. In 1998, a biocontrol programme for CNG was initiated in Argentina by Australia, followed by New Zealand. A rust fungus, *Uromyces pencanus* (Dietel & Neger) Arthur & Holw., was identified as the most suitable biocontrol candidate, with strain UP27 shown to be highly host specific. In 2011, the New Zealand Environmental Protection Authority (EPA) granted a five-year permission to import *Uromyces pencanus*. However, it took over a decade before permission to export the rust fungus from Argentina was finally approved. The holdup was caused by a document called the Convention for Biological Diversity and the Nagoya Protocol, a supplementary agreement to it. By the time a new EPA application was submitted in 2017, further host range testing of *Austrostipa* species native to Australia resulted in the unexpected production of *U. pencanus* spores on two non-target *Austrostipa* species: *A. compressa* (R.Br.) S.W.L. Jacobs & J. Everett and *A. macalpinei* (Reader) S.W.L. Jacobs & J. Everett. While neither of these *Austrostipa* species grow in New Zealand, testing of native Stipoid grasses was recommended. Only three native grasses belonging to the same Stipeae tribe as *Nassella* are present in New Zealand: *Austrostipa stipoides* (Hook.f.) S.W.L. Jacobs & J. Everett, *Achnatherum petriei* (Buchanan) S.W.L. Jacobs & J. Everett and *Anemanthele lessoniana* (Steud.) Veldkamp. Permission to export the rust fungus out of Argentina was finally granted in September 2021 during the Covid-19 pandemic. Due to a restricted number of flights worldwide, the culture took over three weeks to arrive and was no longer viable. In December 2022, a fresh culture was hand-carried from Argentina to New Zealand and has resulted in the successful establishment of *U. pencanus* on New Zealand CNG plants. The CNG rust fungus is the first plant pathogen exported from Argentina for biocontrol. The journey from finding a suitable biological control agent for CNG to getting a culture established in New Zealand is discussed.

Oral presentation

**Sustainable use of resources and services derived from biodiversity: the role of the Instituto Misionero de Biodiversidad (IMiBio), Misiones, Argentina**

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The Paraná jungle, a global biodiversity hotspot, constitutes one of the largest remaining pristine forests in the world. All of what is left of this jungle in Argentina is located in the Province of Misiones, housing 52% of the country's biodiversity. This environment faces serious threats from deforestation due to agricultural expansion and invasive species. In 2019, the Instituto Misionero de Biodiversidad (IMiBio) was created to promote and support policies and legal instruments that guarantee the sustainable use of resources and services derived from biodiversity. The IMiBio constitutes a scientific, technological strategic institution integrated into the educational, social, cultural and economic field for the sustainable use of the natural heritage of the Province of Misiones. Among IMiBio's powers is to create a solid legal framework and update current legal instruments in order to improve the capacities for negotiations to establish the terms and conditions of access and use of genetic resources in the Province of Misiones. In particular, IMiBio is the entity in charge of granting access permits to genetic resources in the territory of the province of Misiones. Currently, of the 56 Internationally recognised Certificates of Compliance issued in Argentina, 4 correspond to the Province of Misiones, of which 1 is for commercial use. The IMiBio plays an important role not only in the preservation of Argentina's biodiversity, but of Latin America's. As such, since 2021, the IMiBio has been participating actively in hosting sessions regarding biodiversity issues in Latin America at the Science Summit United Nations General assembly within the framework of the United Nations Sustainable Development Goals. In addition, the IMiBio is promoting the creation of the Coalition of Biodiversity Research Institutes of Latin America and the Caribbean.



## ARTICULATING CONSENSUS BETWEEN SCIENCE AND ALIEN WEED MANAGEMENT

Moderator: Raelene Kwong (DPI VICTORIA, AU) and G. Cabrera Walsh (FuEDEI, AR)

Oral presentation

### **Biocontrol meets traditional weed management - We have the same goal so how can we engage better?**

**Keynote speaker:** Shaw Richard H.<sup>1</sup>

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It is true that there can be a divide between the classical weed biocontrol practitioners and the traditional weed management community. Weeding is an age-old tradition that predates the use of specialist natural enemies and more recently large businesses have grown up supported by a chemical industry whose product sales for weed control close to insect and disease control combined. The divide between approaches exists in regions where CBC is well established, as well as those, like Europe, where it is a relatively new technology. In this author's experience of raising the profile of biological control in Europe over 25 years, to begin he was as unwelcome as the weeds he was targeting, particularly at conferences run by the burgeoning Japanese knotweed control industry for example, where any natural enemy was a threat to a family business. However, once the concept was explained, the instinctive public resistance to messing with the natural order of things vanished in almost all cases. This is not necessarily so for the learned and influential ecological community who often advise on environmental management plans and could continue to erect barriers to adoption. This paper will consider some of the examples where consensus was lacking between these two communities, whilst suggesting how it can best be built by applying theory and proposing practice. There is extensive literature on the engagement of academics, their departments and Universities with Industry but mainly in the realm of commercialisation. When agriculture is considered, much of the focus is on applying technologies in Least Developed Countries, whereas for biological control, the vast majority of published research is on specific insect pests. In our field of classical weed biocontrol, relatively little is published but it is worth noting that there is also a gap between proponents and users of Integrated Weed Management in agriculture and the reasons behind this could also be informative. The lessons learned from other disciplines could advise us on what barriers to expect, how best to gain influence and how to encourage adoption. Though we are small in number, the biocontrol community is passionate, trustworthy, disciplined and broad-minded and the personal touch will probably open more doors than high ranking publications in the long run.

### **Assessing the outcome of biological control on weed populations**

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A number of approaches have been taken in assessing the success of weed biological control. At an individual level, these include evaluation of the impact of individual biocontrol agents on the fitness of the target weed, the impact of the entire suite of agents established on the weed, and effects on selected biological parameters (such as seed production). Biocontrol success has also been measured at a landscape or whole-species level by estimating the economic benefits accruing, through savings on other control methods and through mitigation of the negative impacts of the target weed on economic activities. Recovery of biodiversity following successful biocontrol has also been measured. From the mid-1990s, in South Africa and subsequently elsewhere, the use of the terms 'complete', 'substantial' and 'negligible' control were applied to individual target weeds to indicate the level of conventional control methods required after biocontrol had reached its full potential. Although these terms proved very useful, they eventually proved inadequate in reflecting the nuances of biocontrol, in terms of variability in effectiveness across habitats and because biocontrol impacts vary across target species depending on the mode of damage inflicted by the individual species of agents. Therefore, we have proposed a new system based on the outcomes of biocontrol at a target weed population level, and tested it on 54 species invasive in South Africa, on which biocontrol is well established. For each target weed, we categorized the impact of biocontrol on four ecological parameters, namely density, biomass, area covered, and rate of spread. Three broad categories were used, namely A, in which the population parameter in question had been reduced below the tolerable economic threshold; B, in which the parameter has been reduced below a 'reversal threshold' i.e., lower than before biocontrol had taken effect; and C, where the parameter had increased compared to the point at which biocontrol had been established, but was still lower than if no biocontrol agents had been introduced. Individual researchers were assigned to each weed species, and to categorise outcomes using either 'expert opinion' or quantitative data. They were also given the latitude to divide outcomes according to variability across habitat or region. Here we present the results of this exercise, and propose that the system provides a more realistic and nuanced reflection of the impact of biocontrol on populations of an individual weed species across its invasive range.

Oral presentation

**Effects of the gall wasp *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae) on the seed production and seed bank of *Acacia longifolia* (Fabaceae)**

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The Australian bud-galling wasp *Trichilogaster acaciaelongifoliae* is an effective biocontrol agent that primarily targets the reduction of *Acacia longifolia* seed formation. Although flower buds of the invasive *A. longifolia* are replaced by *T. acaciaelongifoliae* galls, vegetative buds are also impacted to a lower level. This biocontrol agent was successfully used in South Africa since early 1980s. Despite reduced seed production, negative effects on the seed bank are inconclusive, emphasizing the importance of understanding specific insect-plant interactions. *Trichilogaster acaciaelongifoliae* was released in Portugal in 2015 and preliminary observations indicated the usefulness of the agent against *A. longifolia* in the northern hemisphere. Our work assessed the effect of *T. acaciaelongifoliae* on *A. longifolia* seed production (seed rain) and seed bank replenishment, as these are key factors for invasion persistence. Annual seed rain was studied at four sites in Portuguese coastal dunes with different densities of *T. acaciaelongifoliae* galls and cover of pods. In areas with and without the biocontrol agent, 50 to 75 seed intercept traps were placed at the edge of *A. longifolia* canopies. Trees and traps were monitored since 2019 and checked fortnightly from June to October. Visual cover estimates of galls, pods and ungallo branches were made in each season before seed rain started. The largest decrease in seed production was observed 4 years after the 2016 establishment of the biocontrol agent at two sites. Results also suggested that *T. acaciaelongifoliae* gall cover increased faster in areas where mechanical cuts were executed, which spared some trees. Impact to the seed bank was compared between two sites pre-agent release against two sites each after five- or two-years agent establishment. Seed bank samples were collected at all sites, in areas with and without the biocontrol establishment, before and after seed rain peak. Seed number increased in areas without the biocontrol agent more than in areas with the biocontrol agent. The accumulated seed bank appeared to be stabilizing in areas after five years of establishment. Additional work will be needed before managers integrate biocontrol into their management practices, but these early results are encouraging by showing a decreased seed production and seed bank accumulation at locations of *A. longifolia* attacked by *T. acaciaelongifoliae*.

**Time factor: Biological control of giant salvinia, *Salvinia molesta* (Salviniales: Salviniaceae), leads to restoration of ecological functions in subtropical wetlands**

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Biological control of giant salvinia, *Salvinia molesta*, using the salvinia weevil (*Cyrtobagous salviniae*) has been highly variable in subtropical wetlands. Damage by the weevil ranges from simple yellowing of fronds and thinning of floating mats, to drastic reductions in growth and sinking of plants. The inconsistency in control requires yearly inoculation of mass-reared weevils and results in unpredictability of recovery of ecosystem services. Here, we review the impacts of salvinia on ecosystem services and the timing of biological control in subtropical wetlands. Using field surveys, we quantified how salvinia mats altered water quality, specifically the reduction of dissolved oxygen, pH and light availability, and increased orthophosphate and ammonium in the water column. Compared with wetlands dominated by submerged aquatic vegetation, salvinia wetlands had lowered macroinvertebrate richness, relative abundance, and energetic value. Using mesocosm experiments, we demonstrated that the community of macroinvertebrates was diminished in tanks with 100% cover, while different communities were present at 50, 25 and 0% cover. Regardless of the coverage, salvinia limited aerial colonization of macroinvertebrates. To understand the timing of biological control, a two-year field survey was conducted in coastal wetlands in southwestern Louisiana where salvinia weevils have been established for more than five years. Results demonstrated that densities of more than 40 weevils per kilogram of salvinia early in the season were associated with rapid declines of salvinia coverage. These data were used to build predictive models using weevil densities, habitat type, salvinia coverage, and dissolved oxygen. The models revealed that higher adult weevil densities in June lead to faster reduction of salvinia coverage and recovery of submerged aquatic vegetation, and consequently higher dissolved oxygen levels. Overall, these studies highlighted new negative impacts of salvinia such as internal nutrient loading, and changes in ecosystem and function. The predictive model was an initial step to determine how the timing of biological control can determine the recovery of wetland functions.

**Strategic alliances in the management of *Hydrocotyle ranunculoides* (Apiales: Araliaceae) - using beetles to come together**

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Floating pennywort (*Hydrocotyle ranunculoides*) is a freshwater invasive from the Americas, imported into Europe through the ornamental aquatic plant trade. A relatively recent invader in the UK, it was first reported in the wild in England in the 1990s. Its subsequent spread across the country's slow-flowing waterbodies has been exponential, and the plant's dense, mat-like growth means that it impacts on biodiversity, recreational access and increases flood risks by blocking sluices and drains. The economic cost of management alone exceeds £25.5 million annually, and little progress has been made to curb its spread. Listed on the Invasives Species Order as a plant of European Concern, no single organisation has a duty to manage the plant. Consequently, without consistent, coordinated, well-resourced and meticulous removal efforts from multiple landowners on a catchment scale, conventional management options have been largely unsuccessful. As a result, the plant presents a major and ever-increasing threat to marshes, rivers, canals and lakes across Great Britain, and the deployment of a natural solution has been a much-anticipated prospect. In 2020, a small-scale pilot partnership was launched between Environment Agencies, the Angling Trust and British Canoeing to galvanise volunteers and environmental managers to tackle the plant in the Thames Basin. This was a great success and the following year, a national Great Britain Floating Pennywort Strategy was launched, uniting recreational users, water companies, and government to share best practice and facilitate further management partnerships. In late 2021, after over a decade of government funded research and a year-long consultation period, the petition to release the weevil *Listronotus elongatus* (Coleoptera: Curculionidae) as a biocontrol agent for floating pennywort was granted Ministerial approval. Proactive engagement with stakeholders and information exchange throughout the research phase meant that this approval was positively received by the weed management community. In this presentation, we will highlight the importance of fostering strong relationships across the private, public and government sectors, using media and community engagement to raise the profile of biological control, share knowledge and ultimately contribute to coordinated weed management partnerships.

**Community-led releases and detailed monitoring of the sea spurge biocontrol agent, *Venturia paralias*, provides evidence of agent establishment, spread, and initial impact in Australia**

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Sea spurge (*Euphorbia paralias*) is a highly invasive weed along Australia's southern coastline. The weed invades dune ecosystems forming large infestations that can outcompete native dune flora, reduce available nesting sites for shorebirds and when injured exudes a latex that is toxic to humans and animals. These negative impacts have reduced the amenity value of many beaches and have also threatened native floral diversity. The fungus *Venturia paralias*, collected from diseased sea spurge in Europe, was identified by the CSIRO as a promising highly specific biocontrol agent for sea spurge following studies into its taxonomy and host range. Based on these results, the regulator approved the release of *V. paralias* in Australia in December 2020. Since then, the CSIRO has co-invested in a three-year project to undertake field releases of *V. paralias* along the Tasmania and Victorian coastlines. This project includes extensive community-led releases of the agent and quantitative monitoring of its establishment, spread and initial impact at a few sites. After developing effective field release methodologies, field release kits containing dried fungal spores of *V. paralias* along with detailed release instructions, were distributed to community participants from December 2021. To date, *V. paralias* has been released at 70 sites across Victoria and Tasmania. Furthermore, 9 fixed monitoring sites (3 in Tasmania and 6 in Victoria), each containing 3 10-metre long transects with 5 1 m<sup>2</sup> plots per transect, were established where resident sea spurge plants were inoculated with *V. paralias*. Feedback from community participants within 2-4 months of the releases, indicated that *V. paralias* was present at 41 % of the community-release sites. In contrast, within 12 months post-release *V. paralias* was confirmed as established on sea spurge in 95 % of the plots across the 3 fixed monitoring sites in Tasmania, but only in 38 % of the plots across the 6 sites in Victoria. Observations from the fixed monitoring sites also indicated that the fungus has cycled between two symptom types: development of stem lesions following the release, sporulation of the fungus on these lesions and development of leaf lesions, most likely from spores produced on the stems. This sequence of symptoms contrasts to what was observed during controlled laboratory studies. Such field-based studies have provided a better grasp of the field epidemiology and establishment of *V. paralias* in Australia. Further spread and initial impact on sea spurge in relation to environmental conditions will also be presented.

## INTEGRATING BIOLOGICAL CONTROL, RESTORATION AND MANAGEMENT

Moderator: Melissa Smith (USDA-ARS IPRL, USA)

Oral presentation

### **Integrating biological control, other control methods and restoration in invasive plant management – is it possible?**

**Keynote speaker:** Marchante Hélia<sup>1</sup>.

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Biological invasions are a recognized global threat that often drives local biodiversity loss, habitats degradation and major economic costs. As such, invasive alien species (IAS) management is mandatory in many situations. Management, in a broad sense, includes not only control (perceived as measures to reduce the damage from IAS) but also other stages, with raising awareness and restoration being particularly interesting in the context of this communication. Frequent methodologies used to control IAS can be included in three major categories: physical, chemical, and biological control (BC). BC, in particular of invasive alien plants (IAP), is frequently recognized as safe, sustainable and an important part of management, despite the marked differences that occur around the world. In Europe BC is in its infancy while in other continents BC has more than a century of application. In this context, public awareness raising on BC in Europe is key to gather more support from stakeholders (and others). Depending on the target species, the BC agents (BCA) considered, and even the environmental context, BC alone may control the target species (e.g., *Azolla filiculoides* in South Africa) or may need to be integrated with other control methodologies. Such integration may be necessary, e.g., due to specific contexts (e.g., the plant-target is grown for timber, and only seed predators can be introduced, as is the case with several *Acacia* species), due to limited levels of BCA establishment or difficulties on the BCA spreading. When various methods are integrated good coordination is crucial, particularly when different professional teams are involved. Otherwise, chemical applications may kill the BCA, the use of mechanical methods may reduce the target plant population below that needed for BCA persistence and the sustainability of the method, amongst others. When considering habitat restoration, following initial control but with the IAP still present and BCA action still required to prevent regrowth, the challenges of combining actions may be very similar but achievable. Phenological/ seasonal adjustments can allow BCA to survive well-timed chemical application that remove just the right proportion of invasive populations can allow established BCAs to persist and contain IAP regrowth. Indeed, if done successfully, including the right combinations of different control methods can provide the best outcome in reducing the target species and its impacts and allow successful restoration. Although long-term field data and available publications on the combination of BC with other control methods are scarce, some interesting studies and field experiences reported in grey literature, are available and will be discussed.

### **The impact of mass-rearing biological control agents on invasive aquatic plant control**

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The transition from classical biological control to augmentative control of invasive aquatic macrophytes was prompted by the need to release biological control agents in massive quantities to supplement the small populations already present at invaded sites, and to inundate at newly invaded sites, to improve the prospects of invasive macrophyte control. To do so, the Waainek Mass Rearing Facility (MRF) was established in 2008 in Makhanda (Grahamstown), targeting invasive macrophytes. Since biological control against *Pontederia crassipes* Mart. (Pontederiaceae) (water hyacinth) was initiated in South Africa (SA) in the 1970s, 13 species of aquatic weed biocontrol agents have been released to control five major aquatic weeds, including *P. crassipes*; *Pistia stratiotes* L. (Araceae); *Salvinia molesta* D.S. Mitch. (Salviniaceae); *Myriophyllum aquaticum* (Vell. Conc.) Verd.; and *Azolla filiculoides* Lam. (Azollaceae). Control agents of all of these species, except *A. filiculoides* are mass reared at the Waainek MRF, and released inundatively and frequently, eliminating the need for additional control measures. In addition, *Hydrellia egeriae*, a control agent for *Egeria densa* Planch., the first submerged weed targeted for biological control in SA, has recently been inundatively released, with establishment confirmed at three sites. Over one million agents reared at this facility have been released throughout SA, and also in a number of other African countries. In addition to improving biological control, the Waainek MRF has provided the Makhanda community with employment opportunities, in particular for those who live with disabilities. Given sufficient financial resources, the Waainek MRF will create more employment opportunities to continue to mass rear and release agents for the control of invasive aquatic plants.



## **Does native aquatic plant diversity recover after biological control of invasive floating plants?**

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Invasions of freshwater bodies by floating vegetation, including giant salvinia (*Salvinia molesta*) threaten biodiversity globally. The large, dense mats formed by these plants can reduce light and nutrient availability, change pH and other water quality parameters. These plants are also able to produce and release allelopathic compounds gaining a competitive advantage over other primary producers. The inhibition of aquatic plants, for example, decreases habitat complexity, and complex habitats promote the diversity of species and availability of resources, hence negatively affecting higher trophic levels. Invasive plant control through classical biological control using host specific natural enemies has successfully reduced many of these invasions, and it is often considered one of the first steps in enabling recovery of invader-dominated sites. After successful control, native assemblages are expected to recover on their own, assuming that ecological communities are resilient to invaders and such removal will allow natural communities to recover to a pre-invaded condition. However, management success is often limited to assessing if the plant invader has been successfully controlled. Thus, little is known about the response of aquatic plant communities following this practice. To address this deficit, we investigate aquatic plant community responses to biological control of *Salvinia molesta* by the weevil *Cyrtobagous salviniae* in five freshwater systems located in the Western and Eastern Cape regions of South Africa. We quantified aquatic plant species richness, diversity, and biomass in relation to environmental factors before (December 2021) and after biological control (January 2023) of the target plant. Our main goal is to determine whether native aquatic plant species recover after invasive floating plant control, and determine the environmental factors driving this recovery. To disentangle the factors by which floating invasive plant species and their control affect aquatic plant communities, structural equation modelling (SEM) will be used. SEM is a powerful tool to test and evaluate multivariate causal relationships in complex natural systems. This study can provide management and research recommendations to improve restoration outcomes.

**Managing invasive *Cabomba caroliniana* in Australia: prospects for integrating biological control and chemical control**

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*Cabomba caroliniana* Gray is a submerged macrophyte invasive in the waterways of Australia, and is an invasive alien species worldwide. Its detrimental effects include interference with recreational and commercial use of water bodies, affecting drinking water quality in reservoirs and impacting native flora and fauna. While a suite of control tools such as herbicides, mechanical removal and biological control are available for the management of *C. caroliniana*, use of these tactics can be highly restrictive depending on the management goals and specific circumstances of waterbodies. For example, in waterbodies that are used for recreational activities requiring immediate removal of *C. caroliniana*, biological control will have the least potential as a control tactic. Likewise, treating cabomba infestations with herbicides in dams that supply drinking water is restricted due to the contamination risk. Therefore, developing a multi-pronged integrated approach is crucial to effectively manage *C. caroliniana*. As part of a larger project, we developed biological control and chemical control tactics for the management of *C. caroliniana* in Australia. For biological control, the aquatic weevil *Hydrotimetes natans* Kolbe has been identified as a potential biological control agent based on the preliminary surveys, host specificity tests conducted in the native range (Argentina and Paraguay), and quarantine host-specificity studies conducted in Australia. These studies demonstrated high specificity of *H. natans* towards *C. caroliniana* and negligible risks to non-target plants. As a result, *H. natans* was approved for release in Australia as a biocontrol agent for *C. caroliniana*. For chemical control, the efficacy of the contact herbicide flumioxazin (a protoporphyrinogen oxidase (PPO) inhibiting herbicide) has been evaluated. Due to its high efficacy, the herbicide was subsequently registered by the authorities to treat *C. caroliniana* in Australia. We conducted a series of laboratory trials on the direct interaction between flumioxazin and *H. natans* with a view to develop integrated control tactics for *C. caroliniana*. These studies focused on direct effects of flumioxazin on the biology and behaviour of *H. natans* including changes in the expression of functional genes. The results suggest no or very low detrimental effects of herbicide on *H. natans*. In this talk, we highlight our results and discuss prospects for integrating biological control and chemical control to contribute to the catchment-level management of *C. caroliniana*.

**Impact of the gall-forming rust fungus *Uromycladium morrisii* on the invasive tree *Acacia saligna* in South Africa: 30 years of monitoring**

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First introduced into South Africa in 1987, for the biological control of *Acacia saligna*, the gall-forming rust fungus *Uromycladium morrisii* rapidly spread through the range of its host tree in South Africa. In 1991, ten permanent monitoring sites were set up, each consisting of four transects approximately 10 m apart. Although various sites were destroyed as time went by, by 2020 three of these sites were still being monitored and provide 30 years of continuous data on tree numbers, stem diameter, and gall numbers. Fires that have occurred during these years produced high levels of post-fire recruitment. However, between fires the general trend has been of reduced tree densities. Two of these sites, with high initial tree density, had their densities reduced by 98 and 96% by the end of monitoring. This decline was despite fires occurring within the period of monitoring. The other site, which had a low initial tree density, had a reduction of tree density of only 28% by the end of monitoring. However, this site experienced a fire in 1994 after which tree density was approximately double the initial density, and the tree density declined by 59% from after the fire to the end of the monitoring. Other sites, monitored for shorter periods, show the same trend. Once considered to be potentially the most damaging invasive plant in the Cape Floristic Region, this biological control programme has significantly reduced the impact of this tree.

**Four-trophic level food webs confirm the safety and the neutral impact of a biocontrol agent three years after release**

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Biological control is an essential tool to manage invasive alien species that threaten the conservation of native ecosystems. Post-release monitoring is essential to evaluate the direct and indirect target and non-target effects of biocontrol agents. Nevertheless, rigorous monitoring is still commonly absent or inaccurate. Here, we use an interaction networks approach to unravel the direct and indirect target and non-target interactions of a biocontrol agent for an invasive alien plant widespread in coastal areas. This biocontrol agent is the Australian bud-galling wasp *Trichilogaster acaciaelongifoliae* (Froggatt), used against the invasive *Acacia longifolia* in Portugal since 2015. We compared the structure of empirical four-trophic level food webs assembled before with real webs three-years after the release of the biocontrol agent. After its release, the biocontrol agent has spread to more than 150 km across the Portuguese littoral, and its population has grown exponentially after adjusting to the northern hemisphere seasons, leading to more than 20000 galls in 2020. More importantly, no non-target effects, either direct (on non-target host-plants) or indirect (apparent competition with other galling insects) were detected on the native communities of plants, galls or parasitoids. Additionally, no significant changes were detected on network structure that may be related with the establishment of the biocontrol agent. These results are highly encouraging as they show a rapid establishment of the biocontrol agent with undetected non-target effects on native communities of plants, galls or parasitoids, despite the very large sampling effort implemented. The ultimate effectiveness of the BCA to control *A. longifolia* and restore invaded communities will require future monitoring.

**Integrating classical biocontrol with other control methods for Brazilian peppertree, *Schinus terebinthifolia***

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Integrating classical biological control with other management techniques, such as herbicides, can often be the most effective way to manage invasive plants. However, integrating living biological control agents with other control methods requires special consideration. Control methods that will not harm the biological control agents must be selected, or applied in such a way to reduce negative impacts on the natural enemies. *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae) was released into Florida in 2019 as a biological control agent for Brazilian peppertree, *Schinus terebinthifolia* Raddi (Anacardiaceae), an invasive tree in the United States. While *P. ichini* has established in Florida and is negatively impacting the target plant, biological control alone may not provide enough suppression. Integration with other control methods is likely necessary. We examined the impacts of glyphosate, imazapyr, triclopyr amine, and methylated seed oil applied to *P. ichini* directly and applied to the foliage of plants then fed to *P. ichini*. We also examined plant material that had been treated (basal bark) with triclopyr ester and fed to *P. ichini*. Data showed that thrips survival is significantly impacted by the application of herbicides. However, >75% of thrips survived all treatments up to 120 hours post-application. Only 50% of thrips survived feeding on triclopyr-treated plant material, but thrips demonstrated aversive behaviors toward triclopyr-treated plant material. Due to this aversion, we assessed emigration of thrips from plants treated with triclopyr ester. Results showed that thrips emigrated from herbicide-treated plants to untreated plants and were able to produce offspring, suggesting that under field conditions *P. ichini* are capable of moving from herbicide-treated to untreated plants. These data provide insight into integration of these herbicides and biological control agents in the field.

**Community dynamics in a waterhyacinth, *Pontederia crassipes*, invaded system under integrated management**

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Waterhyacinth (*Pontederia crassipes* Mart.[Solms]) has a worldwide invaded distribution including peninsular Florida and North America's fourth largest freshwater lake, Lake Okeechobee. In Florida, waterhyacinth negatively impacts littoral and limnetic communities, inhibiting the growth of floating aquatic macrophytes and submerged, rooted aquatic plants. To reduce the impacts of waterhyacinth, four biological control agents have been released, three of which are well established in Lake Okeechobee: *Neochetina bruchi* Hustache, *N. eichorniae* Warner (Coleoptera: Curculionidae), and *Megamelus scutellaris* Berg (Hemiptera: Delphacidae). Although biological control significantly impacts biomass and flowering, surface area or acreage covered continues to be problematic and requires repeated treatments with herbicides. Several small mesocosm studies have shown that when biological control is present, herbicide is far more effective for longer time periods and at lower rates. This study was designed to scale up these findings to 24m<sup>2</sup> mesocosm and include common littoral and limnetic community constituents (e.g., *Limnobium spongia*, *Hydrocotyle umbellata*, *Nuphar lutea*, *Nymphaea mexicana*, *Eleocharis cellulosa*, *Sagittaria latifolia*, *Pontederia cordata*, and *Peltandra virginica*). Five treatments were repeated four times within the study: waterhyacinth control (no insects, no herbicide), biological control only, herbicide only, biological control and herbicide, no waterhyacinth. After 12 months, we applied 2,4 – D herbicide in a standard application method (from an overhead spray boom, spray target plants to wet). In all treatments with any control of waterhyacinth, we saw a significant increase in pollinators, floating aquatic plant biomass and area, and submerged aquatic plant leaf area. In treatments with no waterhyacinth control, we found significantly lower dissolved oxygen levels that corresponded to higher fish mortality. Our results suggest that any level and type of control (i.e., herbicide or biological control) yields more positive outcomes for the community, but the use of herbicide when coupled with biological control increases the time between applications (despite plans for subsequent applications, we had insufficient recovery of waterhyacinth in the tanks).

**Biological Control of Weeds in Australia: the last 120 years**

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The development of the field of biological control of weeds in Australia is described, from the first attempts in 1903 to the present day. The interest sparked by the obvious success of the prickly pear program, apparent from 1930-35, resulted in several programs during the next twenty years, followed by a decline in activity until the 1970s when activity increased enormously following the success of the skeleton weed program and the effective use of a plant pathogen for the first time. This momentum was maintained until the beginning of the present century with several successes and was marked by several important advances in genetic profiling, host-specificity testing, economic evaluation, conflict of interest resolution and the ecology of insect/plant interactions, including evaluation of the effectiveness of individual introductions. Biological control has proved to be a valuable and effective approach to weed management in Australia with 39% of all programs considered to produce complete or near complete control, 30.5% partial control and an average benefit-cost ratio of 23:1. Funding for research has been variable with a decline from the late 1990s, but with a significant increase again since 2020.

**Challenges in establishing a new biological control agent, *Aphalara itadori*, against the knotweed species complex, *Fallopia* spp., in the United States**

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The knotweed psyllid (*Aphalara itadori*) from Japan was approved for release in the United States against the knotweed species complex (*Fallopia* [or *Reynoutria*] spp.) in 2020. Field releases of this insect have been made at sites across the country in each year since (under sponsorship of USDA-APHIS-PPQ, the U.S. Forest Service, and state agencies). Here we report on the release and recovery results for a nationwide effort to establish populations of *A. itadori* including 27 releases made in 2020 and 17 made in 2021 that were regularly monitored using timed searches. At best, populations have persisted for up to 2 years following release (4 generations), but many populations fell below detection level before completing a single generation. While the released adults initiate feeding and oviposit abundantly on the field plants, the hatched nymphs exhibit high mortality for reasons only partially understood. We present experimental evidence that ants, which are attracted by the plants extra-floral nectaries, may contribute to nymphal mortality. Other contributing factors may include generalist predators (including mites), desiccation, and the quality of leaf tissue at the timing of nymphal development. A new strain of *A. itadori* originating near the city of Murakami, Japan, is currently under review for a release permit. Evidence from other countries (United Kingdom, the Netherlands, and Canada) suggest that the Murakami strain will have higher nymphal survival because it causes a higher degree of feeding-induced leaf curling, which offers protective shelter.



**High temperatures may limit biological control of Brazilian peppertree in south Texas ecosystem restoration projects**

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Aquatic ecosystem restoration is a major mission of the US Army Corps of Engineers. In southern Texas, USA, restoration of historical waterways of the Rio Grande River Delta, termed resacas, is important to preserve and increase habitat for rare and endemic animals and plants. A large cost component of resaca restoration in these areas is control of the invasive Brazilian peppertree, *Schinus terebinthifolia*. In 2021 we initiated a biological control program of *S. terebinthifolia* in south Texas using the thrips, *Pseudophilothrips ichini* to support ongoing and future restoration efforts. Releases of more than 77,000 thrips since March 2022 have resulted in tentative establishment at most study sites but observations of low summer *P. ichini* abundance indicated that high summer temperatures may be a limiting factor. Therefore, we investigated the upper thermal limits of *P. ichini* and evaluated local (within release sites) and regional weather data (10+ years) in the southern USA, where the majority of *S. terebinthifolia* control efforts are focused. We determined the lower limit of thermal injury zone (LLTIZ) for *P. ichini* by exposing thrips to high temperatures at increasing exposure intervals, then modeling the time-temperature-mortality relationship to identify the upper threshold beyond which thermal injury accumulates. The LLTIZ for *P. ichini* is  $33.05 \pm 0.19^{\circ}\text{C}$ , and exposure to temperatures above that threshold quickly increases the rate of mortality as thermal injury accumulates. By modeling survival using the LLTIZ and actual long-term weather data, we estimated that mean summer survival of thrips would be approximately 30% in south Texas, whereas survival across the entire state of Florida was predicted to be greater than 70%, with most locations much higher. Additionally, temperatures measured within the canopy of our release sites suggest low mean survival, ranging from 4% to 32%. However, microhabitat variation within BPT stands in south Texas may offer a refuge during the most extreme summer temperatures (e.g., survival predicted at the base of trees was up to 80% in one replicate) and allow *P. ichini* populations to recover in the fall each year. Multi-year monitoring is underway to further evaluate seasonal population dynamics of *P. ichini* in the field.

**The pine cone weevil, *Pissodes validirostris* as a potential biological control agent for European pines in South Africa**

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As early as the 1980's South Africa was considering the need to manage invasive alien pine species, particularly *Pinus pinaster*. Due to the success of biological control agents that targeted the seed production of other valuable timber species, such as *Acacia mearnsii* and *Prosopis* spp., researchers considered the possibility of a biological control agent that targeted cones and seeds of *Pinus* spp. In 1997, South Africa received funding to investigate natural enemies that targeted pinecones and seed. Unfortunately, in 2009, concerns about the possible spread of Pine Pitch canker (*Fusarium circinatum*) led to researchers discontinuing the biological control programme. Since then, pines have continued to spread and have dramatically reduced runoff, exacerbating water shortages in south western regions of South Africa. Water managers have calculated that clearing pine trees from catchments is the most cost-effective manner to ensure water security. However, due to the scale, cost and difficult terrain where pines are located, mechanical and chemical clearing options are no longer feasible. Recent engagement with, and resulting support from, South African forestry has allowed for the biological control by the Iberian ecotype of the cone weevil, *Pissodes validirostris* to be revisited. Initial host exclusion tests and choice tests showed that: (i) adults will use a range of pine hosts during maturation feeding; (ii) females under caged-conditions will penetrate and deposit eggs on pine species other than *P. pinaster*; (iii) there is incomplete development of larvae, and hence cone damage, on some of the pinus species on which eggs were laid; (iv) larvae from the Iberian haplotype of *P. validirostris* only survived to become adults on *P. pinaster*, *P. pinea* and *P. halepensis*, confirming that this haplotype is specific to this group of closely-related Mediterranean pine species. Despite these tests, Forestry has requested further research, specifically into underlying mechanism driving *P. validirostris* specificity. Therefore, we aim to investigate the plant volatiles associated with the different pines during pine cone production in an attempt to determine if plant volatiles are driving host selection and oviposition of *P. validirostris*. Initial results suggest volatiles are important in determining host preference. This project highlights the importance of stakeholder engagement as a key success factor in biological control programmes.

**Establishment of the gall-forming rust fungus *Uromycladium woodii* on *Paraserianthes lophantha* in South Africa**

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Permission to introduce the gall rust fungus *Uromycladium woodii* into South Africa against the Australian tree *Paraserianthes lophantha* was granted in late 2015. The first releases were made in 2016, and since then this rust established at 20 sites in the south-western and southern regions of the Western Cape Province of South Africa. From these sites, it is now successfully spreading and has established at localities up to 30 km distant. Once established, high levels of mortality have been recorded within 3 or 4 years of first inoculations. In some cases 100% of all standing trees have died. Although *P. lophantha* is currently a relatively minor environmental weed in South Africa, it is widespread with a persistent soil-stored seed-bank. These properties allow it to establish rapidly following fires, and it has at times been considered a significant environmental weed within certain areas. The introduction of *U. woodii* has ensured that this plant will not become more significant in the future.

**Establishment, spread and early impacts of the first biocontrol agent against an invasive plant in continental Europe: A comprehensive assessment**

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Classical biocontrol is seen as pivotal for the successful management of invasive alien plants, but it is still a relatively new approach in Europe. Post-release monitoring is critical to evaluate the effectiveness of biological control agents, but it is often neglected. Here, we report the detailed post-release monitoring of the Australian bud-galling wasp *Trichilogaster acaciaelongifoliae* (Frogatt) (Hymenoptera: Pteromalidae) and its effects on *Acacia longifolia* (Andr.) Willd; this is the first biocontrol agent intentionally introduced against an invasive alien plant in continental Europe. Preceded by its recognized success in South Africa, this biocontrol agent was released in Portugal in 2015, at several sites along the Portuguese littoral. The establishment, spread and early impacts of *T. acaciaelongifoliae* on target-plants in Portugal, were monitored across 61 sites from 2015 to 2020. Initial release of adults from South African galls and the subsequent releases from galls established in Portugal (2018 onwards) was compared, assessing the implications of the hemisphere shift. The impacts on the reproductive output and vegetative growth of *A. longifolia* were evaluated in more detail at three study sites. Until 2019, 3567 wasps were released at 61 sites, with establishment confirmed at 36 of them by 2020. After an initial establishment success, *T. acaciaelongifoliae* adapted to the northern hemisphere conditions and experienced an exponential growth (from 66 galls in 2016, to 24000 galls in 2018). Galled *A. longifolia* branches produced significantly fewer pods (84.1%), seeds (95.2%) and secondary branches (33.3%) and had fewer phyllodes but more growth of the main branch compared to un-galled branches. Despite the phenological mismatch and adverse weather conditions, *T. acaciaelongifoliae* overcame the hemisphere shift and is now successfully established in the northern hemisphere. To achieve this, it had to synchronize its life cycle with its host-plants' phenology, after which it developed exponentially and began to show significant impacts on the reproductive output of the target plant.

**Assessing the establishment of two biological control agents of Old World Climbing Fern, *Lygodium microphyllum***

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Old World Climbing fern (*Lygodium microphyllum*) is an invasive climbing weed that causes extensive damage by disturbing natural fire regimes, collapsing Everglades tree island canopies, and outcompeting native vegetation. Two biological control agents have been tested for safety and permitted for release by TAG petition. Monitoring the establishment of these agents is imperative for understanding the effects of both *L. microphyllum* and the biological agents in Florida's ecosystems. To evaluate the persistence of both the brown Lygodium moth (*Neomusotima conspurcatalis*), and the Lygodium mite (*Floracarus perrepae*), monthly monitoring was conducted at 5 different sites for 4 years. Ten (10) Pheromone traps along 5-meter transects, and 15-minute visual timed surveys were used to monitor the presence of the brown Lygodium moth, *N. conspurcatalis*. Random samples collected along two transects per site were examined for damage caused by *F. perrepae*. Mid-project we began measuring percent cover of *L. microphyllum* using 1-m<sup>2</sup> quadrats. We found that populations fluctuate interannually. During specific times of the year, each biological control agent species will independently experience population spikes. These population spikes do not correspond to *L. microphyllum* coverage or any single environmental factor but may be a combination of environmental factors including temperature and day length. Quarterly monitoring will continue indefinitely.

**Baseline study for implementing biological control of aquatic weeds in urban wetlands of Resistencia, Chaco, Argentina**

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Urban wetlands are essential because they provide high biodiversity and valuable ecosystem services. In the northeast of Argentina (NEA), particularly in Resistencia city, these wetlands have the crucial role of mitigating floods, however weed invasion can strongly interfere with this ecosystem service. Mechanical removal is currently applied as the unique management method, but this strategy is expensive, and does not provide a long-term solution. Thus, the application of biological control emerges as an alternative to improve weed management. The aim of this study was to carry out a baseline study for implementing biological control, assessing weed invasion as well as biological and physicochemical parameters related to water quality, comparing two urban wetlands in Resistencia City: the Argüello and Francia lakes. Sampling was carried out during June and July 2022. We assessed the invasion status of target weeds, the abundance of biocontrol agents and their natural enemies, as well physicochemical and biological parameters influencing water quality. Our results showed that the water lettuce, *Pistia stratiotes*, was the most abundant and problematic weed in both urban lakes. Water lettuce plants were more vigorous and had a higher reproductive rate in Lake Argüello than in Lake Francia. We found two biocontrol agents associated with water lettuce: *Neohydronomus affinis* and *Lepidelphax pistiae*. Abundance of *N. affinis* and other weevil species was only 11 individuals/m<sup>2</sup> in Lake Argüello, while in Lake Francia 260 insects per m<sup>2</sup> were recorded. *L. pistiae* was only found in Lake Argüello and its abundance reached 42 individuals/m<sup>2</sup>. Abundance of potential natural enemies (Araneae and Braconids) in relation to biocontrol agents tended to be similar in Lake Argüello, whereas in Lake Francia biocontrol agents were 3.8 times more abundant than their natural enemies. Data from physicochemical and biological parameters evidenced contrasting conditions of water quality in both lakes, mainly regarding nutrient content. Our preliminary results suggest that augmentative biological control will require different management strategies and biocontrol insect abundance in both lakes and that in Lake Argüello, these actions should also be complemented by treatments tending to minimize nutrient inputs in water.

### **Spread and impacts of *Tectococcus ovatus* for biocontrol of strawberry guava**

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Impacts of a biological control agent for strawberry guava (*Psidium cattleianum* Sabine; Myrtaceae) have unfolded gradually since it was first released in Hawaii in 2012. Long term monitoring of the Brazilian leaf galling insect *Tectococcus ovatus* Hempel (Hemiptera: Eriococcidae) in demonstration plots reveals suppression of strawberry guava stem growth, leaf cover and fruiting after a period of several years of sustained galling. Impacts vary with tree genotype: although Hawaii's three varieties of strawberry guava are all susceptible to galling, effects have been slower to appear on one variety. In native forest sites across the state, *Tectococcus* has been successfully established and is able to persist and spread in a variety of habitats. Techniques for establishing the biocontrol agent, which disperses as minute eggs and nymphs via crawling or wind, have been refined, simplified, and communicated to managers over time. Natural dispersal of *Tectococcus* has been documented at Hawaii island field sites, averaging 50 to 400 m per year, primarily in the direction of prevailing trade winds.

To attempt widespread suppression of strawberry guava across forested landscapes within the next two decades, broad-scale artificial dispersal of *Tectococcus* to remote forest sites is needed. Aerial methods under development with partners include using a drone for precise placement of the biocontrol onto tops of guava canopies. Similarly, dropping *Tectococcus* from a helicopter has shown promise in preliminary testing, but with less precision in placement. As aerial deployment methods are refined, we expect that they will allow efficient establishment of the biocontrol within a few years across large expanses of difficult to reach forested watersheds.

**Biological control of aquatic weeds: seeking sustainable solutions for the recovery of urban wetlands in northeastern Argentina**

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Urban wetlands are a critical part of urban developments because of the ecosystem services they provide. However, many of them are seriously affected by the presence of invasive plants, as is the case of several urban ponds in northeastern Argentina (NEA). Increases in the biomass of these weeds produce physical and chemical alterations that affect water quality. Also, because of their high reproductive capacity, these weeds displace the rest of the plants, and associated fauna, that coexist with them. This leads to a loss of biodiversity and habitat complexity that alters the structure and functioning of the invaded wetland systems. From an urban point of view, weed invasions represent a relevant problem, since they affect flood buffering capacity. In Argentina, the most harmful aquatic weeds in the world are represented, including, for example, the water lettuce *Pistia stratiotes* (Araceae). Different management strategies are often applied to mitigate the invasion; however, they rarely achieve sustained control. Mechanical control can be ineffective for large areas and is highly costly since fragmentation or seed banks prevent this control from lasting over time. Similarly, chemical herbicide control is also unsuccessful and environmentally unsustainable because it depends on continuous application to control reinfestations, and may be harmful to human health or to biological control agents released as part of integrated management programs. In view of this scenario, a research project has been designed to apply biological control in invaded urban ponds in the NEA, through the introduction of highly specific herbivorous insects, as a harmless, low-cost and sustainable tool. This project will be carried out in collaboration between different research groups in Argentina (HeCoB-CECOAL and FuEDEI) and is intended to: (a) assess the current impact of the weed *Pistia stratiotes* in different ponds of the NEA, (b) perform bioprospecting of natural enemies and potential biocontrol agents of the weed in the field, and (c) conduct experiments to determine the optimal density of agents needed to achieve control. This study will generate knowledge that will contribute to the reduction of *P. stratiotes* invasion levels and the consequent restoration of the ecosystem balance.



**Integrating chemical and biological control for management of alligator weed,  
*Alternanthera philoxeroides* (Amaranthaceae)**

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Alligator weed (*Alternanthera philoxeroides*) is a wetland plant that is invasive in many regions globally. Native to the Paraná River valley of South America, alligator weed has extensively invaded Australia, China, New Zealand, and the United States, and its range is still expanding. The history of alligator weed management is longest in the United States, and chemical and biological control mechanisms are critical tools for plant reduction. Alligator weed is almost always treated with foliar herbicide applications because some biological control agents (*Agasicles hygrophila*) cannot survive cold temperatures in all parts of the invaded range. On the other hand, the alligator weed thrips (*Amynothrips andersoni*) can survive cooler temperatures, but its response to herbicide use and impact on alligator weed reduction are relatively unknown. Alligator weed is sensitive to foliar herbicide treatments, but populations often regrow after treatment due to their extensive networks of stolons. Alternatively, many herbicides that are effective at controlling alligator weed can also be applied to the system as submersed injections. Submersed herbicide applications are available for root uptake and may provide better control than foliar treatments. We tested five herbicides (bispribac-sodium, fluridone, imazamox, penoxsulam, and topramezone) as submersed treatments at two different rates on alligator weed grown in mesocosms in both outdoor and greenhouse conditions. These herbicides have different modes of action targeting different metabolic pathways in plants suggesting they may interact differently with thrips damage to alligatorweed. Our results found multiple herbicides that are highly effective at reducing alligator weed biomass. Our continuation of this study will use our herbicide efficacy data to integrate thrips biocontrol with chemical control to more effectively manage invasive alligator weed.

## THE FUTURE IN BIOLOGICAL CONTROL

Moderator: Kumaran Nagalingam (CSIRO, AU)

Oral presentation

### Weed biological control: Unlocking the future

**Keynote speaker:** McConnachie Andrew J.

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Weed biological control (biocontrol) is an evolving science with a long and distinguished history, having been practiced in several countries for well over 100 years. The discipline has progressively been refined through the adoption of new approaches, not only from a methodological point of view (e.g., improvements in host-range testing), but also from an efficiency/innovation perspective (with the incorporation of novel technologies). Here, I consider several methodological and innovative approaches that have shaped/are shaping the future of weed biocontrol. Selection of weed targets has become more challenging. Contemporary prioritisation frameworks are playing a progressively important role in providing robust, defensible approaches to allocating funding and research efforts for weed targets. Host-specificity testing has seen dramatic improvements over the years. This approach was first adopted in the 1920s to test mainly agricultural species (e.g., for *Opuntia* spp.) and was regularly used from the 1950s, with the first inclusion of choice testing in the 1960s. Coupled with these advances was the refinement of test plant lists following the centrifugal phylogenetic approach (1970s) and the modernization thereof incorporating degrees of phylogenetic separation (2000s). Current developments in digital technologies also support the selection of test plant lists through the development of phylogeny visualization interfaces, producing phylogenetic trees (gathered from the latest gene sequences available) that can be overlaid with other data (relative phylogenetic distances, geographical overlap between species, status as introduced or native, horticulturally useful species). High resolution remote sensing datasets, coupled with machine learning algorithms, allow the identification of weeds in the landscape, while multispectral and hyperspectral imagery are proving to be useful tools in monitoring weed stress due to biocontrol agent activity at a larger scale. The emerging genetic biocontrol approaches (techniques that alter the genetic material of a target organism to control its invasion in the environment) hold significant potential. Embracing gene technologies such as 'gene drive' and 'gene silencing' is gaining momentum in exposing a variety of pathways for the sustainable management of weeds. While established weed biocontrol methods are important in evaluating agent potential and success, meeting needs into the future will require commitment by researchers to embrace and translate new technologies into robust weed management solutions.

**Is inundative classical biocontrol the future for the most difficult weeds?  
The water hyacinth saga**

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Water hyacinth, the world's worst aquatic weed, plagues many of South Africa's waterbodies, and has been the subject of extensive research for decades. Considerable research has focused on understanding the bottom-up effects on top-down regulation of this weed. Nutrients, the main bottom-up driver, result in enriched waterbodies that promote the growth of water hyacinth, to the detriment of control measures, including biological control, the most important top-down driver. South Africa has released more control agents against water hyacinth than anywhere else in the world, however, when water bodies are as polluted as South Africa's are, the efficacy of biological control is reduced. Cold winter temperatures in the country's interior also impact the level of control. In recent years, our approach to biological control has shifted from classical biological control to inundative control, with the support of local stakeholders at invaded sites, in an attempt to increase the top-down pressure from herbivory on water hyacinth. Inundated (or integrated) release of a planthopper, *Megamelus scutellaris*, has provided spectacular results since its release in 2018 at Hartbeespoort Dam, a system plagued by water hyacinth for decades. Water hyacinth cover was reduced from almost 50% (>800 ha) to less than 5% in 2020, through herbivory alone. This pattern was repeated following spring time germination the subsequent growing seasons in both 2021 and 2022, as a result of inundative releases of the hopper from mass rearing facilities.

**Using chemical ecology to enhance weed biological control**

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The use of semiochemicals for pest management programs have become widespread in agricultural systems and have resulted in multiple success stories. However, few semiochemicals are being utilized to improve the performance of weed biological control agents. In this presentation we describe strategies to deploy semiochemicals to manipulate and modify the behavior of weed biocontrol agents, highlighting a case study with the northern tamarisk leaf beetle *Diorhabda carinulata*. Additional studies successfully utilizing semiochemicals to establish agents, increase monitoring efficiency, and enhancing damage potential will also be discussed. In addition, we highlight the application of semiochemicals in weed biological control and call for more widespread adoption and incorporation of chemical ecology into weed biological control programs.

**Microbial genome and community sequencing identifies new potential agents for  
invasive weed biological control**

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Introduced weeds encounter diverse resident microbes during invasion, and successful invasive weeds are expected to form microbial communities through novel association with beneficial and commensal fungi and bacteria. Manipulating these communities or their individual members may improve microbial-based biocontrol practices. Fungal and bacterial communities from two invasive weeds in North America, garlic mustard (*Alliaria petiolata*) and wavyleaf basketgrass (*Oplismenus undulatifolius*), were characterized with community sequencing and whole genome sequencing-based methods. High-throughput, low-depth genome sequencing identified microbial isolates with putative biocontrol applications, and metabarcoding sequencing of bulk microbial communities identified conserved taxa and features that could be leveraged for weed management. The microbes and community structures identified using these methods are proposed for functional characterization and studies involving the manipulation of microbial gene expression to antagonize weed hosts or induce dysbiosis that prevents beneficial microbial community formation.

**Evaluation of a Standardized Impact Monitoring Protocol (SIMP) Pre and Post Release Assessment Tool in the USA**

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A common criticism of biological weed control programs in the USA is the lack of post-release assessments that demonstrate quantitative effects of biological control agents on target weed density, population biology, and/or vegetation response. While quantitative assessments exist, overall conclusions are limited in scope by the spatial scale covered, duration of study, potentially confounding factors, or weed parameters measured. USDA APHIS, the regulatory agency in the USA responsible for biological control agent releases, requires post-release impact monitoring of target weed populations as part of the release permit issued prior to biological control introductions, however, large scale, long-term impact assessments rarely occur for a variety of reasons. To address this problem, a standardized impact monitoring protocol (SIMP) was developed in 2007. The intent was to collect ecological data that are sufficiently robust for quantitative analysis whilst minimizing time spent in the field in an effort to make data collection more manageable. A SIMP collection program has since been implemented for an expanding set of biological control systems in the State of Idaho, and there are now select data sets spanning a fifteen-year period. SIMP data are collected along permanent transects and include weed, categorical vegetation community and biocontrol agent population data. The combination of factors recorded are well suited for dynamic population modeling and can be coupled with environmental factors (e.g., climate or soil type) to analyze how processes like climate (and climate change) can drive variation in weed biocontrol agent interactions. SIMP data can be combined with more detailed plant and/or biocontrol agent surveys in similar ecotypes to add analytical data and observed data trends. Finally, SIMP transects have also been deployed for invasive plants for which biological control agents have not yet been released to provide weed and community vegetation data prior to the introduction of a new biological control agent. Ideally, SIMP transects would be set up across the distribution range of an invasive plant to provide consistent data to generate predictions and identify patterns and gaps in biological control management. Greater coordinated adoption of SIMP across biological control programs in the USA would bridge the persistent gap between pre-release - early program development and post-release implementation success.

**Enhanced detection of the leaf beetle *Gastrophysa atrocyanea* using UV fluorescent pigment in no-choice tests in the laboratory**

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Using whole plants in host specificity testing is critical in determining the host range of biocontrol agents. However, it is unfeasible to design experiments using potted whole plants in some insect systems, and it is often difficult to locate the released insects when using whole plants. We developed a methodology based on ultraviolet (UV) fluorescent pigment to enable rapid spotting of released insects. We applied the fluorescent pigment on the elytra of the leaf beetle, *Gastrophysa atrocyanea*, and used the UV light to detect this agent released onto the potted plants of *Rumex acetosa* (target weed) and *Buxus sinica* var. *insularis* (non-target plant) in the laboratory. The UV application reduced the searching time by 2.7 times and the proportion of missing female leaf beetles by 3.5 times compared to visible light on the target weed. A similar pattern was also observed on the non-target plant under no-choice tests. Additionally, searching time to detect female and male leaf beetles was reduced by 4.8 times on *R. acetosa* using the UV application. We discuss the feasibility of using the UV mark and recapture method in whole-plant testing.

**Increasing production of a univoltine internal-feeding weevil for release: controlling voltinism, infestation rate and using artificial diet**

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When a new biological control agent is approved for release, the next step is to multiply the small population that is brought out of quarantine. For an agent that normally has one generation per year and that develops inside its host plant this presents significant challenges. The weevil, *Ceratapion basicorne* develops in rosettes of the yellow starthistle (*Centaurea solstitialis*), a winter annual. The weevil oviposits in rosette leaves in early spring, larvae mine down to the upper root, where they complete development and pupate. New adults emerge in early summer and remain in reproductive diapause until the following spring. We have made several advancements to manipulate the diapause and reproduction in order to mass produce weevils; 1) We found that exposure to cold (5°C) for 11 weeks was sufficient to induce 95% of females to start ovipositing. Shortening the hibernation period enabled us to produce two generations per year in the laboratory. 2) Emerging adults can be maintained at cold temperature (refrigerator, 5-7°C) for long periods (up to 2 years), and thus can be stockpiled for release in spring. 3) We were able to induce females to begin ovipositing by treating them with methoprene, a juvenile hormone analog (1 µg applied topically). The combination of applying methoprene 2 days after 20-hydroxyecdysone (20E) induced higher fecundity (1.5 eggs/day) compared to methoprene alone (1.0 eggs/day), or no treatment (0.2 eggs/day). 4) We developed a methodology to rear the weevil on potted host plants by caging individual females on leaves to oviposit, moving them to a new plant every 1-2 days. 5) We also developed a larval transfer method to better control the number of larvae developing in a plant, thus reducing cannibalism. Females were held in a small container to oviposit on cut leaves, then eggs were harvested and isolated on moist filter paper in a Petri dish to complete development. Neonate larvae were transferred into an incision in the leaf petiole on a potted plant. This approach made more efficient use of the limited number of eggs available. 6) In order to reduce costs, we are developing an artificial diet to eliminate the need to use plants to rear larvae. We will discuss how the above advancements facilitated the mass production of *C. basicorne* throughout the year.



**Advances in the rearing of two biological control agents against common reed,  
*Phragmites australis***

Häfliger Patrick<sup>1</sup>, Stutz Sonja<sup>1</sup>, Cloșca Cornelia<sup>1</sup>, McTavish Michael<sup>2</sup>, Bouchier Robert<sup>2</sup>, Weyl Philip<sup>1</sup> & Hinz Harriet L.<sup>1</sup>

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European common reed, *Phragmites australis*, has become invasive throughout much of North America, displacing native endemic *P. australis americanus* genotypes. This invasion puts at risk endemic wetland plants and animals, including threatened and endangered species. After 20 years of research on host range testing, life history, impact and behaviour of nine potential agents; the release of two agents, *Lenisa geminipuncta* and *Archanara neurica* (Lepidoptera: Noctuidae) was granted in Canada and recommended for the US. Both species are univoltine. The eggs overwinter under *P. australis* leaf sheaths and hatch soon after the shoots begin to elongate in early spring. Both species have severe impact on plants and early attack can result in stem mortality, while later attack causes wilting of tips. Rearing these biological control agents on cut stem sections is an effective method but very labour intensive and time consuming, and thus not ideal for mass rearing. The aim of this project was to develop more efficient rearing methods using artificial diet. Initial studies using the McMorran artificial diet successfully produced adults of *A. neurica*, however they were not as fit as insects produced on cut stems. *Lenisa geminipuncta* were not able to complete development on diet alone. However, combining both methods, i.e. rearing early instar larvae on stem sections and later instar larvae on artificial diet, proved to be more successful and less time consuming. Using this method, we are currently able to produce over 20'000 eggs and up to 1'000 pupae per year, while on stem sections alone we only produced less than 10'000 eggs and a few hundred pupae. To date, a total of 17'600 eggs, larvae, pupae, or adults have been released at 13 sites in southern Ontario, Canada. Initial survivorship, damage and overwintering results for the insects are very encouraging and monitoring is continuing to confirm establishment.

**Weed biocontrol at the USDA-ARS, Foreign Disease-Weed Science Research Unit**

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Research on the discovery and development of microbial-based biological control agents at the Foreign Disease-Weed Science Research Unit (FDWSRU) employs conventional, molecular, and technology-driven approaches. The combination of these approaches enables FDWSRU to partner effectively with other researchers, refine existing biocontrol methods, and explore novel biological-based solutions to address the challenges of invasive plant management. While foreign plant pathogens evaluated as classical biocontrol agents at the FDWSRU during the last four decades continue to be released, potential applications for endemic microbial communities based on plant-microbe ecology expands the range of available biocontrol agents. Potential sources of plant antagonistic microbes and mechanisms capable of reducing invasive plant fitness are being explored through advanced technologies in microbial genomes and community profiling. Parallel investigation into the application of novel technologies for weed control will produce new genetics-based tools that augment microbial-based classical biological control methods. The integration of these varied approaches will contribute to sustained improvements in biological control programs.

## COMMUNITY ENGAGEMENT AND EDUCATION

Moderator: Alejandro J. Sosa (FuEDEI, AR)

Oral presentation

### **Building social licence and support for weed biocontrol: what I wish I had known 30 years ago!**

**Keynote speaker:** Hayes Lynley M.

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For weed biological control programmes the consequences of a lack of attention to adequate community engagement can range from major to minor. The best-case scenario is that released natural enemies control weeds anyway, without community support and awareness. But that risks missing out on the recognition that biocontrol can be safe and successful, which can make it difficult to secure support and funding for further work. A lack of openness and transparency with communities also risks eroding trust, which may not be that high to begin with since trust in science is generally declining. The worst-case scenario is that major opposition can be triggered, resulting in projects being severely disrupted, delayed or even stopped. Although it seems obvious that adequately resourced efforts to build and maintain social licence to operate, and support for weed biocontrol generally, should be a routine component of all biocontrol programmes, I reflect on why it can be hard to do this in practice. I draw on activities we have undertaken in New Zealand, and the wider Pacific, to engage with communities over the past 3 decades, and consider lessons learnt. Some approaches that have proven useful, and tips for navigating some of the challenges, will be shared. I conclude that efforts to build and nurture a support base, that includes local champions, are unlikely to be wasted. Also proactively engaging constructively at an early stage with groups who fear loss if a biocontrol project goes ahead is recommended.

## **Bugs 2 the Rescue: a citizen science project**

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Citizen science, the intentional engagement of the public in scientific research to increase knowledge around a scientific endeavor, plays an important role in collecting environmental data. Citizen science has already a rich tradition in the inventory of invasive alien species (IAS). At the same time, it can improve communication with the public in ways that can bring about changes in participants' knowledge, skills and attitudes. This is especially needed with regards to classical biological control (CBC) as a management method for invasive weeds. CBC has been practiced worldwide for over 100 years in several regions of the world with a high success rate and cost-benefit ratio but the EU is an exception. The prime reason for this reluctance towards CBC is to be attributed to a general lack of knowledge about it, combined with a high risk-aversion amongst policy makers, the general public and advisors in conservation. The topic of biological invasions has only recently caught the attention of environmental educators. To support the inclusion of IAS in education, we need to develop and implement novel, user-friendly educational materials. In that view, we developed the Bugs 2 the Rescue (B2R) project (<https://www.bugs2therescue.be/>). B2R is a citizen science project that unites researchers, nature volunteers, the local nature and water sector and schools. B2R focuses on three goals: (1) it creates awareness about aquatic invasive plants and biological control, (2) it enables innovative data collection on invasive weeds and insect damage on them and (3) it links schools with citizen science. For the project we developed a Do-It-Yourself kit with field material and a complete lesson package but all the free material is adaptable to the user's own needs or interests. Further it also uses gamification elements and smartphone apps, which have been highlighted to be effective tools for environmental education. To date 5 schools participated in the full project, 50 student-teachers were trained in the field techniques, the lesson package was downloaded > 120 times and the website has 3500 unique visitors. B2R was also present at different science events and had a broad media coverage by which we reached more than 50.000 citizens.

**Perceptions of land-users on the efficacy, benefits and safety of biological control of three invasive alien Cactaceae in South Africa**

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Invasive alien Cactaceae threaten indigenous biodiversity and have negative impacts on agricultural productivity in South Africa. *Opuntia aurantiaca* Lindley, *Opuntia monacantha* Haworth and *Opuntia stricta* (Haworth) Haworth are three of the most problematic invasive alien cactus species. Biological control is regarded as a safe and effective management tool used to reduce the density and negative impacts of these cactus species. The Centre for Biological Control (CBC) mass-rears and releases biological control agents against the targeted invasive alien cactus species throughout South Africa as a government initiative. The aim of this study was to evaluate the efficacy, benefits, and safety of the releases of these agents through the perceptions of land-users. Sixty-two land-users who had received biological control agents for these three cactus species were interviewed with a standardised questionnaire. Most land-users perceived biological control as an effective management option, 70% of land-users reported a decrease in the target weed after release of the agents, and 60% of land-users believed that the reduction in cactus weed densities had resulted in environmental or agricultural benefits. Overall, 95% of the land-users regarded biological control as safe; and said that there had been no non-target effects observed. Eighty-nine percent of the land-users would recommend biological control to other land-users. Biological control of invasive alien plants is a long-term management solution that takes time to show desired results. In many cases, the biological control agents that were released had not had enough time to reach the maximum level of control, so it is anticipated that more of the land-users will observe the benefits of biological control in future. This study has confirmed the efficacy and safety of biological control as a management intervention against these three serious invasive alien plants.

### **Public knowledge on invasive alien plants and biological control in the southern hemisphere**

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Invasive alien plants (IAPs) are one of the causes of massive biodiversity loss and ecosystem imbalances and consequently involve high management costs. Currently, the importance of the social dimension within the problem of IAPs is increasingly recognized, since the inclusion of public opinion and participation is crucial to decelerate the spread of IAPs as well as to demand action from decision makers. Likewise, social perception of IAPs has also a big impact on the success of biocontrol programs. In this study, we assessed i) social perceptions of IAPs and Biological control in four countries of the southern hemisphere, with contrasting situations regarding IAPs management (longer vs shorter history of biological control of weeds applications), and ii) public awareness of the invasion of *Iris pseudacorus* L (Iridaceae), a European IAP that causes major impacts on wetlands of all the evaluated countries. By sharing a questionnaire on social media, 1527 responses were obtained from volunteers from South Africa, New Zealand, Argentina and Uruguay. Respondents from Argentina and Uruguay were less familiar with the terms IAP and Biological Control. Likewise, less than half of them knew of examples of the implementation of biological control against weeds in their country. This number was significantly higher in South Africa and New Zealand. There were also considerable differences between countries in terms of public awareness of the alien invasive status of *I. pseudacorus*, which was lower in respondents from Argentina and Uruguay. This fact becomes more relevant when considering that Argentina and Uruguay are countries with a higher level of invasion and impacts from this plant than South Africa. The greater knowledge about IAPs and biological control in countries such as South Africa and New Zealand can be related to their longer weed biological tradition. Our results show contrasting situations regarding social perception of IAPs and biological control among countries. Likewise, they allow us for a first diagnosis regarding public awareness of those topics, which can be used to demand more investment on environmental education regarding IAPs and biological control in the studied South American countries.

### **Community mass-rearing of waterweed biocontrol agents reaps rewards**

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Biological control is a sustainable, affordable, and eco-friendly alternative to chemical means of controlling invasive species. The ultimate aim of biological control is to get land users to take responsibility for controlling their weeds, but they often are not aware of how to do this, or feel disempowered to implement this science. The Centre for Biological Control (CBC) has developed appropriate strategies to engage communities and empower them to implement weed biological control in South Africa. The CBC offers support in identifying invasive species and gives advice for implementing biological control programmes. The CBC is active on social media platforms and has produced informative documents about the relevant invasive species and their biological control agents which are shared with various stakeholders. Along with this advice offered by the CBC, the recommendation is often to rear the biological control agent near the site of invasion to ensure the frequent and inundative release of agents, particularly after the cooler winter months when agent field populations are low. With over 14 aquatic weed control agent mass-rearing stations set up in the vicinity of invaded sites, and tens of thousands of agents released onto systems, this approach has improved establishment of agents and control at invaded sites where control was usually limited by eutrophication, and cool winters. While the importance of biological control is widely acknowledged, research on how stakeholders involved perceive and value biocontrol as a control method has been lacking. We conducted surveys with community members who are involved with the mass rearing and release of biological control agents and found that the respondents gained a greater understanding of the science and practice of biological control. Respondents valued the process of biological control as a result of first-hand experience with implementation.

**Taking biological control to our communities – use of a rust fungus for the control of *Impatiens glandulifera* (Ericales: Balsaminaceae) in Great Britain**Varia Sonal<sup>1</sup>, Thomas Sarah E.<sup>1</sup>, Wood Suzy V.<sup>1</sup> & Pollard Kathryn M.<sup>1</sup><sup>1</sup>CABI, Egham, Surrey, UK

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The implementation of classical biological control (CBC) in Europe has significantly lagged behind that of the rest of the world and to date only five agents targeting five weeds have been released. Of these, only one is a fungal pathogen, *Puccinia komarovii* var. *glanduliferae* (Pucciniales), first released into Great Britain (GB) for the CBC of Himalayan balsam, *Impatiens glandulifera* (Balsaminaceae) in 2014. Originally introduced as an ornamental plant, Himalayan balsam escaped cultivation and forms dense monocultures along riverbanks, woodlands and disturbed areas across the country, with negative effects across whole ecosystems widely reported. Whilst control of isolated infestations of Himalayan balsam can be achieved using mechanical or chemical methods, both are labour intensive, costly and can be environmentally damaging. A rust-release programme first commenced in 2015 and to date, the rust has been released at over 80 sites across GB. With increasing interest in rust releases and decreasing governmental funding, an improved release strategy was developed to enable a community approach; with site selection, rust release and monitoring taking place with the assistance of local action groups (LAGs). This technique has greatly enhanced both the number and distribution of release sites across the country. Following training by CABI staff, the rust is released three times over the growing season and infection regularly monitored by LAGs. Monitoring takes place in the field using a questionnaire with answers recorded directly into ODK, an open-source data collection platform, and photographs of infected plants and leaves taken. Images are processed using the software ImageJ, enabling a more accurate estimation and comparison of rust infection at each site, where clear photos are taken. Clear and concise instruction is essential, and reminders are necessary to ensure timely releases and monitoring. Whilst there is much interest in the CBC programme, public awareness is still lacking and sometimes met with opposition; in particular from beekeepers, who value stands of Himalayan balsam as a late source of nectar. Expectation management is essential, with the impact of the rust likely only being observable after 5-10 years, in comparison to the “immediate” impact observed by manually removing plants. As a result, a number of release sites have been inadvertently destroyed by local volunteers keen to remove the plant. Nevertheless, in general, infection at sites remains high with the rust frequently overwintering to re-infect seedlings the following year.



**The Gall's Grail: Unlocking the establishment monitoring of *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae) through citizen science in Portugal**

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Post-release monitoring of biocontrol agents is essential to demonstrate the utility of biocontrol within a broader integrated management strategy of the target invasive plant. Such assessments can evaluate the establishment, spread, effectiveness and safety of the biocontrol agent. Unfortunately, long-term post-release evaluation programs are often neglected or underfunded. Citizen science has arisen as a valuable approach to contribute to post-release monitoring assessments. Especially in countries where biocontrol is seldom used, engagement of citizens can additionally raise awareness and improve people's perception of biocontrol. However, engaging people is not an easy task, especially when the target species is inconspicuous, commonly unseen and unnamed by lay people. Indeed, this has been a challenging task in Portugal concerning the biocontrol agent *Trichilogaster acaciaelongifoliae*, a small bud-galling wasp that targets the invasive *Acacia longifolia*. Since its release in 2015, this agent established and its populations exponentially increased along the Portuguese coast, making it virtually impossible to monitor its spread by scientists alone. Therefore, researchers of the Centre for Functional Ecology, University of Coimbra, and Agrarian School of the Polytechnic Institute of Coimbra launched a citizen science campaign in March 2020. Initially a fully customized and dedicated form was created in Epicollect5 app ("Registo de *Trichilogaster acaciaelongifoliae*"). Soon iNaturalist/BioDiversity4All became also used by citizens, and a new project ("*Trichilogaster acaciaelongifoliae* in Iberian Peninsula") was created on this platform. Even considering the high specificity of the Epicollect5 project, in the first two years, 54 users submitted 2557 records of the biocontrol agent. Three years later, and considering all information sources, 153 users submitted 4479 records corresponding to more than 2 million estimated galls. Epicollect5 is the preferred platform with 48% of users, followed by iNaturalist/BioDiversity4All with 36%, and other sources such as email and social networks (namely Facebook) with only 16% of users. As the populations of *T. acaciaelongifoliae* keep increasing and spreading, the participation of citizen scientists is expected to continue to increase, allowing a more complete follow-up of the establishment and effects of this biocontrol agent. Although the number of galls has risen dramatically through time, one should keep in mind that these results are underestimated. Nevertheless, this citizen science project facilitates the monitoring across the entire country by engaging local communities that know the field sites better and can reach areas that would be very difficult to reach by land managers and scientists.

### **Education, outreach and training of human resources to promote weed biological control in Argentina**

Jiménez Nadia<sup>1</sup>, Faltlhauser Ana C.<sup>1,2</sup>, Righetti Tomás<sup>1,2</sup>, Franceschini M. Celeste<sup>3</sup>, Cecere Carla<sup>2,4</sup>, Daddario Juan F. F.<sup>5,6</sup>, Villamil Soledad C.<sup>6,7</sup>, Fernández Souto Adriana<sup>8</sup>, Hill Martin P.<sup>9</sup>, Mc Kay Fernando<sup>1</sup>, Cabrera Walsh Guillermo<sup>1</sup> & Sosa Alejandro J.<sup>1,2</sup>.

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Public awareness is an important component to prevent introduction or resurgence of invasive alien species (IAS). A positive public perception of biological control is essential to support and demand that policy makers implement it in management programmes. Public visibility and research on weed biological control in Argentina have grown in the last decade, mainly due to a series of educational and outreach activities led by research teams from FuEDEI, CONICET, and national universities. Since then, ~6,100 people have participated in outreach activities and environmental education on invasive plants and biological control through our participation in community and science fairs. We provided training courses to 350 school teachers and gave talks about knowledge and perception of IAS to ~1,100 school students. We teach courses on biological control of invasive plants and arthropods every year that have reached, to date, 1,210 undergraduate and graduate students from eight public universities in the country. Furthermore, 30 PhD students and young researchers were incorporated in the national scientific system to investigate different aspects of biological control (e.g., genetics, ecology, social science). A free gamified application called “*Especies*” was developed for teachers and students from secondary schools in Argentina as a friendly learning tool to study the importance of IAS and their management through a United Nations Development Program. We also initiated the implementation of the biological control programme of water hyacinth (*Pontederia crassipes*) in a small lake in San Vicente (Buenos Aires province). Soon after, it became an educational project of the city's agrarian secondary school where students and teachers mass-reared and released host-specific insects to control the water hyacinth invasion. Over time, water hyacinth biological control gained visibility and support among the community, which gave us the opportunity to teach and award a technical diploma in aquatic weed management to 30 people. We are also involved in training and counseling for several national and municipal level government agencies (e.g., FuEDEI provided training courses in the Agriculture Ministry), and international initiatives. All these initiatives could contribute to increasing public awareness about the importance of IAS, raising the profile of weed biological control, and promoting new research opportunities for further growth in Argentina.

## IMPROVING BIOLOGICAL CONTROL STUDIES

Moderator: Julie Coetzee (CBC, SA)

Oral presentation

### **How can we further improve pre-release studies on the safety and effectiveness of weed biocontrol agents?**

**Keynote speaker:** Hinz Harriet L.

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Thorough pre-release studies to determine the environmental safety of biological control agents and to try and predict their effectiveness in controlling the target weed are one of the most crucial parts within a biological control project. Over the last three decades, the scientific vigour of pre-release investigations has continuously improved. Molecular analyses are nowadays more or less routinely incorporated in weed biocontrol projects to confirm agent and weed species identity, describing their genetic variability, detecting species complexes, and analysing plant phylogenies to inform test plant species selection. There have also been efforts to support traditional testing methods using chemical ecology (i.e. analysing chemical signals emitted by target and non-target plants, coupled with behavioural studies of the agents); experimental evolutionary studies to determine the likelihood of host switches; food-web studies to predict potential indirect effects of agent introductions; plant demographic models to establish the most sensitive life stages of the target weed and nontargets; and agent demography in combination with species distribution models to identify where an agent can build up high densities. However, many of these unquestionably valid methodologies have only ever been applied to one or two biocontrol systems and have not necessarily gained mainstream applicability. We must ask ourselves why that is. Some suggestions have only recently been made and technologies might not yet be readily available or still too expensive or time consuming. Scientists might lack the necessary expertise and/or financial resources, and some systems might simply not be amenable to the methods proposed. Reasons will be explored, hands-on recommendations given, and “low hanging fruits” identified. I have no doubt that with further development of above-mentioned methodologies as well as novel technological advances (e.g. gene splicing, machine learning, artificial intelligence), pre-release studies will continue to improve and refine predictions on the environmental safety and impact of weed biocontrol agents and will thus further increase the credibility and success rate of the discipline.

**When a plant invader meets its old enemy abroad: what can be learnt from accidental introductions of biological control agents**

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The steadily increasing globalization of trade and travel facilitates the international movement of pests, but it also increases accidental introductions of weed biological control (BWC) agents. Accidental introductions of BWC agents (i) offer opportunities to assess host use of agents with a potentially broader fundamental host-range than those approved for field release directly in target areas; (ii) urge national authorities to rapidly respond as they may threaten native species or crops, and by this (iii) help advancing post-release studies, a neglected aspect of BWC. Through detailed insights gained from studying the recent accidental introduction of the ragweed leaf beetle, *Ophraella communa*, into Europe, we derive suggestions for overcoming barriers to adoption of BWC by re-evaluating the predictive power of pre-release studies and, thus, the current strict criteria for deciding upon their release that might exclude safe and efficient agents. Our suggestions include focusing on the agent's population growth rate on the target and on selected non-target plants, and identifying host use under various relative abundances of target and non-target plants. To optimize efficacy, we suggest exploring outcomes of the genetic interaction between populations of the target plant and the potential biocontrol agent. Furthermore, we propose experimental evolution studies already pre-release to anticipate potential evolutionary outcomes of the intended species interactions. By using the allergenic weed, *Ambrosia artemisiifolia*, and the accidentally introduced BWC agent *O. communa* as the study system, we also hope to raise the awareness of authorities to consider biological control more prominently as a key approach for pest management in the "One Health" concept in Europe.

**Assessing the risks of biological control to crop and ornamental cultivars**

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Classical biological control is an important tool for long-term, sustainable management of weeds. To be acceptable for introduction, new biocontrol agents must not damage crops, native plants or other non-target species. Host-specificity experiments inform risk assessment of new biocontrol agents by prioritizing and testing non-target plant species. However, current approaches may be inadequate for assessing risks to crop and ornamental species that contain many cultivars, because susceptibility to damage can vary between cultivars of the same species. We reviewed current cultivar selection practice published in prominent biological control journals and government documents. To address perceived gaps, we developed a decision support tool for selecting cultivars for host-specificity testing and applied the tool to a case study. In our review, we found that most papers either did not mention cultivars of the crop or ornamental species being tested, or they provided incomplete descriptions of cultivars without explaining omissions. In most cases, if cultivars were listed then the criteria used to select cultivars were not described, were incorrectly applied or inconsistently applied. Results for individual cultivars were therefore absent or incomplete in most cases. By applying our decision support tool to a case study, we demonstrated how current gaps can be addressed, and how selections could be made through a collaborative and transparent process involving key stakeholders and risk bearers. The decision tool has broad application in weed biological control risk assessment. We argue that our approach, if adopted, will result in more transparent, defensible and reproducible cultivar selection practices leading to greater confidence in biological control risk assessments.

### Do only open field tests matter?

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While recent studies have shown an impeccable track record for released weed biocontrol agents, robust safety testing protocols have been a matter of debate for decades. One of the central issues is the usefulness of the agent's fundamental host range when predicting risk to non-target plant species in the introduced range. Results obtained from no-choice laboratory or greenhouse tests typically guide which plant species to test under choice and/or open-field experiments (ecological host range). Often, this results in contrasted findings. Nonetheless, no-choice evaluations are mandatory for release applications in all countries but the way the results are interpreted, e.g. zero impact required versus minimal, varies amongst countries. We argue here that the ecological host range should be given more emphasis during the evaluation process. To support this, we present three examples of agents; one insect, one mite and one pathogen, which have not yet gone through a release application. First, the beetle, *Chrysochus asclepiadeus*, against swallow-worts (*Vincetoxicum* spp.), was observed to feed on non-target milkweeds in laboratory no-choice tests. However, a three-year open field study showed neither feeding activity nor oviposition on the milkweeds tested. Second, given that eriophyid mites need wind to spread naturally, testing of the mite *Aculus taihangensis* against tree of heaven (*Ailanthus altissima*) followed the opposite strategy, testing first under open-field conditions. Results showed no host use or impact on the 22 North American non-target species, suggesting a narrow ecological host range. These plant species will be tested under laboratory no-choice conditions to assess the fundamental host range of the mite. Third, the rust fungus *Phakopsora jatrophiicola* was assessed against bellyache bush (*Jatropha gossypifolia*) to which three native Australian test species showed weak to moderate susceptibility under greenhouse conditions, but resistance or weak susceptibility, under open field conditions. For the examples given, open-field test results support a decision for release, however, this safety assessment may be compromised by results of no-choice and/or laboratory tests (not known yet for the mite), if these results are not interpreted with care, particularly where the rule is strictly zero impact on native non-target species (USA requirements). We think that the questioning is more a matter of weighing the benefits and risks plus the weight given to each step. Therefore, we push for stronger consideration of open-field or choice tests assessing the ecological host range of a prospective biocontrol agent vs laboratory and greenhouse assessments documenting its fundamental host range.

**Predicting the field host-range of *Leptinotarsa undecimlineata*: a candidate biocontrol agent for *Solanum torvum* in Vanuatu.**

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Candidate agents sometimes feed and develop on plants that they would not attack in the field, particularly in no-choice starvation tests, which can result in safe agents being erroneously rejected. *Solanum torvum* (Solanales: Solanaceae) is an invasive pasture weed in the Republic of Vanuatu. A defoliating beetle *Leptinotarsa undecimlineata* (Coleoptera: Chrysomelidae) was selected as a candidate biocontrol agent for the control of *S. torvum* and screened for non-target attack. A population collected from Jamaica fed and oviposited on eggplant *Solanum melongena* (Solanales: Solanaceae), which also supported development from first instar larvae through to adult in no-choice tests. However, *S. torvum* is reported to be the only host of *L. undecimlineata* in Jamaica, despite hundreds of tons of eggplant being grown there annually. A systematic review indicated that although eggplant is cultivated extensively throughout the native range of *L. undecimlineata*, eggplant has not been reported to be a field host, except for one record of minor, infrequent attack in Central America that likely represents “spill-over” attack from beetles dispersing from nearby native *Solanum* hosts. Given the difficulty of obtaining approval to release agents where host-specificity testing has produced ambiguous results, rare examples, such as *L. undecimlineata*, where there are reliable field specificity data concerning a key test plant in the native range of the target weed, are valuable case studies that should assist the development of more reliable risk assessment techniques. COVID-19 prevented travel to Jamaica to set up field specificity tests, so we conducted additional laboratory tests to investigate the potential risk posed to eggplant should *L. undecimlineata* be released in Vanuatu: the relative performance risk score approach; choice oviposition tests; and ‘multiple generation’ (continuation) tests, where F1 beetles reared from *S. torvum* were returned to *S. torvum* plants and F1 beetles reared from eggplant were returned to eggplant. All approaches indicated that the risk to eggplant is low: the relative performance risk score, calculated for the initial no-choice oviposition and larval starvation tests, indicated that the risk of eggplant being a full field host is low; choice oviposition tests indicated a significant preference for *S. torvum* over eggplant and no-choice multigeneration tests indicated that *L. undecimlineata* populations will decline to extinction when fed exclusively on eggplant. The agreement between the tests provides a compelling case that the worst-case scenario should *L. undecimlineata* be released in Vanuatu will be transient spillover attack on eggplant.

**The challenges of phenological asynchrony in host-specificity tests: case study of a biocontrol agent on *Tanacetum vulgare* (Asteraceae)**

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Host-specificity testing of biocontrol candidates that oviposit on ephemeral plant structures (e.g. flowers) can be challenging, since the acceptance of non-target species as hosts will depend on whether they are in the correct phenological stage. In many cases the phenology of target and non-target species are not synchronous. Thus, non-target species within the fundamental host range of a potential agent may be safe from attack because they are not available when the agent is reproductively active. On the other hand, if an agent becomes reproductively active at a time when its target species is not in the correct phenological stage, it may oviposit on non-target species that would not be accepted in a choice situation. *Gillmeria ochrodactyla* (Lepidoptera: Pterophoridae) is a promising potential biocontrol agent for *Tanacetum vulgare* (common tansy, Asteraceae), a perennial herb native to Eurasia and invasive in the northern USA and Canada. *Gillmeria ochrodactyla* is a univoltine species and females oviposit into open flower heads. Its early instar larvae overwinter in the seed heads and in spring, mine into newly growing shoots, which they can severely damage. In its native range in Eurasia, *G. ochrodactyla* is almost exclusively reported from *T. vulgare*, but no-choice oviposition and larval development tests revealed that it can develop in several congeneric species, including the only North American native congener *Tanacetum bipinnatum*. To assess the overlap in the flowering periods of the native and invasive *Tanacetum* species, we compared their phenologies in a common garden in Europe, and based on photographs submitted to the citizen science platform iNaturalist in North America. To further evaluate the risk of non-target attack by *G. ochrodactyla*, we conducted a series of open-field tests where we either exposed *T. bipinnatum* to a natural population of *G. ochrodactyla*, or artificially synchronized *G. ochrodactyla* with the flowering period of *T. bipinnatum*. We found that *T. bipinnatum* starts flowering a few weeks earlier than *T. vulgare*, and that there is little overlap in the flowering time between the two species in North America. However, should *G. ochrodactyla* females become reproductively active before any flowering *T. vulgare* are available, *T. bipinnatum* may occasionally be accepted for oviposition.



**Determining ecological host range from native and invaded range phylogenies**

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The North American Black locust, *Robinia pseudoacacia* L. (Fabaceae) is distributed in virtually every temperate and subtropical region of the world. An associated gall midge, *Obolodiplosis robiniae* Haldeman (Diptera Cecidomyiidae) has unintentionally followed the tree on its global spread, and is often regarded as a pest. As the midge has not yet been recorded in South Africa, it is being considered as a candidate biological control agent, however, despite several attempts, culturing the species under quarantine conditions has not been successful. We therefore determined the potential host range of the midge using information from experts in the field of galling insects, literature surveys, agricultural pest lists, social science platforms coupled with native and invaded range surveys. The list of non-target species to consider as potential hosts was refined by developing phylogenetic trees of closely related Fabaceae that share the same distribution (native and invaded) as *R. pseudoacacia*. Through the available information gathered, and field surveys of these species, *O. robiniae* has not been shown to utilise any species, other than those from the Robinoide clade. In addition, the midge has also never been recorded on a number of closely related leguminous fodder and horticultural species growing in close proximity at high densities to *R. pseudoacacia* - suggesting negligible risk to South African growers of the same species. Host specificity through field surveys can be regarded as one of the best indicators of the ecological host range, however, this information is difficult to quantify and infrequently available, thus seldom used when determining the safety of a candidate biocontrol agent. In this unique study, using the extensive data collected we are able to show that *O. robiniae* would be potentially safe for release in South Africa. However, open field tests exposing closely related non-target plant species under natural and semi-natural conditions are planned in Switzerland over the next two years aiming to confirm these conclusions.

**Manipulation of release conditions improve establishment of the wasp *Tetramesa romana* (Hymenoptera: Eurytomidae) for biological control of arundo (*Arundo donax*)**

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*Arundo* (*Arundo donax* L.) (Poaceae) reduces water availability, hinders flood control, fuels wildfires, and displaces native species in the arid southwestern United States. Biological control has involved releases of the shoot tip-galling wasp *Tetramesa romana* Walker and the rhizome- and shoot-feeding armored scale *Rhizaspidiotus donacis* Leonardi. Beginning in 2017, we released agents at 11 sites in northern California, including three each in the watersheds of the Sacramento and San Joaquin rivers and five in their Delta. A total of 20,660 wasps collected from populations in the humid subtropical Lower Rio Grande Valley of south Texas were released, as were 800 armored scale adult females. To test whether physical manipulation of the host plant could affect wasp establishment, release plots were treated two months prior to release by cutting all shoots to ground level, topping at 1 m height, or leaving them untopped. In 2018, exit holes made in galls by emerging wasps were 26-fold more abundant in plots that had been cut to ground or topped compared to uncut plots. We surveyed wasp density by counting the number of exit holes for two minutes. In 2019, exit holes were present at 50% or more of survey points at two of the six sites outside of the Delta. From 2019 -2021, the wasp was present each year at 55% to 90% of points at these sites, and we observed dispersal of up to 6.4 km. The wasp had established at all five sites in the Delta by 2021. Adult female armored scales were recovered at nine of the 11 release sites. To establish wasp populations at more sites, new release plots were cut to ground in 2020 and in some plots the regrowth was topped to 1 m height ('double topped') prior to new releases. After one year, wasp exit holes were 15-fold denser in double-cut than in single-cut plots. Wasp source was evaluated as a possible influence on establishment. The wasp *T. romana* is adventive in southern California. Wasps from there and from Texas were released onto bagged arundo stems in the field. Wasps from California produced two-fold more offspring than those from Texas under dry summer conditions in northern California. The results demonstrate the utility of manipulation of host plant physical quality to improve biological control agent establishment, and suggest that arundo wasp populations may adapt to regional conditions. Additional studies are underway to determine agent impact.

**Updates on monitoring *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) field releases and evaluating agent damage to the target weed *Schinus terebinthifolia* (Sapindales: Anacardiaceae) in Florida, USA**

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The thrips *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae) is one of the biological control agents targeting Brazilian peppertree (*Schinus terebinthifolia* Raddi) that have been approved for release in Florida, USA. Through the collaborative efforts of multiple agencies, *P. ichini* is continuing to be mass reared, released, and monitored at field sites across Florida, with over 4 million thrips released across over 700 sites since May 2019. Here we provide updates on the persistence of thrips populations, damage inflicted to Brazilian peppertrees, and outline new avenues of research to better understand how variations in site characteristics affect agent persistence and performance in the field. Local persistence at several field sites across the state has been documented in a previous study and we continue to see persistence without supplemental releases. Recently, a small thrips population was discovered 1 km from the nearest release site - the greatest natural dispersal distance yet recorded in Florida. Thrips damage to Brazilian peppertree canopies has varied among sites, from undetectable to 100% of the canopy with at least some degree of detectable damage. In recent surveys, Brazilian peppertrees with severe damage, canopy thinning, and secondary attack from other phytophagous insects have been noted. Preliminary observations suggest thrips have a greater tendency to persist at sites with other plant species intermixed with the Brazilian peppertree canopy, a more humid microclimate, and soils containing more organic matter and a thicker duff layer. A systematic survey of sites sampling the spectrum of thrips efficacy will be conducted to quantify biotic and abiotic site characteristics, such as canopy diversity, incidence of phytopathogens, water availability, and soil properties, and correlations with thrips efficacy will be examined. The application of this study is to help identify sites optimal for biological control, sites poorly suited for biological control thereby necessitating mechanical or chemical methods of control, and sites suitable for an integrated approach to management techniques.

**Biological control of invasive African grasses: progress and prospects**

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Historically, grasses have been avoided as targets for biological control due to concerns over a lack of host specificity and potential to inflict damage by possible control agents. However, recent work has demonstrated that insects and fungal pathogens can be suitable and highly effective biological control agents. Since 2017, the Centre for Biological Control at Rhodes University in South Africa has initiated several biological control programmes against native African grasses that have become invasive in either the USA or Australia. In this paper, we highlight the progress made towards developing biological control programmes against three key invasive grasses; weedy *Sporobolus* spp. (Giant rat's tail grass) and *Eragrostis curvula* (African lovegrass) in Australia, and *Megathyrus maximus* (Guineagrass) in the USA. We summarise the native-range studies completed for all three species, including describing the natural enemy communities, field host-range surveys, no-choice host-range testing and pre-release efficacy surveys. Across all three programmes, nine possible biological control agents have been identified and assessed to date. Thereafter, we discuss the challenges we encountered during these programmes and measures taken to overcome these issues. Lastly, we provide updates on the possible biological control options available for a range of other invasive African grasses (e.g. *Andropogon gayanus* [Gamba grass], *Hyparrhenia hirta* [Coolataai grass]). Developing biological control programmes for invasive grasses is not without its challenges, but our work has shown that grasses can be suitable targets for biological control.

**Ten years after the release of the balloon vine weevil, *Cissoanthonomus tuberculipennis* (Coleoptera: Curculionidae), in South Africa: an emerging success**

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The seed-feeding weevil, *Cissoanthonomus tuberculipennis* Hustache (Coleoptera: Curculionidae), was first released in South Africa in 2013 as a biological control agent for balloon vine *Cardiospermum grandiflorum* Sw. (Sapindaceae). Here we present a 10-year field assessment of impact by *C. tuberculipennis* conducted at release sites located in eight of the nine South African Provinces. Seed feeding damage by *C. tuberculipennis* was quantified by examining samples of 50 fruits, each containing a maximum of three seeds, from a *C. grandiflorum* stand. The beetle *C. tuberculipennis* has fully established throughout the distribution range of its host in South Africa. Intensive monitoring conducted since the first release of *C. tuberculipennis* shows that infestation levels by the weevil have been increasing at almost all the study sites, reducing the seed production by 25% in Gauteng up to 62% in KwaZulu-Natal (KZN) in 2023. The weevil dispersed at a rate of 33 to 37km/year along the coastal regions of KZN and the Eastern Cape provinces. Furthermore, as a result of seed damage by *C. tuberculipennis* the soil seed bank, seedling recruitment, seed rain, inflorescence and fruit densities have shown a steady decline at all study sites during the past ten years. The decline in soil seed bank and seedling recruitment suggests that *C. tuberculipennis* will eventually cause the decline of *C. grandiflorum* populations in South Africa.

**The importance of long-term post-release studies: insect-plant monitoring and public awareness of water hyacinth management in Dique Los Sauces, Argentina**

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Several key components of classical biological control (CBC) programmes are necessary to assess not only the success of the management strategy (e.g., post-release monitoring) but also to help prevent future reintroductions or resurgences of the invasive species (e.g., public awareness). Water hyacinth, *Pontederia crassipes* (Mart.) Solms (Pontederiaceae) is an aquatic plant naturally distributed in the north-eastern region of the Del Plata basin in Argentina. Reproduction and spread is largely vegetative, however prolific seed production is a source of new or re-infestations. In the 1960s it was introduced into the Dique Los Sauces reservoir located outside of its native range in La Rioja Province, a semi-arid region in western Argentina where it became invasive. The natural enemy, *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) was intentionally introduced in 1974 to control the weed in the reservoir. To assess the success of this CBC programme, a long-term post-release study has been conducted. Between 1965 and 2023, we monitored plant coverage on the water body, estimated *N. bruchi* densities, and quantified the associated damage by reanalysing previously published data and incorporating new field sampling. We also conducted an anonymous online survey to the local community to analyse their knowledge and perception about this programme. Water hyacinth's coverage fluctuated from its first record in 1965 (maximum coverage, 90%) until the complete control of germinated plants in 2018. The plant population decline was accompanied by an increase in the weevil population. In 2023, a few newly germinated plants were recorded following a severe drought that exposed water hyacinth's seed bank. In our survey, out of 325 respondents, only a small group of mostly middle-aged and elderly people knew that the restoration of the water surface had been achieved through a management strategy and even fewer were aware of the biological control approach taken. Respondents who had a positive perception about biological control were more aware about the management plan than respondents who had neutral or negative opinions. *Neochetina bruchi* has played a key factor in the complete control of *P. crassipes*. The intrinsic dynamic of these populations, the dormant seed bank and the lack of public awareness support the need for long-term post-release evaluations including outreach campaigns to ensure a sustainable successful management programme.

**Determining the host range of *Trichilogaster* sp. nov. (Hymenoptera: Pteromalidae), a potential biological control agent of earleaf acacia (*Acacia auriculiformis*) in Florida**

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*Acacia auriculiformis* (earleaf acacia) is a fast-growing, evergreen tree in the Fabaceae family. It is native to Australia and was introduced to the United States as an ornamental plant in the early 1900s. Since then, earleaf acacia has been spreading rapidly throughout Florida, altering native plant communities and ecosystem functions. In 2021, the bud-galling was, *Trichilogaster* sp. nov. (Hymenoptera: Pteromalidae) was imported into containment laboratories in Florida from Australia to assess its potential for biological control of earleaf acacia. Host specificity tests are underway at the UF/IFAS Hayslip Biological Control and Containment Laboratory in Fort Pierce, Florida. No choice oviposition and gall formation tests were conducted on 16 plant species from the list of plant species for host range testing approved by the Technical Advisory Group on the Biological Control of Weeds. So far, no gall formation was observed on any species other than *A. auriculiformis*. More testing is planned to determine if this insect will be safe to release in Florida to control earleaf acacia. However, current results are promising.

**A phylogeny visualisation interface for decision support in weed biocontrol risk analysis**

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The centrifugal phylogenetic method is central to rigorous risk analysis and the experimental design of host-specificity tests in weed biological control research. Native or useful plant species closely related to the target weed are prioritised over more distantly related species in such tests because they are likely to be ecologically, anatomically, and biochemically similar to the target weed and, therefore, at greater risk of non-target utilisation by candidate biocontrol agents that have co-evolved with the target weed. A variety of other factors, such as shared biogeographical ranges, ecological niche overlap and functional trait similarities with the target weed also contribute to the risk profile of non-target species in host-specificity testing. However, to date, biocontrol researchers and practitioners have lacked user-friendly data-integration tools for the exploration of these disparate data streams in the context of biocontrol risk analysis and visualisation of these data for presentation to other stakeholders. We have developed a data integration pipeline deployed using the R Shiny package that visualises phylogenetic trees together with range and ecological trait data for non-target plant species to illustrate the distribution of relevant factors in an evolutionary context. The app itself calculates degrees of phylogenetic separation as a measure of evolutionary relatedness of each species to the target weed and enables the interactive exploration of this relationship for different target species. It displays other data provided by the user, both categorical and numerical, such as geographic overlap between species or ecological traits. The target weed species can be changed, and the phylogeny can be re-rooted by the user. The app provides options for the export of the visualisation as a PNG file. Based on user feedback to date, the app improves the efficiency and confidence with which biocontrol research practitioners can prioritise non-target plant species for deployment in host-specificity testing.



**The biological control agent *Floracarus perrepa* limits growth of Old World Climbing Fern, *Lygodium microphyllum*, sporelings**

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Old World Climbing Fern (*Lygodium microphyllum*) is native to the tropics and subtropics of Australia, Asia, Oceania, and Africa and is considered an invasive plant in North America. The USDA Invasive Plant Research Laboratory first released the biological control mite *Floracarus perrepa* in 2008, and initiated a mass-rearing and release program in 2014. The program has released over 29 million mites throughout south and central Florida. *Floracarus perrepa* is an eriophyid mite that forms leaf roll galls on leaflets and apical meristems of *L. microphyllum*, which can cause leaf necrosis and reduced climbing ability. Additionally, the mite diminishes the plant growth rate, alters plant architecture, and lessens some of the other damaging consequences of *L. microphyllum* on ecosystems. To develop more effective Integrative Pest Management (IPM) for *L. microphyllum*, it is critical to assess the vulnerability of *L. microphyllum* to *F. perrepa* during early life stages. The hypotheses tested in the experiment were A) what life stages of *L. microphyllum* are susceptible to attack by *F. perrepa*, B) because of damage from *F. perrepa*, does sporeling mortality increase, C) can *F. perrepa* damage alter above ground and/or below ground biomass. Sporelings were grouped at the beginning of the experiment by life stages; gametophyte, sporophyte, true leaf, rachis node, and “fits in four-inch pot”. Preliminary results suggest that *F. perrepa* damages *L. microphyllum* as early as the first true leaf stage, but it does not attack the gametophyte or the sporophyte stages. Additionally, at the end of the experiment, the gametophytes, sporophytes, and rachis node groups were significantly more likely to be dead than their control counterparts ( $p < .001$ ,  $p < .001$ ,  $p < .001$ ) and biomass above ground and below ground was significantly different for gametophyte ( $p < .001$ ,  $p < .001$ ), sporophyte ( $p < .001$ ,  $p < .001$ ), rachis node ( $p < .001$ ,  $p < .001$ ), and four-inch pot groups ( $p = .030$ ,  $p = .030$ ). Based on this, we would recommend that these biological control agents be incorporated in IPM at all stages of invasion.

**Make the most of the life history traits of *Aculus taihangensis* (Acari: Eriophyidae) for its evaluation as a biological control agent of *Ailanthus altissima* (Sapindales: Simaroubaceae)**

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Every stage of a biological control program, from the selection and evaluation of a natural enemy to its rearing, release, and prediction of its establishment, requires prior knowledge of its life history and behavioral traits. Despite the increasing interest in the use of eriophyid mites (Acari: Eriophyidae) as biological control agents of weeds, their biology, ecology, and behavior are poorly known. This is mainly due to their microscopic size (around 200 µm) and tendency to hide within plant structures, which make their handling and direct observations particularly challenging. We examined life-history traits of *Aculus taihangensis* (Hong et Xue), an eriophyid mite currently under evaluation in Europe as biological control agent of the global invader *Ailanthus altissima* (tree of heaven) to guide host-range testing, as well as implementation and impact studies. Laboratory experiments were conducted at two different constant temperatures, 26 and 30 °C respectively, by maintaining the mites on a piece of *A. altissima* leaf. No difference was recorded in the developmental time from egg to adult female at the two temperatures investigated, and on average, it took 5.9 and 5.6 days at 26 and 30°C, respectively. The time from the egg to the egg of the next generation (i.e., one full generation) was 1.5 days shorter at 26 than 30°C (i.e., 8.7 and 7.2 days, respectively). The data points out that under proper climatic conditions, *A. taihangensis* populations can rapidly increase in size, which represents one of the preconditions of an effective biological control agent. In addition, the life-history traits of *A. taihangensis* presented suggest for how long a non-target species should be exposed to the mite to guarantee the observation of any potential new generation in case the plant species is a suitable host for its development, thus contributing to the improvement of the methods for host-specificity testing and the interpretation of their results.

**Release and establishment of the shoot-tip galling fly *Parafreutreta regalis* (Diptera: Tephritidae) for biological control of Cape-ivy (*Delairea odorata*) in California and potential ecological limitations**

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Cape-ivy (*Delairea odorata*, Asteraceae), native to South Africa, is invasive along the coast of Oregon and California (USA), as well in Hawaii (USA), Argentina, Chile, western Mediterranean Europe, Australia and New Zealand. In California, Cape-ivy smothers native vegetation in coastal riparian, forest and scrubland habitats. The first biological control agent targeting this weed is the shoot tip-galling fly *Parafreutreta regalis* Munro, permitted for release in the USA in 2016. Releases were initially conducted by caging adults inside small (ca. 1 m<sup>2</sup>) cages, with subsequent releases of uncaged adults at some sites. About 19,000 adults were released between 2016 and 2022 in 131 release events at 12 sites on the northern and central California coast and at seven sites in southern California. Success using caged adults occurred mainly after five staggered releases per site were made in 2018-2019. Starting in 2019, releases were also conducted by planting greenhouse-galled plants at field sites. Through 2022, a total of 3,206 galls were placed in 23 release events at four sites in northern and central California, and in 11 release events at six sites in southern California. Based on counts of galls in monitoring plots at least one year post-release, the galled plant release method was almost two times more efficient in leading to fly establishment (seven of 10 sites, 70% success rate, including the southern California sites) than was the caged adult method (five of 12 sites, 42% success rate). New galls were observed 500 m away from the release location at multiple sites in 2021 and up to 700 away by early 2023. Drought was examined as a cause of establishment failure at some sites. In greenhouse tests, plant water deficit stress reduced fly oviposition by 70%, increased total development time by 10 days, and decreased adult progeny production by up to 80%. Biotic factors that could influence fly populations have been observed. A pteromalid wasp parasitizes larvae or pupae inside 10-15% of field-collected galls, and in lab observations gall probing by female wasps was observed. A native tephritid that galls multiple native and invasive Asteraceae in coastal habitats is also a host. Larvae of a moth, *Spilosoma vagans* (Boisduval) (Lepidoptera: Erebidae) chew holes in mature galls without eating larvae or pupae, but potentially exposing them to predators or desiccation. Current studies are evaluating the impact of the fly *P. regalis* on Cape-ivy in the presence of potential limiting factors.

**Ukrainian vs French chrysomelid beetles for controlling swallow-worts,  
*Vincetoxicum* spp., in North America: Where do we stand?**

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Pale (*Vincetoxicum rossicum*) and black (*V. nigrum*) swallow-worts are herbaceous, perennial, twining vines of European origin. These species are invasive in northeastern North America and dense populations can outcompete native plants, and can disrupt natural and agricultural ecosystems. The defoliating moth *Hypena opulenta*, (Lepidoptera: Erebididae) released in 2014 in Canada and in 2017 in the USA, has not reached outbreak densities yet. *Chrysochus asclepiadeus* (Coleoptera: Chrysomelidae) feed on the root system and foliage of swallow-worts, and could complement the moth in North America. Two populations of the leaf beetle, one from France and one from Ukraine, have been evaluated in laboratory and outdoor conditions. Molecular analyses revealed a substantial genetic divergence based on the mtCOI gene between Ukrainian and Western European populations, suggesting their differentiation at subspecies level, and they were therefore tested separately. However, the host range of both the Ukrainian and the French populations seem to be similar, with partial or complete larval development in no-choice larval transfer tests occurring on a limited number of milkweed species. Milkweed species are essential for the development of the iconic monarch butterfly and therefore critical non-targets. In cage tests exposing non-target species together with *Vincetoxicum* spp. to the Ukrainian population, oviposition and larval development occasionally occurred on *A. tuberosa* and *A. incarnata*. At that stage, host specificity testing was more advanced with the Ukrainian population, however for logistical reasons, it was decided to pursue testing with the French population. In an open-field test in Switzerland exposing *V. hirundinaria*, *Asclepias incarnata*, *Asclepias syriaca* and *Apocynum cannabinum*, adults and larvae were only found on the control. Similar open-field tests over three years in France confirmed these results and revealed a complete absence of feeding and oviposition on *Asclepias tuberosa* and *Asclepias syriaca*, whereas controls (*V. hirundinaria* and *V. rossicum*) were impacted each year. Results obtained so far suggest that non-target species (e.g. milkweeds) are unlikely to be at risk of attack by *C. asclepiadeus* under natural conditions. No-choice larval transfer tests with the French population are being continued. One question arising from our research is whether it is reasonable to infer the attack of the French population on test plants that are not closely related to *Vincetoxicum* spp. based on the data collected with the population from Ukraine.

**A weevil's first year in the UK - a promising start for *Listronotus elongatus* (Coleoptera: Curculionidae), a biocontrol agent for *Hydrocotyle ranunculoides***

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An aquatic invasive plant from the Americas, floating pennywort (*Hydrocotyle ranunculoides* [Apiales: Araliaceae]), has been rapidly invading the UK's slow-moving waterbodies since it first appeared in the wild in the early 1990s. With a suite of promising natural enemies recorded from its native range, the plant was prioritised as a target for biological control due its impacts on biodiversity, recreation and as a flood risk, alongside the difficulties and costs of conventional management approaches. Following over a decade of research and a year-long consultation period for the petition, the weevil, *Listronotus elongatus* (Coleoptera: Curculionidae), was given ministerial approval for release in late 2021, as a biocontrol agent against floating pennywort in England. This is the fourth biocontrol agent approved for release in the UK. Up until that point, the weevil, originally imported from Argentina in collaboration with FuEDEI, had only been bred at optimal temperatures in CABI's quarantine facilities. Following a series of temperature transitions to aid acclimatisation to colder conditions, the first field release of weevils was made in November 2021, as a preliminary overwintering trial. Whilst less than seasonally appropriate for impact or establishment, this provided an opportunity to gain an understanding of the overwintering potential and behaviour of the weevil in the UK climate. In parallel to the field release, a semi-contained experimental study was undertaken in an unheated polytunnel using adult and immature stage weevils to assess survival, behaviour and impact in a semi-artificial setting. This poster presents data gathered from the polytunnel experiment and initial field release monitoring, and demonstrates the complementarity of both approaches for assessing survival and reproductive success of the weevil in its first year of release. Initial monitoring results from field releases made during the spring and summer of 2022 across the country are also presented, providing added insights into temporal and bioclimatic influences on the efficacy of this biocontrol agent across release sites.

**A century of *Azolla filiculoides* biocontrol: the economic value of *Stenopelmus rufinasus* to Great Britain**

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The aquatic fern *Azolla filiculoides* Lamarck (Salviniales: Azollaceae) has been introduced to many countries across most continents and has an array of traits that can lead to it becoming highly invasive when introduced to new regions. The fern has been present in Great Britain (GB) since the end of the nineteenth century and is widespread across the country, but its current impacts are relatively limited. In 1921 a weevil, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae), was recorded on *A. filiculoides* in GB following its unintentional introduction. This same weevil has been used to great success as a classical biocontrol agent against *A. filiculoides* in South Africa where it was introduced in the late 1990s resulting in the water fern's status as a damaging invasive weed being greatly diminished. Although somewhat less effective in GB, likely due to climatic limitations, it is apparent that following a century of residency, *S. rufinasus* plays an important role in mitigating the impacts of *A. filiculoides* that would otherwise be experienced in GB, reducing chemical inputs, lessening biodiversity impacts and reducing economic costs. In addition to the ongoing control of *A. filiculoides* provided by naturalised weevil populations, CABI mass rears *S. rufinasus* for augmentative releases where *A. filiculoides* outbreaks do occur. This study aims to quantify the economic cost savings to the GB economy brought about by the action of *S. rufinasus* in terms of management costs under three scenarios: the first scenario is a theoretical one in which *S. rufinasus* is not present and *A. filiculoides* is widespread with significant potential impacts and therefore subject to extensive traditional management efforts; scenario two is one in which *S. rufinasus* is naturalised and widespread, but mass rearing and augmentative releases of the weevil are not undertaken; and scenario three reflects the current situation in which naturalised and targeted augmentative releases of *S. rufinasus* provide significant control of *A. filiculoides*. The management costs are estimated for each of the scenarios to give an indication of the economic value of *S. rufinasus* to GB, and the non-quantified biodiversity benefits and reductions in negative impacts brought about by the weevil are discussed.

**Impact and potential selection pressure of the leaf-feeding beetle, *Cassida rubiginosa* (Coleoptera: Chrysomelidae), on the pasture weed, *Cirsium arvense***

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The pasture weed, *Cirsium arvense* (Californian thistle, Canada thistle, creeping thistle), is notorious for its ability to tolerate defoliation (i.e., its ability to recover following defoliation). In this contribution, we present data evaluating the impact of the leaf-feeding biocontrol beetle, *Cassida rubiginosa*, and the potential for selection of more tolerant genotypes of the weed. The impact of the beetle was assessed over two years in an established field population of the weed. In the field trial, average shoot defoliation levels of 25% (ranging from 0 to 70%) resulted in population decline and reduced spread of the weed. To investigate if weed genotypes respond differently to defoliation, we imposed artificial defoliation (clipping) on 36 genotypes of the weed. Tolerance to clipping was assessed between clonally replicated genotypes that were clipped three times, or left unclipped, over a six-month growing season. Several plant growth and size parameters varied significantly between the genotypes, confirming genotype-specific tolerances to artificial defoliation. This led to a common garden experiment with eight genotypes of *C. arvense* that ranged in their tolerance to artificial defoliation from 33% reduction in shoot biomass to 16% overcompensation. The common gardens were established in large cages (3m x 1.8m) containing the eight genotypes, with or without *C. rubiginosa*, and were assessed over three years. Despite defoliation reaching levels expected to have a fitness impact on the weed (average of 46%, ranging from 0 to 100%, each year), no effect of beetle herbivory was detected on any of our measured plant responses. This included genotype frequency over time, emergence of new genotypes from seed, shoot size and density. The results suggest that selection pressure from the beetle is likely minimal, and that impacts observed in field studies are probably a result of multiple stress factors including drought and interspecific competition.

**Biology and field performance of the seed-feeding weevil *Smicronyx lutulentus* (Coleoptera: Curculionidae), a biological control agent of *Parthenium hysterophorus* (Asteraceae: Heliantheae) in South Africa**

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The seed-feeding weevil *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidae) has been released in South Africa since 2015, in an effort to curb the prolific seed output of *Parthenium hysterophorus* L. (Asteraceae), a severe terrestrial invader. Despite the contribution of the weevil to the successful management programme in Australia, information on certain aspects of the biology and field performance of the weevil is limited. A laboratory study of its oviposition biology demonstrated that after a pre-oviposition period of two weeks, female weevils up to eight weeks in age were productive, with oviposition tapering off thereafter, thereby informing appropriate timing for mass-rearing and field releases. A laboratory study of the larval damage to seeds at different adult densities demonstrated that five weevil pairs per plant resulted in significantly fewer unexploited flower buds than a single pair. Field studies of the abundance and impacts of *S. lutulentus* at six established sites indicated that weevil abundance varied spatially and temporally. Field populations peaked in the late summer. Up to 30% of developing seeds were rendered inviable when the weevil was present at field sites, through a combination of larval-infested seeds, larval-induced abortion in adjacent infested seeds within the flower head, as well as naturally aborted seeds. Given the high reproductive outputs of the plant and despite a significant reduction in viable seed production by *S. lutulentus*, considerably higher weevil populations are likely required to cause a greater impact. However, such levels of damage recorded within no more than five years since establishment are promising, with future expansion of populations and impact anticipated with time, given suitable conditions. The insights into the reproductive biology, damage potential and realized impact of *S. lutulentus* have provided useful baseline information on the weevil, to improve current understanding and aid future comparisons.



**Investigating the impacts of abiotic factors on *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae), a biological control agent of the invasive Brazilian peppertree**

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The economic damage that invasive species impose on ecosystems by disrupting and dominating native habitats is undeniable. Brazilian peppertree, *Schinus terebinthifolia* Raddi (hereafter BP), is a shrub to small tree native to South America and an aggressive invasive plant that is a threat to natural and agricultural environments, particularly to sensitive habitats in invaded regions. The biological control agent *Pseudophilothrips ichini* Hood (BP thrips) has been released in Florida, USA to manage this noxious plant. As BP thrips pupate in the soil and Southern Florida is prone to hurricanes and heavy rains during the summer and early fall, soil moisture and salinity can be key factors influencing the survival and establishment of this biological control agent. In the present study, we evaluated the direct impact of soil type, moisture, salinity, and immersion survival time on adult emergence. Factorial combinations of fresh or brackish water and five moisture levels (0%, 25%, 50%, 75%, and 100%) were applied to three soil types (fine sand, sand, and muck). We tested larval and pupal survival after being inundated in fresh and brackish water for 0, 1, 2, 3, 5, 8, 16, and 32 hours to simulate the effects of short-term flooding. Moreover, the indirect effects of introducing biological control agents to BP plants watered with four salinity levels (0, 10, 15, and 20 parts per thousand) were evaluated. Results of our study revealed that with the increase of soil moisture, the proportion of adult emergence of BP thrips decreased across all soil types and water types. Neither larvae nor pupae can survive at 32 hours of immersion in either brackish or fresh water. Evaluating a wide range of direct and indirect abiotic factors allows us to better understand thrips biology and ecology, as well as enhance the likelihood of thrips establishment and population growth in the region. Because soil salinities change over time, it is also essential, not only in Florida but also in other coastal regions, to compare BP thrips development to adulthood, survival, and fertility on plants grown under salinity stress to find appropriate release sites and better manage the spread of this invasive species.

**Biological control prospects for African Boxthorn, *Lycium ferocissimum* (Solanaceae), a noxious weed in Australia**

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*Lycium ferocissimum* Miers (Solanaceae) is an invasive weed in Australia that is native to southern Africa. With the ultimate goal of minimizing the weed's negative impacts in Australia, a biological control programme was initiated for this species. Native-range surveys across South Africa (53 sites) revealed more than 105 morpho-species of leaf, fruit, flower and stem feeding arthropods associated with the plant. Four insect species [*Cleta eckloni* Mulsant (Coleoptera: Coccinellidae), *Cleta* sp1 (Coleoptera: Coccinellidae), *Cassida distinguenda* Spaeth (Coleoptera: Chrysomelidae), *Neoplatygaster serietuberculata* Gyllenhal (Coleoptera: Curculionidae)] were prioritised as potential biological control agents owing to their impact on *L. ferocissimum* and their host specificity inferred from the field. No-choice host-specificity experiments showed that all four insect species fed only on plant hosts in the *Lycium* genus. Most notably, an Australian native plant, *Lycium australe* (Muell), was weakly to moderately fed upon and supported reproduction in all four insect species. Choice trials showed that these insects preferred to feed on their original host (*L. ferocissimum*) with negligible oviposition and reproduction on *L. australe*. Although all the insects had a clear preference for the target weed, the non-target damage to the native *L. australe* under choice and no-choice conditions suggests that it may be at risk if these insects were to be released. Multi-choice and multi-generational tests are currently being carried out on one of the insect species, *C. distinguenda*, to determine if this non-target feeding is incidental, exploratory or induced, or if the beetle can successfully live for multiple generations on the non-target species. *Cassida distinguenda* was chosen first over the other three candidate agents due to its relatively wider distribution in the native region and hence presumably better chances of establishing in different climatic zones in Australia if approved for release. Similar studies may be carried out for the other candidate agents in future to assess the risks of non-target attacks over multiple generations.

**Post release monitoring of *Mompha trithalama* along an elevational gradient**

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*Miconia crenata* (formerly *Clidemia hirta*) is a highly invasive tropical shrub that disrupts native ecosystems in Hawaii by outcompeting other plant species and forming impenetrable monocultures. While biological control efforts have been attempted with the release of several agents, including the moths *Carposina bullata* and *Mompha trithalama*, success has been limited, and little is known about factors affecting establishment and impact. To quantify *M. trithalama* impact on seed count and viability, we collected ripe fruit and counted the number of seeds and tested germination rates of fruit infested or not infested with *M. trithalama*. Fruit attacked by *M. trithalama* produced significantly fewer seeds than clean fruit (mean “attacked” =  $390 \pm 28.2$ , mean “clean” =  $734 \pm 35.6$ ,  $p < 0.001$ ). Attack by *M. trithalama* significantly decreased seed germination rates with attacked fruit germinating at  $19\% \pm 3.2$  versus clean fruit at  $50.9\% \pm 3.5$  ( $p < 0.001$ ). In 2021, we began surveys to quantify the presence of *M. trithalama* along an elevational gradient from about 200m to 914m. We collected 50 fruit at each of seven sites along three replicates of the elevational gradient and recorded the number of fruit infested with *M. trithalama*. These surveys were repeated at intervals of 2-4 months for two years to capture seasonal differences. Elevation had a significant effect on biocontrol, with less infestation of fruit by *M. trithalama* at the highest elevations. There also was a significant interaction between elevation and collection date, suggesting seasonal shifts in abundance of *M. trithalama* at higher elevations. Parasitism rates were low, with an unidentified hymenopteran found in 21 out of 8,550 fruit collected. *Carposina bullata* was thought not to have established after its release in 1995, however we recovered a total of 229 *C. bullata* larvae from our fruit collections from multiple locations on Hawaii Island. These results demonstrate the importance of long-term post-release monitoring and climate matching for effective biological control.

**Biological control of Dalmatian toadflax in southern California by the weevil  
*Mecinus janthiniformis***

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Dalmatian toadflax (*Linaria dalmatica*, Plantaginaceae) is a perennial forb that has invaded grasslands in North America. The weevil, *Mecinus janthiniformis* (Coleoptera: Curculionidae), has been released as a classical biological control agent in the western U.S. and Canada. This weed is established in northeastern California, but releases have not been performed in California because of possible nontarget feeding on some native snapdragons (*Sairocarpus* spp.). Nevertheless, *M. janthiniformis* has invaded this area from adjacent states. Meanwhile an isolated population of Dalmatian toadflax was discovered at the Hungry Valley State Vehicular Recreation Area in southern California in 2004. Because this infestation is far from any *Sairocarpus* spp. susceptible to attack, a permit was issued to release the weevil in 2008. Weevils were released at three sites which were paired with nearby check sites. Weevil populations increased at the release sites to an average of 18 weevils per 100 cm of stem length in 2012, and up to 100% of Dalmatian toadflax stems were infested. The weevil also spread to the three check sites that were 212-548 m away (10-72% infestation). A wildfire in May 2013 destroyed the weevil population, but the toadflax recovered by 2014, and new weevil releases were made. By 2017, the weevil populations had again increased at all release sites in the original release area, with up to 47 weevils per 100 cm of stem length and 100% of stems infested. Weevils were found at similar densities at the release and check sites, and had dispersed at least 427 m. The relatively mild climate at this southernmost site in North America probably contributed to both high overwinter survivorship inside the stems (92% survival), and achievement of maximum fecundity. Dalmatian toadflax cover declined 99% in 5 years from 2014 to 2019. Annual and perennial grasses increased significantly during this period, whereas annual forbs fluctuated widely with no consistent trend, and perennial forbs and shrubs were rare.

**Interpreting *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) potential host range in an Australian context**

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Many invasive weed species are shared across climatically similar countries; thus, opportunities exist to adopt similar weed management practices, including weed biocontrol agents. Predicting the risks and benefits of a potential agent in a new environment *a priori* is one of the primary challenges for weed biological control research. The level of uncertainty may be minimised where weed targets are shared between countries, and where an agent has already been released in one country. This is because more is known about the agent and its interaction with the target weed as well as related plant species. *Schinus terebinthifolius* Raddi (Anacardiaceae) is present in over 20 countries outside of its native range in South America. It is an aggressive invader of disturbed habitats and in Australia is predominantly located near water bodies along coastlines. A biological control program for this weed was initiated in Hawaii in the 1950's and has identified several potential agents but this management option had not been explored for the Australian context until recently. *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) was identified through a prioritization process as having high prospects for biological control in Australia because it had already been rigorously risk assessed in the US and found to be sufficiently host-specific. A range of host-specificity testing methodologies have been applied to determine the risk of this insect to native Australian Anacardiaceae species (of which there are relatively few) including no-choice, paired choice, multigeneration and open-field specificity testing undertaken in Florida where *P. ichini* has been approved for release. The results will be discussed in the context of previous risk assessments for *P. ichini*, and how they also contribute to our understanding of host use for this thrips species, considering developments to insect plant host recognition theory that have been published recently. These insights can inform how we can better design and interpret host-specificity testing so that the mechanism of host plant recognition for each insect species being assessed for risk is better understood.

***Egeria densa* (Hydrocharitaceae): The first submerged aquatic weed targeted for biological control in South Africa**

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Managing plant invasions in freshwater systems has proven complex. Following the management of floating aquatic weeds in South Africa, eutrophication and introduced species propagule dispersal have contributed to secondary invasions. The submerged aquatic weed, *Egeria densa* Planch. (Hydrocharitaceae) or Brazilian waterweed is a secondary invader in South Africa after the control of floating aquatic weeds. *Egeria densa* spreads easily through fragmentation, forming dense monocultures in rivers and dams. It is currently the most widely distributed submerged aquatic weed in South Africa. The leaf-mining fly, *Hydrellia egeriae* Rodrigues-Júnior (Diptera: Ephydriidae) was released against *E. densa* in South Africa in 2018. This was the first biological control agent released against *E. densa* in the world, and also the first agent released against a submerged aquatic weed in South Africa. The success of a biological control programme depends on the ability of the biological control agent to survive, reproduce and overcome potential biotic resistance, once it has been released. Climate tolerance studies provide predictability of the establishment ability of an introduced species. The thermal physiology of *H. egeriae* showed that the thermal range of the agent encompassed its host plant's optimal temperature range of 10 to 35°C, with a critical range of 2.6 to 47.0°C, and a lethal<sub>50</sub> range of -5.9 to 40.5°C for adults. Larvae had a wider lethal<sub>50</sub> range of -6.3 to 41.3°C. Cold temperatures (14°C) prolonged the agent's development time to three months, allowing it to only develop through one generation in winter. Nevertheless, post release surveys confirmed establishment at cooler sites, and also revealed that *H. egeriae* has acquired native parasitoids, with parasitism rates of 23.3 % to 53.6 %, and higher levels of parasitism in winter. Despite this, *H. egeriae* has established at release sites, through inundative, frequent releases from mass-reared populations. This paper updates the biological control success of *E. densa* in South Africa, with recommendations for other countries, such as Australia, who are considering control.

**Biological control of prickly acacia, *Vachellia nilotica* subsp. *indica*: host specificity testing of agents from Africa**

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Prickly acacia (*Vachellia nilotica* subsp. *indica*) is a major weed of grazing areas in western Queensland, Australia. Biological control is the most economically viable management option for prickly acacia. Biological control efforts focusing on agents from Pakistan, Kenya, South Africa, and India have had limited success. Hence, the search for new gall-inducing agents was redirected to east and west Africa. A gall inducing thrips (*Acaciothrips ebneri*) from Ethiopia, a gall-inducing tephritid fly (*Notomma mutilum*) from Senegal, and a gall mite (*Aceria* sp.) from Ethiopia have been prioritised for detailed host specificity tests based on field host range and potential impacts. The gall thrips was imported from Ethiopia into a high security quarantine facility in Brisbane, Australia in 2015 and host specificity tests were completed in 2021. No-choice host specificity tests with 55 test plant species confirmed that the gall thrips is highly host specific at subspecies level of the host plant. The gall thrips was approved for field release in Australia in November 2022, and field releases commenced in January 2023. The gall-inducing tephritid fly was imported from Senegal, into a medium security quarantine facility in Brisbane, Australia between 2018 and 2021. No-choice host specificity tests were conducted on 20 test plant species. Oviposition by the gallfly was evident on 13 non-target species, and gall development occurred on eight of them, though adults emerged from only one of the non-target species. In choice tests, oviposition was evident on non-target test plant species. In view of the potential non-target risk, testing of the remaining test plant species was suspended. A gall mite deforming leaflets, rachides and shoot tips in prickly acacia was exported from Ethiopia into a quarantine facility in Pretoria, South Africa for host specificity testing. In preliminary no-choice tests, the gall mite induced galls only on *V. nilotica* subsp. *indica*, and not on other *V. nilotica* subspecies (*kraussiana*, *adstringens* and *tomentosa*). Due to the loss of the culture of *Aceria* sp. in quarantine in Pretoria, South Africa, and the inability to re-collect the gall mites from Ethiopia due to the Covid pandemic, and civil unrest, no further host specificity tests have been conducted. The gall mite from Ethiopia will be imported into the quarantine facility in South Africa as soon as it is safe to conduct field visits in Ethiopia.

Moderator: Freda Anderson (CERZOS, AR)

Oral presentation

**Investigation and evaluation of rust fungi as potential biocontrol agents against  
*Microstegium vimineum***

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*Microstegium vimineum* widely occurs in various habitats across southern and central China. It has invaded the Middle East of the United States and caused seriously negative ecological consequences in the country. So far, there have not been economic and effective control measures available against this invasive alien weed. Biological control using a rust fungus could be an economic and sustainable approach. To that end, a systematic field survey of the natural rust diseases of *M. vimineum* populations was conducted during the summer and autumn of 2014 to 2018 to screen for potentially suitable rust fungal strains. The rust diseases of *M. vimineum* were systematically investigated at 62 sampling sites where it was found in China including Anhui, Jiangsu, Zhejiang, Hunan, Sichuan, Jiangxi, Hubei, and Guizhou provinces. Disease incidence was above 70% in plant populations at 35 sampling sites. Rust fungal samples from 72 diseased plants were collected for isolation and morphological and molecular identification. Pathogenicity was determined using Koch's postulates. A total of 43 isolates and 26 pathogenic rust fungi were isolated belonging to 5 genera. Among them, 3 species of 6 rust strains were reported for the first time in *M. vimineum*. The strain WZ-1 was identified as a novel rust species, *Puccinia microstegiae* Sheng Qiang et Min Tan (unpublished) based on morphological and molecular characteristics. The host specificity of the strain WZ-1 tested on 64 plant species from 12 families revealed that 24 major crops and 35 weed plants were immune to the strain, with only five other gramineous weeds being slightly susceptible but not producing urediniospores. The strain WZ-2 (*Kweilingia divina* (Syd.) Buriticá) shared similar host specificity with the former. Rust epidemiological studies showed that urediniospores from the two strains could infect *M. vimineum* plants in the field, causing severe rust disease symptoms on the leaf surface. The spread of rust disease was positively correlated with humidity, rainfall and wind speed at temperatures ranging from 15 °C to 35 °C. These findings suggest that the rust fungi, *Puccinia microstegiae* and *Kweilingia divina* have potential for use as biological control agents against *M. vimineum*.



**Evaluation of the leafspot *Mycosphaerella polygони-cuspidati* as a potential mycoherbicide for Japanese knotweed, *Reynoutria japonica***

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*Mycosphaerella polygони-cuspidati* (Mycosphaerellaceae, Ascomycota) is a damaging fungal leafspot ubiquitously associated with Japanese knotweed (*Reynoutria japonica*) in its native range in Japan. Evaluation of the fungus as a potential classical biological control (CBC) agent for invasive knotweed in its introduced European and North American ranges showed that it can cause limited non-target impacts under quarantine greenhouse conditions; the pathogen infected and developed fertile spermogonia on *Persicaria hydropiper* and *Polygonum maritimum*, both native to the UK, and on the North American species *Polygonum glaucum*. Based on these findings, and the resultant pest-risk analysis, the leafspot fungus has been ruled out for release as a CBC agent. Nonetheless, *M. polygони-cuspidati* is considered to have potential as a mycoherbicide, especially in problematic urban situations. The pathogen is heterothallic, requiring two complementary mating types to complete its life cycle, and lacks an asexual conidial stage. Therefore, basing a mycoherbicide on a single mating-type isolate would prevent sexual reproduction, and thus the establishment of the fungus in the field. As well as infecting its host via ascospores, *M. polygони-cuspidati* has also been shown to be able to penetrate and colonise Japanese knotweed via mycelial inoculum; both modes of infection cause the same severe herbicide-like symptoms. This concept has been protected by a European patent held by the Department for Environment, Food and Rural Affairs (Defra, UK), with registration in 12 countries. Further international patent applications are pending.

Initial proof-of-concept experiments conducted under quarantine conditions, confirmed that the application of mass-produced mycelial inoculum, based on a single mating-type isolate of *M. polygони-cuspidati*, consistently causes development of characteristic leafspot symptoms on knotweed plants. The research has highlighted the need to maintain pathogen virulence *in vitro*, and demonstrated that ambient relative humidity is an important parameter for disease development. Following Defra approval for release from quarantine in 2019, licensed experimental trials were undertaken with a single mating-type isolate of the leafspot under semi-natural conditions in field tents, from 2019 to 2021. Three experimental treatments – using mycelial-broth inoculum – were trialled and the development of disease incidence and its severity on inoculated knotweed plants were monitored over a 10-week period. Here, we report on the performance of the leafspot fungus under semi-natural in comparison to greenhouse conditions and the methods investigated to preserve its virulence *in vitro*. The need for further research to fully evaluate the potential of *M. polygони-cuspidati* as a mycoherbicide for Japanese knotweed is highlighted.

**Diversity of fungi associated with dieback and stem canker of *Prosopis* tree species in South Africa**

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*Prosopis* is a genus of invasive alien tree species invading the arid and semi-arid regions of South Africa. Here, a complex of hybrids with *Prosopis glandulosa* and *P. velutina*, amongst others, as parental species is involved in the invasion. *Prosopis* species invasions have been reported to cause detrimental effects on biodiversity, ecosystem services and human livelihoods in many countries, including South Africa. Severe symptoms of dieback were recently observed on *Prosopis* trees in the Northern Cape Province, South Africa. In this study, we investigate the possible cause of this dieback across its distribution range in South Africa. Fungi isolated from infected branch samples were identified by partial sequencing of the internal transcribed spacer (ITS) and the large subunit (LSU) gene regions. Eleven species, including *Alternaria alternata*, *Botryosphaeriaceae* sp., *Clonostachys rosea*, *Dothiorella rosulata*, *Dothiorella viticola*, *Eutypella* sp., *Eutypella microtheca*, *Fusarium proliferatum*, *Lasiodiplodia* sp., *Lasiodiplodia exigua* and *Neofusicoccum parvum* were isolated from infected *Prosopis* with dieback and canker symptoms. Results from pathogenicity trials showed variable results for the isolated fungi. *Alternaria alternata*, *C. rosea*, *D. viticola*, *F. proliferatum*, *L. exigua* and *N. parvum* were highly aggressive on *Prosopis* seedlings, producing significantly longer lesions using wounded and unwounded inoculations, while *Botryosphaeriaceae* sp. and *Eutypella* sp. produced significantly smaller lesions and *E. microtheca* did not produce any lesions on *Prosopis*. These fungi will be investigated in the future to determine their feasibility as mycoherbicide candidates against *Prosopis*.

**Difference and cause in pathogenicity of *Bipolaris yamadae* to nine different *Echinochloa* species**

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Barnyard grasses (*Echinochloa* P. Beauv.) are among the most problematic weeds in agricultural systems worldwide, especially in rice paddy fields. Extensive and continuous use of chemical herbicides for their control has caused herbicide-resistance evolution of multiple sites-of-action in many barnyard grass populations around the world. A bioherbicide may be an alternative to chemicals. Our previous research demonstrated that the pathogenic *Bipolaris yamadae* strain HXDC-1-2 has potential as a bioherbicide against *Echinochloa crusgalli*. A bioassay was conducted to evaluate pathogenicity of *B. yamadae* strain HXDC-1-2 against nine different *Echinochloa* species. The results showed that the ED<sub>90</sub> (90% effective dose) values of *B. yamadae* strain HXDC-1-2 on the nine species (*E. phyllopogon* (Stapf) Koss., *E. crus-galli* var. *zelayensis* (Kunth) Hitchcock, *E. oryzoides* (Ard.) Fritsch., *E. crus-galli* var. *mitis* (Pursh) Petermann, *E. colona* (L.) Link, *E. crus-galli* var. *austrojaponensis* Ohwi, *E. crus-galli* (L.) P. Beauv., *E. crus-galli* var. *praticola* Ohwi and *E. glabrescens* Munro ex Hook. f.) ranged from  $4.47 \times 10^3$  to  $2.40 \times 10^5$  conidia ml<sup>-1</sup>. To explain the variation among the nine different barnyard grass species in the ED<sub>90</sub>, the infection process of the fungus on the leaf surfaces was compared. Morphological structure and substance composition of leaves of the nine species were also observed. Conidial germination, mycelial growth and appressorium formation occurred on barnyardgrass leaves within 1 to 6 hours. The hyphae directly penetrated cells and stomata on the epidermis primarily through the appressorium, and necrotic lesions were observed on the leaf surface within 20 to 24 hours. Disease severity was positively correlated with the number of cells per unit area of the leaf epidermis. The rate and severity of infestation were significantly and positively correlated with the number of appressoria and mycelial branches. In addition, ED<sub>90</sub> values were positively correlated with the wax content of the leaf epidermis. The number of appressoria formed was positively correlated with long-chain alkanes. From the above results, it may be concluded that morphological structure and substance composition of leaves influenced the infection speed and consequently caused differences in pathogenicity of *B. yamadae* to the nine different *Echinochloa* species.

**Bioherbicide management of *Cyperus rotundus* (Cyperales: Cyperaceae) – the tropical scourge – right target, wrong agents?**

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Inundative biocontrol started off on the wrong foot in the 1970s. The pioneer products were technical successes but commercial failures. COLLEGO – the *Colletotrichum*-based mycoherbicide, developed against northern jointvetch; Devine – the *Phytophthora palmivora* product for strangler vine and BIOMAL – another *Colletotrichum* mycoherbicide targeting round-leaved mallow, were developed for weeds that did not have enough economical relevance to justify a product market. These initial examples have, to some extent, “tainted the good name” of bioherbicides. Later projects moved to highly relevant target weeds. But perhaps this was too late to attract the interest of the industry. Other problems have also plagued the attempts to develop successful biological herbicides. Here we discuss the works of different research groups that have investigated different fungi as mycoherbicides against, what was often recognized as the “world’s worst weed” – purple nutsedge (*Cyperus rotundus*). Scientists in the University of Georgia have focused their attention to the rust fungus *Puccinia canaliculata* – an unlikely candidate for a mycoherbicide because of its biotrophic nature; the team led by R. Charudattan at the University of Florida investigated the potential of *Dactylaria higginsii*; at EMBRAPA Centro de Recursos Genéticos and Universidade de Brasília (in Brazil) the chosen agent was *Cercospora caricis*; at the Universidade Federal de Viçosa we chose *Duosporium yamadanum* (now recombined as *Curvularia yamadana*). A discussion on the attempts to tackle *C. rotundus* with fungal pathogens of purple nutsedge already present in the Neotropics will be presented. Although *C. rotundus* remains economically highly relevant and a challenging weed to manage through mechanical or chemical methods, and a “good target weed” for biocontrol, particularly when compared with northern jointvetch, strangler vine and round-leaved mallow, it has proven to be a difficult target for biological agents as well. Now, with the quickly increasing interest of the industry in weed biocontrol, it may be the time to revisit each of the past attempts conducted until now and give some of those fungi which were left aside, another opportunity. Their chances may improve with proper research funding from the industry and the novel technologies that became available along the past decades. But, possibly, even more promising, is the possibility of searching for novel strains and novel fungal pathogens of purple nutsedge in the center of origin of the weed in the Old World.

### **The International Organization for Biological Control (IOBC) Global Cactus Working Group Meeting**

Authors: IOBC Global Cactus Working Group Members

The IOBC Global Cactus Working Group aims to raise awareness about biological control as a safe and effective method for managing invasive alien cactus species and to promote the use of biocontrol to manage the pests of beneficial and indigenous cactus populations. The Cactaceae are endemic to the new world, with the exception of a single old-world species, the mistletoe cactus *Rhipsalis baccifera*. Biocontrol agents for both the cactus plants themselves, as well as the pests of the cactus, are therefore sourced in North, Central or South America. The countries with the most active biocontrol programmes against invasive alien Cactaceae are Australia and South Africa, but there are many countries that have serious problems with invasive alien cactus species and very limited or no biological control. One of the aims of the Global Cactus Working Group is to expand the use of cactus and cactus pests biocontrol where it is needed. In many countries in sub-Saharan Africa there are very few cactus agents released outside of South Africa, and in South Europe, where no cactus agents have been intentionally released despite the presence of several invasive cactus populations. On the other side, the cactus moth *Cactoblastis cactorum*, and the cactus mealybug *Hypogeococcus pungens*, are pests of indigenous cacti within the native range, and the cochineal *Dactylopius opuntiae*, is an invasive pest of cultivated cacti in Europe and North Africa. Biocontrol agents are being developed for all three of these insect pests. The Global Cactus Working Group intends to play a role in promoting informed discussion, and developing and implementing biocontrol agents for both cactus species and the pests of beneficial cacti in a way that conflicts of interest are minimized. The next action that the group will take is the development of a paper that will review both cactus biocontrol and biocontrol of cactus pests globally, highlight the need for collaboration between researchers in weed biocontrol and biocontrol of insect pests, and developing a strategy to promote the use of biocontrol where it is most needed.

### International Collaboration for Tamarix Biocontrol

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*Tamarix* spp. comprise a complex of Old-World woody plants that are invasive in North and South America, South Africa, and Australia. A biological control programme in North America has been a politically-charged mixed success that, although far from complete, is still potentially applicable in other regions of the globe. The workshop brought together researchers from both the invasive and native ranges of *Tamarix* to discuss its ecology and management, evaluate the current status and constraints of the biocontrol programme, and, to explore opportunities for international cooperation for a renewed research programme that may help improve the development and outcomes of *Tamarix* biocontrol across different biomes.

The workshop was attended by ca. 20 people representing different institutions, from countries in the invasive and native range of *Tamarix* spp. Tom Dudley and Alex Gafke, who attended virtually, gave a summary of the history and present status of the *Tamarix* biological control project in the USA. Fernando Mc Kay presented on the progress and future plans for a Tamarisk biocontrol project in Argentina. Nic Venter (University of the Witwatersrand, South Africa) commented on the difficulties of finding a sufficiently specific biocontrol agent for invasive *Tamarix* spp. due to the existence of native *Tamarix* species (*T. usneoides*) in South Africa. Sathyamurty Raghu (CSIRO, Australia) indicated that although there is no biocontrol program against Athel pine (*T. aphylla*) at this moment, it is likely that there will be one in the future. As a conclusion, organisers and participants agreed on the need of developing an international consortium for *Tamarix* biocontrol to enhance opportunities for cooperative research and funding.

## Future prospects for biological control of weeds in Europe

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Classical Biological Control (CBC) has been utilised globally for over a hundred years, but although Europe has been the source of both invasive weed targets and biocontrol agents, it is lagging behind. The first intentional classical release against a weed in the EU took place in the UK with the release of the psyllid *Aphalara itadori* against *Fallopia japonica* in 2010. This was followed by further agent releases targeting *Impatiens glandulifera*, *Crassula helmsii* and *Hydrocotyle ranunculoides* in the UK, *Acacia longifolia* in Portugal and *Fallopia japonica* in the Netherlands. Due to these biocontrol programmes, the regulatory processes for CBC agent release have now been established in these countries. There is a growing interest from European governments and stakeholders to implement biological solutions for invasive alien plants problems, which is already paving the way for future opportunities for CBC in Europe. In this workshop we explored how we, as biological control researchers, can capitalise on these opportunities through closer collaboration, both within Europe as well as with counterparts overseas.

To this end there were presentations covering multiple topics including the revival of the European Weed Research Society (EWRS) working group on biological control. The working group will provide a platform for group members to collaborate on all aspects of biological weed control and will also actively engage with the “One Health” concept, the “EU Green Deal” as well as a currently anticipated COST Action on Biocontrol and Invasive Alien Species. The new, yet to be published, EPPO Standard on host range testing was also discussed. This Standard describes the procedure for evaluating the host specificity of non-indigenous (classical) biological control agents (BCAs) for use against invasive alien plants. The Standard covers guidance and best practice on essential elements for host specificity testing of invertebrate and fungal BCAs. In addition to this, EU regulation and the steps involved in releasing an agent in Europe were discussed as well as whether there was a process in place on how BCAs are shared between countries once released. Lastly, perceptions of CBC were discussed with research presented on how different stakeholder communities in France and EU *versus* the rest of the world perceived the risks and acceptability associated with CBC.

## **Are we being too risk averse? Is it time to consider ‘seemingly oligophagous’ agents for release?**

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We define ‘seemingly oligophagous’ agents as agents that have a broad physiological host range, meaning that several species in several different genera in the same family are attacked, when tested under no-choice conditions, but a very restricted host range (though not necessarily monophagous) when tested under more natural conditions in the open-field (ecological host range). We argue that potentially effective agents are currently not being petitioned because of a too strong emphasis on the physiological host range of the agent.

The physiological host range can be reliably predicted using pre-release no-choice tests, and no nontarget attack post-release is expected outside of the physiological host range of an agent. It is also common knowledge and has been confirmed repeatedly, that the physiological host range of agents overestimates their ecological host range under natural conditions. What we know less well is how reliable pre-release studies are to predict the realized host range of agents once released, namely choice and open-field tests, host finding behavioral studies including olfactory and visual cues and additional ecological filters, such as lack of geographical or habitat overlap, asynchronous phenology or inability to produce multiple generations on non-targets.

Opportunities to test the predicting power of these studies are for instance accidentally introduced species, such as *Ophraella communa* on ragweed, studies on released agents with a relatively broad physiological host range, such as *Mogulones crucifer* on houndstongue, and extrapolating from native oligophagous species (e.g. *Chrysochus* on *Apocynum* spp.). The three cases presented confirmed that ‘seemingly oligophagous’ should not be automatically excluded as potential agents. The choice will depend on the system. If a project has a wealth of very specific potential agents to choose from, it will not be necessary to consider agents that bear a higher risk. However, projects against plant invaders with large negative impacts on multiple sectors and with very few candidates, may need to accept greater risk and consider agents with broader host ranges. This will then necessitate more detailed studies to increase the confidence in the realized host range predictions of such agents. Potential risks then need to be weighed against potential benefits, and in case of release, more thorough post-release studies would be required. This in turn could help to improve pre-release studies, with the aim of getting better at predicting the realized host range of agents.



## **Crossing the Equator: Consequences of hemisphere-shift on the establishment and post-release performance of biological control agents**

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The movement and exchange of biological control agents (BCA) within and between hemispheres is a common practice in invasive species management. The success of a biological control program can be influenced by numerous biotic and abiotic factors (e.g., predators, phenological synchronization, climate, habitat quality, etc.). Yet, the implications of the exchanges between hemispheres and the legacy impacts of the potential phenological asynchronization between the BCA and its host (and other limiting factors) are not always evaluated and understood. In fact, a recent evaluation of the success of 377 biocontrol programs at global scale revealed a high proportion of programs (45%) where the limiting factors were considered unknown or undetermined (Schwarzländer, Hinz, Winston, & Day, 2018). The phenological asynchronization may be particularly relevant when the BCA are univoltine and dependent on a particular and ephemeral phase of the host plant life cycle (e.g., flowers or fruits).

A dozen of participants from different continents of both hemispheres, discussed this gap of knowledge during this workshop with fruitful contributions. The workshop started with two interesting presentations. From the insect point of view, F. Grevstad focused on the impact of photoperiod on the insects' life cycle, while F.A. López-Núñez presented two contrasting case studies of BCA imported from South Africa to Portugal: the successful establishment of *Trichilogaster acaciaelongifoliae* and the challenging pre-release quarantine testing of *Melanterius* spp. The presentations were followed by a passionate and fruitful debate, where participants were encouraged to share their experiences and thoughts on the issues raised by the presentations. As main conclusions, participants highlighted the importance of (1) manipulation of BCA dormancy and diapause periods using different stimuli (e.g., photoperiod, temperature, hormones, ...) after hemisphere translocation may be key to mass-rearing agents for specificity testing before (and after) release; consequently, (2) it is necessary to target the life stage of the BCA most susceptible to be influenced by abiotic and biotic conditions, and therefore most likely to undergo changes in its physiology under artificial conditions (e.g., immature stages). Finally, (3) it is critical to invoke one of the fundamental principles of biocontrol: international collaboration and networking to avoid unnecessary expenses and delays during biocontrol programs. As final recension, this workshop not only underlined the importance of better understanding the effects of BCA inter-hemisphere translocation in biocontrol programs; but also paved the way for some promising solutions for a real case - the biocontrol program involving *Melanterius* spp., an univoltine weevil from the southern hemisphere, in Portugal. The solutions discussed can be replicated in other similar situations.

### Catching up on aquatic weed biocontrol

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Aquatic weeds in general pose a significant threat to aquatic ecosystems where monospecific mats may have both socio-economic and ecological impacts. For decades biological control has offered a cost effective and sustainable option for management showing massive successes in several systems. With the current trends of invasion, several invasive aquatic weeds (old and new invaders) are showing up in new places, possibly driven by anthropogenic introductions and a warming climate. In this workshop we aimed to bring together the aquatic weed biocontrol community to foster international collaboration, share research results and learn about participants' interests in different systems. Overall, the workshop was a success with around 50 participants. We believe it was a valuable exercise during which a brief update on some systems was given, highlighting research on old and new invaders with their successes and failures, and, studies on invasion biology of aquatic invaders were commented on.

- Ana Faltlhauser and Alejandro Sosa updated on current projects and agents being studied at FuEDEI, Argentina.
- Grant Martin from the Centre of Biological Control (CBC) in South Africa, gave an update on the success of parrot's feather biocontrol in South Africa.
- Lauréline Humair and Philip Weyl from CABI Switzerland, updated on host range testing and chemical ecology of agents against parrot's feather currently being studied at CABI, Switzerland.
- Preliminary host-specificity results from *Myriophyllum aquaticum* and *Egeria densa* were presented by Kumaran Nagalingam, CSIRO, Australia.
- Rodrigo Diaz, Louisiana State University, USA gave an account of the biological control of *Salvinia molesta* in Lake Ossa, Cameroon.
- Julie Coetzee, Centre of Biological Control (CBC) in South Africa described the success of water hyacinth control in a eutrophic system, by taking advantage of a community-run mass rearing programme for augmentative releases.
- Celeste Franceschini (CECOAL-CONICET-UNNE) presented a new project to control *Pistia stratiotes* in urban water bodies of Chaco, Argentina.
- The invasion of yellow flag iris was presented by Paula Gervazoni (CECOAL-CONICET-UNNE), Gianmarco Minuti (Vrije Universiteit Brussel), and Emma Sandenberg (CBC).

After the engaging interactions observed during this meeting we would like to encourage its recurrence in the next symposium to further enrich the discussions and relationships established.

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