# Proceedings of the XV International Symposium on Biological Control of Weeds

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**Edited by**

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## Symposium Production

Thanks are due to the scientific committee for selecting the talks and reviewing submitted abstracts. Graeme Bourdôt and Maurizio Vurro organized the Bioherbicide session. Lauren Brown (CABI HQ) developed the logo. Joseph Burgoyne, Teddy Searight, Wayne Coles and others from the CABI Marketing Team in the UK helped with the panel, to promote the event, and provided promotional material. Ghislaine Cortat, Céline Martinez and Jonathan Jolidon helped with donor letters. Cliff Moran and Fiona Impson helped compile past symposia data for Table 1 (Preface).

## Symposium Sponsors

Thanks to CABI internal funding through the Action on Invasives Programme, 11 participants from low income countries could be supported to participate, while IOBC supported the participation of 12 young researchers (< 35 years old). Funding from the Swiss National Science Foundation helped to invite keynote speakers and people from the scientific committee. Rachel Winston (MIA Consulting) designed the layout of these Proceedings with funding provided by the USDA Forest Service. In addition, conference funding was provided by CSIRO, the Swiss Federal Office for Agriculture and the Swiss Federal Office for the Environment. All support is highly appreciated and acknowledged. A sincere thank you is extended to George Heimpel and Barbara Barratt for attending the Symposium in its entirety and for presenting IOBC.

CABI

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# Table of Contents

# Preface

The XV International Symposium on Biological Control of Weeds (ISBCW) took place from 26–31 August 2018, at the Hotel Bellevue in the picturesque Swiss Alpine village of Engelberg. The first ISBCW was organized in 1969 by CABI Switzerland (back then the European Station of the Commonwealth Institute of Biological Control) in Delémont, Switzerland. Almost 50 years later, ISBCW came back to its roots, where it has grown from 22 scientists representing 15 institutes and organizations from 11 countries to 205 scientists representing nearly 100 institutes from 25 countries in 2018 (Table 1). The countries most represented were the USA with 56 delegates, followed by South Africa with 30, Australia 21, Switzerland 19, and China and New Zealand with 13 each.

**Table 1.** Summary of attendance and presentations data at ISBCW symposia to date. Data largely from Wu et al. (2013[1]; XIII ISBCW Proceedings: Table 2) with supplementation from Moran and Hoffmann (2015)[2] and C. Moran and F. Impson (pers. comm.). Note: some numbers differ slightly from those presented in Wu et al. (2013) and Moran and Hoffmann (2015).

| Symposium details | | | Attendance numbers by: | | | | | No. papers & abstracts in proceedings |
|---|---|---|---|---|---|---|---|---|
| No. | Date | Location | Countries | Organizations | Participants | % Women | % 1st Timers | |
| I | 1969 | Delémont, Switzerland | 11 | 15 | 22 | 0 | 100 | 21 |
| II | 1971 | Rome, Italy | 9 | 17 | 37 | 0 | 72.7 | 23 |
| III | 1973 | Montpellier, France | 11 | 14 | 25 | 4.2 | 66.7 | 16 |
| IV | 1976 | Gainesville, FL, USA | 11 | 42 | 84 | 1.2 | 76.2 | 45 |
| V | 1980 | Brisbane, Australia | 11 | 52 | 100 | 6.9 | 62.4 | 165 |
| VI | 1984 | Vancouver, Canada | 13 | 59 | 135 | 14.8 | 64.4 | 68 |
| VII | 1988 | Rome, Italy | 20 | 60 | 128 | 11.7 | 65.6 | 96 |
| VIII | 1992 | Canterbury, New Zealand | 18 | 80 | 181 | 14.5 | 55.9 | 96 |
| IX | 1996 | Stellenbosch, South Africa | 25 | 91 | 202 | 17.4 | 60.5 | 139 |
| X | 1999 | Bozeman, MT, USA | 27 | 115 | 308 | 23.6 | 63.9 | 226 |
| XI | 2003 | Canberra, Australia | 20 | 60 | 175 | 27.9 | 45.9 | 177 |
| XII | 2007 | La Grande Motte, France | 32 | 106 | 250 | 32 | 51.5 | 226 |
| XIII | 2011 | Waikoloa, HI, USA | 19 | 78 | 208 | 30.3 | 48.1 | 224 |
| XIV | 2014 | Skukuza, South Africa | 22 | 57 | 154 | 35.7 | 42.2 | 130 |
| XV | 2018 | Delémont, Switzerland | 25 | 94 | 205 | ~ 38 | 46.3 | 195 |

[1]Moran, V.C., Hoffmann, J.H., 2015. The fourteen International Symposia on Biological Control of Weeds, 1969–2014: Delegates, demographics and inferences from the debate on non-target effects. Biol. Control 87, 23–31.

[2]Wu, Y., Johnson, T., Sing, S., Raghu, S., Wheeler, G., Pratt, P., Warner, K., Center, T., Goolsby, J., Reardon, R. (Eds.), 2013. Proceedings of the XIII International Symposium on Biological Control of Weeds. USDA Forest Service, FHTET-2012-07, Morgantown, WV, USA.

Overall, the number of participants appears to plateau at approximately 200 (apart from the peak numbers in Bozeman and Montpellier), and the percentage of first-time participants plateaus just below 50%. This makes for a good mix of experienced biocontrol practitioners and newcomers. The percentage of women has continuously increased but falls short of the desired 50% mark. Of the people that attended the XV ISBCW, Jim Cullen is the most faithful stalwart, participating in 12 symposia, followed by Ernest Delfosse and Alec McClay with 11 participations, and Richard Hill, Peter McEvoy, Louise Morin and Andy Sheppard with nine.

Unfortunately, the Proceedings have undergone a dwindling number of full papers, which is most probably driven by the increasing pressure to publish in peer-reviewed journals.

## Venue of Next ISBCW

The XVI ISBCW will take place in Puerto Iguazú, Misiones Province, Argentina in 2022, and will be co-organized by FuEDEI (Fundación para el Estudio de Especies Invasivas), Argentina, and by the Universidade Regional de Blumenau and the Universidad Federal do Vicosa, Brazil. This is the first time that the ISBCW will be hosted in South America, and it is hoped that this will help to boost classical weed biocontrol in this region of the world.

## Winston et al. (2014) Update

Attendees to this symposium agreed that the weed biocontrol catalog is an important resource for the biological control community and that, at the very least, the online version (https://www.ibiocontrol.org/catalog/) should be regularly updated. Rachel Winston (MIA Consulting) fortunately agreed to continue this work. The following people (entities) graciously volunteered to support her effort financially: Mark Schwarzländer (University of Idaho), Andy Sheppard (CSIRO), Martin Hill (Centre for Biological Control, South Africa), Lynley Hayes (Landcare, New Zealand) and Hariet L. Hinz (CABI). Additional contributions would be highly welcome!

## Creation of IOBC Global Working Group

George Heimpel, the current President of the International Organization for Biological Control (IOBC) and Barbara Barratt, past-President, gave a presentation about the IOBC and the advantages of potentially forming a Global Working Group for Classical Biological Control of Weeds under the umbrella of IOBC. A preliminary vote was positive; either participants voted for the idea or stayed indifferent. An online survey will still follow to allow everybody, including the people who could not attend the Symposium, to have their say. After further discussions and correspondence with George, the group could initially be established as a "Study Group" prior to forming an actual Working Group during the next ISBCW, if warranted. In the meantime myself and Raghu Sathyamurthy (CSIRO) will act as points of contact. We believe that a Global Working Group for Classical Biological Control of Weeds could provide a forum to connect weed biocontrol scientists between the quadrennial ISBCW, facilitate contributions to global discussions on regulations and help to promote weed biocontrol, e.g., by supporting early career scientists, developing country collaborations and providing a mechanism to advocate for its appropriate and sustained use as a vital weed management tool.

## ABS and Nagoya Protocol

Several talks on this subject, as well as a workshop entitled "The Nagoya Protocol and its implications for classical weed biological control," triggered discussions and further actions. For instance, to determine the level of understanding and perception of impacts of the ABS rules on the practice of biological control, the IOBC Global Commission on Access and Benefit-Sharing is developing a questionnaire. An initial draft was circulated to participants at the April 2019 United States Department of Agriculture (USDA) Animal and Plant Health Inspection Agency (APHIS) Technical Advisory Group (TAG) on weed biological control, and feedback is being reviewed. Luciana Silvestri (Instituto de Ciencias Humanas, Argentina) is leading a contribution describing the experiences of providers and recipients of biological control genetic resources. The paper will focus on Argentina, Brazil, South Africa, the United States, Canada and CABI, to discuss how national ABS legislation can negatively impact the effective and efficient access, exchange and utilization of biological control agents (see also Silvestri et al., 2019, these Proceedings). Future work will document examples of experiences by recipients to access biological control agents from countries with and without ABS legislation.

## Special Series in Current Opinion in Insect Science: A close-up on weed biocontrol with insects

A number of short reviews will be published in the 2020 Parasites/Parasitoids/*Biological control* section, edited by Heinz Müller-Schärer (University of Fribourg) and Urs Schaffner (CABI Switzerland), covering recent achievements in our discipline, and as a follow-up of this Symposium and Proceedings. One of the reviews, led by Urs Schaffner, will cover the development of common criteria to monitor the outcomes of biological control and restoration projects, one of the action points discussed during this Symposium.

Hariet L. Hinz
CABI Switzerland
August 2019

# Session 1: Target and agent selection

## Session Chair: Massimo Cristofaro

**Keynote**

# Prioritizing weed targets and agents for biological control: are we getting better at it?

Louise Morin[1]

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Weed classical biological control programs have significant up-front costs and require long-term investments, without guarantees that they will succeed. Considering that resources are ever more limited, there has been a strong push to improve prioritization processes for weed targets and agents in order to make better investment decisions. Recent examples of frameworks used to prioritize weed targets for biological control were discussed, with benefits and pitfalls highlighted. These frameworks have been based on a matrix system that considers the importance of the weed versus the feasibility and likelihood of success of biological control. Investors have welcomed and embraced this approach to guide their decisions. Another challenge encountered by researchers has been the prioritization of potential agents to achieve the goals of biological control programs. This presentation focused on pathogen agents and provided a general overview of the key characteristics to look for in prioritizing them for biological control. A structured approach to select promising pathogens and critical issues to consider was discussed using a series of examples. While predicting the efficacy of agents after release in a new region remains a major challenge, increasing transparency of the decisions we make as biological control researchers can only be beneficial in the long term.

*Oral presentation*

# Geographic population structure in an outcrossing plant invasion after centuries of cultivation and recent founding events

John Gaskin[1]*, Mark Schwarzländer[2], Robert Gibson II[2], Heather Simpson[3], Diane Marshall[3], Esther Gerber[4], Hariet L. Hinz[4]

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Population structure and genetic diversity of invasions are the result of evolutionary processes such as natural selection, drift and founding events. Some invasions are also molded by specific human activities such as selection for cultivars and intentional introduction of desired phenotypes, which can lead to low genetic diversity in the resulting invasion. We investigated the population structure, diversity and origins of a species with both accidental and intentional introduction histories, as well as long-term selection as a cultivar. Dyer's woad (*Isatis tinctoria* L.; Brassicaceae) has been used as a dye source for at least eight centuries in Eurasia, was introduced to the eastern USA in the 1600s, and is now considered invasive in the western USA. Our analyses of AFLPs (Amplified Fragment Length Polymorphisms) from 645 plants from the USA and Eurasia did not find significantly lower gene diversity ($H_j$) in the invaded compared to the native range. This suggests that even though the species was under cultivation for many centuries, human selection of plants may not have had a strong influence on diversity in the invasion. We did find significantly lower genetic differentiation ($F_{st}$) in the invasive range, but our results still suggested that there are two distinct invasions in the western USA. Our data suggest that these invasions most likely originated from Switzerland, Ukraine and Germany, which correlates with initial biological control agent survey findings. Genetic information on population structure, diversity and origins assists in efforts to control invasive species, and continued combination of ecological and molecular analyses will help bring us closer to sustainable management of plant invasions.

*Oral presentation*

# Do host races exist in the Sagittaria fruit-feeding weevil?

Raelene Kwong[1]*, Jackie Steel[1], Mark Blacket[1]

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Levels of genetic diversity in weed populations and compatibility of biocontrol agents to invasive genotypes are two factors critical to the success of biological control. Hence, molecular approaches are being increasingly utilized during the initiation phase of biocontrol research prior to the release of agents to address key issues such as (1) accurate taxonomic identification of the target weed including the identification of novel hybrids, (2) comparison of genetic structure between native and invaded ranges or between different invaded habitats and (3) pinpointing the origin(s) of invasive populations where compatible natural enemies might be found. In this study, we utilized molecular approaches to compare genetic diversity and population genetic structure between native and invasive populations of the aquatic monocot delta arrowhead, *Sagittaria platyphylla* (Engelm.) J.G. Sm. (Alismataceae) to evaluate the likelihood of successful biocontrol. Using Amplified Fragment Length Polymorphism markers (AFLPs) we compared relative levels of genetic diversity and population genetic structure between native North American and introduced Australian and South African populations. After finding three main *S. platyphylla* genotypes in the USA, we then used DNA barcoding techniques to determine if genetic structure exists in natural enemy populations associated with the different host genotypes. This presentation looked specifically at *Listronotus appendiculatus* (Boheman) (Curculionidae), a fruit-feeding weevil under consideration for release into Australia and South Africa.

*Oral presentation*

# Screening for agent selection: genetic diversity and structuring of leaf-tiers and chrysomelids from *Acacia auriculiformis* in Australia

Muhammad Nawaz[1]*, Graham A. McCulloch[1], Ryan Zonneveld[2], Christine H. Goosem[2], Gimme H. Walter[1]

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*Acacia auriculiformis* A.Cunn. ex Benth., also known as earleaf acacia, is a native Australian tree that has become a category 1 invasive weed in Florida, USA. Our research focuses on identifying and prioritizing potential biological control agents for this species. Field surveys were conducted (2016–2018) in its native host range, i.e. North Queensland and the Northern Territory (NQ and NT). Over 600 specimens, comprising a variety of different insect groups, were collected from *A. auriculiformis* and closely related acacia species (distributed mainly in southern Queensland), and identified by COI DNA barcoding. Two insect groups that are highly damaging to the target weed are chrysomelid beetles and leaf-tying moths (mainly belonging to the cosmopterigid genus *Macrobathra*). Phylogenetic relationships within these groups were reconstructed using Bayesian inference. Seven moth lineages were identified from the 50 specimens sequenced, and six beetle lineages from the 30 chrysomelid specimens. Molecular analyses identified genetic divergence between specimens collected from NQ and NT. The Carpentaria Barrier may have isolated these populations from one another by disrupting the distribution of the host plant across Northern Australia. This biogeographical disjunction seems to be reflected in the genetic diversity previously found within *A. auriculiformis* across its distribution. Laboratory colonies of genetically distinct populations of *Calomela* sp. (Chrysomelidae) from NQ and NT have been established for host specificity testing. Future research will assess the necessity of matching the provenance of the weed and its herbivore, by comparing the performance of the two populations of *Calomela* sp. and the damage they cause on *A. auriculiformis* from NQ, NT, Papua New Guinea and Florida.

*Oral presentation*

# Eriophyid mites and weed biological control: does every silver lining have a cloud?

Philip Weyl[1]*, Massimo Cristofaro[2,3], Lincoln Smith[4], Urs Schaffner[1], Biljana Vidović[5], Radmila Petanović[5], Francesca Marini[2], Gorban Ali Asadi[6], Sonja Stutz[1]

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In recent years, eriophyid mites have gained popularity in weed biological control based on a number of assumptions, including high host specificity, short life cycles and high potential impact (Smith et al., 2010). Eriophyid mites are among the smallest plant feeders (100–200 μm). These tiny herbivores are characterized by having an intimate relationship with their hosts, about 80% of currently known species are reportedly associated in nature with a single host (Skoracka et al., 2010). However, despite this very close relationship and potential impact, there are only nine eriophyid species intentionally released against a weed between 1971 and 2016 (Winston et al., 2014) (Table 1). In 72% of the releases, the mites have established in their new range; however, there are often limiting factors to population build up (Table 1). Limiting factors have been identified in 11 of the 18 separate releases and include climate, host plant resistance and predation (Table 1).

Much work to date has focused mainly on the taxonomy of eriophyid mites and relatively less work has been done on understanding the biology and ecology of the group. Often researchers of a weed biological control project will initiate host-range testing and impact studies soon after discovering an eriophyid mite rather than investing time and resources into understanding the biology and ecology of the species, or into elucidating whether infested plants harbor single or multiple mite species. This lack of understanding of the basic mechanisms that underlie the functioning of the organism in the ecosystem leads to difficulties in progressing with the project. Working with eriophyid mites in a classical weed biological control context has posed several challenges in understanding fundamental aspects of their life histories to make an informed decision on the suitability as biological control agents. Some specific challenges commonly encountered include (1) understanding the drivers of host specificity: some eriophyid mites species may be too specific, and only attack certain genotypes of a host, or, on the other hand show broad host range under experimental conditions; (2) identifying potential biotic limiting factors such as the effects of predation on vagrant vs. gall-forming eriophyid mites; (3) recognizing possible abiotic limiting factors such as soil characteristics and climate; and (4) understanding basic life history traits, such as overwintering requirements, dispersal capabilities and the relationship with annual vs. perennial host plants.

The assumption with eriophyid mites (since 80% are only recorded on a single host) is that they are likely host specific. In some cases this is true, and there are examples where the mites may be *too* specific and only attack certain genotypes in the invaded range (see Smith et al., 2010 and example of *Aceria lantanae* [Cook] in South Africa; Mukwevho et al., 2017). However, in many cases, especially in the pre-release testing phase of eriophyid mites, the assumption of host specificity is not met, and usually many test plants appear to be attacked or at least are able to support a population (usually of a few individuals) for an extended period of time (Littlefield and Sobhian, 1999). An example from our own work is the mite, *Aceria acroptiloni* Shevchenko & Kovalev on Russian knapweed (*Rhaponticum repens* [L.] Hidalgo), where under natural field conditions this mite has never been observed causing symptoms or building populations on any other plant species, even closely related Asteraceae plants in the field. However, under open-field experimental conditions, we had extremely low survival on the control plants (<10%) while we recorded mites on several non-target plant species over a five-year period. There are many potential reasons for this which may include accidental alighting and resting of mites on these non-target plants, especially since the mites are extremely mobile using wind for dispersal, or alternatively eriophyid mites may be capable of feeding and surviving on multiple plant species but not able to reproduce on them.

There has been discussion on the suitability of vagrant versus gall-forming mites for weed biological control (Skoracka et al., 2010; Smith et al., 2010). What is clear from many past experiments is that eriophyid mites are extremely sensitive to predation and even low population levels of predators can destroy a culture or ruin a carefully designed experiment. The assumption is that vagrant mites are at a greater potential risk of predation, while gall formers are protected. However, with gall formers, predation can severely

**Table 1.** Eriophyid mite species that have been intentionally released on alien invasive weeds across the world from 1971 –2016. Information adapted from Winston et al. (2014), Smith et al. (2010) and cited references.

| Agent Species | Weed Species | Country released | Year of first release | Established | Limiting Factors |
|---|---|---|---|---|---|
| *Aceria chondrillae* (Canestrini) | *Chondrilla juncea* L. | Argentina | 1989 | Yes | |
| *Aceria chondrillae* (Canestrini) | *Chondrilla juncea* L. | Australia | 1971 | Yes | Host plant resistance |
| *Aceria chondrillae* (Canestrini) | *Chondrilla juncea* L. | USA | 1977 | Yes | Host plant resistance; Predation; Climate |
| *Aceria genistae* (Nalepa) | *Cytisus scoparius* (L.) Link | Australia | 2008 | Yes | |
| *Aceria genistae* (Nalepa) | *Cytisus scoparius* (L.) Link | New Zealand | 2007 | Yes | |
| *Aceria lantanae* (Cook) | *Lantana camara* L. sens. lat. | Australia | 2012 | Unknown | |
| *Aceria lantanae* (Cook) | *Lantana camara* L. sens. lat. | South Africa | 2007 | Yes | Host plant resistance[1]; Climate |
| *Aceria malherbae* Nuzzaci | *Convolvulus arvensis* L. | South Africa | 1994 | No | Land use |
| *Aceria malherbae* Nuzzaci | *Calystegia sepium* (L.) R. Br. | USA | 1993 | Unknown | |
| *Aceria malherbae* Nuzzaci | *Convolvulus arvensis* L. | Canada | 1989 | Yes | Climate |
| *Aceria malherbae* Nuzzaci | *Convolvulus arvensis* L. | USA | 1989 | Yes | Climate; Host plant resistance? |
| *Aceria malherbae* Nuzzaci | *Convolvulus arvensis* L. | Mexico | 2004 | No | |
| *Aceria* sp. | *Chrysanthemoides monilifera* (L.) Norl. subsp. *monilifera* | Australia | 2008 | Yes | Possibly Predation; Climate |
| *Aculus hyperici* (Liro) | *Hypericum perforatum* L. | Australia | 1991 | Yes | Host plant resistance |
| *Cecidophyes rouhollahi* Craemer | *Galium spurium* L. | Canada | 2003 | No | Climate |
| *Floracarus perrepae* Knihinicki & Boczek | *Lygodium microphyllum* (Cav.) R. Br. | USA | 2008 | Yes | Host plant resistance |
| *Colomerus spathodeae* (Carmona) | *Spathodea campanulata* Beauv. | Cook Islands | 2016 | Yes[2] | |

[1]Mukwevho et al., 2017; [2]Paterson, I. (Rhodes University, South Africa) and Paynter, Q. (Landcare, New Zealand) pers. comm.

limit the dispersal capabilities within a plant to new sites or between individual plants. During a heavy infestation of predatory mites on the *Aceria angustifoliae* Denizhan, Monfreda, de Lillo & Cobanoglu culture on Russian olive, predatory mites were observed around the opening of the leaf galls, presumably to pick off any individuals that attempted to disperse. This poses the question that if eriophyids are potentially so sensitive to generalist predation, will they ever be able to build up outbreak densities in the introduced range? Having said this, in the native range of many weeds, eriophyid mites sometimes have been observed to reach impressive populations and appear to have an impact at the population level of the plant. For example, *A. acroptiloni* can reduce the seed production of *R. repens* by over 90% (Asadi et al., 2014).

Subtle abiotic factors can influence eriophyid mites in unexpected ways and often these cannot easily be explained. One of the challenges working with *A. acroptiloni* has been moving the mite from the collection site in Shirvan, northern Iran, 200 km to the southeast to Mashhad where the open field test is conducted. Apart from a slight drop in altitude from roughly 1100 m to 980 m there are no quantifiable differences in abiotic factors (soil structure and type, minimum and maximum temperatures, rainfall, etc.) that could play such a significant role in limiting the establishment of the species. Initially it was assumed that our inoculation techniques were inappropriate; however, over the years we have attempted several different techniques from moving whole plants infested with mites to inoculating individual mites onto plants in the field. Yet, some of these techniques proved successful when infesting control plants in the area where the mites were originally collected.

In conclusion, eriophyid mites may have potential, but in order to give them a viable future in classical weed biological control, we feel that researchers need to take a step back to the basics and fully understand the moving parts of the system. Once we better understand the taxonomy, biology and ecology of this group, we will be better able to design the appropriate experiments to test host specificity and be able to say with certainty that a species is safe for release and will be effective.

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*Oral presentation*

# Prospects for the biological control of invasive giant rat's tail grasses (*Sporobolus* spp.) in Australia

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Giant rat's tail grasses (*Sporobolus pyramidalis* Beauv. and *S. natalensis* [Steud.] Dur. & Schinz., Poaceae, hereafter GRTG's) were introduced from Africa into Australia, where they have become serious pastoral weeds. Mechanical and chemical control options have been largely ineffective, costly and labor-intensive, and thus biological control is being pursued. This study aimed to characterize the phytophagous community associated with GRTG's, in their native distributions, in search of host-specific and damaging control agents. Climate matching, using the MaxEnt software, identified eastern South Africa as the best-suited region to conduct these surveys. Phytophagous assemblages were characterized using (1) surveys across the GRTG's entire native distributions in South Africa, and (2) bimonthly surveys at 22 sites in climatically-matched regions to quantify herbivore abundances, seasonality and potential to inflict sustained damage. Field host-range surveys were performed on 26 sympatric grasses, including seven *Sporobolus* species and 19 non-target grasses with increasingly distant phylogenetic relationships to the GRTG's. Seven natural enemies, all herbivorous insects, were found associated with GRTG's. Two of these are considered promising in terms of their host specificity and damage. Both promising species are stem-galling wasps, a *Bruchophagus* sp. and a *Tetramesa* sp. (Hymenoptera: Eurytomidae), as they have both been recorded only on the two closely related GRTG's. Damage studies were undertaken by comparing plant growth, survival and reproductive capacity between infested and uninfested culms at five sites in eastern South Africa. Plants galled by either *Bruchophagus* sp. or *Tetramesa* sp. demonstrated reduced survival, reproductive capacity and heights. Given their narrow host range and potential to inflict damage to GRTG's under native field conditions, we recommend that both *Bruchophagus* sp. and *Tetramesa* sp. be imported into quarantine in Australia for detailed host-specificity testing.

*Oral presentation*

# Biological control of prickly acacia (*Vachellia nilotica* subsp. *indica*) in Australia: new gall-inducing agents from Africa

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## Abstract

Biological control is the most economically viable management option for prickly acacia (*Vachellia nilotica* subsp. *indica*), a serious weed of grazing areas in western Queensland, Australia. Biological control efforts so far have focused on agents from Pakistan, Kenya, South Africa and India, with limited success to date. Hence, the search for new agents, focusing on gall-inducers, was redirected to Ethiopia and Senegal, based on plant genotype and climate matching. Surveys were conducted on *V. nilotica* subspecies with moniliform fruits including the invasive subspecies *indica*. Prospective biological control agents have been identified based on damage potential, field host range and climate match. A gall thrips (*Acaciothrips ebneri* [Karny]) inducing shoot-tip rosette galls, a gall mite (*Aceria* sp. 3) deforming leaflets, rachides and shoot-tips in Ethiopia and Senegal and a tephritid fly (*Notomma mutilum* [Bezzi]) inducing stem-galls in Senegal have been prioritized for further studies. The gall thrips from Ethiopia has been imported into quarantine in Brisbane, Australia and host specificity tests are in progress. The eriophyid gall mite from Ethiopia has been imported into quarantine in Pretoria, South Africa and host specificity tests are also in progress there. Results to date suggest that both agents are highly host specific at the subspecies level of the target weed. Future research will focus on the host specificity testing of the tephritid gall fly from Senegal which has been imported in quarantine in Brisbane, Australia.

**Keywords:** *Acacia nilotica*, gall thrips, Eriophyidae gall mites, Tephritidae gall fly, Ethiopia, Senegal

## Introduction

Prickly acacia, *Vachellia nilotica* subsp. *indica* (Benth.) Kyal. & Boatwr. (syn: *Acacia nilotica* subsp. *indica*), a Weed of National Significance, is a serious weed of grazing areas in western Queensland and has the potential to spread throughout northern Australia (Dhileepan, 2009). Prickly acacia infests over 6 million hectares of natural grasslands and over 2,000 km of bore drains in Queensland, and costs primary producers AUD 9 million annually in lost pasture production (Dhileepan, 2009). Biological control is the most economically viable management option for prickly acacia.

Biological control of prickly acacia in Australia commenced in the early 1980s, with native range surveys conducted in Pakistan, Kenya, South Africa and India (Dhileepan, 2009; Dhileepan et al., 2014). These surveys resulted in the introduction and establishment of only two agents to date, the seed-feeding bruchid *Bruchidius sahlbergi* Schilsky from Pakistan and the leaf-feeding geometrid *Chiasmia assimilis* (Warren) from Kenya and South Africa. The impact of *B. sahlbergi* on prickly acacia has been insignificant (Radford et al., 2001) while *C. assimilis* has established only at coastal sites, and not widely in the arid inland regions where the major infestations occur (Palmer et al., 2007).

Surveys in India identified five insects and two rust fungi as prospective biocontrol agents (Dhileepan et al., 2013). However, agents from India tested to date are either not sufficiently host specific or are proving difficult to rear in quarantine (e.g., Dhileepan et al., 2014; Taylor and Dhileepan, 2018). There are no other prospective agents identified in India.

A literature search (Dwivedi, 1993) and herbarium records suggested that prickly acacia (subsp. *indica*) and other *V. nilotica* subspecies with moniliform fruits occur in Ethiopia and Senegal. A CLIMEX model has also predicted that areas in Ethiopia and Senegal are climatically similar to the arid inland regions of western Queensland where prickly acacia is a major problem (Senaratne et al., 2006; Dhileepan et al., 2018). Hence, the search for new biological control agents were redirected to Ethiopia and Senegal. In this study, based on field host range, geographic range and damage potential, a gall thrips, a gall mite and a tephritid gall fly have been identified as additional prospective biological control agents for prickly acacia in Australia.

## Materials and methods

### *Prickly acacia*

The multipurpose tree *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb. (syn: *Acacia nilotica*), native to Africa, the Middle East and the Indian subcontinent, is a polytypic species with nine recognized subspecies (Dwivedi, 1993). In Ethiopia, three *V. nilotica* subspecies, subsp. *indica* (Benth.) Kyal. & Boatwr. in the east (Afar and Oromiya regions), subsp. *tomentosa* (Benth.) Kyal. & Boatwr. in the north (Amhara region), and subsp. *leiocarpa* (Benth) Kyal. & Boatwr. in the south (Arbaminch region) were found growing naturally (Figure 1). In Senegal, two *V. nilotica* subspecies, subsp. *tomentosa* along the Senegal River and subsp. *adstringens* (Schumach. & Thonn.) Kyal. & Boatwr. in drier inland areas were found growing naturally (Figure 2). The subsp. *indica* and subsp. *tomentosa* have moniliform fruit pods (necklace-like, narrowly constricted between the seeds); and the subsp. *adstringens* and subsp. *leiocarpa* have non-moniliform fruit pods (not necklace shaped) with their margins straight or crenate or irregularly constricted (Dwivedi, 1993). The invasive prickly acacia in Australia (subsp. *indica*) is similar to subsp. *tomentosa*, but in subsp. *tomentosa* young branchlets and fruits are densely white-tomentose, while in subsp. *indica* young branchlets and fruits are glabrous to sub-glabrous or thinly pubescent (Dwivedi, 1993).

### *Native range survey for potential agents*

In Ethiopia, surveys were conducted in July 2014 (22 sites), December 2015 (41 sites), November 2016 (10 sites) and November 2017 (14 sites) (Figure 1). In Senegal, surveys were conducted in April 2017 (8 sites), October 2017 (18 sites) and April 2018 (12 sites) (Figure 2). In both countries, the subspecies status of the prickly acacia population was recorded and insects and mites associated with various subspecies were cataloged. A greater emphasis was placed on gall-inducing agents, in view of their likely high host specificity. When arthropods were collected from prickly acacia, related co-occurring Fabaceae species were also checked at the survey sites, specifically *Vachellia seyal* (Delile) P.J.H.Hurter, *V. tortilis* (Forssk.) Galasso & Banfi., *V. etbaica* (Schweinf.) Kyal. & Boatwr., *V. abyssinica* (Hochst. ex. Benth.) Kyal. & Boatwr., *V. lahai* (Steud. & Hochst. ex Benth) and *Senegalia senegal* (L.) Britton trees. The insects and mites collected were sent to relevant taxonomic experts in South Africa, Turkey and Australia for identification.

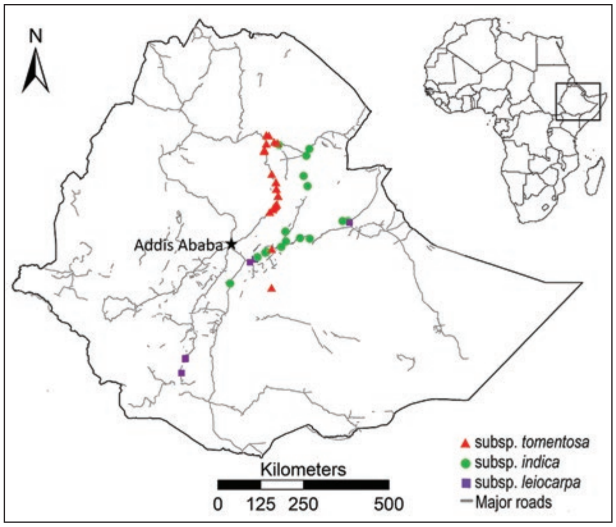


Figure 1. Location of prickly acacia survey sites in Ethiopia.

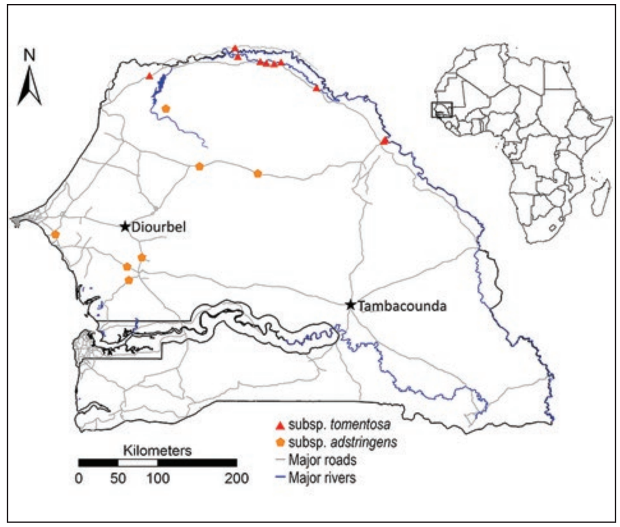


Figure 2. Location of prickly acacia survey sites in Senegal.

### *Host specificity tests*

A subset of the test plants used in the host specificity testing of previous prickly acacia biological control agents from India (Taylor and Dhileepan, 2018) were used for testing the agents in quarantine in Australia and South Africa. The gall thrips and the gall mites from Shewa Robbit, Ethiopia were exported to a quarantine facility at Plant Health and Protection (PHP), Pretoria, South Africa in July 2014 and December 2017, respectively. Based on preliminary host specificity tests in quarantine in Pretoria, South Africa, the gall thrips were imported from Ethiopia into a high security quarantine facility in Brisbane, Australia in December 2015 for detailed host specificity tests.

## Results

### *Gall thrips*

In Ethiopia, the gall thrips *Acaciothrips ebneri* (Karny) (Thysanoptera: Phlaeothripidae) induced rosette galls in the axillary and terminal buds resulting in shoot-tip dieback (Figure 3a). The gall thrips were found only on subsp. *tomentosa* (74% of sites, *n* = 22) and subsp. *indica* (92% of sites, *n* = 24) and not on subsp. *leiocarpa (n* = 8 sites). There was no evidence of the gall thrips on co-occurring *V. abyssinica* and *V. etbaica* trees in Ethiopia. In Senegal, the gall thrips (Figure 3b) was seen on both subsp. *tomentosa* (100% of sites, *n* = 13) and subsp. *adstringens* (88% of sites, *n* = 7), and not on co-occurring *V. seyal, V. tortilis* and *S. senegal* trees.

In no-choice tests in the quarantine facility at PHP, South Africa, adult thrips from Ethiopia induced galls only on Australian prickly acacia (subsp. *indica* with moniliform fruits) and not on South African prickly acacia (*V. nilotica* subsp. *kraussiana* [Benth.] Kyal. & Boatwr. with non-moniliform fruits), indicating a high host plant specificity. Based on these results, a colony of the gall thrips from Ethiopia was established in a high security quarantine facility in Brisbane, Australia in December 2015 and host specificity tests are in progress. To date, no-choice tests have been completed for 50 test plant species, and thus far, the thrips have only induced galls on subsp. *indica* from Australia and subsp. *tomentosa* from Ethiopia (both with moniliform fruits).

### *Gall midge*

In Ethiopia, a gall midge morphologically similar to *Lopesia niloticae* Gagné (Diptera: Cecidomyiidae) induced leaf rachis galls (Figure 3c) on all three subspecies of *V. nilotica*: subsp. *indica* (61% of sites, *n* = 14), subsp. *tomentosa* (83% of sites, *n* = 20) and subsp. *leiocarpa* (63%, of sites, *n* = 5). A morphologically

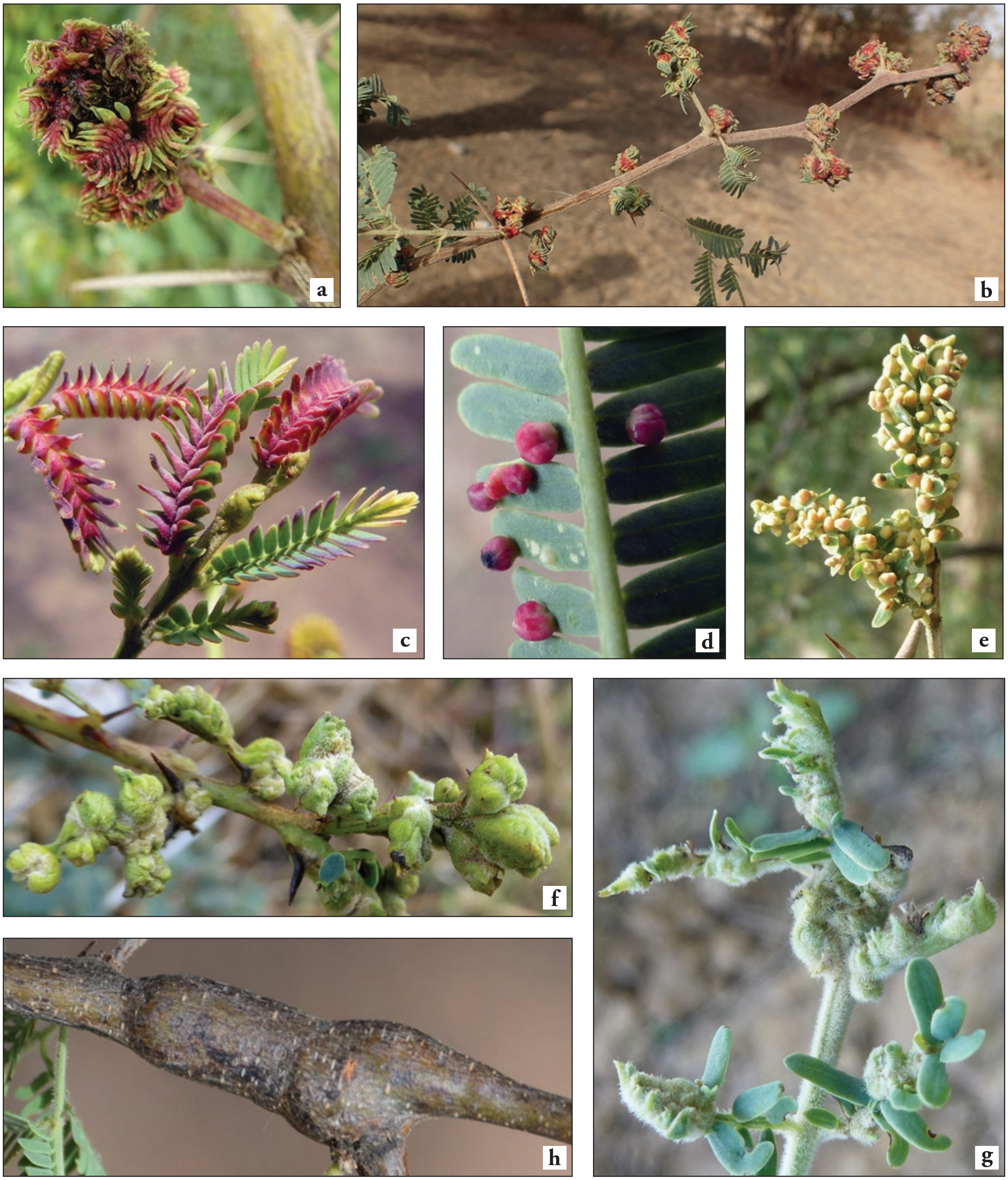


**Figure 3.** Gall-inducing insects and mites associated with *Vachellia nilotica* in Africa. Shoot-tip rosette galls induced by the gall thrips *Acaciothrips ebneri* (a) in Ethiopia and (b) in Senegal; (c) rachis and leaflet gall induced by the gall midge *Lopesia niloticae* in Ethiopia; (d) red spherical leaflet mite gall (type-1) induced by *Aceria* sp. 1 in Ethiopia; (e) creamy-white fluted leaflet mite gall (type-2) induced by *Aceria* sp. 2 in Ethiopia; leaflet, rachis and shoot-tip deforming mite gall (type-3) induced by *Aceria* sp. 3 (f) in Ethiopia and (g) in Senegal; (h) stem galls induced by the tephritid gall fly *Notomma mutilum* in Senegal.

similar gall midge has also been seen on other co-occurring *V. abyssinica* (*n* = 7 sites) and *V. etbaica* (*n* = 4 sites) trees in Ethiopia. There was no evidence of the gall midge on the two subspecies of *V. nilotica* in Senegal.

### *Gall mites*

Three morphologically distinct *Aceria* gall mites were found on *V. nilotica* subspecies in Ethiopia: red spherical leaflet galls (type-1; Figure 3d), creamy-white fluted leaflet galls (type-2; Figure 3e) and hairy mushroom-like galls on leaflets, rachides and shoot-tips (type-3; Figure 3f). Type-1 (Aceria sp. 1) leaflet galls (Figure 3d) were seen on all three subspecies: subsp. l*eiocarpa* (55% of sites; *n* = 4), subs. *tomentosa* (67% of sites, *n* – 16) and subsp. *indica* (56% of sites, *n* = 13). Type-2 (*Aceria* sp. 2) leaflet galls (Figure 3e) were seen only on subsp. *leiocarpa* (75% of sites, *n* = 6) and not on subsp. *tomentosa* (*n* = 24 sites) or subsp. *indica* (*n* = 23 sites). Type-3 (*Aceria* sp. 3) galls on leaflets, rachides and shoot-tips (Figure 3f) were found only on subsp. *tomentosa* (46% of sites, *n* = 11) and subsp. *indica* (57% of sites, *n* = 13) and not on subsp. *leiocarpa* (*n* = 8 sites). Both type-1 and type-3 galls were often found on the same leaves. Galls morphologically similar to the three mite galls found on *V. nilotica* were not seen on co-occurring *V. abyssinica* (*n* = 7 sites) and *V. etbaica* (*n* = 4 sites) trees.

In Senegal, type-2 creamy-white fluted leaflet galls were found only on subsp. *adstringens* in all sites (*n* = 8 sites), and type-3 hairy mushroom like galls deforming leaflets and rachides (Figure 3g) were found on subsp. *tomentosa* (25% of sites, *n* = 2) and subsp. *adstringens* (50% of sites, *n* = 6). There was no evidence of morphologically similar mite galls in co-occurring *V. seyal, V. tortilis* and *S. senegal* trees.

A colony of type-3 gall mites from Ethiopia was established on subsp. *indica* (sourced from Australia) in the quarantine facility at PHP, South Africa in December 2017. To date, no-choice tests have been completed on *V. nilotica* subsp. *kraussiana*, *V. nilotica* subsp. *adstringens, V. nilotica* subsp. *tomentosa, V. sieberiana* (DC.) Kyal. & Boatwr., *V. hebeclada* (DC.) Kyal. & Boatwr., *Senegalia galpinii* (Burtt Davy), and *Paraserianthes lopantha* (Willd.) I. C. Nielson. The type-3 gall mite has induced galls only on subsp. *indica* sourced from Australia (11 ± 5.4 galls per plant) and host specificity testing is ongoing for remaining test plant species.

### *Gall fly*

A stem gall-inducing *Notomma mutilum* (Bezzi) (Diptera: Tephritidae) (Figure 3h) has been identified as a prospective agent from Senegal. This is the first time a gall-inducing tephritid associated with *V. nilotica* has been collected. The gall fly was found in 86% of surveys sites (*n* = 20 sites) in Senegal, on both subsp. *tomentosa* (*n* = 12 sites) and subsp. *adstringens* (*n* = 8 sites), but not on other co-occurring *Vachellia, Acacia* and *Senegalia* species (*n* = 17 sites). The number of galls per shoot ranged from 1 to 12. There was no evidence of the gall fly in Ethiopia.

The gall fly was imported from Senegal, into quarantine in Brisbane, Australia, for colony establishment and host specificity testing. In October 2017, over 800 stem-cuttings with stem galls of *N. mutilum* were imported, but no adults emerged from this material. In April 2018, about 600 stem-cuttings with stem galls of *N. mutilum* were imported again from Senegal, and about 240 adults emerged from this second importation. Potted prickly acacia plants (subsp. *indica*) were exposed to newly emerged adults in insect-proof cages for oviposition and colony establishment.

Adult flies live up to four weeks under quarantine conditions. The adults lay eggs near the meristem of new shoot growth, and the emerging larvae enter through the meristem and induce stem galls. Stem gall development is evident after two weeks. The life cycle from adult to adult is from 4 to 5 months.

## Discussion

The close affinity of prickly acacia to the Australian native *Vachellia* and *Acacia* species makes the biological control efforts difficult. Any potential biological control agent for prickly acacia in Australia needs to be species specific, with no significant risk to Australian native species. Hence, priority was given to galling agents, in view of their likely host specificity. Based on field host range, geographic range and damage levels, the gall thrips and the type-3 gall mite, both from Ethiopia, and the tephritid gall fly from Senegal have been prioritized for detailed host specificity tests.

Field host range in Ethiopia and preliminary host specificity tests in South Africa suggest that the gall thrips is host specific, with its host range restricted to *V. nilotica* subspecies with moniliform fruits (Dhileepan et al., 2018). This is supported by ongoing no-choice host specificity tests in quarantine in Australia; so far the thrips have induced galls only on prickly acacia. Host specificity tests are continuing.

The leaflet and rachis midge galls from various *V. nilotica* subspecies in Ethiopia and from *V. abyssinica* in Ethiopia are yet to be identified, though they appear similar to the gall midge (*L. niloticae*) on subsp. *leiocarpa* in Kenya. Phytophagous Cecidomyiidae generally have narrow host-plant ranges, and therefore, in Cecidomyiidae taxonomy, species distinctions are often made on the basis of host association (Gagné 1986). However, it is essential to ascertain the taxonomy of the midges from various subspecies of *V. nilotica*, before future work on the gall midge is considered.

The eriophyid mites (*Aceria* spp.) inducing the three morphologically distinct galls in Ethiopia appear very similar to *Aceria liopeltus* Meyer (Acari: Eriophyidae) inducing leaflet galls on subsp. *kraussiana* in South Africa (Charnie Craemer, pers. comm.). In both Ethiopia and Senegal, type-1 and type-3 mite galls were often found on the same leaf, suggesting that the distinct gall morphologies are not host related and are likely caused by different gall mite species. In tests in South Africa, gall mite *A. liopeltus* sourced from subsp. *kraussiana* induced galls on subsp. *kraussiana* and not on subsp. *indica* (Witt 2004, Dhileepan et al., 2014). The type-3 gall mite from Ethiopia induced galls on subsp. *indica* from Australia and subsp. *tomentosa* from Ethiopia (both with moniliform fruits) and not on subsp. *tomentosa* from Senegal (with moniliform fruits), subsp. *adstringens*, subsp. *kraussiana* and subsp. *leiocarpa* (all with non-moniliform fruits). This indicates that the gall mites are host specific at the subspecies level. Though morphologically indistinguishable from *A. liopeltus* inducing galls on subsp. *kraussiana*, field host specificity and gall morphology suggest that the mites inducing the three gall types in Ethiopia and Senegal are likely to be distinct species. Future research will focus on using molecular tools in resolving the species status of the mites inducing the three types of galls.

The type-3 gall mite from Ethiopia was prioritized for host specificity testing based on gall morphology, field host range and damage potential. To date there has been no gall development on any non-target plant species. If proven host specific in the preliminary host specificity tests, type-3 gall mite will be imported in to a high security quarantine in Australia for detailed host specificity tests.

*Notomma* Bezzi is a genus of eight described species of tephritid gall fly predominantly from Africa (Munro, 1952; Freidberg and Morgulis 2011). All known hosts are from the Family Fabaceae and species are known to be restricted to a single host species (Hancock 1986; Munro, 1952; Stefan Neser, pers. comm.). This is the first time a tephritid gall fly has been recorded on *V. nilotica* in Senegal. Very little is known about this fly or its biology, as only two specimens had ever been collected before this study (M.W. Mansell, pers. comm.). In Senegal, the tephritid gall fly was found only on *V. nilotica*, with no evidence of the gall fly on other co-occurring *V. seyal, V. tortilis* and *S. senegal* species at the survey sites, suggesting that the gall fly is likely to be host-specific. Host specificity tests for the gall fly will commence once a colony has been established in quarantine in Brisbane, Australia.

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*Oral presentation*

# Open field evaluation of *Aculodes altamurgensis*, a new eriophyid species associated with *Taeniatherum caput-medusae*

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Medusahead (*Taeniatherum caput-medusae* [L.] Nevski) (Poaceae) is an annual grass from the Mediterranean region that is an invasive noxious weed in many western states in the USA. At the beginning of 2000s, a biological control program was started by USDA, combining the screening of different subspecies, populations and biotypes of the target weed with the presence and identification of natural enemies to be used as prospective biological control agents. During an exploration carried out in 2014, a new species of eriophyid mite, *Aculodes altamurgiensis* de Lillo & Vidović was found on medusahead in Southern Italy, and later in Serbia, Turkey, Central Bulgaria and Iran. During our observations, this mite species has always been associated with the target weed, but never with other grass species present in the same area (e.g., *Stipa austroitalica* Martinovský, *Avena sativa* L., *Triticum durum* Desf. and *T. aestivum* L.), suggesting that *A. altamurgensis* may be specific to medusahead. An open field test carried out at the BBCA facilities in Rome with 11 different grass species and/or biotypes confirmed the restricted host range of the mite, which was able to reproduce only on the target weed species. Moreover, the results showed an interesting difference in the preference of the mite among the five different biotypes (two Italian and three from the USA) of medusahead. These data point out *A. altamurgiensis* has the potential of being considered a valid candidate for the biological control of *T. caput-medusae* and encourage further studies to measure the impact of the mite on medusahead.

*Oral presentation*

# Using field host-range testing to prioritize biological control agents

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*Lycium ferocissimum* Miers (Solanaceae) is an invasive weed in Australia that is indigenous to South Africa. Surveys for natural enemies have been conducted throughout the plant's native range. After these surveys three agents were prioritized for further studies according to their damage inflicted, abundance, distribution and other successful examples of biological control agent taxa on Solanaceae elsewhere. The prioritized agents were *Cassida distinguenda* Spaeth (Chrysomelidae: Cassidinae), *Cleta* sp. (syn. *Epilachna* sp.) (Coccinellidae) and *Neoplatygaster serietuberculata* Gyllenhal (Curculionidae). Laboratory no choice tests were conducted on the three species. All three agents were specific to the tribe Lycieae but not specific to *L. ferocissimum*. Australia has one endemic *Lycium* species (*L. australe* F. Muell.) and one economically important species (*L. barbarum* L.). Subsequent open-field choice and no-choice tests were set up at three different climates (50 m, 25 km and 35 km from the coast) in order to further test the environmental safety of the potential agents. The open-field host specificity testing determined not only the ecological host range of the agents but also informed the selection of species that would perform best under different climatic conditions. It suggested that the *Cleta* species would perform better at inland sites, while *N. serietuberculata* would perform better at coastal locations. We suggest an increase of open-field native range testing to help prioritize agents before their introduction into quarantine facilities.

*Oral presentation*

# Integrating results from host range, efficacy and fitness trials to prioritize biotypes of *Dactylopius tomentosus*

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A biological control program targeting eight invasive *Cylindropuntia* species naturalized in Australia began in 2009. Twenty biotypes of *Dactylopius tomentosus* (Lamark) have been imported from Mexico, South Africa and the USA into an Australian quarantine facility to evaluate their potential as biocontrol agents. These biotypes showed significant variability in the level of impact on the naturalized *Cylindropuntia* spp. Exacerbating the issues with selecting the most effective biotype was the large variability in the virility and impact of individuals within a pure biotype culture. Successful establishment of damaging agents is dependent on a systematic methodology to determine the most effective biotype for each of the eight *Cylindropuntia* species. The potential impact of each biotype of *D. tomentosus* on each *Cylindropuntia* species was scored for three indicators: development success, impact of the biotype on potted plants and a measure of fitness on a *Cylindropuntia* species. Both the host range and efficacy trials had a maximum score of 20. Fitness was dependent on egg count and did not have a limit, but the highest fitness score for any biotype tested was 32. For most *Cylindropuntia* species, scores clearly identified the best biotype to release. The final tally incorporating the three scores can be ground-truthed using field observations following release. We conducted two field observations that may provide a perspective of how the final tally can be used to select an agent. The "cholla" biotype, targeting coral cactus, scored 54. Eighteen months after release at a monitoring site, 95% of all plants sampled were dead. A field release of the "imbricata" biotype (final tally of 36) on a clump of *C. kleiniae* (DC.) F.M. Knuth resulted in the death of this clump within 12 months. Our final estimated impact tally for these two field observations may provide a baseline score for what may occur in the field when a biotype is released in the field.

*Oral presentation*

# Brazilian peppertree in Florida, USA: research updates on potential biological control agents

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Introduced into Florida as an ornamental in the mid-1800s, Brazilian peppertree (BP), *Schinus terebinthifolia* Raddi (Anacardiaceae), is an aggressive invasive species in Florida (USA). Several insect herbivores of BP have been investigated as potential biological control agents, but releases have yet to be made. Three gall-inducing psyllids, *Calophya latiforceps* Burckhardt (Hemiptera: Calophyidae), *Calophya terebinthifolii* Burckhardt & Basset and *Calophya lutea* Burckhardt, as well as a thrips, *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae), were discovered in Brazil damaging BP. Two of these species (*C. latiforceps* and *P. ichini*) are awaiting release permits. The objective of these studies was to evaluate the current impact of arthropod herbivores present in Florida on BP prior to the release of biological control agents and to determine the host specificity of *C. terebinthifolii* and *C. lutea*. The impact of herbivory on plant growth and reproduction was assessed from insecticide-protected and unprotected trees every three months for three years in two field plots in Florida. Host specificity experiments were conducted under greenhouse conditions where insects were exposed to 90 plant species belonging to 50 families. Fruit production, chewing damage and plant growth parameters measured were not different between insecticide-treated and untreated plants during the field study. In the host-specificity study, *C. terebinthifolii* and *C. lutea* oviposited on five and seven non-target species, respectively, all in the Anacardiaceae. Oviposition was greater on BP when compared with that on non-target species. Gall development and adult emergence occurred only on BP. These results provide justification for the introduction of these host-specific biological control agents into Florida.

*Oral presentation*

# *Abrostola asclepiadis* (Lepidoptera: Noctuidae) will likely be an ineffective agent due to its impact and diapause traits

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Pale and black swallow-wort (*Vincetoxicum rossicum* [Kleopow] Barbar. and *V. nigrum* [L.] Moench; Apocynaceae, subfamily Asclepiadoideae) are long-lived perennial vines that were introduced from Europe into North America. A potential biological control agent is the defoliating moth *Abrostola asclepiadis* (Denis and Schiffermüller) (Lepidoptera: Noctuidae). It is widely distributed in Europe in open field to forest edge habitats and reportedly has one–two generations per summer. We conducted a greenhouse study to quantify the impact of larval defoliation on seedlings and adult plants at different frequencies (once or twice) and degrees of defoliation (50 or 100% seedlings, one or two larvae/stem-adult plants). We assessed Russian and French populations of the moth for their potential multivoltinism under constant and changing photoperiods ranging from 13:11 to 16:8 hr (L:D) at 20 or 25°C, and we reared the French population outdoors for three years. The various photoperiod treatments are representative of spring and summer photoperiods in northeastern North America. Complete defoliation (100%), and especially repeated defoliation (twice), was needed to significantly reduce plant biomass and seed production. However, for the different photoperiod treatments, no to very few adult moths emerged with few exceptions. We therefore expect *A. asclepiadis* to be univoltine if released, thus greatly limiting its impact. Incorporation of impact data into plant population models suggest that the majority of swallow-wort populations will not be controlled by this agent.

*Poster presentation*

# A paucity of pteridophages: population genetics guiding the biocontrol of invasive *Lygodium microphyllum* in Florida, USA

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*Lygodium microphyllum* (Cav.) R.Br. is a climbing fern native to the tropical and subtropical regions of the Old World (Australasia, Asia and Africa) where it is common in forest understories and riparian zones. The invasive *L. microphyllum* has spread rapidly since it was first recorded in Florida in 1958. It is one of the worst weeds in Florida and is listed on the USA Federal Noxious Weed list. It inhabits wetland and mesic habitats in peninsular Florida and creates a dense mat of live and dead fern fronds. The prolific growth rapidly shades or smothers the underlying vegetation including tall trees, promoting fire in plant canopies, changing fire regimes, altering the habitat structure and reducing native plant diversity. Biological control is considered to be an essential element towards the control of this weed. Biocontrol of *L. microphyllum* has been challenging due to a limited suite of potential agents; across ten countries over 20 years of research, most of the 40 herbivore species found are generalists, with only 18 candidates requiring any assessment as potential agents. Two equally-specific and damaging Musotiminae agents have showed disparate establishment after their respective releases in Florida. Post-hoc genetic analyses of *L. microphyllum* and its agents has highlighted the value of applying this approach early in a biocontrol program—an integrated approach of genetics and field research can maximize the chances of successful, sustainable control.

*Poster presentation*

# A needle in a haystack: targeted exploration for biocontrol agents of monoecious *Hydrilla verticillata*, an aquatic weed in the United States

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Hydrilla is a submerged aquatic macrophyte that is native to Asia, Australia, Europe and Africa. It was introduced into North America in the early 1950s and has since become highly invasive. Both monoecious and dioecious forms exist in the USA, likely due to separate introductions. Management using chemical control, mechanical control and introduced herbivorous fish are expensive and ineffective. Worldwide surveys for insect biological control agents of hydrilla have been conducted since the 1970's in Africa, Asia and Australia and briefly in Panama. Two leaf-mining *Hydrellia* flies were released and established in the United States. Only *Hydrellia pakistanae* Deonier is widespread and damaging, but its impact on hydrilla in the field is limited; more agents are required, especially on monoecious hydrilla which is spreading into the cooler regions of the USA and threatens the Great Lakes. Since 2013, extensive hydrilla surveys have been conducted in China and the Republic of Korea where a small number of sites were located with the monoecious form that seems to be a match to USA monoecious populations. A leaf-mining *Hydrellia* sp. genotype, possibly highly specific and adapted to the USA monoecious form, has been collected from these plants and targeted for further evaluation.

*Poster presentation*

# Biological control of common sowthistle: what is known, what is new and what is still missing?

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Common sowthistle, *Sonchus oleraceus* L. (Asteraceae), is a widespread agricultural and garden weed in its native range of Europe and northern Africa. Now widely distributed, this invasive species is an increasingly important weed in Australia. Development of herbicide resistance is making populations extremely difficult to manage in cropping systems. As an alternative method to herbicides, CSIRO, in collaboration with Montpellier SupAgro (France), has initiated a classical biological control program for this weed. Characterizing the natural enemy community associated with *S. oleraceus* is crucial to further select potential biological control agent(s). However, this community is surprisingly poorly understood across its native range. From a literature review and early field collections, two flies, the leaf-gall former *Cystiphora sonchi* (Bremi) (Cecidomyiidae) and the bud-gall former *Tephritis formosa* (Loew) (Tephritidae), are considered as promising candidates. Their host specificity is currently being investigated by testing a series of key plants (i.e. economically important species and Australian native species). Moreover, native range surveys, guided with a climate-matching approach, were carried out in Morocco and Western Europe in 2017 and 2018. To date, more than 40 arthropods species and at least five species of fungi have been collected. Potential new candidates (one rust and one hoverfly) have been identified and are currently maintained at the laboratory for host testing.

*Poster presentation*

# What informs our decisions when choosing the best species to control a weed pest?

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Herbivorous insects represent a large community among ectothermic organisms. According to their host ranges, these insects can sometimes be used in biological control programs against various weeds and/or invasive plants. In assessing the fitness of more than one species as potential biological control agents for the management of pest populations, a detailed knowledge of each insect species is essential. The capacity of insect biocontrol agents to effectively reduce and suppress the weed host depends on the successful establishment of the insect population. Despite the success of biological control agents against weeds in some areas, they have been less successful in others (Winston et al., 2014). This has been ascribed to several factors, both abiotic and biotic, including the local climate, nutrient status affecting plant quality and interaction between species.

There are three possible scenarios regarding interacting populations/species within biological control programs (Ismail and Brooks, 2018): (1) the interaction does not lead to different outcomes from those in which each population is released individually; (2) the interaction of two populations/species may increase the impact of biocontrol, in which case it would be advisable to release both populations/species; and (3) the interaction of two populations/species could reduce the overall impact, making it more effective to release only the population/species that is better adapted to the respective field conditions. In addition, within and between species interactions can have a strong impact on mating behavior and reproduction, and can thus have an impact on the effectiveness of biological control.

The invasive aquatic weed, *Eichhornia crassipes* Mart. (Solms) (Pontederiaceae), commonly known as water hyacinth, has been widely distributed from its origin in South America throughout tropical, subtropical and some warmer temperate regions of the world. It is extremely invasive and can grow rapidly, forming expansive colonies and interrelated floating mats, covering the water bodies and consequently affecting biodiversity, fishing, freshwater ecosystems and water supply (Winston et al., 2014). The most promising method for reducing populations of water hyacinth is through the release of host specific biological control agents, including the mirid *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae: Bryocorinae), a leaf-sucking insect (Hill et al., 1999). Both adults and nymphs feed on water hyacinth, causing severe damage to the plant through loss of chlorophyll (Hill et al., 1999). There are two geographically isolated populations of the mirid, originating from Brazil and Peru, respectively (Hill et al., 2000, 1999). Because of the apparently identical morphology, the two populations were originally considered to be one species, but Paterson et al. (2016) have confirmed that they are cryptic species and more recently, Henry (2017) described the Peruvian population as *Eccritotarsus eichhorniae*.

The studies presented here focused on three main aspects potentially affecting the biological control program for water hyacinth: temperature, mating preferences of males of the two species and the impact of the combination of temperature and water nutrient level.

Results on the influence of temperature showed that fitness traits (lifetime fecundity, egg hatching rate and nymphal survival rate) for both species were significantly reduced at 30°C compared with 25°C and 20°C (Figure 1 a,b,c). Nonetheless, Peruvian individuals continued their development at 30°C, whereas Brazilian individuals that successfully emerged did not continue their development. In contrast, sex ratio

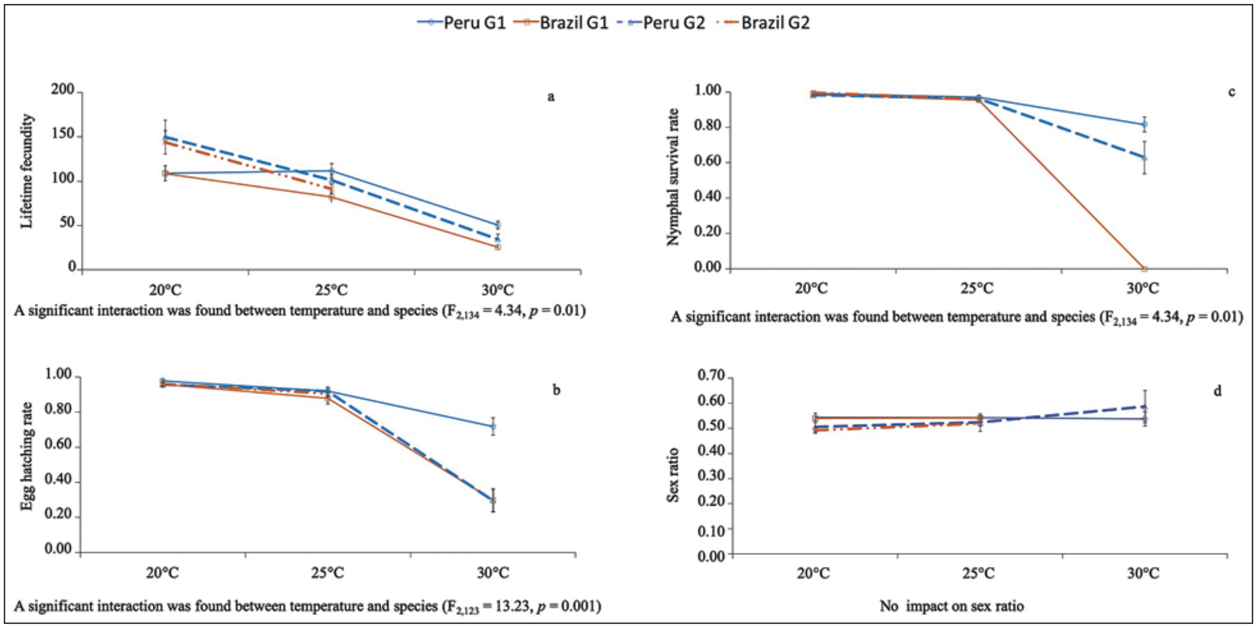


**Figure 1.** Responses of two generations (G1 and G2) of *Eccritotarsus catarinensis* (Brazil) and *Eccritotarsus eichhorniae* (Peru) on various fitness traits at three temperatures (from Ismail and Brooks, 2016, reprinted with permission).

was unaffected by temperature (Figure 1d). In addition, the Peruvian species was able to reproduce for two successive generations at 30°C (Figure 1c) (Ismail and Brooks, 2016).

*Eccritotarsus eichhorniae* (Peru) presented different phenotypes and was better adapted to extreme high temperatures than *E. catarinensis* (Brazil) (Ismail and Brooks, 2016). The tropical origin of the species most probably enables the insect to better tolerate extreme high temperatures. The results support the "warmer is better" hypothesis which predicts that species living at low latitudes, implying stable climate with warmer temperatures, will show higher maximum performance than those living at high latitudes where climate is variable and temperatures colder (Huey and Kingsolver, 1989).

The reproductive compatibility between the two species was evaluated by performing inter- and intra-species mating experiments to investigate whether or not males from each species would be able to distinguish between partners from either species, and if they would mate with partners from the opposite species (Ismail and Brooks, 2018). The results showed a decrease in lifetime fecundity, and most importantly, a lack of production of offspring from eggs resulting from forced hybridization. In addition, the Peruvian males mated only with females from their own species, and did not mate with females from the Brazilian species. In contrast, Brazilian males did not distinguish between partner females, and mated equally with females from both species (Ismail and Brooks, 2018). The results followed the third of the scenarios of interaction among species. This implies that the interaction created by mixing the two species would have a negative impact on biological control, because of the negative impact on reproduction in the case of mixed crosses.

The interaction between temperature and water nutrient level, which dictates plant quality, was evaluated at three temperatures (20, 25 and 30°C) on water hyacinth plants grown at three nutrient levels (low, medium and high). Several fitness, morphological and physiological traits were measured, only on *E. eichhorniae* from Peru (previously referred as *E. catarinensis*). The results showed that combining the two factors (temperature and nutrient level) was more detrimental and had the highest negative effects on fitness traits compared to applying each individual factor alone. Individuals reared at 30°C and fed poor quality plants exhibited the smallest size with reduced fitness traits and had proportionately less lipid content. The study demonstrated that reproduction of *E. eichhorniae* was most effective on plants grown in water with high and medium nutrient levels, at 20 and 25°C (Ismail et al., 2017). The results concur with the Plant Vigor Hypothesis of Price (1991) and with the Nitrogen Limitation Hypothesis of White (1993), proposing that herbivorous insects will perform better on vigorous plants.

In conclusion, the most advantageous biocontrol agent would be one that is successful despite temperature changes and varying host quality while displaying no negative interaction with other species or sub-species present in the field after release. According to conditions in the areas targeted for biocontrol in this study, *E. eichhorniae* (Peru) may be considered a better choice than *E. catarinensis* (Brazil).

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*Poster presentation*

# Preliminary observations on the impact of *Aculus mosoniensis*, perspective biological control agent of *Ailanthus altissima*

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*Ailanthus altissima* (Mill.) Swingle, tree of heaven is an introduced tree species in Europe, Africa, South America and North America. Seeds were introduced from China to France in the middle of the 1700s and in North America as an ornamental shade tree during the late 18th century from Europe. It is a serious threat to ecosystems in introduced areas, as the plant is very competitive through allelopathic chemicals that may inhibit growth of surrounding native plants. It has a complex of secondary chemicals that make it unpalatable to most of the phytophagous generalist arthropods. Management of this species has been very difficult because of its fast growth and production of root-suckers. Europe uses the classic approach of mechanical and chemical treatments which provide only short-term control; however, this usually exacerbates the problem. One potential agent for *Ailanthus*, the eriophyid mite *Aculops mosoniensis* Ripka (Acari: Eriophyoidea), has already been recorded in six European States: Hungary, Italy, Serbia, Austria, Croatia, Macedonia and Greece. In addition to the classic symptoms associated with the mite infestation (leaf rolling), an important impact has been recorded on the growth and the survival rate of young plants. This poster provided life history data and described the results of impact bioassays carried out in field and laboratory conditions, comparing the effects of classic biological control alone and in combination with other management approaches.

*Poster presentation*

# Artificial diet rearing of a cerambycid beetle (*Oberea shirahatai*, Ohbayashi, 1956)

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Surveys in the native range of Japanese honeysuckle (*Lonicera japonica* Thunb.) in Honshu, Japan revealed a natural enemy biota attacking the plant that was rich in potential biocontrol agents for New Zealand (NZ), including a stem-boring cerambycid beetle (*Oberea shirahatai*, Ohbayashi, 1956). *Oberea* females saw a slit in the larger stems of *L. japonica* and then turn around and slide a single egg down the stem just under the cambium bark layer. After approximately seven days, the egg hatches and the larva begins to feed around the stem under the surface bark layer before entering the main pith of the stem to feed and develop to pupation. Adults emerge from the pupal case inside the stem and take several days to harden before chewing their way out and feeding on new leaves. The time taken for larvae to develop to pupation and adult emergence in the field is around two years and two over-wintering periods. This extended life cycle proved very difficult to maintain inside containment on potted plants. We used an artificial diet developed for a closely related cerambycid species (*Prionoplus reticularis* White, a NZ native stem borer) and began rearing first-instar larvae extracted from excised stems. After approximately 30 days, the weight of larvae from excised stems averaged 0.003 g, whereas larvae reared on the artificial diet for the same time averaged 0.07 g in weight. After approximately 60 days, larvae reared on the diet were 0.16 g and appeared to be inactive, non-feeding and pre-pupal. After a further 120 days in over-wintering conditions, larvae were placed in warm conditions with long day length where pupation occurred and new adults emerged.

*Poster presentation*

# The potential for classical biological control of *Sagina procumbens* in the UK Overseas Territory of Tristan da Cunha

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*Sagina procumbens* L. (Caryophyllaceae), procumbent pearlwort, is an invasive species in the UK Overseas Territory of Tristan da Cunha (Tristan and Gough Island). This species is native to a wide geographical range in Eurasian temperate regions. *Sagina procumbens* is particularly widespread in rock cliff habitats on Gough Island and is known to form dense matting within upland habitats on the South African Marion Island, where the species is also invasive. It is feared that a spread to similar habitats on Gough Island would pose a threat to nesting sites of endangered seabirds. Therefore, the potential for classical biological control of the weed using coevolved natural enemies from the native range is currently being investigated. A preliminary review of natural enemies attacking *S. procumbens* throughout Europe showed only a few fungal pathogens to be recorded: *Asteromella saginae* Urries, *Mycosphaerella saginae* Urries, *Puccinia arenariae* (Schumach.) G. Winter, *Puccinia saginae* Fuckel (synonym of *P. arenariae*) and *Macrophoma sagina* (Rostr.) N.F. Buchw. This suggests that the mycoflora of this species has been little studied. The review did not reveal any immediately promising herbivores suitable as control agents. Survey work has commenced in the UK and other European countries to collect recorded fungal pathogens and identify additional natural enemies associated with the weed to assess their potential as classical biological control agents. To date, the rust pathogen *P. arenariae* and a *Cercosporella* sp.—likely to represent the asexual stage of *M. saginae*—as well as two *Phomopsis* spp. and one *Phoma* sp. have been collected and brought into culture. Out of these, *P. arenaria* and the *Cercosporella* sp. are considered to hold the most potential as classical biological control agents, and host specificity evaluation of these two pathogens has commenced.

*Poster presentation*

# A beautiful flower with a bitter taste, *Allium triquetrum* L., angled onion: a new biological control program

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It is now well recognized that many invasive plants have escaped from gardens and landscapes where they were originally planted for ornamental purposes. Angled onion, *Allium triquetrum* L. (Amaryllidaceae), is another example of such an escapee. This bulb-forming herbaceous perennial plant, native to the west and central Mediterranean, is now widespread and invasive in different parts of the world. In Australia, the weed is declared noxious in southern parts of the country, and its uncontrolled spread threatens biodiversity in natural environments. In addition, *A. triquetrum* is also problematic in agriculture, causing milk and meat taint with strong onion odor. Because of the lack of effective control methods, a classical biological control approach has been initiated by CSIRO and Montpellier SupAgro (France). The first phase of the program includes native range surveys for characterizing the natural enemies community (arthropods and fungi) associated with the target plant, which seems to be poorly understood across its native range. This poster summarized the relevant information from a biological control perspective based on literature review and presented the results of the first field surveys carried out in the native range.

*Poster presentation*

# Host-specificity testing and taxonomy of *Passalora euphorbiae*, a potential biological control agent of sea spurge (*Euphorbia paralias*) in Australia

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Sea spurge (*Euphorbia paralias* L.) is an invasive plant infesting beaches and dunes across southern Australia. It outcompetes native dune and foreshore flora while simultaneously disturbing the nesting habitats of coastal bird species. Sea spurge is progressively advancing its invasion fronts northwards along the Western Australia and New South Wales coastlines. Surveys undertaken for potential biological control agents in Mediterranean Europe, the native range of sea spurge, identified several natural enemies. One of these is the fungus *Passalora euphorbiae* (Karak.) Arx, which causes leaf and stem lesions on sea spurge. Initial host testing performed in France was promising, indicating that the fungus may be highly specific towards sea spurge. The fungus has now been imported into quarantine in Australia for further, more comprehensive host-specificity testing on a broader range of Euphorbiaceae species, spanning the four *Euphorbia* subgenera *Esula, Athymalus, Chaemsyce* and *Euphorbia*, as well as selected species from the Picrodendraceae and Phyllanthaceae families. Results obtained so far on the host-specificity were presented. In addition, due to recent taxonomic revisions of the fungal family Mycosphaerellaceae, we have sought to clarify the taxonomy of *P. euphorbiae* by analyzing DNA sequence data from several informative nuclear and protein-coding gene regions in tandem with informative morphological characteristics.

*Poster presentation*

# Exploration for natural enemies associated with Guineagrass (*Megathyrysus maximus*, syn. *Panicum maximum*) in Central Kenya

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Guineagrass, *Megathyrsus maximus/infestus,* is a very serious alien weed invading rangelands, agroecosystems, urban landscapes and natural areas of South Texas. A biological control program was started in 2016 with the aim to (1) discover and evaluate the herbivore arthropods associated with this perennial grass and (2) conduct an Africa-wide survey of *M. maximus* and determine the origin of the invasive Texas Guineagrass. Intensive surveys of the arthropod herbivores have been conducted at the Mpala Research Station in northern Kenya. Among several arthropods associated with *M. maximus*, three stem-boring moth species and one new species of eriophyid mite recorded in Kenya are considered possible biocontrol agents due to their specificity and impact on the target grass species. A similar but separate suite of herbivores has been collected from *Pennisetum ciliare* (L.) Link, buffelgrass, which is sympatric with *M. maximus* in Kenya. Molecular characterization of the *M. maximus* plant samples is underway comparing Lrn-L chloroplast DNA and using AFLP. Based on these results, we will focus arthropod surveys on the match location.

*Poster presentation*

# Initiating biological control of *Iris pseudacorus*: a north-south collaboration

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The European wetland yellow flag iris, *Iris pseudacorus* L. (Iridaceae), is a new target for biological control in South Africa. Since its first record outside of cultivation in 2004 in South Africa, it has spread to at least five of the nine provinces. While it remains an eradication target under current legislation, the extent and rate of spread warrants further control interventions. Through collaboration with the Vrije Universiteit Brussel and the Centre for Biological Control, Rhodes University, a biological control program has been initiated. The iris flea beetle *Aphthona nonstriata* (Goeze) (Coleoptera: Chrysomelidae) and the flower feeding weevil *Mononychus punctumalbum* (Herbst) (Coleoptera: Curculionidae) have been prioritized as suitable control agents to reduce this damaging weed of wetlands in several southern hemisphere countries, including Argentina and New Zealand.

*Poster presentation*

# From genomic analysis of the rapid colonization of the exotic Sahara mustard in the United States to the search of its natural enemies

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Sahara mustard (*Brassica tournefortii* Gouan 1773) is a winter annual that is believed to have been introduced in California in the 1920s. Since then, it has rapidly invaded the southwestern United States and northern Mexico, displacing natives and altering these water-limited landscapes. We sequenced genome-wide single nucleotide polymorphisms to identify the population structure and the spatial geography of Sahara mustard using 760 individuals from 52 sites across its invaded range. Herbaria records were also used to model the species' expansion rate since its presumed introduction. Overall, results showed that Sahara mustard experienced atypical expansion patterns with a relative constant rate of expansion since its introduction and that Sahara mustard exists as three genetically distinct populations in the USA, and with low levels of diversity likely the result of self-fertilization. The origin of these populations was unknown but was hypothesized to be linked to the importation of date palms from North Africa and the Middle East. Sampling of Sahara mustard was initiated in early 2015 across Western Europe, Northern Africa and the Middle East in order to pinpoint the origin of the USA populations. We identified as many as seven distinct populations in Sahara mustard's native range. Ancestry analyses suggest that populations from one site in Morocco and two sites in Jordan are the putative origin(s) of Sahara mustard in the USA. Little is known about natural enemies of Sahara mustard in the native range, providing a high potential for new discoveries. Several natural enemies were collected during our surveys, most of which were Coleoptera, mainly root-galling weevils. We also observed one flea beetle, two galling midges and a stem-mining moth. Knowing the genetic diversity, probable regions of origin and preliminary knowledge on prospective agents provides a strong foundation for developing a classical biological control program.

*Poster presentation*

# Is being a specific and damaging insect enough to be considered as a good candidate for the biological control of water hyacinth?

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*Cuernavaca longula* (Remes Lenicov) is a phloem-feeding insect associated with the aquatic invasive weed *Eichhornia crassipes* (Mart.) Solms, and is distributed in South America from Peru to Northern Argentina. It was studied and proposed as a potential biocontrol agent demonstrating its host affinity to water hyacinth through host specificity and damage studies. However, the complications in maintaining a colony of *C. longula* under laboratory conditions, due to failure of mating in captivity and the lack of information about the effect of this agent on natural populations of *E. crassipes*, make difficult its final consideration as a biological control agent. A population study was carried out throughout one year in two different localities with the aim of studying the reproductive biology of *C. longula* and its phenological relation to abiotic and biotic parameters. Morphological and physiological aspects of the insects were also considered. Interestingly, this species (as with many dictyopharids) presents two pores, one for mating and one for ovipositing. We found that females have the mating pore open and visible only in mated females (mature) with developed ovarioles. The largest proportion of mature females was found in summer. Despite many females being collected in autumn, almost all of them were immature. The population dynamics of the planthopper were slightly associated with abiotic parameters (temperature and evapotranspiration) but not with biotic parameters (plant features). The pattern found throughout the year could indicate that the species has one, maybe two generations a year, even in tropical areas. In addition, thermal tolerance studies helped us to understand and model its distribution and phenological patterns found in the field. Although mating has still not ever been observed in nature, these results will help to improve and detect rearing features that will enable us to obtain a reproductive laboratory colony.

*Poster presentation*

# Performance of the gall midge *Orseolia javanica* (Diptera: Cecidomyiidae) on two Florida biotypes of cogongrass, *Imperata cylindrica*

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Cogongrass (*Imperata cylindrica* [L.] P.Beauv.) is a diploid $C_4$ rhizomatous grass that is a noxious weed in over 70 countries where it threatens global biodiversity and sustainable agriculture. Recent genetic analyses identified four distinct non-hybridizing clonal lineages of cogongrass in the USA. In Florida and other southeastern states, this invasive grass infests cattle pastures and pine plantations and thrives in poor soil conditions such as ditch banks, roadsides, railroad rights-of-way and reclaimed phosphate-mining areas. Control of cogongrass relies primarily on mowing and herbicide applications. The Indonesian gall midge *Orseolia javanica* Kieffer & van Leeuwen-Reijinvaan is a potential biological control agent of cogongrass. Larval feeding induces the formation of hollow, sterile shoot galls in which one larva develops. These galls serve as nutrient "sinks" that divert rhizome resources away from normal shoot production. According to literature, the only reported host plant for *O. javanica* is cogongrass. However, it is not known the extent to which *O. javanica* will develop and reproduce on the Florida peninsula or Gulf Coast (Florida Panhandle) clones of cogongrass. We collected/propagated cogongrass from two different geographic locations in Florida and shipped healthy rhizomes under permit to Bogor Agricultural University, West Java, Indonesia, for clonal testing. Performance of *O. javanica* on each cogongrass clone (no. of galls and adults produced, development time to adult stage) will be compared. Although the experiments are still in progress, we anticipate that gall production and development of *O. javanica* on the two Florida clones of cogongrass will be comparable to Indonesian cogongrass. Based on these findings, importation permits will be requested to establish a colony of *O. javanica* in a Florida quarantine laboratory for detailed biological studies and extensive host-range testing.

*Poster presentation*

# *Doassansia niesslii* (white smut pathogen): a new potential biological control agent for flowering rush in North America

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Flowering rush (*Butomus umbellatus* L.) is a perennial aquatic plant of European origin that was introduced to North America (NA) as an ornamental over 100 years ago. It has developed into an aggressive invader of freshwater systems, especially in the midwestern and western states of the USA and western Canada. Since 2013, CABI in Switzerland has been conducting surveys for natural enemies of flowering rush in Europe. Currently, this work focuses on a weevil species in the genus *Bagous*, two species of Diptera and, since 2016, on the white smut *Doassansia niesslii* De Toni, a hemibiotrophic fungal pathogen. A strain of this pathogen was collected in northern Germany and has been tested against two genotypes (1 and 2) of the flowering rush from NA. This strain was only able to infect genotype 2 from British Colombia, demonstrating the specificity of the agent at the plant population level. More strains are now required from throughout its native range to identify strains that can attack all the plant genotypes present in its invasive range, especially the most common genotype 1. Infection studies have identified two infective spore states: pycnidiospores (asexual state) for within-season infection and dispersal; and telial spore balls (sexual state) which develop within the plant tissue for overwinter survival. Infection of flowering rush by the overwintering state has been found to occur underwater, which increases the value of this agent since the plant also grows as a submerged weed in its invasive range. Pycnidiospores produce masses of sporidia in culture that are highly infective, causing death of all aerial parts of the plant. This may also allow the fungus to be developed as a mycoherbicide. The white smut has been tested against three closely related non-target plant species, and no symptoms were observed. The work undertaken thus far has demonstrated that the white smut pathogen has excellent potential as a classical biological control agent for flowering rush in NA.

*Poster presentation*

# Exploration of natural enemies of calotrope in its native range: predispersal seed-predation in Pakistan

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*Calotropis procera* (Aiton) W.T. Aiton (Apocynaceae), commonly known as calotrope, Indian milkweed or Aak, is a spreading shrub or medium-sized tree native to South Asia, West Asia and North Africa. It is a serious weed of rangelands and is a target weed approved for biological control in Australia (Dhileepan, 2014). Surveys in its native range in Pakistan yielded several common herbivores (Table 1) (Ali and Shabbir, 2017). The pre-dispersal seed-feeding Aak fruit fly, *Dacus persicus* Hendel (Diptera: Tephritidae), and Aak weevil, *Paramecops farinosus* Schoenherr (Coleoptera: Curculionidae), have been identified as prospective biological control agents due to records of their restricted host range (Dhileepan, 2014) (Figure 1; Table 1).

Life history, seasonal dynamics and damage potential of both agents were studied under laboratory and field conditions in Lahore, Pakistan. Populations of *D. persicus* began to increase in summer (June) and peaked in August to September before starting to decline in autumn (November). The duration of the life cycle of *D. persicus* (egg to adult) was 42.2 ± 0.4 days (mean ± SE) with a range of 38 to 50 days. The average life span of an adult fly was 16.4 ±0.7 days. The Aak fruit fly larvae destroyed all immature seeds and internal tissue of infested pods of the host plant (Figure 1).

In contrast, Aak weevil populations built slowly during spring and early summer (March to May) and peaked in late summer (August). Like the Aak fruit fly, the larvae of the Aak weevil also destroyed all immature seeds of infested pods (Figure 1).

**Table 1.** Common herbivores associated with *Calotropis procera* in Pakistan.

| Species | Order | Family | Feeding habit | Host range [a] |
|---|---|---|---|---|
| *Platycorynus peregrines* (Herbst) | Coleoptera | Chrysomelidae | Leaf (adult); Root (larva) | Generalist |
| *Paramecops farinosus* Schoenherr | Coleoptera | Curculionidae | Leaf (adult); Fruit and seed (larva) | Specific to *Calotropis* spp. |
| *Niphona indica* Breuning | Coleoptera | Cerambycidae | Stem borer | Specialized |
| *Poekilocerus bufonius* (Klug) | Orthoptera | Pyrgomorphidae | Leaf | Generalist |
| *Poekilocerus pictus* Fab. | Orthoptera | Pyrgomorphidae | Leaf and fruit | Generalist |
| *Spilostethus hopes* (Fab.) | Hemiptera | Lygaeidae | Seed | Generalist |
| *Aphis nerii* Boyer de Fanscolombe | Hemiptera | Aphidae | Leaf and fruit | Generalist |
| *Dacus persicus* Hendel | Diptera | Tephritidae | Fruit and seed | Specific to *Calotropis* spp. |

[a] information taken from Ali and Shabbir (2017) and Dhileepan (2014)

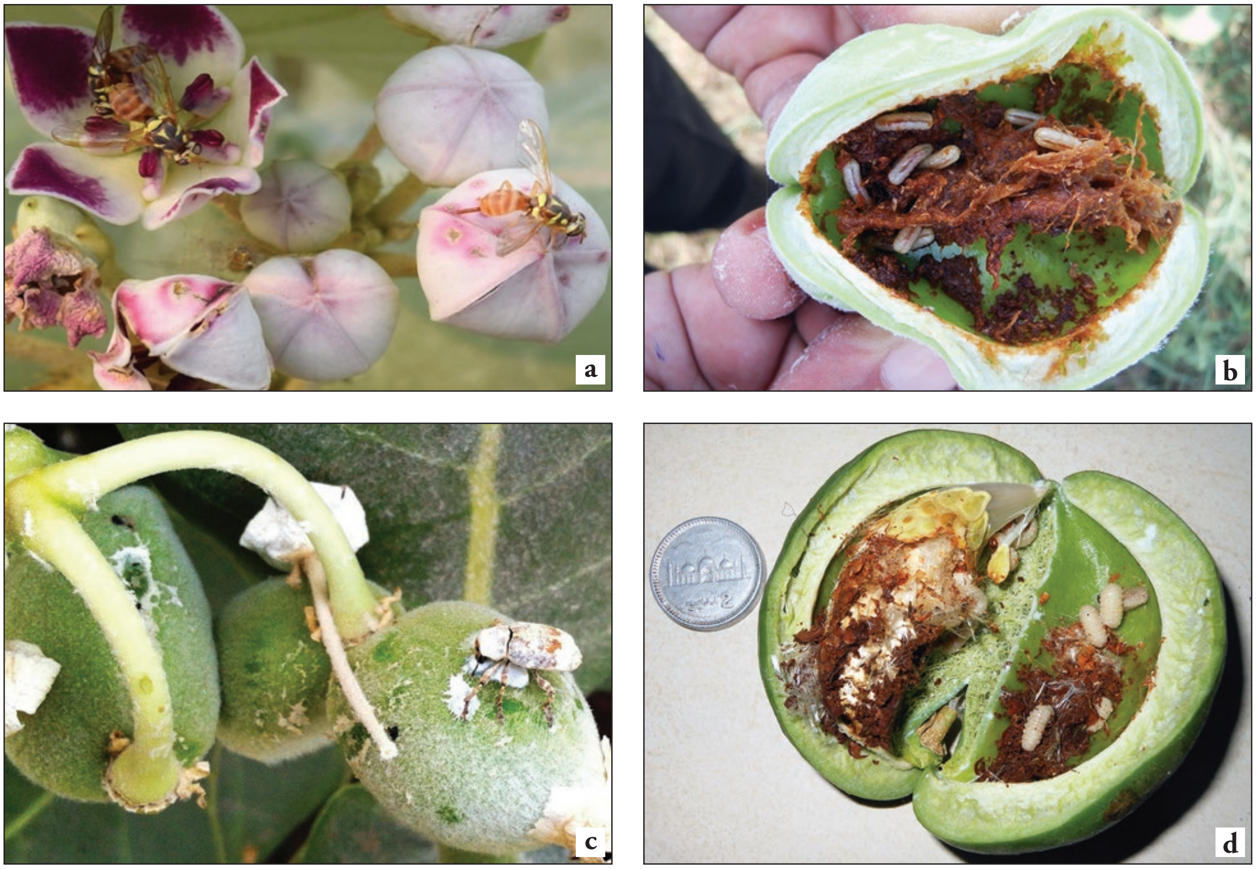

**Figure 1.** Prospective biocontrol agents of calotrope. (a) Adults of the fruit fly *Dacus persicus* sitting on flowers of *Calotropis procera*; (b) seed damage caused the larvae of *D. persicus*; (c) an adult weevil, *Paramecops farinosus,* on young fruit of *C. procera*; (d) seed damage caused the larvae of *P. farinosus*.

Field host specificity, fast development and damage potential of the fruit fly and weevil indicate that these agents hold promise to be considered as potential candidate agents for biological control of *C. procera* in Australia and other parts of the world where *C. procera* and the closely related *C. gigantea* (L.) Dryand. are problem weeds. Both agents are planned to be imported into quarantine for further testing in Australia.

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*Poster presentation*

# Recognition of natural enemies and genetic variability of *Conyza bonariensis* (L.) Cronquist (Asteraceae) in its area of origin

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The plant popularly known in Brazil as Buva, *Conyza bonariensis* (L.) Cronquist, is native to South America. Although considered native throughout Brazil and other parts of South America, this plant is also an important weed of the main agricultural crops because of its resistance to herbicides. In Australia, Buva is known as flaxleaf fleabane and is considered an important invader in agricultural and non-agricultural areas. A cooperation between CSIRO and the Regional University of Blumenau, initiated in 2017, aims to select potential natural enemies for the accomplishment of classic biological control of this weed in Australia. To date, extensive surveys have been conducted in southern Brazil, where 54 sites were sampled and are being monitored for the presence of different natural enemies. At each site, in addition to the natural enemies surveyed, herbarium and DNA samples of the different *Conyza* species (10 sub-samples/area) were collected to better understand the taxonomy of this genus and to elucidate the origin of the material introduced in Australia. Through the first half of 2018, 17 sampling areas were visited in the state of Paraná, 20 in the state of Santa Catarina and 17 in the state of Rio Grande do Sul. In addition to surveys in southern Brazil, two collection sites were sampled in the state of Bahia in the Northeast region. Across all these surveys, the main insects associated with the genus *Conyza* were: root mealybugs (Pseudococcidae), among them the species *Pseudococcus viburni* (Signoret) and *Spilococcus* sp. (likely a new species); a *Lixus* sp. (Coleoptera: Curculionidae); and the stem-galling fly *Trupanea bonariensis* (Brèthes) (Diptera: Tephritidae). Another potential agent found for the control of flaxleaf fleabane is a beetle from the family Mordellidae that is consistently present in the roots and stems in about 90% of *Conyza bonariensis* plants sampled.

*Poster presentation*

# Preliminary studies on *Oporopsamma wertheimsteini* and *Sphenoptera foveola*, two potential biological control agents of *Chondrilla juncea*

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Field collections and preliminary host-specificity, survival, fecundity and impact testing of two potential biocontrol agents of rush skeletonweed, *Chondrilla juncea* L., were conducted between 2014 and 2017. Root cases with pupae of the Chondrilla crown moth, *Oporopsamma wertheimsteini* (Rebel), were collected in the Gorovan Sands desert in Central Armenia, and no-choice tests were conducted at the Montana State University containment facility (Bozeman, Montana). Adults of the root-boring buprestid *Sphenoptera foveola* (Gebler) were collected in the Kulanbasy desert hills in the Almaty province of southeastern Kazakhstan, and no-choice host-specificity tests as well as survival and fecundity tests were carried out at the Zoological Institute St. Petersburg, Russia. Based upon no-choice tests, *O. wertheimsteini* appears to be host-specific and has potential to be an effective biocontrol agent. The suitability of *S. foveola* for biological control of rush skeletonweed also appears promising but needs further investigation.

*Poster presentation*

# *Acinia corniculata* (Diptera: Tephritidae): a new potential biocontrol agent for spotted knapweed?

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Spotted knapweed, *Centaurea stoebe* L., is a species native to Asia and eastern Europe. Two cytotypes of *Centaurea stoebe* L. sens. lat. are recognized as different species: *C. stoebe* L. is the appropriate name for the diploid form present throughout Europe while the appropriate nomenclature for the tetraploid form invasive in North America remains to be resolved but is referred to as *C. stoebe* L. sens. lat. until the resolution is made. Spotted knapweed is considered invasive in much of North America and is able to quickly establish itself and spread, adversely affecting native biota as well as ecosystem processes. Several biocontrol agents have been introduced to date, but in general, they have not been shown to be efficient against *C. stoebe*. The fruit fly *Acinia corniculata* (Zetterstedt) (Diptera: Tephritidae) is a rare species, being known in several countries in central and northern Europe. Literature records indicate that adults are associated with *Centaurea* species, especially the brown knapweed complex (*C. jacea* L.). It was hypothesized that *A. corniculata* larvae develop in flower heads, like other species of fruit flies. However, I obtained adults of *A. corniculata* by rearing larvae and pupae from galls collected at the shoot base of *C. stoebe* at sites in northeastern Romania. I also placed adults of *A. corniculata* together with young *C. stoebe* flowers, but did not obtain any galls, further confirming that the species does not develop in flower heads. The fact that the attacked shoots remain smaller and no longer produce flowers, or are completely destroyed, indicates that *A. corniculata* could be an additional, effective biocontrol agent for *C. stoebe.*

*Poster presentation*

# Gathering weapons to fight stinking passionflower in Australia: fungal pathogens of *Passiflora foetida* from Brazil

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*Passifloa foetida* L. (PF), stinking passionflower (Passifloraceae), was recently chosen as a classical biocontrol target in Australia because of the environmental harm caused by invasions of natural habitats. It has a broad distribution in its tropical native range in the American continent. In Brazil, it has been more commonly recorded in semi-arid and sand dune situations, but never forms dense stands as observed in Australia. Considering the extent of invasion, biocontrol is considered the most sustainable approach for its management. Nevertheless, the taxonomic proximity between PF, cultivated passionfruit (*Passiflora edulis* Sims) and native *Passiflora* species in Australia, may make finding sufficiently host-specific biocontrol agents a challenge. Historically, the pathogenic mycobiota of weeds have been a valuable source of highly host-specific biocontrol agents. A project was recently started with Australian funding to search for potential pathogen biocontrol agents from the neotropics to be deployed against PF in Australia. Here the results of the initial searches for fungal pathogens of PF in Brazil are discussed. Surveys were guided by botanical record databases available for the Brazilian flora. These indicated that PF is common in the northeast of Brazil. Localities in the states of Bahia, Sergipe, Alagoas, Pernambuco, Paraíba, Rio Grande do Norte and Ceará were visited. Two groups of diseases have so far been found: anthracnose (*Colletotrichum* spp.) and leaf spots (*Alternaria* spp., undetermined cercosporoids, *Cladosporium* sp., undetermined coelomycete asexual morphs, undetermined helmithosporoid, undetermined hyphomycete asexual morph and *Pythomyces* sp.). The precise identity of each of these fungi is being clarified. Field surveys are also being expanded to encompass new areas in the Brazilian northeast. Observations on the impact of the diseases in the field and results of pathogenicity tests under controlled conditions will be discussed in a biocontrol context. As no fungi have ever been recorded from PF in Brazil, it is likely that the surveys will reveal taxonomic novelties, as commonly occurs during this kind of study.

*Poster presentation*

# Knowledge on life history improves rearing success and informs host-range testing of a semi-aquatic weevil

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Flowering rush (*Butomus umbellatus* L.) was introduced from Eurasia to North America as an ornamental aquatic plant more than 100 years ago. It escaped cultivation and spread in the wild to become a severe problem in freshwater systems of the midwestern/western states of the USA and in western Canada with multiple impacts (Jacobs et al., 2011). A biological control project was initiated in 2013. The semi-aquatic weevil *Bagous nodulosus* Gyllenhal is currently our most promising insect biocontrol candidate (Häfliger et al., 2018).

Studies in the lab and common garden and field observations revealed that adults of *B. nodulosus* spend most of their life below water (Figure 1). Larvae feed and develop in the leaves and rhizomes of flowering rush. Although it is reported to be rare, we have collected it at over 20 sites in Germany, Poland, Czech and Slovak Republics, Hungary, Serbia and Georgia. In our initial rearing trials, we covered potted plants of flowering rush with gauze bags (mesh width 1 mm) and either released egg-laying females or transferred larvae. We also exposed infested plants to different water levels, different types of water (tap or rain), and used different populations of flowering rush. Nevertheless, we always experienced problems with high larval mortality and very low rates of successful development to adults.

**Figure 1.** *Bagous nodulosus* under water. Photo by Tim Haye, CABI, used with permission.

Since early instars do not leave obvious traces in the leaves, not much is known about the larval behavior of *B. nodulosus*. Similar to many terrestrial weevils, we assumed larvae do not change plants once females have inserted an egg into the plant tissue. However, we found that gauze-covered control plants (no weevils released) were attacked when randomly arranged among infested plants (with weevils released) in the same pool. In addition, we observed an increasing number of adults developing on uncovered plants in our artificial pond, where some flowering rush plants were exposed to adult *B. nodulosus* covered with gauze bags (Häfliger et al., 2018). Thus, larvae of *B. nodulosus* must be able to swim and to actively move from plant to plant. Observations in the lab confirmed that larvae can float on the water surface and also actively move below water for many hours.

The knowledge that larvae are commonly moving from leaf to leaf and from plant to plant helps to explain the low larval development rates on gauze covered plants in our rearing. There is a high probability that larvae leave through the gauze or lose orientation and desiccate on the gauze. Providing larger water surfaces with more and uncovered *Butomus* should increase the number of successfully developed weevils as the chances for larvae reaching new leaves increase. This new knowledge also has implications for the methodology of host-specificity tests. Thus far, we have only conducted sequential no-choice oviposition tests with *B. nodulosus* females. Of 41 plant species tested, only one (the introduced *Baldellia ranunculoides*

[L.] Parl.) was accepted once for oviposition. Now that we know that larvae are more mobile, we also started testing larval host choice on 15 different plant species. Thus far, none of the larvae were able to develop on test plants. Most larvae were dead after five days, while larvae on flowering rush usually developed to second instars. This confirms the very narrow host range of *B. nodulosus*.

In conclusion, larvae of *B. nodulosus* are much more mobile than previously assumed and commonly switch plants during development. Methods for rearing and host-specificity tests have been adapted accordingly. This case confirms that detailed knowledge on the life history and biology of agents is a pre-requisite to develop effective rearing methods and conduct meaningful host-specificity tests.

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*Poster presentation*

# Biological control of garlic mustard with *Ceutorhynchus scrobicollis*, an update

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Garlic mustard (*Alliaria petiolata* [M.Bieb.] Cavara & Grande) is an invasive biennial plant and in North America, poses a threat to native herbaceous and woody plants in the forest understory. *Ceutorhynchus scrobicollis* Neresheimer & Wagner is a crown-mining weevil that is native to northern temperate regions of Europe and is being developed as a biocontrol agent for garlic mustard in North America. In the USA, a petition for the field release of *C. scrobicollis* was recommended for release by the Technical Advisory Group for the Biological Control Agents of Weeds (TAG) in 2017. We are conducting additional impact and single-choice development tests with several Brassicaceae species native to the western USA to ensure compliance with the Endangered Species Act through the U.S. Fish and Wildlife Service (USFWS) and the National Environmental Policy Act through Animal Plant Inspection Service (APHIS) as well as tribal compliance. Results of these tests are presented.

*Poster presentation*

# A new biological control program for South Africa: high elevation weeds

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The high altitude mountain catchments of South Africa are the most important systems to water security as they provide nearly 50% of all water run-off. In South Africa, there has been greater focus on invasive alien plant management in riparian areas than on the invaders of mountainous catchments. Currently, biological control of invasive species in mountain catchments is limited to a few species. These are usually widely distributed species that also grow in high altitude areas, such as *Acacia mearnsii* De Wild. Biological control can offer an effective, sustainable management option for high altitude species. Additionally, many of these high elevation weeds grow on steep slopes and dangerous terrain where conventional control methods are difficult and expensive. No high altitude mountain catchment invasive species has been targeted for biological control programs in South Africa. Therefore, a project has been initiated against a number of the worst invasive species growing at high elevations with a particular focus on reducing the number of viable seeds being produced. The program aims to include biological control programs against the invasive trees *Robinia pseudoacacia* L. and *Gleditsia triacanthos* L., and biological control feasibility studies into *Pyracantha angustifolia* (Franch.) C.K.Schneid., *Populus alba* L., *Populus canescens* (Aiton) Sm., *Salix fragilis* L., *Salix babylonica* L., *Rosa rubiginosa* L., *Cotoneaster* spp. (*C. franchetii* Boiss. and *C. pannosus* Franch.), *Acer* spp. (*A. negundo* L. and *A. buergerianum* Miq.) and *Fraxinus* spp. (*F. americana* L., *F. angustifolia* Vahl and *F. pennsylvanica* Marshall [Oleaceae]).

*Poster presentation*

# The rust fungus *Puccinia rapipes*: a potential biological control agent of African boxthorn (*Lycium ferrocissimum*) in Australia

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Suitability of the rust fungus *Puccinia rapipes* Berndt & E. Uhlmann as a biological control agent for African boxthorn (*Lycium ferocissimum* Miers) is being assessed. African boxthorn is a major environmental and agricultural weed in regional Australia and a Weed of National Signficance (WoNS). Studies with *P. rapipes* are being undertaken to elucidate its life cycle, genetic diversity and specificity towards *Lycium* species that occur in Australia. The relationship between different rust spore stages observed on African boxthorn in the field in South Africa are being established using sequencing in order to confirm that the fungus is macrocyclic and does not have an alternate host (i.e. autoecious). Sequencing is also being used to assess the genetic diversity of the fungus across its native range. Multiple haplotypes of African boxthorn, as well as the closely related non-target species, the native Australian boxthorn (*L. australe* F.Muell.) and exotic goji berry (*L. barbarum* L.) are being tested with Eastern and Western Cape accessions of the rust fungus to obtain initial data on their host ranges. Results of this preliminary host-specificity testing and molecular characterization will be used to decide which of the rust accessions studied should be selected for more comprehensive testing to fully assess risks to non-target plant species.

*Poster presentation*

# Asynchrony in phenology of target and non-target plants: implications for host-specificity testing with *Platyptilia ochrodactyla*, a potential biocontrol agent for common tansy

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Common tansy, *Tanacetum vulgare* L., is a perennial plant native to Eurasia that has become an increasing weed problem in pastures, riparian areas and forest margins across Canada and the northern USA (McClay and Gassmann, 2013). The plume moth *Platyptilia ochrodactyla* (Denis & Schiffermüller) (syn: *Gillmeria ochrodactyla* Denis & Schiffermüller) is currently being studied as a potential biological control agent for *T. vulgare* (Stutz et al., 2019). Its life cycle is rather unique; females oviposit into the flower heads of *T. vulgare* where the early instar larvae are overwintering. The larvae leave the flower heads the following spring and burrow mines into newly growing shoots. No-choice tests revealed that *P. ochrodactyla* can develop on several congeneric species, including the North American native *Tanacetum camphoratum* Less. and *T. huronense* Nutt., the medicinal plant *T. parthenium* L. and the European *T. corymbosum* (L.) Sch.Bip. A common next step in evaluating the risk of non-target attack in the field would be to expose target and non-target species simultaneously under multiple-choice conditions. However, since the congeneric non-target species flower several weeks earlier than the target weed, *P. ochrodactyla* may not experience a choice-situation under natural conditions. Early flowering non-target species may either be safe from attack by *P. ochrodactyla* or, if *P. ochrodactyla* become reproductively active before any *T. vulgare* are flowering, they may accept non-target species that would not be accepted if flowering *T. vulgare* were present (e.g., Sheppard et al., 2006; Paynter et al., 2008).

To further evaluate the risk of non-target attack by *P. ochrodactyla*, we therefore exposed non-target species along a transect next to a natural population of *T. vulgare* and *P. ochrodactyla* for one month. We recorded the phenology of the exposed plants and of the *T. vulgare* plants growing naturally across the field site and checked the plants for adults of *P. ochrodactyla* using a sweep net in two-week intervals. At the end of the experiment, the flower heads of all plants and approximately 2,000 additional open flower heads of *T. vulgare* from across the field site were dissected for eggs and larvae.

*Platyptilia ochrodactyla* larvae were found in the flower heads of half of the exposed potted T. vulgare plants, but no *P. ochrodactyla* larvae were found in any of the test plant species exposed. No *P. ochrodactyla* larvae were found in the naturally occurring *T. vulgare* within the transect or within any of the *T. vulgare* flower heads that were collected across the field site. Three males of *P. ochrodactyla* were collected at the end of the experiments, but no adults were observed on any of the earlier dates. The first observation of *P. ochrodactyla* coincided with the observation of the first flowering *T. vulgare* plants on the field site. There was only very little overlap between the flowering time of *T. camphoratum, T. huronense* and *T. corymbosum* and the flowering time of *T. vulgare* (Figure 1), and at the time the first *P. ochrodactyla* were observed, all the flower heads of the three non-target species had already senesced. The flowering period of the medicinal herb *T. parthenium* partly overlapped with the flowering period of *T. vulgare* and the occurrence of *P. ochrodactyla*.

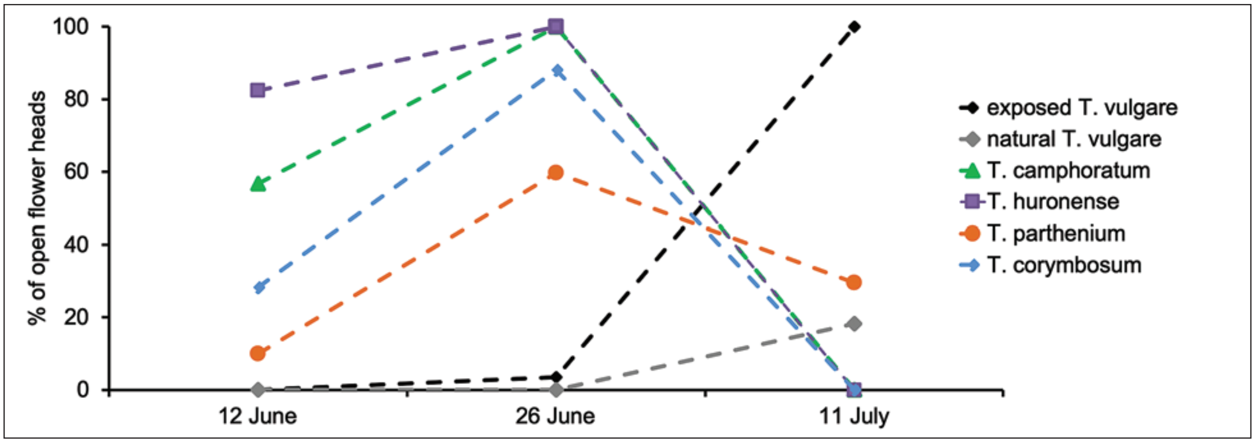


**Figure 1.** Percentage of open, non-senescent flower heads of *Tanacetum vulgare* and non-target species in the open-field test conducted with *Platyptilia ochrodactyla*.

We conclude that the risk that the North American native *T. camphoratum* and *T. huronense* will be accepted for oviposition under natural conditions is low. The risk that the medicinal herb *T. parthenium* will be accepted for oviposition cannot be excluded, but damage is expected to be negligible because plant harvesting likely disrupts the life cycle of the moth. It also remains unclear whether *T. camphoratum* and *T. huronense* would be accepted for oviposition if *P. ochrodactyla* females were active before any flowering *T. vulgare* are available and how likely it is that this would happen in North America. We therefore plan to conduct an additional open-field test, where we will artificially advance the emergence of *P. ochrodactyla* and release the species when *T. camphoratum* and *T. huronense* are flowering and again when *T. vulgare* is flowering.

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*Poster presentation*

# Molecular studies on *Arundo donax* and an adventive population of a stem-galling wasp, *Tetramesa romana*, in South Africa

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Giant reed, *Arundo donax* L. (Poaceae), is a widespread and highly invasive weed in South Africa (SA) that negatively impacts native biodiversity and the country's scarce water resources. Several genetically-distinct genotypes of a stem-galling wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), are being considered as biological control agents to help manage the weed. However, in 2010, an adventive *T. romana* population of unknown origin, but of known genetic distinction from wasp genotypes released in the USA, was found to be present on *A. donax* infestations throughout the country. Molecular studies were undertaken to investigate the genetic variation present in the SA *T. romana* population across the distribution of the species. Additionally, we investigated genetic variability within the SA *A. donax* population and compared samples to native and introduced populations from Mediterranean Europe and the USA, respectively. In total, 135 *T. romana* wasps collected from all nine provinces in SA were genotyped using a panel of three microsatellite markers that had previously been used to detect variation within the native range populations of *T. romana*. The results revealed two genetically distinct *T. romana* populations occupying climatically different regions of the country, suggesting two separate introductions. The *A. donax* molecular studies used a phylogenetic approach and haplotype diversity was determined by genotyping three chloroplast markers in 26 samples from SA, the USA, Spain, France, Greece and Mexico. South African *A. donax* populations were found to be genetically similar to *A. donax* from Spain and the USA and SA samples shared the same haplotypes as samples from Spain, the USA and France. Further molecular studies using microsatellite markers will be undertaken; however, these preliminary results strongly suggest that Spain will be an excellent source for biological control agents for *A. donax* in SA. Further, the highly successful Granada *T. romana* genotype used for biocontrol of *A. donax* in the USA is a promising candidate agent for SA, since these countries share genetically similar host-plant genotypes.

*Poster presentation*

# Potential biological control agents of some invasive and noxious weed species in south eastern Anatolia region of Turkey

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This study was carried out to determine the potential biological control agents of some invasive and noxious weeds in agricultural areas of Southeastern Anatolia Region, Turkey during 2015–2017 through exploratory surveys. Ninety-six different fungal pathogen species were detected on eighty-four weed species occurring in different field crops in the region. Sixteen of these were observed to suppress the growth of weeds under field conditions, and thus could be potential biological control agents of host weed species. These sixteen fungi were the most common species observed in the region and included *Cercospora centaurea* Died., *C. sorokinii* Sacc., *Coleosporium datiscae* Tranzschel, *Erysiphe convolvuli* DC., *Passalora ferruginea* (Fuckel) U. Braun & Crous, *Puccinia xanthii* Schwein., *P. aristolochiae* (DC.) G.Winter, *P. bromina* Erikss., *P. calcitrapae* DC. var. *calcitrapae*, *P. montana* Fuckel, *P. malvacearum* Bertero ex Mont., *P. phragmitis* (Schumach.) Körn., *Pyrenophora chaetomioides* Speg., *Uromyces glycyrrhizae* (Rabenh.) Magnus, *U. acetosae* J. Schröt. and *U. vesicatorius* (Bubák) Nattrass. The respective host weed species of these fungi were: *Centaurea behen* L., *Convolvulus arvensis* L., *Datisca cannabina* L., *C. arvensis*, *Artemisia vulgaris* L., *Xanthium strumarium* L., *Aristolochia bottae* Jaub. & Spach, *Bromus sterilis* L., *Echinops orientalis* Trautv., *Serratula cerinthifolia* (Sm.) Boiss., *Centaurea balsamita* Lam., *Malva* sp., *Phragmites australis* (Cav.) Trin. ex Steud., *Avena sterilis* L., *Glycyrrhiza glabra* L., *Rumex crispus* L. and *Leontice leontopetalum* L., respectively. The current study adds valuable information, which can be used in future studies dealing with biological weed control. The observed fungal pathogens could be potential biological control agents of the respective weed species. However, thorough investigations such as host specificity bioassays are needed in this regard.

*Past Proceedings correction*

# Correction to abstracts published in the Proceedings of the XIII International Symposium on Biological Control of Weeds, Waikoloa, Hawaii

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In Dolgovskaya et al. (p. 188: *Abrostola clarissa* [Lepidoptera: Noctuidae], a new potential biocontrol agent for invasive swallow-worts, *Vincetoxicum rossicum* and *V. nigrum*) and Sforza et al. (p. 200: Towards biological control of swallow-worts: the ugly, the bad and the good), the moth *Abrostola clarissa* Staudinger (Lepidoptera: Noctuidae) was reported as a new agent under study for invasive *Vincetoxicum* species. It was later determined that the insect was misidentified, and that the species was *Abrostola asclepiadis* (Denis and Schiffermüller). Thus, any reported information on host range refers to a Russian population of *A. asclepiadis*.

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# Session 2: Opportunities and constraints for classical weed biocontrol in developing countries

**Session Chair: Urs Schaffner**

**Keynote**

# Opportunities and constraints for classical weed biocontrol in developing countries

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Classical biological control is one of the foremost activities to be considered for tackling invasive weeds. This technology has become well entrenched in many developed countries located in temperate regions such as Australia, Canada, New Zealand, South Africa and the USA, but its adoption and implementation in developing countries predominately located in the tropics is limited and lacks momentum.

Opportunities for biological control of weeds in developing countries is as abundant as in developed countries. Historically, the first documented example of biological weed control involved *Opuntia monacantha* (Willd.) Haw. (Cactaceae), which was introduced to India from the Americas in the eighteenth century for production of cochineal dye. However, because of the mismatch of the host plants and the dye-producing cochineal insects, the cactus became invasive and occupied a vast area stretching from Punjab to Assam in northern India. In 1795, a cochineal insect, *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae), was introduced from Brazil for cochineal dye production, which did not contribute much to the cochineal dye industry, but killed the cactus and effectively cleared it from the infested area. Even though it was not a deliberate attempt of biological control of a weed, it brought the potential of classical biological control of weeds to light (Pruthi, 1969). Subsequently, based on this success, *D. ceylonicus* was introduced to Sri Lanka from India around 1865 for control of *O. monacantha*, constituting the first international transfer of a natural enemy for biological control of a weed (Goeden, 1988).

Either the serendipitous control of *O. monacantha* in 1795 in India or the intentional introduction of *D. ceylonicus* from India to Sri Lanka around 1865 should be considered as the first biological control of weed activity in the world. However, there are several publications in the literature of the biological control of weeds that wrongly report the introduction of natural enemies of *Lantana camara* L. in 1902 into Hawaii as the first instance in history.

Developing countries, in general, lack financial, institutional and human resources to take up exploration, host specificity screening and regulatory policies for primary biological control activities, but they are very amenable for technology transfer, as is, for instance, being done in Ethiopia. USAID is funding implementation of biological control of *Parthenium hysterophorus* L. (Asteraceae) in Ethiopia, transferring technologies developed in Australia and South Africa with minimal research activities on host specificity screening of local plant species and training local scientists. It also established collaboration between scientists from the USA, Australia and South Africa with scientists in Ethiopia, Kenya, and Uganda. Several such collaborations occurred in the past on many invasive weeds such as *Ageratina adenophora* (Spreng.) R. M. King & H. Rob. (Asteraceae), *Chromolaena odorata* (L.) R. M. King & H. Rob. (Asteraceae), *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae), *Lantana camara* L. (Verbanaceae), *Mimosa diplotricha* C. Wright (Mimosaceae), *Opuntia* spp., and *Salvinia molesta* D.S. Mitch. (Salviniaceae), to name a few, and currently some are ongoing.

Most biological control of weeds programs in developing countries are sporadic, predominately conducted with external assistance and little or no follow up. For example, biological control of *L. camara* in Ghana, West Africa, began in 1971 with the assistance of the International Institute for Biological Control

(IIBC, now CABI). Of the five natural enemies introduced (*Diastema tigris* Guenée [Lepidoptera: Noctuidae], *Leptobyrsa decora* Drake [Hemiptera: Tingidae], *Octotoma scabripennis* Guérin-Méneville [Coleoptera: Chrysomelidae], *Teleonemia scrupulosa* Stål [Hemiptera: Tingidae], and *Uroplata girardi* Pic [Coleoptera: Chrysomelidae]), the first two did not establish and the last three established (Winston et al., 2014). After 1973, no follow up or consideration for additional agents was given.

In Tanzania, East Africa, four natural enemies were introduced with the assistance of IIBC. In 1958, *T. scrupulosa*, and in 1967, *U. girardi* were introduced and established. In 1967–68, *D. tigris* and *Salbia haemorrhoidalis* Guenée (Lepidoptera: Pyralidae) were introduced, but they failed to establish. Since then, no further activity on biocontrol of *L. camara* has been carried out (Winston et al., 2014).

In Zambia, Southern Africa, *T. scrupulosa* was introduced in 1962 by the Ministry of Agriculture, but it did not establish. In 1969, IIBC introduced *T. scrupulosa* and *U. girardi*, and in this case, the first established and the second did not. In 1970, introductions of *D. tigris* and *S. haemorrhoidalis* by IIBC, and *L. decora* and *Teleonemia elata* Drake (Hemiptera: Tingidae) by CSIRO, Australia did not establish (Winston et al., 2014). Here, too, no additional work has been carried out since 1970.

In India, most of the biocontrol activities for *L. camara* were carried out by local government agencies. The program started in 1918 with a survey of local natural enemies by Rao (1920) supported by the Government of India, while the sequence of introduction of natural enemies progressed from *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae) in 1921 by the entomologist in the State of Mysore (Subramanyam, 1934), to *T. scrupulosa* in 1941 by the Forest Research Institute at Dehra Dun (Roonwal, 1952), to *D. tigris*, *U. girardi* and *S. haemorrhoidalis* from 1968–1969 by the CIBC and supplied to the Central Plant Protection Institute in Hyderabad (Rao et al., 1971), to *O. scabripennis* and *U. girardi* in 1971 and *L. decora* in 1977 by the Forest Research Institute, Dehra Dun. Of these introductions, *L. decora*, *D. tigris* and *S. haemorrhoidalis* did not establish, and not all established species were widely dispersed in India (Muniappan and Viraktamath, 1986; Winston et al., 2014). Even though the program was carried out for about 50 years, there was limited coordination between the agencies and the weed is still a serious problem.

Misinformation on biological control activities in developing countries is mostly unattended and not corrected. For example, Winston et al. (2014) mentioned that *Insignorthezia* (*Orthezia*) *insignis* (Browne) (Hemiptera: Ortheziidae) was introduced to India from Hawaii*. Also, Julien and Griffiths (1998) stated Sri Lanka to be the source for the introduction of this insect to Hawaii. Both of these statements require substantiation. One of the common names of *I. insignis* is Kew bug and it was accidentally introduced from Kew Gardens in England to the Botanic Garden in Perdeniya, Sri Lanka in 1893 (Rao, 1920). From Sri Lanka, it was accidentally introduced to Nilgris, India in 1915. It is worth checking whether Kew Gardens, the West Indies or Sri Lanka are the source of introduction of *I. insignis* to Hawaii.

There is a need to address conflicts emerging in developing countries from the activities of scientists in developed countries. For example, *Opuntia ficus-indica* (L.) Mill. (Cactaceae) is cultivated for food and fodder in Ethiopia, Israel, Lebanon, Morocco and other countries in North Africa and the Middle East. *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopidae) was released in Saudi Arabia in 2010 and Kenya in 2014 for control of *Opuntia stricta* (Haw.) Haw. (Cactaceae). To protect *O. ficus-indica* from *D. opuntiae*, Israel imported and released a lady beetle, *Hyperaspis trifurcata* Schaeffer (Coleoptera: Coccinellidae) in 2017 (Protasov et al., 2017). Scientists involved in biological control of the cactus *O. stricta* and the cochineal insect *D. opuntiae* should communicate, address interests of individual countries and the region, and prevent counterproductive activities. The recently formed IOBC Global Working Group on Management of Cactus Species will hopefully address this conflict and confirm an amicable solution.

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*This error has since been corrected in the online version of the weed biological control catalog (https://www.ibiocontrol.org/catalog/).

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*Oral presentation*

# Weed biocontrol in India: opportunities and constraints

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With more than 250 invasive alien plants identified in India, *Chromolaena odorata* (L.) R.M.King & H.Rob., *Eichhornia crassipes* (Mart.) Solms, *Lantana camara* L., *Leucaena leucocephala* (Lam.) de Wit, *Mikania micrantha* Kunth, *Mimosa diplotricha* C. Wright, *Parthenium hysterophorus* L. and *Prosopis juliflora* (Sw.) DC. cause the most significant economic and ecological harm. Conventional control is not feasible and biocontrol is deemed the only long-term cost-effective management tool. Biocontrol has been attempted on 10 species, including *L. camara*, *C. odorata* and *P. hysterophorus* with limited success. Biocontrol of *Salvinia molesta* D.Mitch has been the most successful program, while the recent attempt on biocontrol of *M. micrantha* was unsuccessful. Reasons for the lack of success of several biocontrol programs include a lack of capacity and infrastructure to fully implement programs. However, there is also a fundamental lack of awareness on the efficacy and benign nature of classical biological control among policy makers, scientists, foresters, quarantine officials and agriculturists. Skepticism is prevalent on the value of centrifugal phylogenetic testing of host specificity and the minimal chance of an agent spreading to non-target hosts through mutation. There is also a lack of clarity on the procedures for introducing classical biological control agents, even among researchers, although the Plant Quarantine Order of India has empowered the Plant Protection Advisor to accord permission for importing biocontrol agents based on risk analysis. Other challenges include inefficient networking between different government departments which deal with invasive alien species management. Utilizing experience from elsewhere, India has enormous opportunities for weed management using biocontrol, including: a more suitable pathotype of *Puccinia spegazzinii* De Toni for *M. micrantha*, *Heteropsylla spinulosa* Muddiman, Hodkinson & Hollis for *M. diplotricha* and *Neohydronomus affinis* Hustache for *Pistia stratiotes* L. Developing an efficient framework will assist the advancement of weed management through biocontrol in India

*Oral presentation*

# Prospects of classical biological control of weeds in Pakistan: challenges and opportunities

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## Abstract

Alien invasive weeds are recognized as a global threat to natural and agro-ecosystems. The list of alien weeds of Pakistan is extensive, with many of these having become invasive posing a serious threat to agricultural production, biodiversity and human and animal health. Some of the worst affected environments are forest ecosystems, where woody invaders have transformed the native vegetation by altering native species composition and diversity. *Lantana camara, Prosopis juliflora, Broussonetia papyrifera, Eucalyptus* spp. and *Leucaena leucocephala* are considered the worst woody invaders while *Parthenium hysterophorus, Conyza bonariensis* and *Soliva anthemefolia* are some examples of problematic herbaceous invasive weeds. Aquatic weeds such as *Salvinia molesta, Pistia stratiotes, Alternanthera philoxeroides* and *Eichhornia crassipes* have established in many wetlands in the Sindh and Punjab provinces. Despite the number of invasive weeds in Pakistan, management has lagged behind, with the only classical biological control example being *Cactoblastus cactorum* released against *Opuntia* cacti in the 1990s. CABI has a long history and contribution in global efforts for the biological control of weeds in different parts of the world and now, with the help of key stakeholders, has recently started the "Action on Invasives" program which in Pakistan is focused on management of *P. hysterophorus* (parthenium weed) as a pilot project. This program includes awareness campaigns about the weed as well as the initiation of classical weed biological control projects against parthenium weed. Classical biological control offers a sustainable, long term control strategy against invasive weeds that needs to be employed, especially in developing countries such as Pakistan.

**Keywords:** Alien invasive weeds, spread, biological control, insects, management, developing country

## Introduction

Pakistan occupies a wide biogeographic region with a diversity of landscapes. The elevation ranges from sea level to 8,611 m (K-2, world's second highest peak). This natural elevation gradient, running from south to north, provides some of the most diverse environments, each with a unique flora and fauna. There are more than 6,000 species of vascular plants reported from Pakistan with a high degree of endemism, especially in the northern regions of the country. Many alien plant species are also reported from Pakistan (Shehzadi, 2018), of which at least 30 are considered invasive. Despite the high number of invasive species, there is a scarcity of scientific data available on economic losses caused by invasive alien species in Pakistan; however, they are expected to be extensive.

Invasive weed species are difficult to manage, mainly because they are occupying large geographic areas and because of a limited availability of resources for management, especially in developing countries. A lack of awareness about invasive species and their impacts among the general public as well as a non-existent policy on invasive species at a national level are important reasons for their introduction and subsequent spread. In Pakistan, there is some good basic research, mainly done by universities, on the ecology and management of some invasive species, but research on invasive species is not a priority for national and provincial research organizations. For this reason, CABI has initiated the "Action on Invasives" program funded through UK Aid (DFID) and the Directorate-General for International Cooperation (DGIS, Netherlands), as well as CABI's national and international partners. This program is coordinated by CABI but has joined with partners in Pakistan in a collaborative effort to control invasive species through an integrated pest management approach in which biological control will play an important role. The program will create a truly integrated and sustainable framework for tackling the problem of invasive species to generate growth, create jobs and help reduce poverty. The program's goal is to protect and restore agricultural and natural ecosystems, reduce crop losses, improve health, protect trade and reduce degradation of natural resources and protected areas. This will be done by supporting local, national and regional activities for prevention, early detection, and control of invasive species. The control and management of parthenium weed in Pakistan is the first pilot study to create awareness through large scale campaigns and build capacity through training courses.

## Invasive alien weeds in Pakistan

A large number of alien weeds are reported in Pakistan with some of these becoming invasive and threatening natural and agricultural ecosystems (Nasim and Shabbir, 2012; Shehzadi, 2018). Lantana (*Lantana camara* L.), mesquite (*Prosopis juliflora* [Sw.] DC.), paper mulberry (*Broussonetia papyrifera* [L.] L'Hér. ex Vent.), *Eucalyptus* spp., and leucaena (*Leucaena leucocephala* [Lam.] de Wit) are considered the worst woody invaders, while parthenium weed (*Parthenium hysterophorus* L.), fleabane (*Conyza bonariensis* [L.] Cronquist) and *Soliva* spp. are problematic herbaceous invaders (Table 1). Invasive vines or woody climbers, such as cat's claw creeper (*Dolichandra unguis-cati* [L.] L.G. Lohmann) and ivy gourd (*Coccinia grandis* [L.] Voigt.) are threatening forest trees in protected areas and parks. Finally, aquatic invasive weeds such as giant salvinia (*Salvinia molesta* D.S. Mitch), water lettuce (*Pistia stratiotes* L.), alligator weed (*Alternanthera philoxeroides* [Mart.] Griseb.) and common water hyacinth (*Eichhornia crassipes* [Mart.] Solms) are rapidly invading wetlands in Sindh and Punjab provinces (Table 1).

Among the herbaceous plant invaders, parthenium weed is considered one of the worst weeds in Pakistan, threatening diverse ecosystems. Parthenium weed is an annual herb of the Asteraceae family, originating from the tropical Americas. It has since become a weed of global significance with as many as 48 countries invaded around the world (Shabbir et al., 2018). Parthenium weed significantly reduces crop and pasture productivity, impacts native plant communities and biodiversity and negatively affects human and animal health (Adkins and Shabbir, 2014). In Pakistan, this weed was first reported from the Gujarat district of Punjab province in the 1980s (Razaq et al., 1994); since then it is rapidly spreading throughout the province of Punjab, the Islamabad Capital Territory (ICT) and parts of Khyber Pukhtunkhwa (KP) province (Shabbir et al., 2012).

The spread of this weed was initially limited to wastelands and along roadsides, but it is now infesting field and horticultural crops and native forests. If not controlled, parthenium weed is responsible for significant yield losses in maize and sorghum crops grown in Punjab, Pakistan (Asif et al., 2017; Safdar et al., 2015) and is responsible for transforming the native vegetation and community structure of some protected areas in the Punjab region (Mujahid, 2015). Human allergy problems due to parthenium weed are on the rise in Pakistan (Nadeem et al., 2005). Similarly, livestock feeding on the weed may develop severe allergenic reactions, their meat and milk become tainted (Tudor et al., 1982) and it could be fatal for young calves (Ahmed et al., 1988). In spite of the significant impacts of parthenium weed in Pakistan, the levels of awareness in the general public are low.

**Table 1.** Common invasive plant species of Pakistan.

| Category | Common name | Scientific name | Origin | Invaded habitats |
|---|---|---|---|---|
| Woody | lantana | *Lantana camara* L. | South America | Forests, protected areas and national parks in Punjab, Khyber Pakhtunkhwa (KP) Islamabad Capital territory (ICT) and Kashmir |
| | mesquite | *Prosopis juliflora* (Sw.) DC. | Mexico, South America and the Caribbean | Forests, protected areas and wastelands in Sind, Punjab and KP |
| | paper mulberry | *Broussonetia papyrifera* (L.) L'Hér. ex Vent. | South East Asia | Protected areas and urban forests in ICT, Punjab and KP |
| | eucalyptus | *Eucalyptus* spp. | Australia | Forest areas in KP |
| | leucaena | *Leucaena leucocephala* (Lam.) de Wit | Southern Mexico and Northern Central America | Protected areas and along roadsides and motorways |
| | farnese wattle | *Vachellia farnesiana* (L.) Wight & Arn. | Southern USA, Mexico, Central America | Forests and protected areas in Punjab |
| | cactus | *Opuntia* spp. | South America | Most of arid parts of Punjab and KP |
| Climbing | cat's claw creeper | *Dolichandra unguis-cati* (L.) L.G. Lohmann | Mexico, Central America and Tropical South America | Protected areas in Punjab and ICT |
| | ivy gourd | *Coccinia grandis* (L.) Voigt. | South East Asia | Forests and parks in Lahore |
| Herbaceous | Parthenium weed | *Parthenium hysterophorus* L. | Mexico, South America | Wastelands, roadsides, agricultural lands, protected areas in Punjab, KP, ICT and Kashmir |
| | fleabane | *Conyza bonariensis* (L.) Cronquist | South America | Wastelands, roadsides, urban areas |
| | Button burweed | *Soliva anthemfolia* (Juss.) Sweet | South America | Weeds of turf as well as recreational parks |
| | roundleaf bind-weed | *Evolvulus nummularius* (L.) L. | South America | Grasslands, turf areas in Punjab |
| Aquatic | giant salvinia | *Salvinia molesta* D.S. Mitch | Southeastern Brazil | Ponds, waterways in Punjab |
| | water lettuce | *Pistia stratiotes* L. | Southeastern North America | Waterways in Punjab |
| | alligator weed | *Alternanthera philoxeroides* (Mart.) Griseb. | Temperate regions of South America | Water bodies in Punjab |
| | common water hyacinth | *Eichhornia crassipes* (Mart.) Solms | South America | Water bodies in Punjab, Sind and KP |

## Management options

Several control strategies have been suggested to manage invasive weeds; however, each has limitations. Chemical control of invasive weeds under certain circumstances can be effective, but it is usually expensive and can only be used in specific situations. Mechanical control involving slashing or uprooting is labor intensive and may not be effective, creating massive regrowth and/or germination due to disturbance, and exposure to some weeds such as parthenium weed increases health concerns due to their allergenic nature (Adkins and Shabbir, 2014). Classical biological control, although usually slow, is an effective, safe and sustainable control strategy against many alien invasive weeds. For example, the biological control program against parthenium weed released nine insects and two pathogens (rusts) in Australia. Most of these agents are now well established in Australia and have a measurable impact on the growth and spread of parthenium weed (McFadyen, 1992).

There are several common problematic weeds across geographic regions for which some effective biological control agents are available at a fraction of the cost of a typical classical biological control initiative. Successful management of parthenium weed through biological control has been achieved in Australia and South Africa. This provides a great opportunity for developing countries like Pakistan to benefit from these successes.

## Biological control of weeds in Pakistan

Under the Pakistan Plant Quarantine Act of 1976 and Plant Quarantine Rules of 1967, importation of insects and other organisms are allowed for scientific and allied purposes. Once a request for an import permit is lodged, the Plant Protection Department may issue the import permit after a review process. Additional approvals may be required from the Ministry of Environment and Climate Change for the release of an agent. However, the whole procedure is sometimes lengthy.

Several biological control agents have been released against insect pests in Pakistan; however, only one deliberate introduction of a biological control agent has been made against an alien weed. The cactus moth (*Cactoblastis cactorum* [Berg.]) was released against *Opuntia* spp. in Rawalpindi and Chakwal districts of the Punjab province in 1994. Egg sticks of the agent were sourced from Australia, and a culture was established in the laboratory before larvae were released in the field. However, it is not certain if the agent has established after its release (Zimmermann et al., 2000).

Despite the lack of deliberate introductions in Pakistan, classical biological control agents such as *Zygogramma bicolorata* Pallister (a leaf-feeding beetle) have fortuitously arrived in Pakistan. It is believed that *Z. bicolorata* arrived from India where it was released as a classical biological control agent against parthenium weed in 1984 (Javaid and Shabbir, 2006). The beetle is very effective and can curtail parthenium weed populations in its introduced range by reducing its growth and seed production. Most of the central and northern parts of Pakistan are climatically suitable for *Z. bicolorata* (Dhileepan and Senaratne, 2009).

CABI, under the program “Action on Invasives,” is considering the release of *Listronotus setosipennis* (Hustache), a new biological control agent against parthenium weed in Pakistan. *Listronotus setosipennis* is a small nocturnal stem-boring weevil native to Argentina and Brazil that only feeds on parthenium weed. This agent was already released in Australia, South Africa, Uganda and Ethiopia. Adult weevils feed on leaves and flowers while newly emerged larvae tunnel directly from the peduncle into the stem to feed. Larval feeding can kill parthenium weed seedlings and rosettes.

A proposal to the Plant Sciences Division (PSD) of the Pakistan Agricultural Research Council for the import of *L. setosipennis* from South Africa was submitted on 24 August 2017. The PSD committee recommended the importation of the agent for small-scale testing on parthenium weed and other non-target plants. In light of the committee’s recommendations, a request for an import permit was lodged with the Plant Protection Department under the Plant Quarantine Act. The competent authority approved to issue an import permit subject to the condition that the agent will be kept in a quarantine facility. CABI is currently building such a facility at its regional office in Rawalpindi in order to commence work on the host-range testing of *L. setosipennis* in 2019.

## Conclusions

Many alien invasive plants are reported in Pakistan; however, the problem of alien invasive weeds is not well recognized due to lack of awareness in the general public and effective policies. Biological control is a safe and sustainable option for the management of invasive weeds in Pakistan. We believe once implemented successfully, CABI’s program “Action on Invasives” will pave the way and remove barriers for the commencement of new biological control programs against other problematic weeds in Pakistan.

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*Oral presentation*

# Introducing *Zygogramma bicolorata* and *Listronotus setosipennis* for biological control of *Parthenium hysterophorus* in Ethiopia

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The invasive annual weed parthenium (*Parthenium hysterophorus* L.) damages agriculture, adversely impacts biodiversity and is hazardous to human and animal health in Ethiopia. This invader has been successfully managed in Australia and India using selected host-specific natural enemies. Two natural enemies, a leaf-feeding beetle *Zygogramma bicolorata* Pallister and a stem-boring weevil *Listronotus setosipennis* (Hustache), were evaluated for biological control of the weed in Ethiopia. The specificity of *Z. bicolorata* and *L. setosipennis* were tested against 29 non-target plant species. The host range of *Z. bicolorata* and *L. setosipennis* was assessed using both no-choice and choice tests to examine their oviposition and feeding response on non-target plants. Both biocontrol agents were unable to complete development on any test plants offered in no-choice or choice tests. Based on these results, and in combination with host range data from Australia and South Africa, permission for the release of *Z. bicolorata* and *L. setosipennis* in Ethiopia was granted in 2013. *Zygogramma bicolorata* and *L. setosipennis* are reared in large numbers at three sites in Ethiopia and are being released at different locations throughout the country to assist in the management of this serious invader. The challenges faced in securing the permit to introduce the biocontrol agents to Ethiopia include lack of regulation governing importation, no established procedure to assess their host specificity and inadequate awareness about biological control.

*Oral presentation*

# Weed biocontrol in Vanuatu: progress to date and new activity

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Vanuatu is a tropical mountainous archipelago of volcanic origin, consisting of over 80 islands in the South Pacific. As a developing country, Vanuatu faces many challenges, including serious weeds, and is ranked number one in the world for natural hazard risks. Vanuatu has embraced biological control, releasing the first agent *Teleonemia scrupulosa* Stål for *Lantana camara* L. in 1935. Since then nine biological control agents have been introduced against eight weeds species, mostly with Australian assistance. Seven of the nine agents are known to have established and another six have spread into the country unintentionally. The impact of biological control has been variable. The most successful agent, *Calligrapha pantherina* Stål, provides complete control of *Sida acuta* Burm. f. and *S. rhombifolia* L. Control of two water weeds, *Eichhornia crassipes* (Mart.) Solms by *Neochetina bruchi* Hustache and *N. eichhorniae* Warner and *Pistia stratioties* L. by *Neohydonomus affinis* Hustache, has also been fairly good in most areas. Anecdotal evidence suggests the rust *Puccinia spegazzinii* De Toni, released in 2012, is having an impact on *Mikania micrantha* Kunth. More agents are needed to help manage other weeds, both existing and new targets. A new 5-year project, aimed at key pasture weeds affecting the beef industry, will begin in 2018 and is funded by New Zealand's Ministry for Foreign Affairs and Trade. This project will seek agents for three novel targets: *Solanum torvum* Sw., *Urena lobata* L. and *Senna tora* (L.) Roxb. Known agents available in Australia will also be released against *L. camara*, *Parthenium hysterophorus* L., and possibly *Mimosa diplotricha* C. Wright. Agents will also be released against two environmental weeds: *Dolichandra unguis-cati* (L.) L.G. Lohmann and *Spathodea campanulata* Beauv. Lessons learned from previous projects in Vanuatu and other Pacific countries are being incorporated into this project, such as the need for substantial capacity building and planning for tropical cyclones. It is hoped that ultimately this project will not only benefit Vanuatu, but the wider Pacific region.

*Oral presentation*

# Sharing the success of cactus biological control across borders

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In most cases, biological control is the most effective method of managing invasive alien cactus species. The Cactaceae are almost exclusively indigenous to the Americas, so agents used in one country are likely to be suitably specific to release in other countries outside of the native distribution of the family. The Nagoya Protocol encourages the sharing of benefits derived from indigenous biodiversity, including the country where agents were sourced, the country developing the agents and other countries that could benefit. There is a long history of collaboration in cactus biological control between South Africa and Australia, with both countries benefiting from agents developed by the other. There is scope to grow this collaboration and encourage biological control of common invasive plant species to other countries. The thistle cactus, *Cylindropuntia pallida* (Rose) F.M. Knuth, is problematic in both South Africa and Namibia and has recently been targeted in Australia using a new lineage of the cochineal *Dactylopius tomentosus* (Lamark) "californica var. parkeri." Similarly, the creeping cactus *Pereskia aculeata* Mill. is problematic in Australia and a new agent, the stem-wilter *Catorhintha schaffneri* Brailovsky & Garcia, has recently been released in South Africa. There are also many cacti in Namibia that could be controlled from effective and freely available agents already introduced into South Africa. An application for the release of three cactus agents (*Dactylopius opuntiae* [Cockerell] "stricta" for *Opuntia stricta* [Haw.] Haw.; *Dactylopius tomentosus* [Lamark] "imbricata" for *Cylindropuntia imbricata* [Haw.] F.M. Knuth; and *Hypogeococcus* sp. for *Harrisia* spp.) has been submitted to the relevant authorities in Namibia. There are significant benefits to sharing successful agents for cactus weeds: expensive pre-release research is not repeated, experience and expertise are shared, and countries that have been difficult to work in may become more open to prospecting for new potential agents.

*Poster presentation*

# Embarking on classical biological weed control in Brazil: the rust fungus *Maravalia cryptostegiae* versus *Cryptostegia madagascariensis*

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The woody vine *Cryptostegia madagascariensis* Bojer ex Decne (common name Madagascar rubber vine) is an invasive alien species in the semi-arid northeast region of Brazil, where it is known locally as devil's claw or, in Portuguese, "unha-do-diabo." The tropical genus *Cryptostegia* belongs to the Apocynaceae plant family and is endemic to western Madagascar. It accommodates only two recognized species, *C. madagascariensis* and its sister species *Cryptostegia grandiflora* (Roxb.) R. Br. (common name rubber vine) (Klackenberg, 2001). Within its native range, *C. madagascariensis* has a more northerly distribution, while *C. grandiflora* is common in the south of the island. Hybridization of the two species is known to occur where their ranges overlap and they grow sympatrically (Marohasy and Forster, 1991; Klackenberg, 2001). Both *Cryptostegia* species are now widely distributed in the tropics following their introduction for ornamental purposes, as well as for their potential as an alternative source of rubber, especially during World War II (Tomley, 1995).

The initial introduction of *C. madagascariensis* as an ornamental into Brazil is posited to have been made at the Botanical Gardens, Rio de Janeiro, via the USA (H.C. Evans and R. W. Barreto, pers. obs.). From there, the vine was taken to northeastern Brazil where it has become highly invasive and a threat to the unique Caatinga ecosystem (da Silva et al., 2008). Caatinga, the "white forest" (from the native Tupi language with "ka'a" meaning forest and "tinga" meaning white), is an extensive dry forest region covering an estimated 11% of Brazil and spanning several federal states. With its high percentage of endemism, the biodiversity of the Caatinga, home of iconic species such as the carnaúba palm (*Copernicia prunifera* [Mill.] H.E. Moore) and the three-banded armadillo (*Tolypeutes tricinctus* [Linnaeus]), is of high conservation significance (Leal et al., 2005). Devil's claw infestations smother vast areas of pristine riverine habitats in the Caatinga, as well as deplete the scarce water resources (Bonilla and Major, 2006). Besides its major environmental impact, *C. magadascariensis* also poses an economic threat in the region as it overgrows and destroys natural stands of the carnaúba palm, which is the sole source of the carnaúba wax (da Silva et al., 2008). This "queen of waxes," extracted from carnaúba leaves, is used for a wide range of industrial applications and products and supports rural livelihoods and an important local industry worth up to USD 120 million annually (source: Brazilian Ministry of Industry, Foreign Trade and Services http://www.mdic.gov.br/index.php, 2014). To date, the invasion of *C. madagascariensis* in the Caatinga is still at an early stage; however, the vine is extremely difficult and hazardous to control using conventional methods (particularly owing to the production of abundant toxic latex) and, based on climatic matching models, it has the potential to spread over a much wider area in the near future (R. Bourchier, pers. comm.).

Brazil has as yet to take advantage of classical biological control (CBC) as a strategy to manage invasive alien weeds. In order to select potential target weeds for this approach, a prioritization tool—which takes into account the weed's suitability for biocontrol, its impact in the affected region(s) and the ease of implementing a control program as well as its likely success (Paynter et al., 2009)—was used to assess more than 100 non-native invasive weeds in Brazil (Barreto et al., 2014). Out of these, *C. madagascariensis* was identified as the top target given the severity of its current and predicted impact, the fact that there is no conflict of interest associated with its biocontrol, and the opportunity to benefit from an "off-the-shelf" biocontrol agent previously used to successfully control its sister species *C. grandiflora* in Queensland, Australia (Tomley and Evans, 2004). The highly damaging rust pathogen, *Maravalia cryptostegiae* (Cummins) Ono, closely associated with both *Cryptostegia* species in Madagascar, had been identified as a potential biocontrol agent for invasive *C. grandiflora* in northern Queensland. Following a thorough assessment of its host specificity and safety, the rust was imported into Australia in 1994, and the biocontrol program against rubbervine has since been ranked among the most successful of all Australian weed biocontrol projects with a cost-benefit ratio of 1:109 and a net benefit of AUD 230 million in 2005 (Page and Lacey, 2006).

Following prolonged efforts in project development, lobbying and liaison with key stakeholders, the first classical weed biocontrol project targeting the invasion of *C. madagascariensis* commenced in Brazil at the beginning of 2018. Funding for the program has been secured from the Government of Ceará through the Development Agency of Ceará (ADECE) and the Ceará Sindicate of Carnaúba Wax Refiners (SINDCARNAUBA), and its implementation has also been made possible through a vital grant from SC Johnson in the USA. The four-year collaborative project will be undertaken by scientists from four Brazilian Universities, the Universidade Federal de Viçosa, the Universidade Estadual do Ceará (UECE), the Universidade Federal do Ceará (UFC) and the Universidade Estadual de Feira de Santana (UEFS), as well as from CABI and also members of the Associação Caatinga's team. The research to be conducted will establish ecological baseline data of the weed invasion by setting up monitoring stations at selected sites in the State of Ceará, and the current impact of devil's claw on local livelihoods will be assessed using a socio-economic survey. Finally, piggy-backing on the success of the Australian biocontrol program, the suitability of the rust *M. cryptostegiae* as a CBC agent for devil's claw will be evaluated.

To date, a bespoke monitoring protocol has been devised for the invasive vine to enable the start of the field work at three established monitoring stations in Ceará. In parallel, the socio-economic questionnaire is being fine-tuned for the planned survey which will target land-owners and collectors involved in the Carnaúba wax production. Concerning the assessment of the *Maravalia* rust, a list of selected non-target species within the Apocynaceae and related families, as well as other safeguarding species with economic and ecological relevance to Brazil, including carnauba, cashew (*Anacardium occidentale* L.) and oiticica (*Licania rigida* Benth.), has been compiled for its host-range testing in the UK. Several of these test species have already been sourced and established in the CABI quarantine greenhouse. In order to re-collect the pathogen, a field survey was conducted in western Madagascar in May 2018, in collaboration with scientists from the University of Antananarivo. A total of 18 distinct rust strains were collected from *C. madagascariensis*, and its putative hybrids with *C. grandiflora*, and hand-carried to the UK following the issue of export permits. Using inoculation studies, mass spectrometry and molecular techniques, these strains are currently being assessed at CABI in order to identify the one best matched to the invasive weed biotype in Brazil and to the local climatic conditions. The selected *M. cryptostegiae* strain will then undergo extensive host-specificity testing under quarantine conditions against the established test plant list.

Conditional to the evaluated pathogen strain being considered suitable and safe for use as a CBC agent for devil's claw in northeastern Brazil, any future importation of *M. cryptostegiae* will need to be approved by the quarantine authorities of the Brazilian Ministry of Agriculture (MAPA) and brought into quarantine through the Laboratório de Quarentena Costa Lima (EMBRAPA Meio Ambiente), which has been involved since the start of the initiative. If successful, this pioneering project could pave the way for future management of invasive alien weeds in Brazil using the CBC approach.

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*Poster presentation*

# A weed biocontrol program for the Cook Islands: progress report

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## Abstract

Much of the Cook Islands' natural habitats and agricultural land are threatened by invasive weeds. A five-year weed biocontrol program for the Cook Islands started in November 2013. We present information on the establishment, impact and field specificity of agents released against six weed species, including "novel" programs for red passionfruit, *Passiflora rubra*, and African tulip tree, *Spathodea campanulata*.

Overall, the program has been highly successful and looks likely to deliver important benefits to the Cook Islands and the wider Pacific as novel agents released during this program are now available for redistribution to other countries.

**Keywords:** Rarotonga, Pacific, Invasive species

## Introduction

Invasive plants threaten biodiversity and agricultural production in the Cook Islands, where 287 native seed plant and fern species are outnumbered by 333 naturalized alien species (Paynter and Dodd, 2012). Draft IUCN Red List assessments were recently made for 19 plant species that are endemic to Rarotonga (the largest of the Cook Islands); one species was assessed as "Extinct," nine species "Critically Endangered," one species "Vulnerable," three species "Data Deficient," and five species "Least Concern" (Martin et al., 2012).

Key habitats for endemic flora are chronically threatened by weed invasion, for example, *Cyrtandra rarotongensis* Cheeseman relies on lowland habitats that are being severely modified by invasive plants and has a high chance of extinction in the next 20 years unless conservation management is implemented soon (Martin et al., 2012). Martin et al.

(2012) also noted that several invasive weed species (e.g., *Mikania micrantha* Kunth) are establishing on the remote mountain peaks that have acted as refuges for other critically threatened endemics (e.g., *Sclerotheca viridiflora* Cheeseman).

Agriculture and fisheries only account for 3% of the Cook Islands GDP (Anon, 2018) and there is little published information on the impacts of weeds on agricultural production. However, a priority for the Cook Islands agricultural sector is to increase the local production of livestock (pigs, poultry, goats), fruits, vegetables and root crops to cater for the increased demand on the local market brought about partly by the expanded tourism industry. Improved management of key agricultural weeds should assist this goal (Paynter and Dodd, 2012).

A scoping study (Paynter and Dodd, 2012) used Paynter et al.'s (2009) scoring system, modified by using Paynter et al.'s (2012) predictions of biocontrol impact, to rank weed biocontrol targets in the Cook Islands. Final rankings were adjusted according to local expert advice. For example, *Eichhornia crassipes* (Mart.) Solms was rejected as a biocontrol target as it is valued as a nitrogen-fixing plant and source of green manure in Rarotonga, which lacks major water bodies typically associated with problems caused by *E. crassipes* invasion in other countries.

A five-year weed biocontrol program, based on the scoping study recommendations, started in November 2013. Seven weed species were initially targeted (giant reed *Arundo donax* L.; grand balloon vine *Cardiospermum grandiflorum* Sw.; mile-a-minute vine *Mikania micrantha*; red passionfruit *Passiflora rubra* L.; strawberry guava *Psidium cattleianum* Sabine; African tulip tree *Spathodea campanulata* P. Beauv., and cocklebur *Xanthium strumarium* L. sp. agg). Work on *A. donax* was subsequently terminated when surveys indicated that its abundance had been greatly overestimated due to confusion with elephant grass *Pennisetum purpureum* Schumach. and it was decided that biocontrol was unnecessary.

A genetic study was also conducted to investigate the origin of *Decalobanthus peltatus* (L.) A.R.Simões & Staples, comb. nov. ≡ *Merremia peltata* (L.) Merr. in the Cook Islands. *Decalobanthus peltatus* is distributed from the Indian Ocean Islands, throughout Malesia, eastwards into Polynesia to the Society Islands. Dovey et al. (2004) listed *D. peltatus* as an important weed affecting Pacific island countries and territories for which the potential of biological control should be explored. However, *D. peltatus* was present on many Pacific islands when the first European botanists documented the regional flora (including Rarotonga; Cheeseman, 1903), and there is controversy regarding whether it was an early Polynesian introduction to the region or if the *D. peltatus* invasion is a symptom of environmental degradation. If it is native to the region, biological control may be an inappropriate management option (Paynter et al., 2006). This study is ongoing, and results are not reported here.

## Materials and Methods

Environmental impact assessments were prepared in accordance with the Cook Islands Environment Act 2003 and the Cook Islands Biosecurity Act 2008, allowing public comment prior to a decision by the Cook Islands National Environment Service on whether to approve proposed biocontrol agent introductions.

Host-range testing performed by Landcare Research in New Zealand and Rhodes University, South Africa is outlined below.

### Grand balloon vine *Cardiospermum grandiflorum*

Two species, previously investigated as candidate biocontrol agents for *C. grandiflorum* in South Africa, were considered for release in Rarotonga: the rust fungus *Puccinia arechavaletae* Speg. and the seed weevil *Cissoanthonomus tuberculipennis* Hustache. *Cissoanthonomus tuberculipennis* was demonstrated to only feed and develop on *C. grandiflorum* (Simelane et al., 2014) and *P. arechavaletae* isolates obtained from *C. grandiflorum* plants in South America were also highly host-specific (Mc Kay et al., 2010) and only damaged *Cardiospermum* species (Dr Alan Wood, ARC-PPRI, pers. comm.). Nevertheless, given there are records of *P. arechavaletae* attacking other genera within the Sapindaceae (Farr and Rossman, 2015), a decision was made to test all three native Cook Islands members of the Sapindaceae as possible hosts for the rust.

***Host-range testing of Puccinia arechavaletae***

*Cardiospermum grandiflorum* leaves infected with mature *P. arechavaletae* pustules were shipped from Paraguay in January 2017 and reared in containment in Auckland to provide material for host-range testing. Twelve *C. grandiflorum* leaf sections with *P. arechavaletae* pustules were placed onto water agar plates and suspended above young healthy test plants: *Sapindus saponaria* L. (six plants); *Dodonaea viscosa* Jaquin (only two plants, but this taxon was also tested in South Africa), *Allophylus timoriensis* (DC.) Blume (only one plant survived shipping to Auckland) and five *C. grandiflorum* controls in a closed Perspex box at 24°C with a humidifier. After 24 h the plants were removed from the box, assessed for the presence of pustules after 21 days and subsequently monitored weekly for any additional symptoms. Lack of replication for *A. timoriensis* was tolerable because two other *Allophylus* spp. tested in South Africa were not hosts.

### Mile-a-minute vine *Mikania micrantha*

The rust fungus *Puccinia spegazzinii* De Toni was imported from Vanuatu, where it was introduced to control *M. micrantha* in 2012 (Day and Bule, 2016). Past testing (Ellison et al., 2008) indicated that *P. spegazzinii* is specific to the genus *Mikania* and no further specificity testing was required.

### Red passionfruit *Passiflora rubra*

No native Cook Islands plant species belong to the Passifloraceae. *Passiflora* species of the subgenus *Passiflora* are, however, cultivated for their edible fruits. *Heliconius* spp. butterfly larval host plants are restricted to the Passifloraceae (Benson et al., 1975). *Heliconius erato cyrbia* Godart (Figure 1a,b) was prioritized for testing because host records are confined to *Passiflora* subgenus *Decaloba*, including *P. rubra* (Jiggins et al., 1996).

***Host-range testing***

**Larval starvation tests.** *Heliconius erato cyrbia* pupae were obtained from a commercial supplier (Heliconius Butterfly Works, Quito, Ecuador).

*Heliconius erato cyrbia* eggs were collected from *P. rubra* foliage in a 4 $m^3$ breeding cage (1.2 m wide x 2.4 m long x 1.4 m tall) containing adult butterflies. Neonate larvae were transferred to stem tips cut from mature test plants and placed in plastic containers (one larva/stem tip per container). Plant material was replaced with fresh foliage, as required. Three of four *Passiflora* species cultivated in the Cook Islands were obtained for host-range testing: 30 replicates of the most important test plant *Passiflora edulis* Sims were performed, and 10 each of *P. ligularis* A. Juss and *P. quadrangularis* L., with corresponding (50) *P. rubra* controls. *Passiflora laurifolia* L. could not be sourced but is of minor importance in the Cook Islands.

**Choice oviposition tests.** Two vases, each containing one 50 cm (approximately) long stem tip of *P. edulis* and one of *P. rubra*, were placed in the 4 $m^3$ breeding cage containing 12 *H. erato cyrbia* adults. Potted lantana (*Lantana camara* L.) plants and vases of cut flowers provided shade, perching sites, nectar and pollen.

After 24 h the stems were removed, any eggs present were counted, and fresh stems were placed in the vases. Tests were conducted over 12 days, so that 24 stems of both *P. rubra* and *P. edulis* were exposed to ovipositing *H. erato cyrbia*.

### Strawberry guava *Psidium cattelianum*

The scale insect *Tectococcus ovatus* Hempel was imported from Hawaii, where it was introduced to control *P. cattleianum* in 2011 (Johnson, 2016). *Tectococcus ovatus* is highly host-specific (e.g., Wessels et al., 2007) and further host-range testing was unnecessary.

### African tulip tree *Spathodea campanulata*

Two candidate agents, an eriophyid mite *Colomerus spathodeae* (Carmona) and a flea beetle *Paradibolia coerulea* Bryant (Figure 1c,d), were imported into South Africa from Ghana in March 2014 following genetic matching to identify where to source agents for invasive *S. campanulata* populations in the Pacific (Sutton et al., 2017b). Host-range testing of *C. spathodeae* was reported by Paterson et al. (2017). Testing of *P. coerulea* is almost complete and, thus far, indicates it should be suitably specific for release in Rarotonga (see Sutton et al., [2017a] for preliminary results).

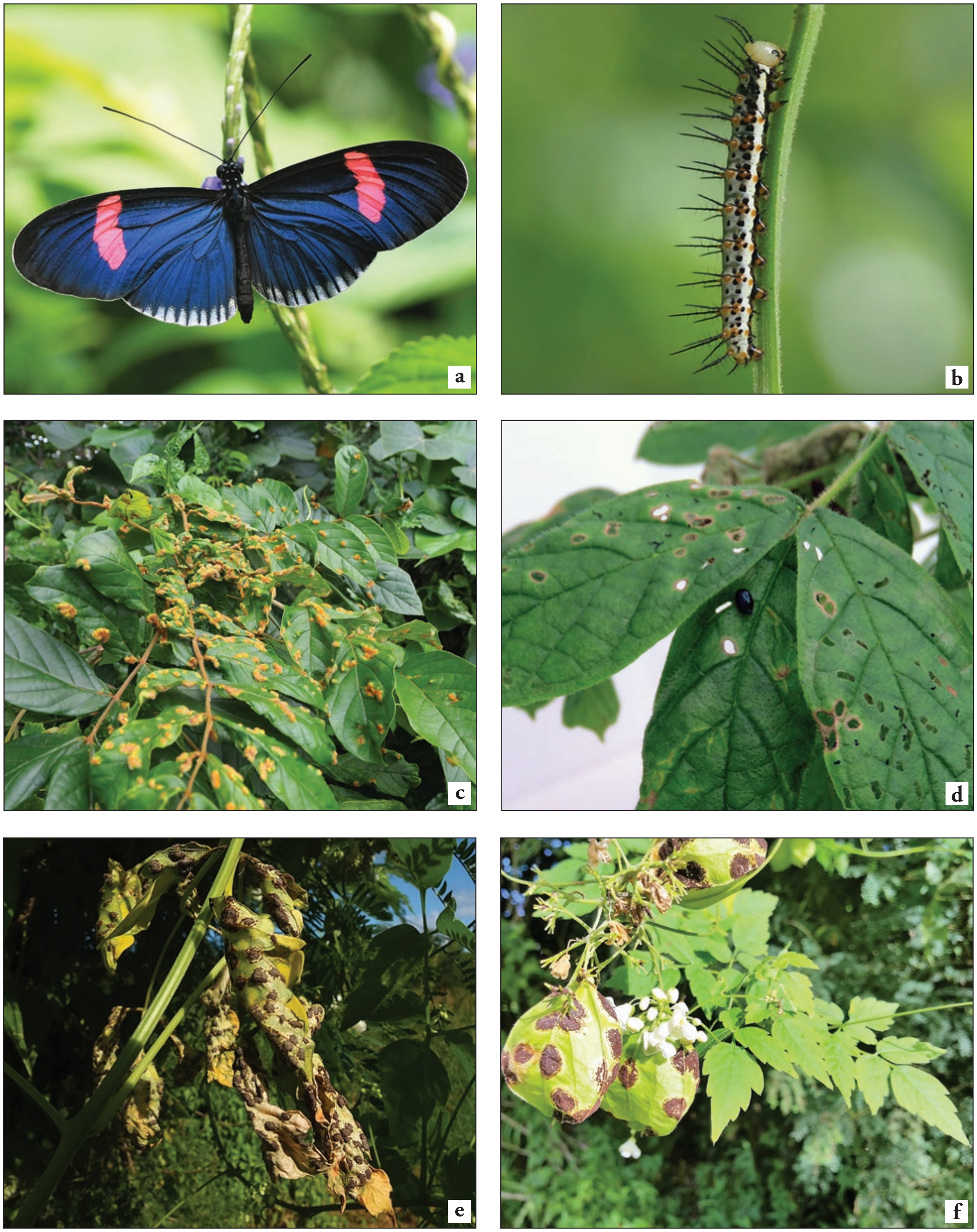


**Figure 1.** Novel biocontrol agents developed or released for the first time during the project: (a) *Heliconius erato cyrbia* adult; (b) *Heliconius erato cyrbia* larva; (c) *Colomerus spathodeae*; (d) *Paradibolia coerulea* adult; (e) *Puccinia arechavaletae* pustules on balloon vine foliage; (f) *Puccinia arechavaletae* pustules on balloon vine pods.

**Cocklebur *Xanthium strumarium***

A North American rust fungus *Puccinia xanthii* Schwein., which is responsible for complete control of *X. strumarium* across most of eastern Australia (Van Klinken and Morin, 2012), was prioritized for introduction to the Cook Islands and sourced from Queensland, Australia.

***Host-range testing***

*Puccinia xanthii* only attacks plants within the Asteraceae tribe Heliantheae (Seier et al., 2009). The only native representative of this tribe in the Cook Islands is *Melanthera biflora* (L.) Wild, which was not included in past host-range testing. Specificity tests were conducted within a mist chamber to produce optimal inoculation conditions (25°C; 24 h dew point; Morin et al., 1993) using the "leaf disc method" described by Morin et al. (1993) where discs bearing mature telia were cut from infected plants and placed onto the surface of 2% water agar in Petri dishes (telia uppermost). Plates containing the telial discs were inverted (after the lid was removed) and attached to the top of a mist chamber to allow spores to drop onto the test and control plants within the mist chamber. Two tests were performed in November 2014; the first consisted of three *M. biflora* plants and three *X. strumarium* control plants and the second used two *M. biflora* plants and four *X. strumarium* controls. After 24 h the plants were removed from the box, assessed for the presence of pustules after 21 days and subsequently monitored weekly for any additional symptoms.

## Results

**Grand balloon vine *Cardiospermum grandiflorum***
***Host-range testing***

Testing confirmed *P. arechavaletae* will not harm native Cook Island Sapindaceae. All *C. grandiflorum* controls became heavily infected, the *A. timoriensis* plant displayed no symptoms, three *S. saponaria* plants displayed no symptoms and three displayed minor chlorotic spots on the leaves with no pustule formation. Small, barely visible spots formed on *D. viscosa* leaves, but did not develop, consistent with observations in South Africa.

***Release and establishment***

*Puccinia arechavaletae* was released in Rarotonga in December 2017 and was widespread by March 2018 (Figure 1e,f). Analysis of 20 photo points established in December 2017 indicated that by June 2018, mean *C. grandiflorum* cover had declined from 54% to 5.4% (Chi-Squared: 12.80, d.f. = 1, $P < 0.001$) and mean competing vegetation cover had increased (from 35% to 73%; Chi-Squared: 12.80, d.f. = 1, $P < 0.001$). Native and exotic species benefited from *C. grandiflorum* control although the greatest increase was recorded for *M. micrantha* (from 18% to 34%). Notably, *P. rubra* did not increase following *C. grandiflorum* control.

Due to the success of *P. arechavaletae*, it was decided that release of *C. tuberculipennis* was unnecessary.

**Mile-a-minute vine *Mikania micrantha***
***Release and establishment***

*Puccinia spegazzinii* established in the Avarua District of Rarotonga, following release in January 2017. By June 2018, it was present up to 3 km from where establishment was first detected, and many *M. micrantha* plants were heavily infected. By November 2018, *P. spegazzinii* was widespread throughout Rarotonga, and *M. micrantha* had declined markedly in some inland areas.

**Red passionfruit *Passiflora rubra***
***Host-range testing***

All *H. erato cyrbia* larvae confined on *P. edulis, P. ligularis* or *P. quadrangularis* died within a few days. Most (76%) larvae that fed on *P. rubra* successfully pupated (Kruskal-Wallis one-way analysis of variance, H = 60.68, d.f. = 3; $P < 0.001$). In choice oviposition tests, no eggs were laid on *P. edulis* stems while 21 of the 24 *P. rubra* stems were oviposited on (average no. eggs/stem = 0 and 1.83 on *P. edulis* and *P. rubra*, respectively; Friedman chi-squared = 12, d.f. = 1, $P < 0.001$).

***Release and establishment***

*Heliconius erato cyrbia* was released in Rarotonga in August 2016 and rapidly became abundant. Detailed impact monitoring has not been done (but see *C. grandiflorum*, above). In Rarotonga, *P. rubra* commonly coexists with more dominant *M.*

*micrantha* and *C. grandiflorum*. Impacts should be easier to measure if *H. erato cyrbia* is redistributed to other islands, such as Mangaia and Ātiu, where *M. micrantha* and/or *C. grandiflorum* are absent and *P. rubra* is often the dominant invasive vine.

Non-target attack on *P. edulis* reported in April 2018 was investigated in June 2018. Field surveys indicated that approximately 1.5% of *P. edulis* stem tips had *H. erato cyrbia* eggs or larvae present (in contrast to approximately 60% of *P. rubra* stem tips). Only small larvae were found on *P. edulis*, and feeding damage was trivial.

**Strawberry guava *Psidium cattleianum***
***Release and establishment***

*Tectococcus ovatus* was first released in November 2016 and has tenuously established. Initially, few plants growing in full sun were inoculated and it was found that *T. ovatus* performs poorly on shaded seedlings in the forest understory (which are easiest to access for releases).

***African tulip tree Spathodea campanulata***
***Release and establishment***

*Colomerus spathodeae* was released in January 2017 and had dispersed throughout Rarotonga by November 2018. A petition to release *P. coerulea* will be submitted after the completion of host-range testing.

***Cockleburr Xanthium strumarium***
***Host-range testing***

All inoculated *X. strumarium* control plants were infected with *P. xanthii*, while *M. biflora* plants were completely resistant, displaying no symptoms of infection.

***Release and establishment***

*Puccinia xanthii* established following releases in September and October 2016. Periodic epidemics have subsequently severely damaged *X. strumarium* plants. Detailed impact monitoring has not been done, but a farmer in the Matavera District of Rarotonga reported that problems caused by *X. strumarium* have declined since the establishment of *P. xanthii*.

## Discussion

Several agents are having major impacts against their target weeds within a short period of time. The impact of *P. xanthii* on *X. strumarium* appears similar to that reported in eastern Australia by Van Klinken and Morin (2012), and *P. arechavaletae* has been spectacularly devastating to *C. grandiflorum*. Moreover, although *M. micrantha* cover increased following *C. grandiflorum* control, the establishment of *P. spegazzinii* has ensured that this should be only temporary as *M. micrantha* comes under control. Furthermore, *H. erato cyrbia* was already widespread and abundant when *P. arechavaletae* was released and *P. rubra* did not increase following the reduction in *C. grandiflorum*, validating the decision to target multiple vine species.

*Colomerus spathodeae* also dispersed and proliferated rapidly, although it is too early to assess its impact on *S. campanulata*. Establishment of a second agent, *P. coerulea*, is considered desirable to increase the likelihood of successful control of *S. campanulata*.

Progress against *P. cattleianum* has been slower, and further redistribution of *T. ovatus* onto plants growing in full sun is required.

Non-target attack has not been detected for any of the agents released except *H. erato cyrbia*, which was found to rarely oviposit on *P. edulis*. We consider the attack to be minor spillover, likely resulting from the high density of butterflies and a lack of suitable host plants on which to oviposit. The trivial larval feeding damage is consistent with host-range testing that indicated the growing tips of mature *P. edulis* plants are toxic to *H. erato cyrbia* larvae.

Overall, the program has been highly successful and looks likely to deliver important benefits to the Cook Islands and the wider Pacific as novel agents released during this program are now available for redistribution to other countries.

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*Poster presentation*

# Preliminary study on the intrinsic mechanism of appressorium formation in *Exserohilum monoceras*

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*Exserohilum monoceras* (Drechsler) K.J.Leonard & Suggs is a phytopathogenic fungus on barnyardgrass (*Echinochloa crus-galli* [L.] P. Beauv.), which can control the weed while being safe to rice. Our study, comparing appressorium formation of *E. monoceras* on different hydrophobic surfaces, showed that leaf outermost hydrophobicity of host plants can be recognized as surface signals by *E. monoceras*. In addition, we showed that epicuticular wax of non-host plant leaf surface had an adverse effect on appressorium formation of *E. monoceras*. The components of the surface wax in the leaves of barnyardgrass and rice are roughly similar, mainly linear long-chain aliphatic compounds and cyclic terpenes, but the relative content of each component is different. The percentage of alkanes of barnyardgrass epicuticular wax was (with 30.0%) significantly higher than the one of rice (with 20.0%). Aldehydes of rice wax were (with 22.7%) significantly higher than in barnyardgrass wax (with 15.2%). In general, these results showed that the epicuticular wax of barnyardgrass significantly enhanced the appressorium formation, while the epicuticular wax of rice inhibited the appressorium formation of *E. monoceras* conidia. There was a significant difference in the relative contents of alkanes and aldehydes between the compounds of barnyardgrass and rice epicuticular wax.

*Poster presentation*

# Water turbidity affects the establishment of *Neochetina eichhorniae*: implications for biological control of water hyacinth

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Water hyacinth is an invasive aquatic macrophyte associated with major negative economic and ecological impacts in the tropics and subtropics. It has been the target of eight biological control agents in South Africa including *Neochetina eichhorniae* Warner. However, these weevils have failed to control water hyacinth invasions in Rwandan water bodies where water is often turbid. The aim of this study was to investigate the influence of water turbidity on the performance and establishment of *N. eichhorniae*. Water hyacinth plants were maintained in clear water and at three different turbidity levels: high (2,000 NTU), medium (850 NTU) and low (160 NTU). Three larvae were inoculated into each plant. Plant growth parameters were measured weekly for three months, while adult emergence was observed and recorded from the 56$^{th}$ day of the experiment. 70% of adult weevils emerged from the low turbidity treatment compared to the medium treatment with 40%, while no adult weevils emerged from the plants grown at high turbidity levels. Weevil larvae pupate under water on the roots of water hyacinth and may be affected by the low dissolved oxygen recorded in the water at high and medium turbidity levels. In contrast, the plant weight, number of leaves, leaf length and number of ramets were significantly enhanced by increased water turbidity.

*Poster presentation*

# Current and future work to control the spread of invasive *Tamarix* in South Africa

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At least five species of *Tamarix* trees have become invasive in the Americas and Australia, and two species are invasive in South Africa (SA), where biocontrol has only recently been considered as a control option. The successful biocontrol program against invasive *Tamarix* in the USA, using several spp. of *Diorhabda* beetles, is being used as a guide in SA. Finding suitable biocontrol agents for invasive *Tamarix* in SA is complicated by the presence of the indigenous *T. usneoides* E.Mey. ex Bunge which, together with the invasive species, is being used for phytoremediation of mine tailings dams. A further complication is that the various *Tamarix* spp. have hybridized, which may be promoting invasiveness. As part of a pre-release study, we are investigating various aspects of the *Tamarix* invasion in SA, as well as conducting host-specificity tests. Thus far we have confirmed that Hyperspectral Remote Sensing (HRS) can be used to differentiate our *Tamarix* spp., and currently we are using HRS to map the extent of their distributions. Plant physiology studies have been used to compare flood tolerance between these species, which surprisingly revealed that the invasive *Tamarix* and their hybrids are less tolerant to inundation than the indigenous species. Host specificity tests with *D. carinulata* (Desbrochers) have shown that the beetle feeds and reproduces successfully on the indigenous *T. usneoides*, and is thus not safe for release in South Africa. Therefore, new agents are being sought through collaboration with Italian, American and Kazakhstani researchers.

*Poster presentation*

# Host specificity of the stem-boring weevil, *Listronotus setosipennis* (Hustache)

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The annual weed, parthenium (*Parthenium hysterophorus* L.) causes crop yield loss, invades pasture lands and is a threat to biodiversity in Ethiopia and other eastern African countries. The stem-boring weevil *Listronotus setosipennis* (Hustache) is one of the biocontrol agents released in Australia and South Africa to manage parthenium. Host-range evaluation of *L. setosipennis* was conducted in a quarantine facility on 39 non-target plant species, mostly belonging to the Asteraceae family. Nineteen major crops mostly used as food and export were also included in the host-range evaluation using no choice tests. No feeding or eggs were recorded on any of the non-target plant species, while an average of 39.0 ± 3.4 eggs was laid on parthenium. Based on these results, as well as host range data from Australia and South Africa, permission for the release *L. setosipennis* in Ethiopia was granted. Presently, *L. setosipennis* is being reared and released in Ethiopia.

# Session 3: Bioherbicides

## Session Chair: Graeme Bourdôt

**Keynote**

# Navigating the bioherbicide success pathway

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Bioherbicides are a desirable weed management solution that could reduce the ecological footprint of synthetic herbicides. Yet, navigating the pathway to produce bioherbicides for widespread use has been limited with the business model template where success is defined as using an organism with herbicidal properties that is registered to a company to be manufactured as a weed control product for delivery and sale to consumers (Ash, 2010).

Under this model, there are five forces that may impact success, either positively or negatively: science, industry, regulation, consumers and markets. The past has shown that some bioherbicides are registered but are never sold, whereas others get sold but later fall to one or more of the forces described (Table 1). Presently, only three registered bioherbicides are for sale in the marketplace and two are under active development by companies. This paper examines the role of the five forces on the development of *Phoma macrostoma* Mont. and provides insights on future directions to achieve bioherbicide success.

**Science:** The science development of *P. macrostoma* considered the interests of industry, regulators and consumers to build a story that answered "How, What, When, Where, and Why" and described the overall vision. Studies showed that when introduced to soil, this fungus differentially affected plant species using biological and chemical modes of action (Bailey et al., 2011a; Bailey et al., 2011b; Hubbard et al., 2016). Genetic profiling distinguished unique biotypes of *P. macrostoma* from *Cirsium arvense* (L.) Scop. that had bioherbicide activity (Pitt et al., 2012). Defining all possible use patterns (i.e. commercial and domestic consumers in agriculture, horticulture, turfgrass and agro-forestry for broadleaved weed control) showed market opportunities attracting industry and consumer support. Fungal biology showed no sexual reproductive stage, minimal dispersion, weakly competitive behavior, and macrocidin formation only in mycelium which limited spread and survival in the environment. A growth process based on the mushroom spawn industry produced a granular formulation that was applied to target weeds in selective cropping situations as pre- and post-emergent or spot treatment applications. Field trials confirmed >80% weed reduction lasting over the growing season (Hynes, 2018). Knowing that industry demands exclusive licensing for investment, patents were filed.

**Industry:** Once industry was involved, the science was directed to studies supporting a regulatory submission (Bailey and Falk, 2011). Other business priorities focused on marketing and economic projections. Efficacy data were collected over seven years in 129 efficacy and 69 phytotoxicity trials to determine weed reduction and consistency of response. Weed reductions required environmental temperatures between 15–30°C and moist soil at application. Additional post-application moisture was not required. Comparisons to herbicide standards showed similar efficacy although slower acting.

The manufacturer adapted the growth process to increase scale while maintaining product stability (shelf life, storage conditions at room and high temperatures, packaging) and assessing costs. Despite achieving a base production price of USD 2.50/kg, the markups required by manufacturer (2X base cost) and wholesaler, marketer, distributer and retailer added another 4–8X increase to the price. There was more work to be done to raise yields and lower costs to fulfill all market opportunities.

**Regulation:** Regulatory issues placed demands on time and cost to the business and collection of scientific data (Bailey et al., 2009). Information on the criteria required to support a science-based regulatory

**Table 1.** Business model forces impacting the commercial outcome of registered bioherbicides.

| Trade name® and Origin | Organism | Targets | Business model forces [1] | |
|---|---|---|---|---|
| | | **Never commercially sold** | | |
| BioMal *Canada* | *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. | Round-leaved mallow | Low-cost herbicide released; needed moisture after application | -M -S |
| Dr BioSedge *USA* | *Puccinia canaliculata* (Schwein.) Legerh. | Yellow nutsedge | Production economics; weed biotype resistance | -S -I |
| Smoulder *USA* | *Alternaria destruens* E.g., Simmons | Dodder | Production economics | -S -I |
| Woad Warrior *USA* | *Puccinia thlaspeos* Ficinus & C. Schub. | Dyer's woad | No industry backer; researcher provided to users for a while | -I |
| | | **Under company development for commercial sale** | | |
| Opportune *USA* | *Streptomyces acidiscabies* Lambert & Loria[2] | Broadleaf weeds | Lower production costs | -S -I |
| Bio-Phoma *Canada* | *Phoma macrostoma* Mont. [2] | Broadleaf weeds | Lower production costs | -S -I |
| | | **Commercial sales in past** | | |
| DeVine *USA* | *Phytophthora palmivora* (E.J. Butler) E.J. Butler | Strangler vine | Efficacy was too long lasting; produced on demand for a while | -S -C |
| Camperico *Japan* | *Xanthomonas campestris* (Pammel) Dowson | Annual bluegrass | Short shelf life and frozen storage costly for supply chain delivery | -S -I |
| Chontrol *Canada* | *Chondrostereum purpureum* (Pers.) Pouzar | Woody vegetation | Niche commercial market for 9 yrs with Mycologic; licensed to Lalle-mand 2013, no current sales | -I -M |
| MycoTech *Canada* | *Chondrostereum purpureum* (Pers.) Pouzar | Woody vegetation | Short shelf life; competing markets with Chontrol; de-registered in 2008 | -S -I -C |
| Sarritor *Canada* | *Sclerotinia minor* Jagger | Broadleaf weeds | Low cost Fiesta® released; short shelf life; cold storage; moisture needs; poor consumer experience | -S -C -M |
| | | **Commercial sales in present** | | |
| Lockdown[3] *USA* | *Colletotrichum gloeosporoides* (Penz.) Penz. & Sacc. | Northern joint vetch | Small-niche market; produced on contract direct to growers | +S +I +C |
| BioProtec *Canada* | *Lactobacillus* spp. [2] *Lactococcus* spp. [2] | Broadleaf weeds | Small-niche market; only partial weed suppression in turf | +S +I +C |
| SolviNix *USA* | TMGM virus | Tropical soda apple | Small-niche market; produced on demand for clients; exploring other uses | +S +I +C |

[1] S = Science, I = Industry , R = Regulatory, C = Consumer, M = Market, (–) = Negative, (+) = Positive
[2] Bio-activity based on chemistry
[3] Original trade name Collego®

decision by The Pest Management Regulatory Agency within the Government of Canada was published in the Regulatory Directive DIR2001-02 Guidelines for the Registration of Microbial Pest Control Agents and Products on March 30, 2001 which is online at https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/cps-spc/alt_formats/pacrb-dgapcr/pdf/pubs/pest/pol-guide/dir/dir2001-02-eng.pdf. Enthusiastic scientists provided more information than requested which only led to more questions from regulators, taking more time and money to resolve. Tier 1 toxicology testing was done by approved GLP labs and cost about USD 400,000. Fortunately, no studies triggered Tier 2 testing which would have been a much costlier process. The process to regulatory approval should take about two years, but for *P. macrostoma* it took four years to get conditional registration, plus two years to collect the final data, and one year after re-submission to get full registration in Canada.

**Consumers:** Consumers proved to be friends and foes to the product development (Bailey et al., 2010). The researchers encountered many skeptics and naysayers. To counter these attitudes, friendly supporters were cultivated by repeatedly telling the story to wide-ranging groups (city managers, professional organizations, schools, commodity groups). The use of friendly cooperators (plant pathologists, entomologists, turf advisors, innovative farmers) to conduct field testing spread positive third-party messaging and avoided traditionally-trained agronomists and herbicide scientists who frequently had negative views. This approach was effective as consumers learned about the technology from experts and peers allowing them to contribute ideas through the development process.

**Markets:** Market forces play the wild card to success which can unexpectedly make or break a project. Legislation preventing the use of herbicides in urban environments and finding specific markets where herbicide options did not exist had positive effects on pursuing development of *P. macrostoma*. Retail pricing and familiarity with traditional herbicides had negative effects. The problem with market forces is the unpredictability of competitors and consumers actions, which can change swiftly and in surprising directions.

Hindsight evaluation of the business model forces encountered with *P. macrostoma* and other bioherbicides (Table 1; Bailey, 2014) led to the conclusion that demands of science, industry and consumers must be positively met in order to attain commercial success. Regulatory forces presented no obstacles that could not be overcome.

## Conclusions

To advance bioherbicide development, there are four areas to consider:

(1) Look beyond traditional plant pathogens as the source of new agents. Saprophytes and endophytes may also display bioherbicidal traits. Do not pursue agents with moderate activity levels. Choose only those with strong bioactivity, preferably agents with modes of actions using chemistry and biology to target the host and decipher the role of microbial physiology and gene function in the bioactivity. (2) Prioritize work on a production system early in the science phase with the view to maximizing yield and reducing cost. Move quickly from lab scale to a small-scale industrial prototype including formulation, shelf life and storage assessments. Determine the true active ingredient (living entity versus its chemistry) and then choose the best industrial process that will maximize yield. (3) Reconsider whether a high-profit business model is the right one for the specific project or whether alternative models would work better. A lifestyle-business model is more suited to niche market bioherbicides taken on by smaller companies who are invested in the product for the benefits it returns to society along with a reasonable, but not excessive profit. A lifestyle co-operative model would involve specialized or commodity groups who organize to undertake contracted manufacturing to produce a bioherbicide at cost for distribution to the co-op membership. All models must meet basic costs but vary on profit expectations. (4) Find champions for the vision among the science, industry and consumer communities. The scientist has to sell the vision, the industry has to buy the vision, and the consumer must adopt it. Building a well-defined product package from the start is crucial in getting support from industry, aiding in positive interactions with regulators, and keeping consumers engaged throughout the development cycle to commercialization. But in the end, the bioherbicide must work at a price point the consumer will buy.

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*Oral presentation*

# From classical to inundative control: *Mycosphaerella polygoni-cuspidati* as a potential mycoherbicide for Japanese knotweed

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Japanese knotweed (*Fallopia japonica* [Houtt.] Ronse Decr., Polygonaceae), listed as one of the hundred worst invasive alien species (Lowe et al., 2000) has been widely documented to exert detrimental impacts on both the biodiversity and local infrastructures in Europe, North America and parts of Oceania. Native to Japan, *F. japonica* was originally brought to most of its adventive range as an ornamental in the 19th century. However, the plant's ability to form dense monocultures and to regrow from tiny fragments of rhizome has made Japanese knotweed a troublesome invader wherever it has been introduced. Classical biological control (CBC) programs targeting the weed were initiated in the UK and the USA in 2000 and in Canada in 2007. From the suite of natural enemies associated with Japanese knotweed in its center of origin, the psyllid *Aphalara itadori* Shinji (Homoptera: Psyllidae) and the leaf-spot pathogen *Mycosphaerella polygoni-cuspidati* Hara (Mycosphaerellaceae, Ascomycota) were selected for full evaluation as CBC agents (Djeddour et al., 2008). Having undergone the pest risk assessment (PRA) process and a public consultation, the selected strain of the psyllid received ministerial approval for release against Japanese knotweed in the UK in 2010, becoming the first weed CBC agent in the EU. The psyllid was also subsequently released in Canada and has been petitioned for release in the USA.

The Mycosphaerella leaf-spot is a damaging pathogen of *F. japonica* and is common and widespread on its host in the native range. Thorough host-specificity assessment of *M. polygoni-cuspidati* under quarantine conditions at CABI UK showed that the pathogen is able to infect and develop fertile spermogonia producing spermatia on the non-target species *Persicaria hydropiper* (L.) Delarbre and *Polygonum maritimum* L., both native to the UK, as well as on *Polygonum glaucum* Nutt., the sister species of *P. maritimum* and a native of North America (Seier et al., 2014). Spermogonia and spermatia, which constitute the first stage in an ascomycte life cycle, developed regularly on inoculated *F. japonica* plants under greenhouse conditions. However, to date all attempts to induce *M. polygoni-cuspidati* to complete its life cycle on its natural host under experimental conditions have failed. This then leaves the question as to whether the pathogen could complete its life cycle on the affected non-target plants unanswered, and thus currently precludes the fungus from use as a CBC agent. Nevertheless, the unique biology of *M. polygoni-cuspidati* lends itself to the potential development as a mycoherbicide. The Mycosphaerella leaf-spot is only known in its sexual form and lacks an asexual conidial stage (Kurose et al., 2009) which, in other *Mycosphaerella* species, is the spore type predominantly responsible for repeated infection cycles and disease spread. Furthermore, the pathogen has been shown to be heterothallic (Kurose, 2016), meaning it requires two complementary mating types to complete its life cycle and develop sexual structures and ascospores as the primary and only infective and dispersal propagules in the field. However, akin to a number of other *Mycosphaerella* species, *M. polygoni-cuspidati* has a dual infection mechanism; it is also able to infect through mycelial fragments under experimental conditions (Kurose et al., 2015). Such mycelial infection has been shown to cause the same herbicide-like disease symptoms on Japanese knotweed as seen with ascospores, and this infection route could be exploited by mass producing mycelium fragments of the pathogen in liquid culture for spray application. *Mycosphaerella polygoni-cuspidati* is not able to grow saprophytically and does not persist in its host by means of systemic infections. Therefore, using a single mating type isolate would prevent the fungus from reproducing, spreading and persisting in the field and allow for targeted spray application against invasive Japanese knotweed stands, especially in urban situations, while minimizing any non-target impacts. This concept has now been protected through UK and International patent applications held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK (DEFRA), and "proof of concept" research has commenced. This research is focusing on improving the shelf-life of mycelial fragments as well as assessing the impact of the Mycosphaerella leaf-spot on its host Japanese knotweed.

Mycelial fragments of *M. polygoni-cuspidati* produced in liquid medium have a limited shelf-life during which they retain viability and infectivity. Freeze-drying, routinely used to store fungal spores, has a poor track record to preserve fungal mycelium, although some success has been reported for *Claviceps* spp. (Pertot et al., 1997), some mycorrhizal fungi (Tommerup, 1988) and certain basidiomycetes (Singh et al., 2004). Experiments showed that mycelial fragments of the Mycosphaerella leaf-spot can retain viability and infectivity during freeze-drying when lyoprotectants, such as sorbitol, inositol or trehalose, are added and combined with 10% skimmed milk. Sorbitol, particularly at higher concentrations of up to 15% (w/v), gave the best results. Reducing the rehydration time of freeze-dried fragments to 0.5 hours was also found to improve mycelial survival rate. However, the highest percentage viability of mycelial fragments after freeze-drying was found to be 27% after one week of storage, but this declined over longer storage periods (assessed up to six weeks after freeze-drying). Consequently, this methodology will need to be optimized to better preserve mycelial fragments as the active ingredient of any future mycoherbicide in order to increase shelf-life.

Impact assessments of the pathogen on Japanese knotweed are currently still limited to quarantine greenhouse conditions. A PRA for the release of a single-mating type isolate of *M. polygoni-cuspidati* from quarantine to conduct experimental field trials has been submitted to the respective UK authorities. However, while this documentation is under evaluation, the pathogen is classed and treated as a quarantine organism in the UK. Studies showed that inoculations with mycelial concentrations of the pathogen at 107 fragments/ml led to leaf-drop in inoculated plants while concentrations of 105 to 106 fragments/ml reliably caused disease symptoms in the form of necrotic leaf-spots. However, disease severity was variable and strongly dependent on the age of the leaf-spot culture used for mass production, as well as the post-inoculation ambient relative humidity; fungal cultures older than three months and lower relative humidity impacted negatively on disease expression. Inoculation with *M. polygoni-cuspidati* stimulated the production of new shoots linked with an increased length of and number of leaves on these shoots in treated Japanese knotweed plants. This observation can currently only be documented as a trend due to high variability, but it suggests that the pathogen interferes with shoot apical dominance and thereby activates the growth of rhizome buds, a phenomenon also seen in Japanese knotweed plants as a result of cutting or poisoning of aerial shoots (Bashtanova et al., 2009).

It is hoped that an approval of the PRA for the Mycosphaerella leaf-spot and permission for its release from quarantine will allow experimental field trials to take place in order to assess the efficacy of the pathogen under field conditions. If mycelial applications of *M. polygoni-cuspidati* prove to give good control, and the concept of a mycoherbicide for Japanese knotweed based on a single mating type of the pathogen is to be taken forward, a partnership with the private sector will be crucial and talks with industry regarding this are already in progress.

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*Oral presentation*

# Plant-associated bacteria as control agents for weeds

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Plant-associated microorganisms play different roles in the growth and health of plants. They are generally well adapted to their host. Changes in the amount of certain microorganisms due to different environmental factors can change the interaction from neutral or beneficial to deleterious. Such microorganisms can be used as inundative biocontrol agents against specific weeds. Nevertheless, the vast majority of microbes in the environment are unexplored and represent a major reservoir for new bioactivity. In various research projects, plant-associated bacteria were isolated and characterized for the control of specific weeds. In one of these projects, we isolated plant-associated bacteria from *Ambrosia artemisiifolia* L. and tested their effects on their hosts and on sunflower. From three different locations in Burgenland, bacteria were isolated from the rhizosphere and endopyhtes from the roots and stems. A total of 296 unique isolates were tested for different function including tests on ragweed and sunflower seeds. Twenty-five of the isolates reduced seed germination by up to 32%. Three of these isolates also inhibited the germination of lettuce seeds. It was observed that different *Pseudomonas* strains showed different modes of action on ragweed plants, including growth inhibition, germination reduction and inhibition of re-sprouting after wounding. We analyzed in more detail one strain from *Lepidium draba* L. (CDR14c), isolated from a plant in a vineyard, which showed herbicidal activity against several Brassicaceae species. We observed for strain CDRTc14 a stop in germination, like strain WH6, but CDRTc14 does not contain the genes for the FGV cluster. The reaction of CDRTc14 against its host depends on the amount of bacteria applied. The host specificity was not in accordance with the centrifugal phylogenetic method from Wapshere (1974). Therefore, the method has to be reviewed for biological weed control.

*Oral presentation*

# Requirement of mycotoxin TeA-activated singlet oxygen signaling for disease development induced by necrotrophic fungus *Alternaria alternata*

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Tenuazonic acid (TeA) is a nonhost-specific phytotoxin produced by several necrotrophic fungi including *Alternaria alternata* (Fr.) Keissl., *A. longipes* (Ellis & Everh.) E.W. Mason and *A. tenuissima* (Nees) Wiltshire. TeA has been patented as a new bioherbicide. Previous studies show that TeA is a photosystem II inhibitor, leading to cell necrosis by triggering chloroplast-derived reactive oxygen species burst. Our recent research with *Arabidopsis* seedlings reveals that TeA-induced singlet oxygen (1O2) activated a signaling pathway that depends on the two EXECUTER (EX) proteins EX1 and EX2 and triggered a programmed cell death response. Experiments with mature plants of *Arabidopsis* show 1O2-mediated and EX-dependent signaling is also involved during the development of disease caused by *A. alternata*. That 1O2 signaling was activated was indicated by the rapid and transient up-regulation of 1O2-responsive genes in wild type (Col-0) and the conditional fluorescent mutant (flu) as well as its suppression in EX1/EX2 mutants. Furthermore, inactivation of EX1 and EX2 significantly suppressed cell death of flu and Col-0 mature plants. High light-triggered 1O2 acclimation could, we speculate [BG1] , confer cross-protection against *A. alternata*-induced disease in mature plants of *Arabidopsis*. Activation of 1O2 signaling in mature *Arabidopsis* plants is due to TeA accumulation in leaf tissues during *A. alternata* disease development [BG2] . Thus, the role of TeA-triggered 1O2-mediated and EX-dependent signaling is to promote disease development.

*Oral presentation*

# Effects of medium composition on chromatography and toxicity profiles of extracts of *Stagonospora cirsii* S-47, a pathogen of perennial sowthistle

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The toxigenic potential of the fungus *Stagonospora cirsii* Davis, a possible biocontrol agent against *Sonchus arvensis* L., was evaluated. For this purpose, we determined the effects of some standard liquid (Czapek media, YMG, soy meal–sucrose) and solid (rice, pearl barley, millet) substrates on growth of *S. cirsii* S-47, yield extractive matter (YEM) from fungal cultures, a spectrum of biological activity of the extracts and their chromatography profiles. Maximal YEM from the culture filtrates (ca. 300 mg/L) was obtained from the two-week culture of *S. cirsii* S-47 on soy meal-sucrose liquid medium. When the fungus was grown on the solid substrates, maximal YEM was about 1.5 g/kg for the two-week culture on pearl barley. The highest level of phytotoxic (on leaf segments of *Sonchus arvensis*) and antimicrobial activity (against *Bacillus subtilis* [Ehrenberg]) was from a dichloromethane (DCM) extract from the three-week culture grown on modified Czapek medium (CM). Maximal cytotoxic activity (on U251 cell line) was demonstrated using an ethyl acetate extract from a three-week culture grown on liquid YMG medium. Esterase activity inhibition was maximal for the two-week culture grown on CM. Cultivation technique and, to a lesser extent, medium composition caused significant effects on quantitative composition of the fungal extracts. The extracts from the culture filtrates showed a broader spectrum and higher level of biological activity than the extracts from solid cultures of *S. cirsii* S-47. Main phytotoxic and antimicrobial metabolites found in the extracts were identified as stagonolide A and herbarumin I while some compounds remained unknown. It seems that the inoculum production of the fungus on solid substrates would be less dangerous from a toxicological point of view than inoculum produced in liquid culture. Further evaluation of the ecological safety of a mycoherbicide based on submerged mycelium of *S. cirsii* as well as waste culture filtrate is necessary in the future.

*Oral presentation*

# Setting the mood: initiating herbivory response increases impact of fungal pathogens on Canada thistle

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*Cirsium arvense* (L.) Scop., Canada thistle, is one of the most detrimental weeds for agricultural production and rangeland health. It is common in temperate zones globally and difficult to eradicate due to its clonal and resilient root system. The fungal pathogen *Puccinia punctiformis* (F. Strauss) Röhl., or CT-rust, systemically infects and kills only Canada thistle. However, CT-rust rarely reaches epidemic proportions in natural populations. The objective of this study was to determine if manipulating plant defense hormones affects host susceptibility to the rust, making it a more effective biocontrol agent. A foliar spray of water, jasmonic acid (JA), or salicylic acid (SA) was applied to greenhouse-grown Canada thistle plants during a two-incubation period. Half of the plants were inoculated with CT-rust teliospores prior to incubation; others acted as a control and to determine the effect of plant hormones alone on thistle growth. Plants were monitored for a period of ten weeks post incubation and then harvested for above and belowground biomass. All inoculated plants exhibited symptoms by week ten. No control plants exhibited symptoms. Plants produced multiple ramets, and infection rates for ramets were 48% for water, 64% for JA and 39% for SA. Infection reduced root biomass by 62% for the water treatment, 79% for JA and 51% for SA relative to the uninoculated water control plants. The increased rate of symptomatic infection and decreased root biomass in JA-treated plants indicate that a boost of JA at the time of inoculation likely reduces SA-dependent defense responses increasing the impact of the rust. Therefore, it is likely that the efficacy of the CT-rust would increase when used in conjunction with other insect biological control agents as JA is released in plants in response to insect herbivory.

*Oral presentation*

# Isolate differences in *Sclerotinia sclerotiorum* applied as a bioherbicide to *Cirsium arvense*

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*Sclerotinia sclerotiorum* (Lib.) de Bary has been proposed for use in a bioherbicide formulation to control *Cirsium arvense* (L.) Scop. populations in pastures in New Zealand. Our research has investigated whether there is a difference between isolate S36 (the currently favored isolate) and another potential candidate, S37. Mycelial growth and morphology of the two isolates were compared on potato dextrose agar. A detached leaf bioassay was conducted on two populations of the thistle in Canterbury, New Zealand: one at Irwell and another at Lincoln. The fungus was applied as an oat formulation or as sclerotia. Lesion development on detached leaves was compared between the isolates, and molecular analysis was undertaken to determine if any genetic differences existed. S36 exhibited greater colony growth than S37 but no morphological differences were observed. Using the oat formulation on the Lincoln population of the thistle, S37 was more virulent than S36, forming substantially larger lesions. This difference was not observed on the Irwell population. In the detached leaf assays with the Irwell population, negligible (0.04%) germination of the S37 sclerotia occurred compared to 30% of the S36 sclerotia. The latter were more virulent, as assessed by lesion development. Random amplified polymorphic DNA indicated genetic differences between isolates S36 and S37 but whether these polymorphisms are responsible for virulence differences is unknown. This work indicates that the two isolates could be used together in a bioherbicide to overcome differences in virulence on different genotypes of *C. arvense*.

*Oral presentation*

# Persistence and effectiveness of Sarritor (*Sclerotinia minor* [IMI 344141]) in the southeastern USA

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Experiments were conducted to evaluate the efficacy of Sarritor on broadleaf weeds common to turfgrass in North Carolina, USA and to monitor the persistence of Sarritor using a lettuce plant bioassay. Significant differences were observed between spring and fall experiments. In the spring experiment, Sarritor treatments resulted in little or no control of broadleaf weeds and very short persistence (as detected by the lettuce transplant bioassay). In contrast, lettuce transplant survival in the fall study was reduced for at least eight weeks following Sarritor treatment. Additionally, in the fall experiments, control of field madder (*Sherardia arvensis* L.) with Sarritor was similar to that provided by an auxin herbicide mixture. However, the results were quite variable with some replicates exhibiting nearly complete control and other replicates of the same treatment with poor control. Dandelion plants were infected by Sarritor, but no reduction in dandelion populations were observed. Fall applications of Sarritor provided temporary control of white clover, similar to Fiesta (FeHEDTA), but by spring white clover had re-grown. These data suggest that environmental conditions in North Carolina will have very significant impacts on Sarritor efficacy and persistence. Variable weed control among replicates was also observed.

*Oral presentation*

# Development of a granular bioberbicide using *Sclerotium rolfsii* for biocontrol of broadleaf weeds

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*Sclerotium rolfsii* Sacc., isolated from a destructive stem rot of diseased goldenrod plants (*Solidago canadensis* L.), has been attempted to be developed into a mycoherbicide for biocontrol of broadleaf weeds and sedges. The effectiveness and specificity tests showed that the mycoherbicide could control most of the broadleaf weeds and some sedges in fields, turfgrass and invaded habitats. The mass production of a granular formulation was established through submerged and solid substrate fermentation using crop straw and hull as a carrier. A series of field trials was conducted to evaluate the effectiveness and safety of this product and develop its application techniques in paddy fields, corn fields, turfgrass and goldenrod-invaded habitats. It was applied to turfgrass to effectively control perennial weeds with rhizomes. Its application to paddy fields demonstrated stable weed control efficacy over 75%, regardless of field environmental conditions. In addition, it promoted wheat straw rotting when the wheat straw was fed back to fields. Therefore, the *S. rolfsii* product may be potentially developed into a novel mycoherbicide for biocontrol of broadleaf weeds and sedges in paddy and corn fields, turfgrass and invaded habitats.

*Poster presentation*

# Could fungi stop buddleia in its tracks?

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*Buddleja davidii* Franch. (buddleia, butterfly bush) is a large perennial woody shrub or small tree native to China and Japan that was imported into the UK in the 1880s as an ornamental. Cultivars of the shrub are popular with horticulturalists due to their colorful, showy flowers which are attractive to butterflies. The species can grow up to 2 m in height annually and produce millions of highly-dispersible seeds. Buddleia compensates well for physical damage through re-sprouting, and its seedlings are drought-tolerant. Once escaped from the garden, wild buddleia populations can colonize derelict land and are known to outcompete native vegetation and reduce biodiversity. Buddleia is able to grow in many difficult-to-access sites throughout urban and rural environments such as chimney stacks and railway sidings and is capable of inflicting considerable structural damage. Consequently, in the UK, the Department for Environment, Food and Rural Affairs (DEFRA) now regards *B. davidii* as an invasive, non-native species. Network Rail has the responsibility to maintain the railway infrastructure and to deal with weeds on or near tracks, and buddleia impacts on their ability to do this by blocking train lines, interfering with overhead power lines, obscuring visibility of train signals and hindering safety inspections. Due to buddleia's woody nature and its ability to grow in inaccessible places, chemical or physical control methods can be difficult. In 2017, Network Rail funded a two-year research project to investigate the potential of native fungi for use as a cut-stump mycoherbicide for biological control of buddleia. Field surveys to collect fungi on buddleia stumps were conducted in urban and rural areas across England and Wales. *Chondrostereum purpureum* (Pers.) Pouzar, the causal agent of silver leaf disease on fruit and ornamental trees, was the main target of these surveys; however, all isolated pathogens were recorded. The fungi were identified by morphological and molecular methods and the most promising were selected and are being assessed for their ability to prevent regrowth of buddleia.

*Poster presentation*

# Herbicidal activity of crude ethanol extracts from *Humulus scandens* (Lour.) Merr. and the possible mechanisms involved

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The herbicidal activity of ethanol extracts from *Humulus scandens* (Lour.) Merr. was evaluated for inhibition of four weed species: *Echinochloa crusgalli* (L.) P.Beauv., *Beckmannia syzigachne* (Steud.) Fern., *Leptochloa chinensis* (L.) Nees and *Lindernia procumbens* (Krock.) Philcox. The chemical constituents of *H. scandens* were preliminarily identified using GC-MS. The possible mechanism of inhibition of *E. crusgalli* was studied through comparing the activities of phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) between ethanol *H. scandens* extracts treated and untreated. The 0.1, 0.5, 1, 3 and 5 g/20 ml (concentrations of extractum) of ethanol extracts from fresh leaves and stems of *H. scandens* were tested for their impacts on germination and seedling growth of the four weeds.

*Poster presentation*

# Fungal pathogens and their bioactive metabolites for controlling *Ailanthus altissima*

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Among the woody invasive alien species colonizing non-crop areas, *Ailanthus altissima* (Mill.) Swingle (tree of heaven) is one of the worst and most damaging weeds. It spreads everywhere in urban, suburban and natural areas creating dense stands and causing much damage including biodiversity loss. Its control is very difficult because of its fast growth, its capability of regenerating as "suckers" from buds on the roots and stem after mechanical interventions, and its production of large numbers of seeds that are easily scattered in the environment. Within the "LIFE Alta Murgia" Project, founded by the European Commission and aimed at eradicating *A. altissima* from the Alta Murgia National Park using innovative and eco-friendly control techniques, the suitability of potential microbial biological agents were explored. Two fungal pathogens were isolated from diseased plants, identified, tested for pathogenicity and aggressiveness, and studied for the production of bioactive metabolites. The first results of the studies on the two fungal agents, including the chemical and biological characterization of the metabolites and their potential as natural herbicides, are presented.

*Poster presentation*

# Molecular cloning of genes involved in ophiobolin A biosynthesis pathway from bioherbicidal fungus

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Ophiobolins are sesterterpenoid-type phytotoxins produced by fungi. They are active on a broad spectrum of organisms including plants, fungi, bacteria, nematodes and tumor cells, so they may be considered as important candidates for the development of new crop-protection and pharmaceutical products. However, due to the low content of bioactive components in ophiobolin-producing fungi, the commercial applications of ophiobolins are restricted. To significantly increase yields of ophiobolin A from the ophiobolin-producing fungus *Bipolaris eleusines* J.H.Peng &J.Y.Lu (Be), transcriptome sequencing was used to produce a substantial expressed sequence tags (EST) dataset for the fungus. Based on the analysis of EST, molecular cloning by RACE technology and analysis of related key genes involved in ophiobolin A biosynthesis pathway were conducted. BeHMGR, BeIPPI, BeFPPS and BeGGPPS of four key enzymes involved in ophiobolin A biosynthesis pathway were, for the first time, isolated from an ophiobolin A-producing fungus strain of *B. eleusines*. This information might be helpful not only for theoretical research of further defining the mechanism of ophiobolin A biosynthesis, but also supplying more clues about target enzymes that might enhance the production of ophiobolin A for practical application.

*Poster presentation*

# Biological control of *Fusarium avenaceum* (Fr.) Sacc. on *Avena fatua* L. in Qinghai, China

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Mycoherbicides using microbial metabolite toxins, especially those from plant pathogenic fungi, have shown good potential and have become one of the hot spots in the field of microbial herbicides. *Fusarium avenaceum* (Fr.) Sacc. is a pathogenic fungus that causes disease in *Avena fatua* L. Our study aimed to optimize the production of herbicidal compounds from *F. avenaceum*. The main results are as follows: (1) The optimization of culture condition for herbicidal toxin production was investigated by single factor experiments and an orthogonal design method. The optimal culture conditions of *F. avenaceum* GD-2 to produce herbicidal toxin were corn solid medium, alternative illumination with 25°C during the day and 15°C at night. In the condition of optimizing fermentation parameters, the production of herbicidal toxin could reach 16.3 mg/kg. The herbicidal bioactivity of its crude toxin extracted using butanol was the strongest among all tested solvent extraction methods; (2) By bioassay-guided separation, the compounds were isolated and purified from the n-butanol extract of *F. avenaceum* GD-2 by column chromatography, thin layer chromatography, reverse phase HPLC. Three compounds were obtained and identified: cyclo-(L-leucyl-L-proline), Fusaric acid and 9, 10-Dehydrofusaric acid. The compounds showed activity against seed germination of *A. fatua*. The shoot and root length inhibition rates in seedlings of *A. fatua* were, respectively, 82 and 85% for cyclo-(L-leucyl-L-proline), 79 and 91% for Fusaric acid, and 81 and 84% for 9, 10-Dehydrofusaric acid when the seed was treated; (3) This project focused on the isolation and structure elucidation of secondary metabolites and culture conditions in the production of bioherbicidal substances. Our results provide an important reference for further study on the development and utilization of strain GD-2 and also highlight the potential of research into microbial metabolites as bioherbicides.

*Poster presentation*

# Could *Phytophtora* species associated with declining populations of invasive European blackberry be used for biological control?

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European blackberry (*Rubus fruticosus* L. agg.) is a thorny invasive shrub that grows primarily in southern areas of Australia where annual rainfall exceeds 700 mm. It readily invades land along watercourses, competing against native plants and pasture and preventing access to the water by native fauna and livestock. *Phytophtora* species have been found to be associated with severe blackberry dieback in Western Australia. The objective of this study was to determine the biological control potential of two *Phytophthora* species by comparing their pathogenicity on blackberry and testing the specificity of the most promising species on a range of non-target plants. The two species, *P. bilorbang* Aghighi, G.E. Hardy, J.K. Scott & T.I. Burgess and *P. pseudocryptogea* Safaiefarahani, Mostowfizadeh, G.E. Hardy & T.I. Burgess, have been found to occur naturally in soil in other states. Under glasshouse conditions, *P. pseudocryptogea,* grown on solid substrate and applied to the soil, killed or significantly reduced biomass of blackberry plants when exposed to simulated flooding events. In contrast, plants treated with *P. bilorbang* did not differ from untreated control plants. In a series of subsequent experiments, *P. pseudocryptogea* was found to adversely affect a range of non-target species, including some *Acacia* and *Eucalyptus* species. On the basis of these results, it was decided not to proceed with field trials.

*Poster presentation*

# Biological control of *Solidago canadensis* using a bioherbicide increases the biodiversity in invaded habitats

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Goldenrod (*Solidago canadensis* L.) is native to North America and has become one of the most serious invasive plant species in China. Herbicide-dependent control of this weed has caused new ecological consequences, such as biodiversity loss and environmental pollution. Biological control may mitigate such adverse ecological impacts. Five different goldenrod-invaded habitats were plowed and treated using the SC64 bioherbicide during the different growth stages of this weed. The densities of goldenrod and other plants were surveyed after treatment to evaluate the adaptability and influence of this biocontrol technique on plant diversity in the spring and autumn. The results showed that a combination of plowing and bioherbicide technology presented a controlling effect of 89% on average in different habitats and different seasons, while the chemical herbicide demonstrated only a controlling effect of 70% on average. Biological control caused significant increases in the total number of plant species, and the importance value of *S. canadensis* decreased by 70% on average, regardless of spring or autumn seasons, which was significantly higher than that of chemical control (32%). Bioherbicide treatment caused significant increases in the Patrick richness index, Simpson diversity index, Shannon-Wiener diversity index and Pielou evenness index, and the average increase rates were 100%, 295%, 100% and 267%, respectively. In conclusion, compared with chemical herbicides, bioherbicide treatments significantly improved the plant community structure and markedly increased the biodiversity in habitats invaded by *S. canadensis*.

*Poster presentation*

# Optimization of fermentation process of (*Serratia marcescens*) Ha1 and bioassay of its herbicidal activity

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Corn is the third largest food crop in China. Ensuring its production is a prerequisite for maintaining food security. Weeds can lead to a decline in the yield and quality of corn and their effective control is essential. Chemical herbicides have many advantages but also some problems such as toxicity to non-target species, weed resistance and soil residues. To reduce the usage of chemical herbicides and delay the occurrence of resistance, microbial herbicides have become important measures in corn production. They have many advantages including being environmentally friendly, highly efficient and easily degradable. In order to search for herbicidal substances, *Serratia marcescens* Bizio Ha1 from marine microorganisms was used as the experimental material. In this study, the fermentation medium and environmental conditions for *S. marcescens* Ha1 strain were optimized through single-factor tests and orthogonal experiments, respectively. The bacteria produced were tested for herbicidal activity by leaf spray of fermentation broth and soil treatment using infected granules. The results showed that (1) the optimal carbon source was 10 g/L sucrose, nitrogen source was 20 g/L peptone, and inorganic salt was sodium chloride 10 g/L, CaCl2 3 mol/L. The optimum fermentation conditions to produce 1.69 g bacterial biomass/100 ml were: temperature 20°C, pH 7.0, inoculum amount 1%; shaking culture rotation speed 200 rpm, fermentation time of 46–48 h; (2) the non-sterilized fermentation broth spray-applied to *Digitaria sanguinalis* (L.) Scop., *Amaranthus retroflexus* L. and *Pharbitis nil* (L.) Choisy was better than the sterilized fermentation broth, reducing the fresh mass of these weeds 62, 77, and 47%, respectively. The effect of the sterilized fermentation broth on stems and leaves of *Echinochloa crusgalli* (L.) P.Beauv., *Abutilon theophrasti* Medik., and *Chloris virgata* Sw. was 38, 56, and 30% respectively; (3) granule application to foliage was superior to that of soil treatment giving 92, 91, and 74% reduction in *D. sanguinalis, A. retroflexus*, and *A. theophrasti,* respectively. By contrast, the inhibition of these weeds by soil-applied granules was 90, 89 and 70%, respectively.

*Poster presentation*

# Isolation and structure identification of herbicide-active compounds from *Phoma herbarum*

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Safer and more environmentally-friendly natural herbicides would be considered favorably for weed management, particularly in those habitats where the use of chemicals is restricted or banned. Natural herbicidal compounds from plant pathogens may be effective as novel herbicides. We isolated strain SYAU-06 of *Phoma herbarum* Westend. from diseased leaves of dayflower (*Commelina communis* L.) and found that the metabolites from this strain inhibited seed germination, restrained plant growth and caused necrotic spots on the leaves of dayflower. The herbicidal activity increased with the metabolite concentration. The herbicide-active component 5d6 was isolated and purified from the metabolites by Thin-layer Chromatography (TLC), High Speed Counter-current Chromatography (HSCCC) and High Pressure Liquid Chromatography (HPLC) using a bioassay. Structural analysis showed that 5d6 was a 2-methyl-3, 5-dinitro-benzoate ester.

# Session 4: Novel methods to determine efficacy and environmental safety of agents

**Session Chair: Michelle Rafter**

**Keynote**

# The value of novel approaches in the development of weed biological control programs

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## Abstract

The overlap of chemical ecology and biological control of weeds presents a rich opportunity to exploit potentially coevolved relationships between agents and plants where chemical factors mediating these interactions are important. Knowledge of these interactions can direct the selection of potential agents and assist in the prioritization of species in test plant lists. Several topics were integrated in this presentation, which could improve the predictability of host range determination, agent establishment and impact on the target weed. The host secondary plant chemistry and a potential biological control agent's response to that chemistry can be exploited to improve predictability of potential agent host range. Variability of weed secondary chemistry can be an important factor that potentially influences the performance of biological control agents and their impact. Finally, biological control may be improved by prioritization of agents that are protected against generalist predators by sequestration of secondary plant chemistry or synthesis of similar protective compounds. Recognition of these patterns and processes can help identify the factors that impart success to a biological control program. Several studies are highlighted below that demonstrate examples of these interactions that could be widely adopted by researchers.

**Keywords:** attractants, chemical ecology, host selection, olfactory cues, pheromones, sequestered plant compounds, host specificity

## Introduction

Novel approaches to biological control of weeds, including chemical ecology, have sought to improve our understanding of these complex insect-plant interactions and hopefully to provide tools that make this weed control technique safer and more predictable. Examples covered here include both laboratory and field, new and old, published and unpublished. Examples will be drawn from several weed projects on which we have worked at the USDA-ARS in Fort Lauderdale including paperbark trees (*Melaleuca quinquenervia* [Cav.] S. T. Blake; Myrtaceae), Brazilian peppertree (*Schinus terebinthifolia* Raddi; Anacardiaceae) and Old World climbing fern (*Lygodium microphyllum* (Cav.) R.Br.; Lygodiaceae).

As this symposium is entitled "novel" approaches, this will be emphasized as breakthroughs that have led to new methods that improve the way we conduct biological control. However, it needs to be recognized that while novel approaches may lead to discoveries that are useful, other times the value of these discoveries might not be immediately apparent. Three topics will be covered here where chemical ecology has interfaced with biological control. These include (1) agent chemical defenses, (2) chemical ecology assisting in predicting host range and (3) assisting in the monitoring of released agents.

## Biological control agent chemical defenses

The Florida Everglades is a unique ecosystem that includes 500,000 ha of subtropical wetland. This threatened "river of grass" at the southern tip of Florida is a national conservation priority. Threatened by many factors, among the most damaging are invasion from alien weeds such as *M. quinquenervia*. Biological control of this weed has been a high priority for the USDA-ARS at least since 1986 and has resulted in the release of several agents (Center et al., 2012). Among the most successful has been the Australian leaf-feeding weevil *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae), released in 1997 (Center et al., 2000). Since this initial release, *O. vitiosa* has become widely established throughout the invaded range of *M. quinquenervia* and made significant impacts on the target weed populations (Balentine et al., 2009). The *O. vitiosa* agent has been successful for several reasons, among them is the protection derived from the sequestration of plant compounds that coat the larval cuticle. The larvae are covered naturally with a shiny oily substance that ranges from orange to yellow (Montgomery and Wheeler, 2000). This excretion smells much like the *M. quinquenervia* leaves upon which the larvae feed. The chemical nature of this larval defensive secretion was characterized and its defensive properties against generalist predators evaluated.

One of the most common antagonists of *M. quinquenervia* biological control in the invaded range is the generalist red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). When fire ants were presented with early and late instars of the *M. quinquenervia* biological control agent *O. vitiosa*, they seldom attacked (Montgomery and Wheeler, 2000). When the larval excretions were removed with organic solvent and applied to bait at physiological concentrations, fire ant visitation and consumption were significantly reduced (Wheeler et al., 2002). The chemical constituents, or terpenoids, in these *O. vitiosa* larval extracts matched the foliar components of *M. quinquenervia* consumed by the larvae. These results indicated that the *O. vitiosa* larvae sequestered foliar terpenoids from the *M. quinquenervia* plant leaves. These sequestered terpenoids coated the larval cuticle, repelling and protecting them from mandibulate natural enemies like fire ants. This antipredator protection was a significant factor in the success of this biological control agent as it achieved outbreak populations and reduced the density of the weed *M. quinquenervia*.

Although the larvae of *O. vitiosa* are well protected from mandibulate, generalist natural enemies (Wheeler et al., 2002), one North American predator with haustellate mouthparts, *Podisus mucronatus* Uhler (Hemiptera: Pentatomidae), was able to penetrate these defenses and attacked *O. vitiosa* larvae (Wheeler et al., 2002). The terpenoids sequestered by *O. vitiosa* larvae provide protection from mandibulate predators, but not those with piercing/sucking mouth parts (Tipping et al., 2013). Similarly, the coevolved parasitoid *Anagonia* sp. (Diptera: Tachinidae) in the native range was apparently able to circumvent these defenses (Purcell and Balciunas, 1994). In one field survey, adult *Anagonia* flies emerged from 29% of the larvae and resultant pupae ($n$ = 101).

These herbivore chemical defenses are not always desirable qualities for potential biological control agents as some may synthesize toxins that poison livestock and wildlife. Knowledge of these chemical defenses early in a project could save considerable time and expense. Two examples come from the Melaleuca and Brazilian peppertree biocontrol projects. Both projects had completed host-range testing and were considering releases of sawflies from the family Pergidae (Hymenoptera) (Medal et al., 1999; Buckingham, 2001; Hight et al., 2003). Field and quarantine tests indicated that the Melaleuca sawfly, *Lophyrotoma zonalis* (Rohwer) (Hymenoptera: Pergidae), was a narrow specialist (Burrows and Balciunas, 1997; Buckingham, 2001). Biological control releases were authorized but canceled when the larvae were reported to contain several D-amino acid-containing peptides including lophyrotomin and a mixture of pergidin and val-pergidin (Oelrichs et al., 2001). These peptides were well known to be responsible for livestock poisonings in Australia and South America (Dutra et al., 1997; Oelrichs et al., 1999). Another example was found with the Brazilian peppertree (*Schinus terebinthifolia*) sawfly, *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae). Host-range testing indicated *H. hubrichi* was safe for release in the continental USA (Medal et al., 1999). Larvae of this (*H. hubrichi*) and another *Schinus* herbivore, *H. jorgenseni* (Jorgensen), produce similar toxic peptides, pergidin, 4-valinepergidin, dephosphorylated pergidin and lophyrotomin (Boevé et al., 2018). However, behavioral bioassays with captive blue jays (*Cyanocitta cristata* [L.]) showed the birds avoided the sawfly larvae. When offered a choice between mealworms (*Tenebrio molitor* L; Coleoptera: Tenebrionidae) and incapacitated sawfly larvae of either the Melaleuca *L. zonalis* or the Brazilian peppertree *H. hubrichi*, the birds always selected the mealworms. Additional toxicological studies showed no toxicity when laboratory mice were fed *L. zonalis* larvae or injected with larval extracts (G.R. Buckingham, pers. comm.). Even though there were no signs of toxicity from these Pergidae sawfly larvae, possibly because they contained sublethal concentrations (Dittrich et al., 2004; Boevé et al., 2018), they were never released.

## Chemical ecology assisting to predict host range

Host range of biological control agents is highly predictable and very accurate (Suckling and Sforza, 2014). Factors that influence specificity are important as they assist in the development of the test plant list, the list of plants that are expected to be most vulnerable to non-target attack. Phylogeny is the best predictor known for host range, and compiling test plant lists depends heavily on this potentially coevolved relationship. Among the first to notice the relationship between butterflies and plants, Ehrlich and Raven recognized these patterns and proposed a coevolutionary relationship between herbivores and plants (Ehrlich and Raven, 1964). The species most closely related phylogenetically to the weed share similar traits and thus deserve close scrutiny as they are thought to be most at risk from non-target attack of biological control (Futuyma and Agrawal, 2009). Molecular phylogenies assist in the prioritization and selection of plant species for testing (Briese, 1996; Briese and Walker, 2008).

Though not mutually exclusive, plant secondary chemistry has also been shown to influence host range (Becerra, 1997; Agrawal, 2007; Gershenzon and Dudareva, 2007). This relationship between secondary chemistry and host range was examined for the Brazilian peppertree herbivore *Nystalea ebalea* Stoll (Lepidoptera: Notodontidae) (Wheeler et al., 2014). Neonates were offered two haplotypes of the target weed and five species that were close and distant relatives within the family Anacardiaceae. Analysis included the nutritional constituent (nitrogen) and non-nutritional components, such as 79 terpenoids and four pentadecyl (C15) and four heptadecyl (C17) urushiols. The results indicated that urushiol diversity and concentration best predicted host range of *N. ebalea* larvae (Wheeler et al., 2014). Despite these results for one herbivore species, phylogeny within members of the Anacardiaceae predicted host range much better when analyzing all the 17 potential biological control agents tested (Wheeler and Madeira, 2017).

Old world climbing fern *L. microphyllum* is among the most invasive weeds that invades the Florida Everglades. Biological control has been a high priority for the USDA-ARS lab in Fort Lauderdale since at least 1998 (Pemberton and Ferriter, 1998). A number of moths (Lepidoptera: Crambidae) have been released or are in development to control this invasive weed. These include *Austromusotima camptozonale* (Hampson) released in 2004, *Neomusotima conspurcatalis* (Warren) released in 2007, and *Lygomusotima stria* Solis & Yen. still in quarantine (Solis et al., 2004; Boughton and Pemberton, 2008; Boughton and Pemberton, 2009). The most important test plant species of these and other *L. microphyllum* biological control agents are congeneric species of the weed. These include the weed *L. japonicum* (Thunb.) Sw., a North American species *L. palmatum* (Bernh.) Sw., and the Caribbean species *L. cubense* Kunth, *L. oligostachyum* (Willd.) Desv., *L. venustum* Sw., and *L. volubile* Sw. Thus, as these important non-targets are all close relatives, phylogeny plays a limited role separating these species.

To examine the volatile chemistry of these species, aerations were performed on excised leaves of each species (G. Wheeler, unpub.). The volatile constituents of *L. microphyllum* and all six *Lygodium* non-target species were analyzed with 10 replicate collections. The volatiles were obtained by trapping the filtered air on an adsorbent material (SuperQ). The trapped volatiles were eluted with a minimum of $CH_2Cl_2$ solvent and analyzed by GCMS. This analysis revealed green leaf volatiles (1), monoterpenes (2), organic alcohols (3), a ketone (1), alkenes (6) and sesquiterpenes (7). The volatile results were analyzed with principal component analysis (PCA). The analysis indicated that 37% of the variance could be explained by the first principal component (PC), whereas 21% of the variance could be explained by the second PC (Figure 1). The first PC separated the *L. oligostachyum* and *L. palmatum* volatiles from the remaining species. The second PC separated the *L. volubile* and *L. venustum* from the other species. These PCs appear to be influenced by several volatile components, namely 1-octen-3-ol, 3-octanone and 3-octanol, while all were found in high concentrations in *L. japonicum* and *L. microphyllum* compared with *L. oligostachyum* and *L. palmatum*. The volatiles viridiflorene, d-cadinene and cadalene were in relatively high concentration in the headspace of *L. oligostachyum* and *L. palmatum* (G. Wheeler, unpub.).

Once the volatile constituents that separated the different species were identified, their means and variance could be plotted and compared. These results show the relatively high concentrations of 1-octen-3-ol in several species, but this constituent was nearly absent from *L. palmatum*. Several of these compounds found were already known to be active attractants in insects, for example 1-Octen-3-ol (mushroom alcohol) known to attract biting mosquitoes. Similarly, *L. japonicum* and *L. microphyllum* had the highest concentrations of 3-octanone compared with the other *Lygodium* species.

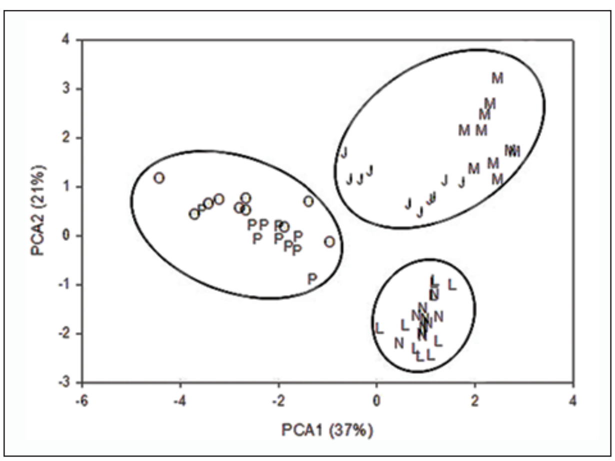


**Figure 1.** Results of principal component analysis of 39 volatile compounds emitted by *Lygodium* spp. PCA1 explains 37% and PCA2 explains 21% % of the variation of the volatile profiles. The letters denote *Lygodium* spp. J: *Lygodium japonicum*; L: *Lygodium volubile*; M: *Lygodium microphyllum*; N: *Lygodium venustum*; O: *Lygodium oligostachyum*; and P: *Lygodium palmatum*. Each circle encloses *Lygodium* species with similar volatile profiles.

To examine the *N. conspurcatalis* moth attraction and their ability to distinguish between them, extracts were applied to filter paper and tested in a flight tunnel. Ten mated, one-day-old females were released into the flight tunnel, and volatile extractions from either *L. microphyllum* or *L. palmatum* were blown into the tunnel. Moth activity and the frequency of moths approaching the source of each odor was recorded over a 24 h period. These bioassays were conducted in IR with an IR video camera.

Moths approached the *L. microphyllum* odor more often (64.1% ± 1.2%) compared with *L. palmatum*. However, when these results were compared with moth oviposition responses during host-testing studies, they laid 7.8 times more eggs on *L. microphyllum* plants than *L. palmatum* (Boughton et al., 2009). These comparisons suggest the moths received additional cues from the whole plant bioassays, such as additional plant chemistry, morphology and visual cues.

These results provide mechanistic details that help explain the host discrimination by this biological control agent toward members of the *Lygodium* genus. Possibly, they will lead to additional tools that will assist in the determination of potential agent host range. Finally, they may provide the opportunity to test plant volatiles when the test plants themselves are difficult to collect and grow or are otherwise unavailable (Park, 2017).

## Using chemical ecology to monitor biological agents

The monitoring of field populations of insects is one of the fundamental components of modern pest management. The use of traps, baited with chemical sex attractants or pheromones, has become an important means of estimating field populations of insects. Though excellent examples exist (Suckling et al., 2000; Cosse et al., 2005; Bartelt et al., 2006; Suckling et al., 2006; Bartelt et al., 2008), their widespread use to estimate populations of released weed biological control agents has yet to be fully exploited. The application of pheromone-baited trapping of biological control agents could be useful to detect nascent populations of newly released agents. Obtaining accurate post-release estimates of agent densities in time and space could assist in understanding seasonal or regional fluctuations. This method could assist in improving weed control by better understanding agent limitations and capabilities.

The *L. microphyllum* biological control agent *N. conspurcatalis* was examined for sex-specific attractants. To determine if the females elicit a response in male moths, a dual-choice flight tunnel was used. This presented a choice to the males between clean filtered air (control) or the odor of five one-day-old virgin females. The results of 12 h recordings showed that males more frequently (66.4%) oriented toward female volatiles, remained in the area where the female odor was emitted (2.2 X sec) and took less time to appear in this female odor (0.5 X sec).

These volatiles were trapped on SuperQ adsorbent (described above), but until these studies can be completed, field densities of moths were estimated by baiting sticky traps with live females (Hight et al., 2002). Two young unmated females were placed in vented containers located inside wing traps (Pherocon 1-C, Trece Inc). Baited traps ($n$ = 10) were placed in five sites every month and monitored. The results of this ongoing study indicate that moth densities can be monitored consistently with live female baited sticky traps (A. David, unpub.). Nascent populations are detected and seasonal trends show greater densities during the winter months. These methods can be improved with synthetic lures as they are currently subject to variation due to the short lifespan of the females and the effect of weather on moth behavior. However, until this method is improved, live female baited sticky traps provided the first evidence of *N. conspurcatalis* seasonal population trends.

## Conclusions

Novel approaches that include the methods of chemical ecology have the potential to provide useful developments to biological control of weeds. These approaches can be helpful when prioritizing potential agents during the early stages of a project. Those agents that are protected from generalist natural enemies may rapidly achieve outbreak densities causing significant reduction to the target weed. The results of chemical analysis and bioassays can reveal the mechanisms of host selection. This information can complement traditional host-testing protocols to better understand agent host range. Sampling methods that include pheromone-baited trapping of biological control agents could detect nascent populations and monitor populations in time and space. These approaches have the potential to improve the predictability of host range determination, agent establishment and impact on the target weed.

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*Oral presentation*

# The potential role of targeted and non-targeted metabolic profiling in host-range testing

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Phylogenetic distance from a target weed is usually one of the most important criteria for the selection of species for host-specificity testing of biological control agents. However, not all results of host-range testing, especially when conducted under confined conditions (fundamental host range) can be explained by phylogeny. Targeted and/or non-targeted metabolic profiling may help improve our understanding of the mechanisms underlying acceptance or rejection of critical test plant species. The weevil *Ceutorhynchus cardariae* Korotyaev is being considered as a biological control agent against the invasive Brassicaceae *Lepidium draba* L. Here we studied insect performance on plant species in two genera, *Lepidium* and *Streptanthus*, which contain species that are most suitable for development of the weevil. In parallel, we performed targeted and non-targeted metabolomic profiling. The targeted profiling focused on glucosinolates (GLS), which are the main class of defensive compounds of the plants tested (all belonging to the family Brassicaceae). Non-targeted profiling encompassed the entire metabolome of each species. Results showed that both the targeted metabolic profiling and the full metabolic data could explain a significant amount of variation in insect performance. Importantly, GLS profile similarity with the main host (*L. draba*) was better correlated with insect performance than phylogenetic distance at the inter-genus level (*Lepidium* and *Streptanthus*). This result was also mostly consistent at the within-genus level. These results have important implications for classical biological control and show that chemical ecology may be used to improve host plant selection for specificity testing, and help explain the underlying mechanisms of no-choice tests results.

*Oral presentation*

# Integrating sensory ecology to complement pre-release risk assessments for biological control candidates

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Current pre-release host-specificity testing protocols in biological weed control focus on feeding and development tests on non-target plant species for prospective candidates. Here we present data supporting our contention that sensory ecology based on olfactory and visual plant cues can be an important tool to advance pre-release host-specificity testing and to explain the underlying mechanisms for host finding. We investigated behavioral and electrophysiological responses of the seed-feeding weevil *Mogulones borraginis* (Fabricius) to olfactory and visual cues emitted from *Cynoglossum officinale* L., a rangeland weed in North America, and eight confamilial, native North American non-target plants including four considered threatened and endangered (T&E) in the USA. *Mogulones borraginis* reacted indifferently to *Andersonglossum occidentale* (A.Gray) J.I.Cohen, and was repelled by all other confamilials, including the four T&E plant species. Further, behavioral bioassays were consistent with single-choice and no-choice oviposition tests that could be conducted for non-targets. Using gas chromatography-electroantennographic detection and mass spectrometry, ten electrophysiologically-active volatile organic compounds in *C. officinale* were identified for *M. borraginis*. Among all plant species tested, (-)-α-copaene and (E)-β-farnesene were unique to *C. officinale*. Using electroretinography and a photoradiometer, four electrophysiologically-active wavelengths of light were identified for *M. borraginis* at 350, 430, 640 and 830 nm. Relative reflectance spectra from flowers of tested non-target species differed from *C. officinale* with regard to the bioactive wavelengths. We argue that behavioral ecology and its underlying electrophysiological mechanisms can advance our understanding of the mechanisms for discrimination by specialists among confamilial non-target species and thus improve the predictability of post-release host ranges in biological weed control.

*Oral presentation*

# The role of thistle phylogeny on long-range and short-range host selection behavior of the biocontrol agent *Cassida rubiginosa*

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The oligophagous thistle tortoise beetle, *Cassida rubiginosa* O.F. Müller, was introduced to New Zealand to control the noxious weed *Cirsium arvense* (L.) Scop. We used a phylogenetic approach to study the importance of plant volatiles in the long-range host selection process of female *C. rubiginosa* beetles, and investigated the effect of phylogeny on their feeding and oviposition behavior. A series of dual choice olfactometer experiments testing the target plant *C. arvense* against 15 species of Cardueae and three non-Cardueae species was carried out. Dual choice cage experiments using the same species were performed to test the feeding and oviposition preferences of the beetle. The results showed that as phylogenetic distance from the primary host plant increases, the beetles' preference for alternative hosts decreases. This was the case for all three parameters: olfactory choice, feeding and oviposition preference. Furthermore, larval performance and adult beetle preference were correlated, suggesting that adult choices are adaptive, and that host plant utilization is a highly conserved trait. The emerging picture is a predictive pattern to host utilization and potential impact on non-target plants, or secondary weed targets, across the entire Cardueae tribe.

*Oral presentation*

# Phylogenetic reasoning secures release approval for a biocontrol agent in South Africa, thus circumventing conventional host-specificity testing

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Many species of Australian trees in the genus *Acacia* have become invasive in South Africa and are targets for biological control. Historically, African acacias were considered to be at risk; however, these species have now been assigned to separate phylogenetically-discrete taxa. In 2015, permission was obtained for the release of a midge, *Dasineura pilifera* Kolesik (Cecidomyiidae), in South Africa. The larvae of this midge develop in the flowers of their Australian *Acacia* host plants and induce galls which prevent seed production. In-quarantine testing of these flower-galling midges is not feasible. Further, testing of two related *Dasineura* species had demonstrated that unrealistically stringent containment measures would be required in order to safely conduct out-door sleeve tests. Thus, the case for the release of *D. pilifera* was based on (1) studies of the realized host-range of the midge in Australia, (2) the phylogenetic relatedness of *D. pilifera* to a clade of its congeners and (3) an improved understanding of the patterns of host-use displayed by these midges, including experience gained with two closely-related and highly effective *Dasineura* agents used in South Africa against Australian acacias. Peer-reviewers and regulatory authorities accepted these arguments as evidence of the safety and potential efficacy of *D. pilifera*. Consequently, a rapid turnaround time of less than a year was achieved from inception to importation and release of the midge in South Africa.

*Oral presentation*

# Understanding limits to species-wide demographic generalizations: the ecology and management of *Parkinsonia aculeata*

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The search for generalizations in the face of complex species-environment interactions is particularly important for minimizing the cost of managing populations of species. We tested whether we could generalize, at various nested scales, the species-level demography of the widely invasive plant species *Parkinsonia aculeata* L. (Fabaceae), and whether these generalizations were representative of the demography observed locally. Demographic surveys of all life stages of the species were conducted in 23 Australian sites over seven years (from 2001 to 2007) across a 1,000 km climatic gradient. Sites were nested across four climate regions (arid, semi-arid, semi-wet/dry tropics, wet/dry tropics) and three habitat types (upland, wetland and riparian). We estimated the vital rates at all life stages and size classes (growth/retrogression, survival, fecundity) and combined them to create 91 site-year demographic matrix population models. With these models we then estimated site-year specific asymptotic population growth rates and their corresponding prospective elasticity values to perturbation of the vital rates. We then developed a nested retrospective elasticity analysis (nested LTRE) to test whether and how up-scaling the results (i.e., from site to habitat, to climate region, and to the invaded range) produced information loss, which could lead to spurious interpretations of the relationships between the retrospective elasticity values. The prospective analysis highlighted that site-year variation in the models, population growth rates and corresponding elasticities could not be well summarized by a single species-level analysis, and that the spread was as diverse as found in previously reported multi species demographic analyses. The nested LTRE analysis showed that up-scaling demographic models introduced, for most sites, new sources of errors in the estimation (in terms of magnitude and sign of retrospective elasticities), which increased drastically as we progressively aggregated the demographic information between nested scales of observation. We present our findings in the context of management (including biological control) of *P. aculeata.*

*Oral presentation*

# Demographic modeling of a prospective biological control agent of a weed, the case of *Ophraella communa* and *Ambrosia artemisiifolia* in Europe

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One of the key factors affecting the outcome of weed biological control programs is the ability of the biological control agent (BCA) to build up high population densities in the novel environment. We use the *Ophraella communa-Ambrosia artemisiifolia* system to illustrate how survival, growth and fitness of a BCA vary along the environmental gradient in the introduced range. *Ophraella communa* Le Sage, a chrysomelid beetle native to North America, is considered a successful BCA of *A. artemisiifolia* L. in China. The species was first recorded in Europe in 2013 in northern Italy, from where it has started spreading to neighboring countries. We collected climate-dependent demographic data of *O. communa* on *A. artemisiifolia* in field and laboratory experiments. Along an altitudinal gradient in northern Italy (including temperature differences of approximately 8°C), generation time ranged from 28–73 days, resulting in 1–4 generations per year. Adult mortality and pre-oviposition period were not influenced by climate, while total number of eggs per female per site increased with increasing temperature. Low relative humidity had a highly negative effect on egg hatching success. The variable responses of different life stages of *O. communa* to climate indicated that demographic models of *O. communa* populations need to be stage-structured. The potential implications for management and challenges for predicting the demography of *O. communa* and its potential impact on *A. artemisiifolia* in Europe will be discussed. We stress the importance of humidity-dependence and stage structure in demographic modeling of insects. We furthermore propose that incorporation of the climate-dependent performance of BCAs into process-based demographic models should be used more often to make BCA releases in classical biological control programs more predictive.

*Oral presentation*

# Biological control of *Parkinsonia aculeata*: niche model-based identification of climatically suitable areas for agent release in Australia

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Biological control of invasive weeds relies on the intentional release of host specific natural enemies from the native range of the weed to the invasive range. Climatic conditions can significantly affect distribution and efficiency of biological control agents in the invaded range, limiting their ability in controlling target weeds. The use of climate matching techniques provides an effective tool to prioritize climatically suitable regions in the invasive habitat for the release of biocontrol agents. Native to the Americas, the neotropical tree *Parkinsonia aculeata* L. (Fabaceae) has a wide geographic distribution in its invaded range in Australia, and is considered a significant economic and environmental weed. Among the several candidate agents identified during extensive surveys in the native range in Central America, two geometrid moths, *Eueupithecia cisplatenisis* Prout and *E. vollonoides* Hausmann, were approved for release in 2002. We developed ecological niche models using the occurrence records of the two moths in its native range to project areas in the invaded range of *P. aculeata* likely to be climatically suitable for release of these agents. Using MaxEnt we developed a modeling scheme to optimize selection of model background and other parameter settings. Projected distribution of *E. cisplatensis* in Australia revealed that a small region of southwestern Queensland and parts of northern New South Wales and eastern parts of South Australia within known occurrences of *P. aculeata* were highly suitable for this moth. In contrast, the projected distribution of *E. vollonoides* suggested that it might perform better across northern parts of Queensland, southern parts of Northern Territory, as well as parts of Western Australia. The projections generated in this study are guiding ongoing releases of *E. cisplatensis* and *E. vollonoides* in Australia, and the establishment data gathered as part of post-release monitoring will be used to test these model projections.

*Oral presentation*

# Strengthening the bonds? Investigations into the *Puccinia chondrillina*-*Chondrilla juncea* pathosystem in Australia

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Great efforts are taken to ensure that weed biological control agents are highly host-specific prior to their release into a new country. The long-term genetic stability of the agent is often questioned but rarely scientifically tested. Here, we use the *Puccinia chondrillina* Bubák & Syd. (rust fungus) and *Chondrilla juncea* L. (skeleton weed) pathosystem in Australia to explore such issues. Three clonal forms of *C. juncea* exist in Australia: narrow-, intermediate- and broad-leaf forms. Isolates of *P. chondrillina* specific to the narrow- and intermediate-leaf forms were previously released in Australia as biological control agents against skeleton weed. *Puccinia chondrillina* isolate IT32, released in 1971 to target the narrow-leaf form, showed exceptional impact in controlling this form. Three additional isolates targeting the intermediate-leaf form were subsequently released: TU21 in 1980, recorded soon after as not having persisted; IT36 in 1982, recorded as having a moderate impact; and TU788 in 1996, for which the impact is unknown. Illumina genome sequencing of these isolates and of isolates collected on both narrow- and intermediate-leaf forms in 2007 and 2016 is being used to determine the lineage of the contemporary *P. chondrillina* isolates, and to identify genetic changes that may have occurred over time. Concurrently, each contemporary isolate has been tested on the three forms of the weed to confirm its pathogenicity phenotype. Results indicate that all six narrow-leaf form isolates were specific only to this form. The five intermediate-leaf form isolates were highly pathogenic to the intermediate-leaf form, but four of them also infected, albeit to a much lesser extent, the narrow-leaf form. Infection of the narrow-leaf form by the intermediate-leaf form isolate (IT32, IT36) was observed during testing in a controlled-environment prior to its release in Australia. None of the isolates infected the broad-leaf form.

*Oral presentation*

# Molecular investigations into the association of cactus biotypes and cochineal lineages: implications for biocontrol

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Molecular-based approaches have much to offer in providing both pre- and post-release information, clarifying organisms' identities, addressing agent selection prior to release and predicting agent establishment. Taxonomic clarification of both the target weed and the control agent status is an important aspect for understanding plant-insect associations in biocontrol efforts. *Opuntia engelmannii* Salm-Dyck ex Englem. (Cactaceae), native to North and Central America, is a problematic invasive species in South Africa, Kenya and other countries. Although no specific biocontrol agents have been introduced against *O. engelmannii* in South Africa, the cactus moth (*Cactoblastis cactorum* [Berg]) and cochineal insects (*Dactylopius* spp.) have colonized it. However, neither of these agents causes sufficient damage to reduce *O. engelmannii* infestations. Therefore, there is need to find effective host-specific biocontrol agents against this weed. Microsatellite markers were used to clarify the taxonomy of morphologically similar *O. engelmannii* biotypes, investigating their genetic diversity in South Africa and Kenya (introduced ranges), and North America (native range). Similarly, genetic diversity and characterization of *D. opuntiae* (Cockerell) biotypes collected from ten different *O. engelmannii* populations in the USA (native range) was done using amplified fragments length polymorphisms (AFLPs). This will shed light on either a new association or long-term established relationship between the various *O. engelmannii* biotypes and the different lineages of the cochineal insects. Moreover, this information will lead to a better understanding of the underlying reasons for successful biocontrol of *Opuntia* using cochineal insects.

*Oral presentation*

# Molecular analysis of ecological interactions for optimizing biocontrol of the invasive weed *Sonchus oleraceus* L. (Asteraceae) in Australia

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Community ecology is a promising approach for optimizing biological control of weeds. The characterization of ecological interaction networks associated with the target plant in its natural context allows deciphering the complexity of interactions within arthropod communities and gives precious clues on potential undesirable effects induced by perturbing the communities through the introduction of biocontrol agents. Based on this approach, several research questions are addressed: (1) What is the diversity of members of the community, and what is the specificity of the herbivores feeding on the plant? (2) Is there a top-down control of herbivores by natural enemies? (3) Are there differences in network structure between geographical areas? Our study targets sowthistle, *Sonchus oleraceus* L., which is native to Europe but invasive in Australia. The development of herbicide resistance makes it extremely difficult to manage in its invasive area. As an alternative to pesticide use, biocontrol solutions are being explored via a collaborative research program between Montpellier SupAgro and CSIRO. First steps of the project focus on the molecular characterization and comparison of the ecological networks among three different climatic regions in its native range. This is done using cutting-edge molecular approaches (i.e. multiplex metabarcoding through NGS) to reveal interactions from arthropod gut-content. Network metrics such as species richness, linkage density or connectance are computed and compared between sites to assess differences in levels of complexity among changing environments. The study will be further expanded to Australia in order to confront results between invasive and native ranges, and contribute to a better assessment of risks and potential changes induced by classical biocontrol introductions.

*Oral presentation*

# Insect thermal tolerance: methodologies, trait plasticity and links to biocontrol agent efficacy

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Biological control practitioners rely heavily on the determination of insect thermal tolerance to predict post-release establishment and, to some extent, post-release agent success. Traditionally, this has been achieved through the use of critical thermal range and lethal limit testing, as well as developmental rate determination at varying temperatures. Predictive efforts using these methods have been mostly accurate, but some examples exist of unpredicted establishment successes or failures. With the large effect that thermal regimes have on insects, many other entomological fields, most notably agricultural and medically important pest outbreak modeling, also investigate thermal tolerances. These researchers seem miles ahead of biocontrol practitioners, however, as they seem to focus more on the effects of temperature on individual physiology over the much broader-reaching aspects of tolerance investigations completed by the latter biologists. Specifically, the thermal effects on metabolism, a commonly investigated topic in pest management, is one not touched on in biocontrol literature. This is surprising, as metabolic changes have significant effects on feeding behavior, an important component of agent efficacy. Preliminary investigations completed by the author into metabolic rates over a range of temperatures in a field-established South African biocontrol agent (*Neochetina eichhorniae* Warner [Coleoptera: Curculionidae]) of water hyacinth (*Eichhornia crassipes* [Mart.] Solms) have illustrated that metabolic rates can differ between populations, despite no clear differences in their thermal tolerances as determined using classical methods. This raises questions as to the efficacy of these different agent populations in controlling their target weed, as well as the extent of plasticity of their physiological traits. Currently, a variety of other investigations are being completed by the author and other collaborators to corroborate the recorded metabolic differences and determine whether they do have an effect on feeding and digestive traits of the different populations. Furthermore, the work aims to determine how much this metabolic rate can be forcibly adapted to produce more effective agents for release elsewhere.

*Oral presentation*

# Gene tech-based next generation biological control of weeds: what could it look like?

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After over one hundred years of successful classical biological control using natural enemies, we are now on the cusp of a potential new era for biological control with the advent of access to easy and accurate gene-editing technologies. It is now possible to develop biocontrol agents that are small pieces of RNA of targeted changes to DNA either exogenously applied onto or endogenously incorporated into the target host and propagated through the invasive populations. Groundbreaking new genetic approaches like RNAi and gene drive open new possibilities for tackling intractable weeds species and resistant genotypes. This presentation explored these technological approaches in the context of weeds and imagine the future possibilities for next generation weed biological control. Future possibilities for managing weeds are now possible in ways we wouldn't have dreamed of even at the start of this millennium. Is this a chance for biologists to provide solutions to control weeds once and for all?

*Poster presentation*

# Agent specificity: can genetic signatures help us to select specialist agents?

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Risk assessment for non-target effects of biocontrol agents is an important component in classical weed biocontrol. Substantial resources are spent on risk assessment alone; host-specificity testing is often the most time-consuming and expensive task in weed biocontrol programs. A large number of studies are focusing on diet breadth in insects to understand, for example, what makes them a generalist/specialist by profiling genes/gene families involved in the detoxification process of toxic phytochemicals (e.g., cytochrome p450). Similar approaches may help us distinguish specialist from generalist agents, and this can inform the likelihood of their specificity. However, in an evolutionary context, insects are regarded as specialist even if they feed on a few genera or on plants within a single family, whereas the regulatory requirement for biocontrol agents require the agent to be almost restricted to the target weed species. In this poster, we highlighted other genetic signatures, in addition to detoxifying enzymes, which can possibly help us anticipate (and maybe even predict) specificity of insects in the context of weed biocontrol.

*Poster presentation*

# A biogeographical comparison of herbivory on *Phragmites australis*

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Biological control of the European subspecies of *Phragmites australis* (Cav.) Trin. ex Steud. that has invaded across much of North America is promising. As a result, a petition is underway to approve field release within the USA of two extremely host-specific stem-mining noctuid moths: *Archanara geminipuncta* Haworth and *A. neurica* Hübner. However, before introducing biocontrol agents to a novel range, we must first broadly understand how herbivores attack and impact *Phragmites* within natural field settings. To this end, stems were collected and dissected from more than 75 *Phragmites* populations across Europe and North America. Damage on each stem was then attributed to specific herbivore species (> 20 monophagous species within Europe, but < 5 monophagous species within North America). Within both ranges, we estimated attack rates (for each sampled quadrat) and impact (height of attacked vs. unattacked stems within each quadrat) of each herbivore species. By analyzing how biogeography, origin, stem and population characteristics influence herbivore attack rates and impact, we can better determine where releases of *Archanara* will have the highest efficacy, as well as whether (and where) non-target impacts on the native subspecies should be of concern. Moving forward, our data can be integrated with other pre-release studies to better inform demographic models, which represent an important tool for predicting the efficacy of biocontrol agents and their environmental safety both pre- and post-release.

*Poster presentation*

# Risks and decisions: is *Leptinotarsa texana* suitable for biological control of silverleaf nightshade in Australia?

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The leaf beetle *Leptinotarsa texana* Schaeffer was introduced to South Africa from the USA to control silverleaf nightshade, *Solanum elaeagnifolium* Cav. Subsequent post-release studies in South Africa found the beetle to be an effective, host-specific biological control agent of silverleaf nightshade. *Leptinotarsa texana* has potential for biological control of silverleaf nightshade in other countries where the weed adversely impacts agricultural production, including Australia. However, *L. texana* can only be introduced to Australia if risk analysis demonstrates that the agent poses a negligible or very low risk to the environment and economy. We collated evidence of the agent's possible impact in Australia by conducting quarantine laboratory experiments on 49 Australian native species and economically important plant species and cultivars, and field experiments with eggplant *S. melongena* L. in the beetle's native range of Texas, USA. In laboratory experiments, we observed feeding damage greater than 50% leaf area removed on plants of 12 Australian *Solanum* spp. and two crop species (eggplant and potato [*S. tuberosum* L.]). *Leptinotarsa texana* successfully developed from first instar larva to adult on 15 Australian *Solanum* spp. and two crop species (a single eggplant cultivar and four potato cultivars). When given a choice of silverleaf nightshade and 10 Australian *Solanum* spp. in a large cage experiment, *L. texana* oviposited on silverleaf nightshade and three of the non-target Australian *Solanum* spp. Despite utilizing eggplant in quarantine laboratory experiments, *L. texana* did not oviposit on eggplant in a native-range field experiment. We conclude that *L. texana* is not suitable for introduction to Australia. Nonetheless, we propose novel methods to assess and communicate the risk of introducing *L. texana* to other countries where silverleaf nightshade occurs.

*Poster presentation*

# A weevil's choice: how accurately do pre-release behavioral bioassays predict post-release host selection?

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Discrepancies between fundamental and ecological host ranges of biological control candidates speak to the importance of comprehensively understanding the host plant selection behavior of the candidate. Though such host selection behavior may be tested mechanistically or physiologically within the lab, corroborating lab results with field behavior is critical to delineating the ecological host range and its pre-release predictability. Here, we test whether chemically based inferences drawn from laboratory studies of host selection by a potential weed biological control agent can be validated in a field setting. *Mogulones crucifer* (Pallas) (Coleoptera: Curculionidae) is a root-mining weevil that has been released in Canada for the biological control of *Cynoglossum officinale* L. (Boraginaceae), yet is still prohibited for release in the USA. Behavioral bioassays have revealed that volatiles of certain native North American plants within *M. crucifer*'s fundamental host range are not attractive or are repellent to *M. crucifer* under laboratory conditions. To test the weevil's responses to these plants in the field, we conducted choice tests in which *M. crucifer* adults could settle on plants within pure stands of *C. officinale* vs. within mixed stands of *C. officinale* and one of four confamilial plants found to be repellent or unattractive in the laboratory. This poster reported settling by the weevils on *C. officinale* and the alternative hosts in these stands, interpreted in light of the prior laboratory results. The implications for the accuracy and applicability of laboratory bioassays in predicting post-release pre-alightment host selection behavior of biological control candidates were discussed.

*Poster presentation*

# Volatiles from congeners of its host plant are repellent to a candidate biological control agent

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Host-specificity testing of weed biological control candidates relies on bioassays to assess their feeding and development on potential non-target plant species. The ecological host range of a potential agent, however, also depends upon its pre-alightment responses to cues from target and non-host species. Delineating these responses could improve the accuracy of pre-release assessments. Previously, we found that during host finding *Mogulones crucifer* (Pallas) (Coleoptera: Curculionidae), a root-miner that was released in Canada in 1997 for the biological control of the invasive plant *Cynoglossum officinale* L. (Boraginaceae), strongly preferred volatiles from *C. officinale* over those from eight North American non-target plant species in a four-arm olfactometer bioassay. The non-targets evaluated included four that are threatened and endangered in the USA. Here, we present data using the same experimental design detailing the responses of *M. crucifer* to volatiles of three Eurasian congeners of *C. officinale*: the Mediterranean-distributed *C. creticum* Mill., the ornamental *C. amabile* Stapf & Drummond and *C. germanicum* Jacq., which overlaps in distribution with *C. officinale*. We found that *M. crucifer* females were repelled by volatiles from all three congeners relative to *C. officinale* or purified air in the bioassay. Although these congeners of *C. officinale* are within the fundamental host range of *M. crucifer*, there are no reports of them being used as hosts by *M. crucifer.* Our data propose a mechanistic explanation of why in its native range, *M. crucifer* is a near-monophagous specialist on *C. officinale* that discriminates against the closest relatives of its host plant prior to alightment by being repelled by volatiles of those closely-related non-hosts. These findings show how ecological host ranges can be maintained and call into question the reliance on the fundamental host range when assessing the environmental safety of biological control candidates.

*Poster presentation*

# Influence of host plant on wind dispersal by an eriophyid mite, *Aceria salsola*

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Host specificity testing of prospective biological control agents typically involves choice and no-choice testing. However, eriophyid mites normally disperse by wind, so it is difficult to design an experiment that would realistically enable a mite to choose between two host plants. It may be more practical to measure the propensity of mites to disperse away from preferred or non-preferred host plants. *Aceria salsolae* de Lillo and Sobhian is a prospective biological control agent for Russian thistle, *Salsoa tragus* L. We tested adult mites in a small wind tunnel and recorded the number of mites remaining on five species of plants. Wind reduced the number of mites on all plants, but had a greater effect on non-target plants. The population remaining after 42 hours on the natural host plant, *S. tragus*, was 13% lower in the wind treatment than in absence of wind. On the non-target plants, the population was reduced by 52 to 86%. The results indicate that mites more readily disperse from non-target plants, but that many remain even after a 43-hour period under laboratory conditions.

*Poster presentation*

# Demographic matrix model for knapweeds (*Centaurea* spp.)

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Plant demographic models, such as stage-based matrix models, are useful in identifying life stage transitions that contribute the most to population growth of an invasive plant and hence should be targeted for disruption by biological control and/or other control tactics. This information can inform the selection of effective biological control agents in either a prospective or retrospective manner. We are in the process of parameterizing a four life-stage matrix model (seeds, seedlings, vegetative juveniles and flowering plants) in order to quantify reductions in various demographic or vital rates that are needed to cause population decline in two *Centaurea* species in the northeastern United States. Spotted knapweed (*C. stoebe* L. sens. lat.) is a short-lived perennial that is widespread in North America, although not as common in New York State. Meadow knapweed (*C.* x *moncktonii* C. Britton = *C. jacea* L. nothosubsp. *pratensis* [W.D.J. Koch)] Čelak) is a long-lived perennial hybrid that is abundant in New York State in moist pastures and grasslands. Biological control agents currently established in the region are ineffective. We are quantifying demographic transitions over three years of both knapweed taxa in New York State (*N* = seven populations). Vital rates being estimated include survival, germination, transitions to other life stages and fecundity (filled seeds produced per plant). This poster presented data on model parameters derived to date.

*Poster presentation*

# *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae): reassessing its potential as a Brazilian peppertree biological control agent

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Brazilian peppertree (BP), *Schinus terebinthifolia* Raddi (Anacardiaceae), is a South American weed invading natural and agricultural areas of Florida, Hawaii and Texas (USA). Biocontrol research of BP began in the 1950s, continued in Florida between 1980s–1990s and again from 2004 to present. The defoliating sawfly *Heteroperreyia hubrichi* Malaise has been studied as a promising BP biocontrol agent in Brazil, Hawaii and Florida. Like other Pergidae species, *H. hubrichi* was known to contain toxic peptides, which delayed its further consideration as a biocontrol agent for its potential for poisoning wild and domesticated animals that may consume the insect larvae. Toxicity studies, native range host plant use and adult oviposition no-choice tests on previously untested Anacardiaceae/Rutaceae species were conducted. Several peptides (e.g., pergidin) were found in H. hubrichi, but in much lower concentration compared with other toxic sawfly species (e.g., *Lophyrotoma*). Toxicity and behavioral studies will be needed on vertebrates and invertebrates to determine environmental safety of *H. hubrichi*. Four field trips visiting 21 sites along BP's native range in Northeastern Argentina were made between 2016 and 2017. A total of 399 *H. hubrichi* larvae were found by checking 815 *S. terebinthifolia*, 86 *S. lentiscifolius* Marchand, 34 *S. longifolia* (Lindl.) Speg., 200 *S. molle* L., 86 *S. weinmannifolius* Engl., 40 *Astronium balansae* Engl., 300 *Lithrea molleoides* (Vell.) Engl. (Anacardiaceae) and 39 *Zanthoxylum rhoifolium* Lam. (Rutaceae). Larvae of *H. hubrichi* were only found feeding on BP and the closely related species *S. weinmannifolius*. Laboratory no-choice oviposition tests on cut shoots revealed that *H. hubrichi* can oviposit on native South American Anacardiaceae (*Schinus* spp., *Lithrea* sp. and *Astronium* sp.) and on the exotic economically important *Pistacia integerrima* J. L. Stewart. To evaluate the risk posed by *H. hubrichi* on non-target species, further testing including no-choice oviposition and larval development tests on live plants of South and North American Anacardiaceae species is needed

*Poster presentation*

# Defoliating Geometridae caterpillars as potential weed biological control agents

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*Schinus terebinthifolia* Raddi (Anacardiaceae) is a fast-growing invasive species that decreases biodiversity in its invasive range. Because mechanical and chemical means of control prove to be costly and time-consuming, classical biological control is being considered as an ecologically sound and cost-effective supplement to these methods. In *Schinus*' native range, the weed is estimated to be consumed by more than 150 phytophagous species. Of the many insect families observed feeding on *Schinus*, numerous species of Geometridae (Lepidoptera) have been recorded. The Geometridae are the second largest family of Lepidoptera, consisting of approximately 23,000 species. As defoliators, geometrid caterpillars are potential biological control agents of weeds. Worldwide, a total of nine geometrids have been released against six species of weeds, including releases in the United States, Canada and Australia. Four species have established field populations and safely damage their respective target weeds to varying degrees. Surveys of *Schinus* in its native range have revealed approximately 20 geometrid species. Due to the relatively large size of the larvae and their capacity for severe defoliation of the weed, several were investigated as potential biological control agents. Four of these geometrids—*Oospila pallidaria* Schaus, *Oxydia vesulia* Cramer, *Hymenomima* nr. *memor*, and *Prochoerodes onustaria* (Hübner)—were successfully colonized in quarantine at the USDA IRPL. In order to test their specificity for *Schinus*, all species were subjected to no-choice starvation tests involving native and economically valuable members of the Anacardiaceae. Unfortunately, all species exhibited broad feeding habits and developed to adult on many of the plants tested. Therefore, these species will not be released as biological control agents on *Schinus* in the United States.

*Poster presentation*

# Assessing efficacy and risk with plant demographic models: examples from the water chestnut biocontrol program

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Outcomes that need to be evaluated during any biological control program include the demographic impacts an agent may have on the target organism as well as potential population-level risks to non-target species. We argue that use of plant demographic models to project the population growth rates of target and non-target species in the presence and absence of herbivore attack offers a defensible and quantitative approach for evaluating both of these outcomes. Here, we detail our work applying these concepts to the biological control program for water chestnut, *Trapa natans* L., an aquatic annual plant species that is problematic in northeastern North America. The life cycle of water chestnut was collected by monitoring individual plants over two growing seasons. The data was then integrated into periodic matrix models to forecast the population-level response of water chestnut to varying intensities of herbivory by *Galerucella birmanica* Jacoby, the leaf-feeding beetle and potential biocontrol agent. Previous native range laboratory studies demonstrated that the beetle can reduce seed output of water chestnut by 80%. Our model results indicate that even a 40% reduction in seed output will result in target population growth rates below 1, signaling a declining population and a biological success. We also discuss how demographic models can augment traditional risk assessments to non-target species and help determine ecological successes of biocontrol programs.

*Poster presentation*

# WILL PARASITOIDS RELEASED TO CONTROL *LILIOCERIS LILII*, LILY LEAF BEETLE, ATTACK CONGENERIC WEED BIOLOGICAL CONTROL AGENTS?

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Biotic resistance may prevent the establishment of a weed biological control agent or reduce its efficacy. We investigated the potential for biological control agents of *Lilioceris lilii* Scopoli (Coleoptera: Chrysomelidae) to impact congeneric weed biological control agents. The Eurasian insect *L. lilii*, lily leaf beetle, was introduced to North America in 1945, where it is a pest of cultivated and native lilies and *Fritillaria* spp. Three parasitoid species have been released for control of *L. lilii* in the USA since 1998: *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), *Lemophagus errabundus* Gravenhorst and *Diaparsis jucunda* (Holmgren) (Hymenoptera: Ichneumonidae). Releases of *L. cheni* Gressitt & Kimoto, a biological control agent of the invasive weed air potato, *Dioscorea bulbifera* L. (Dioscoreaceae), began in Florida, USA in 2011. Although the ranges of these beetles and parasitoids do not yet overlap, they are all undergoing range expansion, and the *L. lilii* parasitoids can attack a few *Lilioceris* species in their native range. Our objectives were to determine if parasitoids of *L. lilii* could successfully parasitize *L. cheni*, and, if so, to design a strategy to decrease the impact of the parasitoids on L. cheni. We conducted no-choice and choice tests with the three parasitoid species and larvae of the two *Lilioceris* species. In no-choice tests, up to 17.5% of *L. lilii* larvae were parasitized, with successful attack by all three species, but no *L. cheni* larvae were parasitized by any of the parasitoid species. In choice tests, parasitoids attempted to oviposit in up to 53.3% of *L. lilii* larvae but no *L. cheni* larvae. These results suggest that the parasitoids for *L. lilii* will not pose a risk to the effective air potato biological control agent *L. cheni*. Similar tests will be conducted with the parasitoids and *L. egena* (Weise), which is under consideration for release as an additional biological control agent of air potato.

*Poster presentation*

# *Generations*: understanding weed-herbivore interactions using Python

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Tools for modeling plant-herbivore interactions help to predict the establishment and impact of imported biological control agents on target weed populations. These interactions are often strongly affected by complex life-history traits of invasive plants and associated herbivorous agents. *Generations* is an open-source Python package containing customizable modules for understanding weed-herbivore systems. *Generations* employs functional programming to provide a set of equations and parameters to model the response of a seedbank-dependent plant to an herbivorous biological control agent. The package includes a baseline model for a coupled plant-herbivore interaction described by Buckley et al. (2005; J. Appl. Ecol. 42:70-79), along with a modified version of that model incorporating stage-structured interactions with a biennial plant. We present an example of *Generations* applied to interactions between the invasive biennial weed, garlic mustard (*Alliaria petiolata* [M.Bieb.] Cavara & Grande), and a univoltine biological control weevil, *Ceutorhynchus scrobicollis* Neresheimer & Wagner.

*Poster presentation*

# Multiple Crossed Generations, a novel method to evaluate the performance of a thrips on two invasive species of *Ludwigia* (Onagraceae)

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*Ludwigia grandiflora* subsp. *hexapetala* (Hook. & Arn.) G.L. Nesom & J.T. Kartesz and *L. peploides* subsp. *monteviensis* (Spreng.) Raven are two invasive weeds of South American origin that have been taken as ornamentals to different regions. In France, *L. g. hexapetala* is considered as the worst invasive aquatic weed. In the USA, these plant species alter aquatic environments by obstructing lakes, ponds, irrigation canals and other sensitive wetlands. In 2007, FuEDEI began the search for natural enemies of *L. g. hexapetala* highlighting *Liothrips ludwigi* Zamar as the most promising candidate. In host specificity tests, *L. ludwigi* was highly specific for *L. g. hexapetala* and *L. p. montevidensis* and was able to persist on both species, completing their development. Both of these species coexist as invasive plants in the USA, France, England, Belgium, Italy and the Netherlands. Therefore, the objective of this work was to evaluate the performance of *L. ludwigi* on these two species to improve management strategies in a more efficient and safe way. When confined to the laboratory for host specificity testing (either in the native range or quarantine), potential biological control insect agents are reared only on the target weed for multiple generations. If the insect is influenced by the plant on which the previous generation was reared, the results can be biased. Understanding this effect is important in order to correctly interpret the experimental results and to study potential biological control agents in particular. Hence a novel method called Multiple Crossed Generations was developed to evaluate the survival and fecundity of *L. ludwigi* that was reared on *L. g. hexapetala* for several generations. The mortality and fecundity recorded indicated a better performance of the thrips on *L. p. montevidensis*. The interaction between crossed generations was not significant suggesting that, except on a specific combination, maternal effects did not influence the results.

*Poster presentation*

# The integrative taxonomy in classic biological control of weeds: *Metaculus* sp. on *Isatis tinctoria*, a case of study

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Classical biological control of weeds depends on finding agents that are host-specific, and this requires a correct understanding of their identity. Integrative taxonomy, which combines genetic and morphological analysis and sometimes other characters such as behavior and physiology, makes it possible to verify the traditional morphological identifications as well as to reveal that some arthropod species previously thought to be polyphagous really consist of multiple cryptic species, or biotypes, some of which are more host-specific than others. *Metaculus rapistri* (Acari: Eriophyoidea) was initially described by Carmona (1969) from samples of *Rapistrum rugosum* (L.) All. collected in Portugal (hereinafter *M. rapistri* ex *R. rugosum*). A supplementary morphological description of this species was provided by Monfreda and de Lillo (2012) from samples of *Isatis tinctoria* L. collected in Turkey (hereinafter *M. rapistri* ex *I. tinctoria*). These authors avoided the institution of a new species on *I. tinctoria* because not enough morphological differences were recorded. Therefore, an integrated approach based on the combination of morphological and genetic analyses was chosen to further investigate these *Metaculus* species. In the molecular analysis, there was an 8.4–9.0% divergence (p-distance of 580 bp of the COI region) between *M. rapistri* ex *R. rugosum* and *M. rapistri* ex *I. tinctoria*, indicating two distinct host-associated species are present. Moreover, in 2017 *M. rapistri* ex *I. tinctoria* was recorded on *I. tinctoria* in two other localities, Italy and Germany. These new results indicate that a species thought to be oligophagous could be a complex of host-associated cryptic species. *Metaculus rapistri* ex *I. tinctoria* is a new species considered as a potential biological control agent of *I. tinctoria*. This new record points out the importance of an integrative taxonomy approach in the biological control of weeds to avoid the rejection of some potentially good agents or the release of the wrong ones.

*Poster presentation*

# Examining pre-alightment host selection of a potential biological control agent of dyer's woad to cues of non-target confamilial plants

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Pre-release assessments of the host range of biological weed control candidates typically rely on choice and no-choice feeding and development tests. However, determining a candidate's fundamental host range with no-choice tests provides a limited understanding of its pre-alightment host-selection behavior and may exclude environmentally safe biocontrol candidates from consideration because in such tests, candidates may develop on plants they would not seek out post-release. Accurately determining a candidate's post-release host range, and thus its potential as a biocontrol agent, requires understanding its pre-alightment host selection behavior. Here we assess the host selection behavior of the seed-feeding weevil *Ceutorhynchus peyerimhoffi* Hustache, a potential biological control agent for *Isatis tinctoria* L., a Eurasian mustard that is invasive in the western USA. Oviposition and developmental tests of 114 test plant species indicate *C. peyerimhoffi* has an extremely narrow fundamental host range. Development occurred on three confamilial plant species of *I. tinctoria*: *Braya alpina* Sternb. & Hoppe, *Caulanthus heterophyllus* (Nutt.) Payson, and the federally-listed threatened and endangered *Boechera hoffmannii* (Munx) Al-Shehbaz. To determine the risk of post-release non-target attack on these species, we compared behavioral responses of *C. peyerimhoffi* to olfactory and visual cues from these non-targets, *I. tinctoria* and purified air using a double-stacked y-tube device (D-SYD). We also identified bioactive volatile organic compounds (VOCs) in the headspace of *I. tinctoria* for *C. peyerimhoffi* by combining gas chromatography-mass spectrometry (GC-MS) with electroantennographic detection (EAD). We discuss our results in the context of the environmental safety of *C. peyerimhoffi*.

*Poster presentation*

# A novel approach to host-specificity testing for non-target plant species restricted to highly specialized soil types

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The Eurasian weevil *Ceutorhynchus cardariae* Korotyaev is a stem and petiole gall-forming biological control candidate of the clonal Eurasian mustard *Lepidium draba* L. which is invasive in the western USA. Conventional pre-release feeding, oviposition and development tests have been conducted on 156 test plant species to assess the environmental safety of the weevil. Based on these tests, *C. cardariae* is considered safe for release, with the potential exception of development on certain species in the native North American genus *Streptanthus*. Many *Streptanthus* species are endemic to serpentine soils, which are characterized by low-nutrient content and high concentrations of various heavy metals. However, for all pre-release host-specificity tests, *Streptanthus* plants were propagated in nutrient-rich, standardized horticultural soils, ignoring research that has shown that serpentinic soils have herbivory-repelling effects. Here, we present data on the performance of *C. cardariae* on four native North American *Streptanthus* species propagated in their native serpentine soils versus the standardized horticulture soils: *S. inflatus* (S. Watson) Greene, *S. flavescens* Hook., *S. anceps* (Payson) Hoover and the threatened and endangered *S. albidus* Greene subsp. *albidus*. In addition, we measured the performance of *C. cardariae* on *L. draba* propagated in both soil media as control. This poster discussed the effects of serpentinic soil on both plant and biocontrol candidate performance, as well as implications for the environmental safety of *C. cardariae* with regard to *Streptanthus* species.

*Poster presentation*

# Alternative methods to evaluate the host range of *Melanagromyza albocilia* for the biological control of field bindweed in North America

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Field bindweed, *Convolvulus arvensis* L., is a perennial vine native to Eurasia. It was introduced into North America in the 18th century. It outcompetes native North American forbs and grasses and is considered a noxious weed in agricultural fields. *Melanagromyza albocilia* Hendel is a small agromyzid fly that is being evaluated for the biological control of field bindweed in North America. Females oviposit under the epidermis of field bindweed leaves. Larvae mine towards the base of the stem or into the root where they pupate. The fly has only been recorded from field bindweed in Europe. Studies on the physiological host range of *M. albocilia* under confined conditions have proven challenging. Several test plants were accepted for oviposition, but larval development in captivity is unreliable. Rearing in the lab has thus far failed, and the process of obtaining adults for testing is very time consuming. We therefore started conducting open-field tests at a site in southern Germany where the fly is abundant. In 2017, none of the four exposed test plant species was attacked, and attack rates of exposed field bindweed plants were similar to attack rates of naturally-growing field bindweed. A similar test was established in 2018, and results were presented in this poster. Also discussed was the option to determine the host range of *M. albocilia* using open-field tests as an alternative to caged development tests.

*Poster presentation*

# Using native congeners as "surrogates" to identify false positives in host specificity testing

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An insect's fundamental host range, defined by host-range testing, is usually broader than its ecological host range. However, without field releases, it can be difficult to identify which plants are viable ecological hosts and which plant species are only accepted as hosts within the testing environment (i.e. false positives). When false positives from testing are suspected, additional insight into the ecological host range of an agent may be available by considering the host range of congeneric species. Here, we compare the host-testing results of a potential European biocontrol agent and two North American (NA) congeneric species. The European species is the beetle *Chrysochus asclepiadeus* (Pallas), which was initially considered for biocontrol of swallow-worts, *Vincetoxicum* spp. The two congeneric species are *C. auratus* (Fabricius), which feeds on dogbane in eastern NA, and *C. cobaltinus* J.L.LeConte, which is found on native milkweeds and dogbane in western NA. As a root feeder, *C. asclepiadeus* has significant impact on target weeds. However, host testing was stopped when results showed it was able to oviposit, develop and feed on NA milkweeds. Host specificity testing of NA *Chrysochus* spp. demonstrates that both species can feed, oviposit and develop on milkweeds in choice and no-choice tests. However, only *C. cobaltinus* uses milkweed as an ecological host, and *C. auratus*' use of milkweed in tests is a clear "false-positive." The host-specificity results of the European *C. asclepiadeus* align more closely with *C. auratus* than with *C. cobaltinus*. Additionally, both our field surveys and greenhouse experiments demonstrate that ovipositional preference by NA *Chrysochus* spp. under confined conditions is not a good indicator of ecological host use. These results suggest that additional open-field testing of *C. asclepiadeus* on NA milkweeds in Europe is merited; comparing these results with field testing of NA congeners may better define the risks of release of the European species.

*Poster presentation*

# Geographic and genetic variability of disease resistance in flowering rush in the USA

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Large-scale plant invasions may reflect regional heterogeneity in biotic limiting factors such as plant competition, natural enemy attack or genetic variation within and between invading populations. Information on biotic resistance varies spatially and can be especially valuable when implementing a biological control program because introduced agents may have differential impacts through interactions with host genotype, local environment or novel enemies. We conducted field surveys and greenhouse/laboratory studies to determine whether there was evidence of biotic resistance, in the form of disease, in two introduced genotypes (G1, G4) of the Eurasian wetland weed *Butomus umbellatus* L. in the USA. We tested whether genotypes differed in disease attack and whether spatial (latitudinal and longitudinal) patterns emerged for either genotype. First, we surveyed 27 populations (17 G1, 10 G4) across the USA distribution to document disease occurrence and pathogen species associated with plants. For a subset of populations, we isolated pathogenic foliar fungi and then tested pathogenicity of three isolates in laboratory and greenhouse assays. After accounting for location, G1 plants had a lower disease incidence than G4 plants in the field (38% vs 70%) but no difference in fungal richness. Despite these results, our bioassays revealed that G1 plants consistently received a higher damage score and larger leaf lesion regardless of pathogen isolate. The seemingly contradictory results between pathogen incidence in the field and pathogeneticity in the laboratory/greenhouse may be due to differential susceptibility during *B. umbellatus* development stages or environmental differences between areas that limit the regional pool of pathogens or their effect on plant genotypes. These results demonstrate that the two widespread *B. umbellatus* genotypes have differential susceptibility to the pathogens tested here and that potential pathogen biological controls may be more effective against G1 plants.

*Poster presentation*

# Some like it hot, some like it cold: thermal plasticity of biological control agents enhances establishment

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Aspects of the thermal physiology of the water hyacinth mirid, *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), have been extensively investigated over the past 20 years in an attempt to understand and improve establishment. Recently, we incorporated the plastic nature of insect thermal physiology into models of agent establishment. This study determined whether season and locality influenced the thermal physiology of two field populations of *E. catarinensis*: one collected from the hottest establishment site, and one collected from the coldest establishment site in South Africa. The thermal physiology was significantly influenced by season and site, demonstrating that the thermal physiology of *E. catarinensis* is plastic under field conditions. We then determined whether cold hardening under laboratory conditions was possible. Successfully cold-hardened *E. catarinensis* had a significantly lower critical thermal minimum ($CT_{min}$) compared to the population from the coldest establishment site. This suggests that cold hardening of agents could be conducted before release to improve their cold tolerance and increase their chances of establishment, allowing for adaptation to colder climates in the field. Increasing establishment of the most effective agents will decrease the number of agent species needed in a biological control program, thus encouraging a more parsimonious approach to biological control.

# Session 5: Making classical biological control more predictive: moving from ecological to evolutionary processes

**Session Chair: Marie-Claude Bon**

**Keynote**

# Predicting benefits and risks of biological control of the invasive common ragweed in Europe: from ecological to evolutionary studies

Heinz Müller-Schärer[1]*, Yan Sun[1], Maria Litto[1], Sarah Bouchemousse[1], Suzanne Lommen[1], Urs Schaffner[2]

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Balancing benefits with risks is key in developing a successful biological control program. In 2013 we were confronted with the accidental introduction of the North American native ragweed leaf beetle *Ophraella communa* Le Sage (*Ophraella* in the following) into Europe, which needed an urgent decision on how to respond to this unforeseen arrival of an oligophagous insect that is potentially an effective biological control agent of common ragweed, *Ambrosia artemisiifolia* L. (*Ambrosia* in the following). In the frame of the then newly formed EU-COST SMARTER consortium (FA1203 on "Sustainable management of *Ambrosia artemisiifolia* in Europe"), we immediately reacted to this event by creating an Ophraella Task Force composed of specialists in weed and invasive species management, ecology, aerobiology, allergology and economics (Müller-Schärer et al., 2018).

Like no other plant, the North American-native *Ambrosia* has raised the awareness of invasive plants in Europe, causing great damage to our society due to its highly allergenic pollen and as an important and hard-to-control crop weed (Essl et al., 2015). *Ophraella* has the potential to reduce the population density and pollen production of ragweed in Europe and to halt its further spread, but it also bears the risk of attacking the taxonomically closely-related sunflower and native endangered species (Müller-Schärer et al., 2014). From the locations in the Milano area where the beetle was initially found in 2013, *Ophraella* expanded its range by 2017 to some 500 km eastwards to Slovenia and Croatia, 200 km westwards to the French border and approximately 150 km both north to near Bellinzona in southern Switzerland and south to near Ravenna in Italy.

Firstly, we summarize our findings on the beetle's potential benefits, ranging from its impact on ragweed performance, demography, spread and aerial pollen concentrations, up to its potential to reduce health costs. Field studies in Italy and data from a pollen monitoring program provided evidence that *O. communa* can reduce *A. artemisiifolia* pollen production and aerial pollen concentrations by >80% (Bonini et al., 2015; Lommen et al., 2017). In a multidisciplinary approach, we recently assessed the potential economic benefits of an establishment of *Ophraella* in the heavily invaded Rhône-Alpes region in southeastern France. We estimated that the number of days with a ragweed pollen risk at which sensitive people express symptoms would be reduced by 50% and the medical costs due to common ragweed would subsequently decrease by € 5–7 million annually (Mouttet at al., 2018). Similarly, at the European level, we projected a reduction in the number of patients by about 2.6 million (19.1 %) and the health costs by € 1.7 billion annually by modeling the number of generations of *Ophraella* across its suitable habitat range in Europe (Schaffner et al., unpub.).

We further present our results on the risks of the beetle for non-host plants in Europe. During 2013–2016, we conducted extensive host specificity studies, both under controlled (in the quarantine facility at the University of Fribourg) and open field conditions (in Switzerland, Italy and China). Biosafety studies included egg and larval transfer tests in the quarantine and in the field, and host choice experiments at field sites in Northern Italy where *Ophraella* has now established. All these experiments used a series of different designs both in the presence and absence of *Ambrosia*. We exposed the test plants at three sites and during three time periods (cohorts in early May, mid-July and early September, each lasting nine weeks) to mimic different levels of *Ophraella* densities and ratios of co-occurrence with the target species, including the late season conditions when availabilities of *Ambrosia* are lowest and beetle densities highest. In addition, we performed extensive non-target field surveys on a total of 25 phylogenetically closely related plant species in 55 localities (including crops, other exotic species and native endangered species) across southern Switzerland and northern Italy to monitor potential *Ophraella* occurrence and damage of non-target species under natural conditions.

So far, we detected no impact on commercially grown sunflower, mainly because the window of vulnerability of this crop does not coincide with high beetle densities at the end of the growing season. Similarly, no significant damage was found on taxonomically closely related ornamental and endangered native plant species (Müller-Schärer et al., 2017).

Secondly, and in view of improving predictions for future long-term benefits and risks of this potential biological control organism, we initiated a novel experimental evolutionary approach to (1) assess the beetle's potential to select for resistant/tolerant ragweed populations, as well as (2) the beetle's potential for evolutionary adaptation to novel biotic (host plants) and abiotic (colder temperature for the yet unsuitable habitats in northern and western Europe) conditions. We use sequential next-generation sequencing (pool-seq) and genome scan analyses to detect selected alleles/genomic regions over time in both *Ambrosia* (study 1) and *Ophraella* (study 2), and use behavioral bioassays to evaluate population differentiation in *Ophraella* host choice and larval performance over time. We initiated two field studies in the Magnago, Milano region of northern Italy in 2016 and 2017 and a bioassay experiment under controlled conditions in 2017 and 2018 for the two studies, respectively.

For study 1, we established in 2016 twenty 2×2×2 m caged plots (two herbivore treatments × two warming treatments × five blocks) with genetically similar *Ambrosia* plants from a wide range of populations. In the herbivore cages, we released approximately 100 *Ophraella* adults, and we used Plexiglas in the elevated temperature plots to increase the temperature by 2.6°C. In the $F_2$ *Ambrosia* generation, both seed number and seed quality were greatly reduced in the *Ophraella* cages. Furthermore, preliminary population genomic analyses revealed many more SNP-positions with significantly higher population differentiation ($F_{st}$ values) after one generation in the Plexiglas and *Ophraella* as compared to the control cages, indicating potential selection (Figure 1). Further analyses for specific SNPs under selection are on-going.

For study 2, we installed 12 experimental cages of 4m$^3$ (2x1x2 m), in which we grew either sunflower (eight cages) or *Ambrosia* (four cages). We subsequently released in the sunflower and *Ambrosia* cages, respectively, approximately 1,200 and 800 beetles that had been widely collected across the present distribution in Europe. In August 2018, i.e. after one year and 4–5 generations, beetle numbers have drastically decreased in the sunflower cages to a total of <200 individuals in the six cages, with two cages showing no sign of *Ophraella* survival (Table 1). Both population genomic (pool-seq) and phenotyping analyses are presently ongoing to detect signatures of selection and to test the preference and performance of the beetles from the two cage treatments after five generations of exposure to *Ambrosia* and sunflower.

This is the first attempt to rigorously and simultaneously assess the evolvability of a biological control agent and its target weed. We advocate such experimental evolution studies be conducted pre-release in order to advance biological control towards a more predictive, efficient and sustainable management strategy under changing climatic conditions.

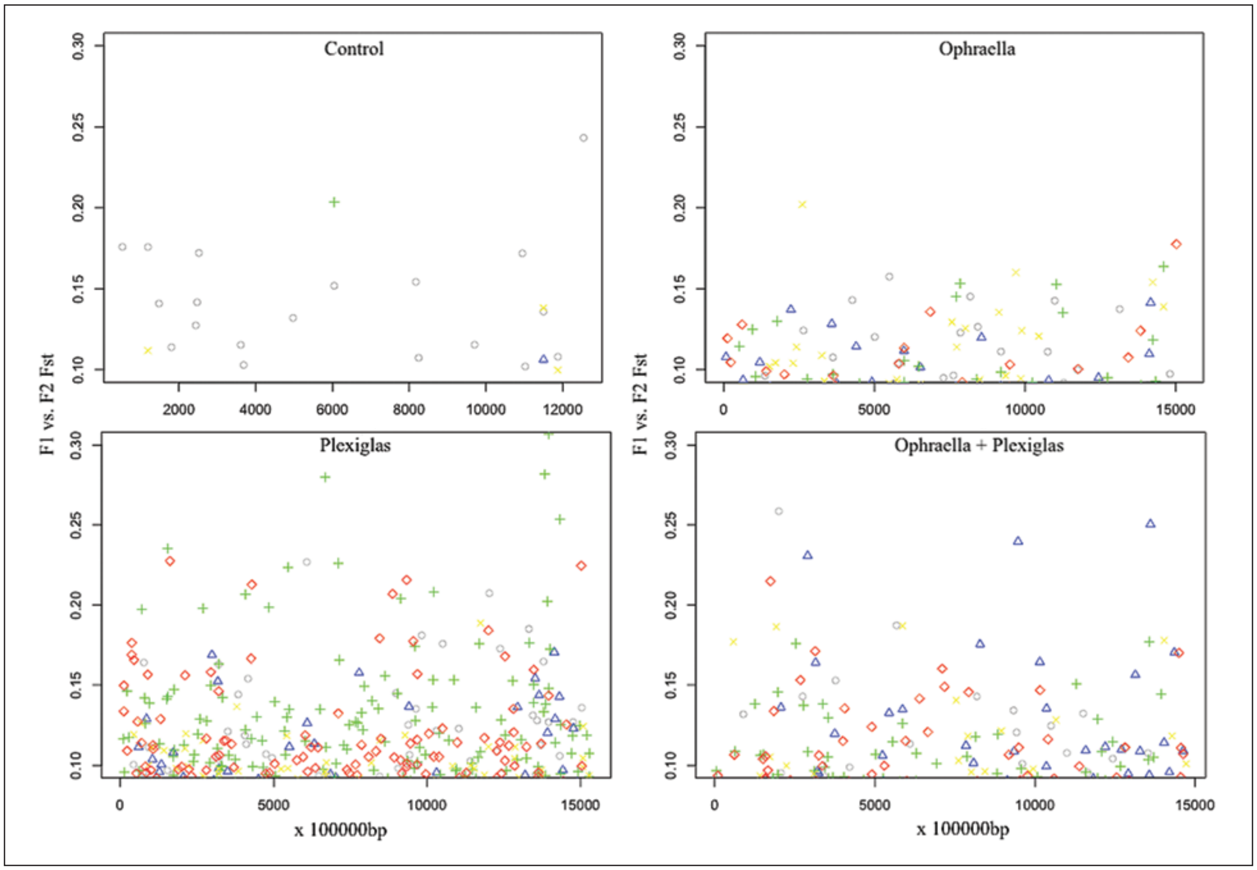


**Figure 1.** Genetic differentiation ($F_{st}$ values) between *Ambrosia* plants initially placed in the cages ($F_1$ generation) and those after one generation ($F_2$) based on whole genome scans (pool-seq) for the four experimental treatments. Colors indicate five blocks (sliding window of 100,000 bp).

**Table 1.** Mean number of *Ophraella communa* individuals in August 2017 (first release), September 2017 (first monitoring) and August 2018 (one year after release, ~4–5 generations) in *Ambrosia* and sunflower cages.

| Life stage | August 2017 | | September 2017 | | August 2018 | |
|---|---|---|---|---|---|---|
| | *Ambrosia* | sunflower | *Ambrosia* | sunflower | *Ambrosia* | sunflower |
| Egg batches | | | 427 | 107 | 154 | 94 |
| Larvae | ~ 800 | ~ 1,200 | 375 | 273 | 1,141 | 63 |
| Pupae | | | 50 | 125 | 965 | 46 |
| Adults | | | 310 | 408 | 201 | 82 |

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*Oral presentation*

# The potential role of rapid eco-evolutionary dynamics in biological control

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The role of rapid evolution, both adaptive and non-adaptive, in biological control has been a core part of the field nearly since its inception, and is an area of increasing focus today. Rapid evolution can drive establishment, population growth or decline. Growing experimental evidence shows that the effects of rapid evolution on ecological dynamics can be quite large. In this presentation, I show evidence from work in my laboratory using *Tribolium castaneum* (Herbst) as a model species to study rapid eco-evolutionary dynamics, as well as review evidence from the literature. Experiments in which evolution (both adaptive and neutral) is prevented show that evolving populations can spread further, grow larger or decline to extinction more rapidly than non-evolving populations. The implications for the evolution of both the dynamics that occur in populations of invasive pests and those that occur in populations of biological control agents are profound.

*Oral presentation*

# Rapid evolution in biological control: implications for safety and effectiveness

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Biological control is often portrayed as a self-sustaining and environmentally-friendly alternative to chemical control. Yet biological control is not a panacea, nor is it risk free. Our research addresses the inherent properties of biological control systems that make an assessment of risks, costs and benefits difficult: biological control organisms (like chemicals) can harm other organisms (be they targets or non-targets of control), but control organisms (unlike chemicals) can multiply, spread by autonomous dispersal and evolve. We show that populations of the cinnabar moth undergoing range expansion from low to high elevations have rapidly evolved changes in the timing of life cycle events from emergence of adults at the start of the growing season to arrival at the pupa stage at the end of the growing season. These changes speed phenological development within the growing season and likely improve the odds of persistence in the short growing season found in mountain environments. In mountain environments, genetic and phenotypically plastic changes in phenological development facilitated control of the target host *Jacobaea vulgaris* Gaertn. (a benefit) but also led to acquisition of a non-target host *Senecio triangularis* Hook. (a potential risk). The strength of interactions between the cinnabar moth and its hosts varies with the phenological synchrony between insect and host, and phenological synchrony varies with host species and climatic conditions. This research is relevant for policy; we show how understanding these complexities is essential for evaluating both the benefits and risks of biological control.

*Oral presentation*

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

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Invasive alien plants and their natural enemies used as biocontrol agents are ideal study systems to address questions of whether and how fast organisms adapt to novel environments. Climate change is likely to further shape the interaction between the plant invader and its biocontrol agent. In 2016, we initiated a study to get insights into the evolvability of the European plant invader *Ambrosia artemisiifolia* L. to a biocontrol insect and global warming. In an ongoing field-selection experiment in northern Italy, we grow artificial populations of *A. artemisiifolia* exposed to the recently introduced and potential biocontrol herbivore *Ophraella communa* Le Sage, and a warming treatment in a 2*2 experimental design with five replicates. To test for evolutionary changes in *A. artemisiifolia*, pooled samples from each of the 20 experimental populations will be analyzed over four years (a) for their genetic composition using next-generation sequencing (pool-seq) and (b) in various bio-assays. To date, we have collected seeds from the first ($F_1$) and second ($F_2$) generations and grown them alongside their parents in growth chambers under two experimental temperature conditions to assess the beetle's performance and plant competitive ability. We found high variation in trait means and plasticities to temperature treatments in performance variates among $F_0$ mother plants, indicating high initial standing genetic variation. We already found $F_1$ plants from elevated temperature in the field plots to perform differently in phenology, growth and plasticities as compared to plants from the ambient temperature plots. Moreover, results from quarantine preference and performance studies with *O. communa* on $F_2$ plants from the field selection populations are in line with the results of competitive ability of $F_2$ plants from greenhouse studies, supporting the growth-defense trade-off in $F_2$ plants under herbivore selection. These studies will improve forecasting of the biocontrol efficiency in a changing world.

*Oral presentation*

# Characterizing hybridization in the tamarisk leaf beetle

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When effective, classical biological control is an excellent management tool for long-term control of invasive species. However, achieving success in weed biocontrol is an elusive task, with only 27% of programs leading to successful establishment of the introduced agent. To combat potential failure, biocontrol scientists can release several different ecotypes to better match the novel environment and to increase genetic variation of agent populations. Hybridization between ecotypes can increase the chances of establishment, or allow for transgressive phenotypes to emerge that are outside the range of the parents. In the case of the tamarisk leaf beetle, four ecotypes of *Diorhabda* were released and later determined to be different species. Here we show the effects of hybridization between three different species on several different life history traits in *Diorhabda* spp. We show that important life history traits, such as oviposition rate, development time and, importantly, host specificity can be affected by hybridization. The genetic distance between species affects the outcome of hybridization, and thus the effects of hybridization were not consistent between crosses. We also quantified the occurrence of hybridization in the southwestern USA through next-generation sequencing, RADseq. Incorporating potential effects of hybridization could be beneficial for biocontrol programs, but the associated risks must be fully examined before releasing different ecotypes.

*Oral presentation*

# Prospects in understanding the role of eco-evolutionary dynamics in an admixed, range-expanding biocontrol agent

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Biological control agent populations are crucibles for rapid evolution. Despite rigorous host-specificity testing prior to release, rapid evolution of these systems may be inevitable due to small effective population sizes, perhaps even requisite to establishment, persistence and spread. The resolution and functional context provided by genomic tools have revealed the pervasiveness of rapid evolution in the wild; yet it remains a challenge to identify the relevant patterns and processes to inform management. Using the introduced tamarisk beetle species complex (*Diorhabda* spp.) as a case study, this presentation demonstrated how we can use restriction site-associated DNA sequencing (RADseq) and a de novo genome assembly to test theoretical expectations regarding rapid evolution and inform management decisions in weed biocontrol systems. Since 2001, six source populations have been introduced to North America for biological control of the riparian shrub tamarisk (saltcedar; *Tamarix* spp.): *D. carinata* (Faldermann) from Uzbekistan, *D. carinulata* (Desbrochers) from China and Kazakhstan, *D. elongata* (Brullé) from two sites in Greece, and *D. sublineata* (Lucas) from Tunisia. It is unknown which source populations established, spread, and how rapid evolution, in concert with range expansion and admixture, have impacted risk and agent efficacy. This presentation discussed our results of genotyping over 500 individuals across both introduced and native range populations. We assessed genome-wide diversity and quantified the proportion of ancestry across six clusters, which reflect source populations and lab cultures. We found evidence of serial bottlenecking at the edge of range expansion, differential establishment across source populations, extensive admixture and hybridization across source populations, and showed that proportions of ancestry do not necessarily reflect introduction history. This presentation introduced our cost-effective de novo assembly of *D. carinulata*, the first weed biocontrol agent reference genome, and illustrated its potential as a resource for weed biological control to identify genomic changes and the genomic architecture of rapid evolution in ecologically relevant traits.

*Oral presentation*

# Admixtures of Chinese and Nepalese *Lilioceris* species: helpful or harmful for USA air potato biocontrol efforts?

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Weed biological control practitioners have often championed the release of multiple biotypes of a proposed agent to enhance the potential for a program's success. In principle, such releases take advantage of intraspecific variability in an agent's life history characteristics and climatic tolerances to address the target weed's variability throughout its adventive range. Outcomes of the use of such admixtures have been inconstant. Further, whereas pre-release studies of life history traits and host fidelity of agent biotypes are common, investigations of the life history characteristics of the hybrids produced by such admixtures are rare and generally *post hoc*. We investigated potential hybrid vigor or depression, as measured by fecundity, that might arise from the admixtures of Nepalese (N) and Chinese (C) biotypes of the approved air potato biocontrol agent *Lilioceris cheni* Gressitt & Kimoto and its congener *L. egena* (Weise). The trials with *L. cheni* were conducted after field release of the Chinese biotype, and to allow us to make an informed decision regarding whether to release the Nepalese biotype. The trials with *L. egena* (awaiting regulatory approval for release) were conducted in tandem with its host range trials. Crosses included: C♀c♂, C♀n♂, N♀c♂, and N♀n♂. For *L. cheni*, $F_1$ generation fecundity was significantly higher in the N♀n♂ line than the C♀c♂ line, and the hybrid lines did not differ from either. In the $F_2$ generation, the N♀n♂ and C♀n♂ lines produced significantly more eggs than the C♀c♂ line. We concluded that hybridization would not result in hybrid depression, and field releases of the Nepalese biotype commenced. For *L. egena*, the pure lines did not vary within either the $F_1$ or $F_2$ generation. Likewise, the hybrid lines were neither more nor less fecund than the pure lines within either generation. We conclude that hybridization will not result in hybrid depression, and so we will conduct field releases with both biotypes once we have final regulatory approval.

*Oral presentation*

# Anticipating cryptic species and determining their host associations in weed biological control

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The presence of unrecognized cryptic species confuses ecological interpretation and practice. In weed biological control, the consequence is that desirable and potentially useful agents may be overlooked. More serious is the potential for an unrecognized complex to be investigated and host tested as a single species or introduced as an agent. Molecular methods have seen the increasing detection of cryptic species within "generalist" herbivorous insects, although not all results are as unequivocal as portrayed. Some cryptic species have proven to be host-associated specialists and of value to biological control. Can cryptic species be anticipated? And can biological control benefit? Typically, cryptic species have been recognized only incidentally, at least initially, and resolution is not always achieved. That is, the design of tests to clarify the status of populations suspected to harbor unrecognized host-associated species is not always straightforward. In addition, species problems are not always obvious, and this is likely to cause issues when insects from an unrecognized species complex are included in agent surveys and host-testing experiments. The recognition concept of species was developed to accommodate the existence of cryptic species. This concept acknowledged, for the first time, that morphological diversification across species was not inevitable, especially in organisms whose sexual signaling involves modes of communication unrelated to morphology. If those signals are cryptic to our senses, we can expect to find cryptic species complexes. Current evolutionary theory can thus be used to anticipate when cryptic species should be considered a possibility. This same theory should also inform how ecological sampling, population genetic assessments, laboratory mating tests and host-use assays should be structured. The examples used also demonstrate how multiple lines of investigation can deliver the information required to resolve not only the question of cryptic species, but also provide information about how the host plant associations of any species should be delimited.

*Oral presentation*

# Bacterial symbionts as potential drivers of biotype formation within the hawkweed gall wasp *Aulacidea pilosellae*

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The European gall wasp *Aulacidea pilosellae* (Kieffer) (Hymenoptera: Cynipidae) is a candidate biocontrol agent being assessed for release in North America against multiple invasive hawkweed species (*Pilosella*; Asteraceae). Previous field studies identified populations either specific to *P. officinarum* Vaill., or which occur on several *Pilosella* spp. excluding *P. officinarum*. Earlier molecular analyses also identified genetic differentiation between the two host-affiliated types, indicative of two separate biotypes: Biotype 1 = ex. *Pilosella* spp., Biotype 2 = ex. *P. officinarum*. This study sought to further explore the differences between biotypes to provide a greater understanding of the genetic identity, life history and evolution of *A. pilosellae*. Using laboratory-reared populations, studies were conducted on the voltinism, sex ratio, fecundity and adult emergence patterns of the biotypes. Females from populations were also used in next-generation sequencing (NGS) of DNA from the entire microbiome harbored by the wasps. Results showed populations of Biotype 1 to be univoltine (obligate diapause) and bisexual, whereas those of Biotype 2 are multivoltine (facultative diapause) and parthenogenetic. Biotype 1 also produced approximately 30% more eggs/female, and adults emerged as a distinct peak of shorter duration compared to Biotype 2. NGS of the wasps' microbiomes showed Biotype 1 populations with relatively few individuals infected with the bacteria *Wolbachia.* In contrast, all Biotype 2 individuals were uniformly infected with this common insect endosymbiont. Because the maternally-inherited *Wolbachia* are known to induce parthenogenesis in other wasp species, our results suggest that *Wolbachia* infections have done likewise in a subset of *A. pilosellae* populations to potentially cause genetic divergence in host-range, voltinism and other biological traits of importance to the species' efficacious use in biological control.

*Oral presentation*

# *Rhinusa pilosa*: a case study of environmental bottleneck

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The shoot gall weevil *Rhinusa pilosa* (Gyllenhal) (Mecinini, Curculionidae) was first released in Canada in 2014 for biological control of *Linaria vulgaris* Mill. (Plantaginaceae). *Rhinusa pilosa*, which has a western Palearctic distribution, is characterized by deep intraspecific mitochondrial divergence that exceeds 4.5% on the cytochrome oxidase subunit II gene (*COII* gene). Consequently, two haplotype groups have been designated as Rp-A and Rp-B. Furthermore, significant mitochondrial divergence is also present within these two haplotype groups with 0.2 and 0.7% for Rp-A and Rp-B, respectively. Population density of *R. pilosa* in Europe is dramatically influenced by its inquiline species *Rhinusa eversmanni* (Rosenschöld), which reduces population size of *R. pilosa* leading to an erosion of genetic diversity of the species with a high probability of inbred mating. Further genotyping of *R. pilosa* metapopulations in Europe revealed the existence of only one (rarely two) haplotypes in each studied metapopulation. Reduced genetic diversity may have strong implications for successful biological control by reducing the adaptability of the weevil to new environments. Overall, low metapopulation genetic diversity and the occurrence of two highly-diverged haplotype groups represent a critical determinant in the biology of this species as a biological control agent. Thus, preserving and increasing the *mt*DNA variation of introduced populations by rearing individuals carrying different mitochondrial haplotypes is crucial for successful establishment and efficacy of *R. pilosa* in its new environment. In the case of *R. pilosa*, haplotype-based rearing methods and the absence of the inquiline species *R. eversmanni* in North America could potentially help to increase the genetic diversity of the weevil over time and contribute to its success as a biocontrol agent of *L. vulgaris*.

*Oral presentation*

# DNA barcoding determines the native field host range of endophagous insects associated with *Senecio madagascariensis*

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Fireweed, *Senecio madagascariensis* Poir. (Asteraceae), is a short-lived perennial plant from southern Africa that incurs high economic losses for livestock farmers in eastern Australia and Hawaii and is currently the focus of a biological control program. Demonstrating host specificity of biocontrol agents is crucial for Australia, which harbors 87 native *Senecio* species, several of which co-occur with fireweed. Host-specificity requirements are less stringent for Hawaii, where there are no native species in the tribe Senecioneae. Studies on the native field host range of biocontrol agents can eliminate non-specific candidates at an early stage, thus reducing reliance on time-consuming laboratory studies. Endophagous insects from families that have shown host specificity in past biocontrol programs against Asteraceae were targeted during this study. Stem-boring Curculionidae and stem-boring and capitulum-feeding Lepidoptera and Diptera were surveyed across 18 native *Senecio* species in KwaZulu-Natal, South Africa to assess their host specificity and suitability as candidate agents. Since a clear morphological separation of insect larvae to species level is not possible, DNA barcoding was used to differentiate between species associated with different *Senecio* species and thereby determine their host range. The Curculionidae, Lepidoptera and capitulum-feeding Diptera that were associated with fireweed all contained one or more species that displayed restriction to plants in the *S. madagascariensis* species complex. In contrast, none of the stem-boring Diptera were confined to fireweed or its species complex. DNA barcoding has thus narrowed the search for potentially host specific insects that could be deployed for the biocontrol of fireweed in Australia and Hawaii and which ones should be prioritized for more intensive host-range assessments.

*Oral presentation*

# The fundamental and ecological host ranges of the crown-boring weevil *Listronotus sordidus*, a proposed biocontrol agent for the aquatic weed delta arrowhead, *Sagittaria platyphylla*

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## Abstract

Appropriate host-specificity testing in laboratory trials can elicit the fundamental host range of a candidate biocontrol agent—the range of plant species capable of supporting an insect's life cycle with a high degree of certainty. However, the fundamental host range almost always overestimates the ecological host range—the range of plants that are utilized in the field.

Natural enemy surveys give an indication of the ecological host range of candidate biocontrol agents, and larval specimens dissected from a range of related plant host species can be identified using molecular barcodes. We utilized this method when undertaking natural enemy surveys for the aquatic weed delta arrowhead, *Sagittaria platyphylla* (Alismataceae), in its native range of the southern USA. In this paper, we discuss preliminary information from barcoding identification of field-collected insects to provide the ecological host-range context for examining the results of the laboratory trials. Results from host-specificity tests of the crown-boring weevil *Listronotus sordidus* on 14 plant species indicate that its fundamental host range includes other *Sagittaria* species, as well as other Alismataceae genera. Preliminary data from a field survey in the native range confirmed that the weevil is not monospecific and includes congeneric species other than the target weed, and at least one other species in the family Alismataceae. Further laboratory tests and barcoding results from more of the field-collected samples are necessary to provide a better characterization of both the fundamental and ecological host ranges of this weevil.

**Keywords:** ecological host range, fundamental host range, molecular identification, *Listronotus sordidus, Sagittaria platyphylla*

## Introduction

Delta arrowhead, *Sagittaria platyphylla* (Engelm.) J.G.Sm. (Alismataceae), is an emergent macrophyte native to the southern USA (Adair et al., 2012) and a target for biological control in Australia (Kwong, 2016) and South Africa (Martin et al., 2018). Natural enemy surveys in North America identified a crown-boring weevil, *Listronotus sordidus* (Gyllenhal) (Coleoptera: Curculionidae), as one of three prioritized candidate biocontrol agents for this weed (Kwong et al., 2014). Prior to finding this weevil on *S. platyphylla*, it had only been recorded from two other *Sagittaria* species: *S. engelmanniana* J.G.Sm. and *S. filiformis* J.G.Sm. (Blatchley and Leng, 1916; O'Brien, 1981), indicating that it may be restricted to this genus (Kwong, 2016).

Host-specificity testing under laboratory conditions is used to determine the fundamental host range (also referred to as physiological host range) of a potential biocontrol agent—the range of plant species that can support the life cycle of the insect (Schaffner, 2001). However, the ecological host range (i.e. the range of plant species that an agent fully utilizes as a host under field conditions) is often a subset of the fundamental host range (Hinz et al., 2014).

Our main aim was to use laboratory trials to characterize the fundamental host range of *L. sordidus*. To improve our understanding of how the insect's behavior in the laboratory relates to the ecological host range of *L. sordidus*, we compared laboratory results of host acceptance with preliminary information from field studies conducted in the native range. This paper reports on a series of laboratory studies that investigate the fundamental host range of *L. sordidus* and contextualizes our results with data generated thus far from the field with a range of Alismataceae species sampled from across the southern USA, using DNA barcoding methods to identify endophagous insect larvae to species level.

## Methods

### *Listronotus sordidus* culture

*Listronotus sordidus* larvae were collected from western Tennessee (Reelfoot Lake), northeastern Texas (Caddo Lake) and western Mississippi (TARA Wildlife Reserve). Larvae were reared to adults at the Engineer Research and Development Center, U.S Army Corps of Engineers in Vicksburg, Mississippi prior to introduction to the AgriBio quarantine facilities in Melbourne, Australia. Insects collected from Texas and Mississippi were combined to maintain viable culture numbers, hereafter referred to as "mixed colony," and kept separately from the Tennessee colony. The mixed colony was used in each trial, except where explicitly indicated.

The insect culture was maintained in ventilated rectangular plastic containers lined with moist paper towels, with approximately ten males and ten females in each. A bouquet of fresh leaves was fed to adults every three to four days, and segments (approximately 8 cm long) of dried leaf petioles were provided as an oviposition substrate. Each week, petioles were collected from rearing containers and placed at the base of *S. platyphylla* plants grown in standard potting mix. Upon hatching, larvae migrate from the dried petioles and burrow into the plant where they feed within the crown, roots and tubers before pupating in the soil (Martin et al., 2018; Kwong, 2014). Pots were placed in trays of standing water to keep the soil moist but not water-logged and enclosed in nylon-mesh rearing tents within the quarantine glasshouse at approximately 24°C. Supplemental lighting was used to maintain a daylength of 14 h light. Adult emergence occurred five to six weeks later.

### Test plants

The plant list for testing the fundamental host range of *L. sordidus* was developed based on currently accepted phylogenetic information of the Alismataceae family (Chen et al., 2012). Test plant species were selected using the phylogenetic relationship of test plants to the target weed—the centrifugal phylogenetic method (Briese, 2005). In Australia, the only *Sagittaria* species present are introduced ornamental plants, some of which have become naturalized and invasive, such as *S. calycina* Engelmann (Adair et al., 2012).

The taxonomy of the *Sagittaria* genus and of Alismataceae is currently under review (Keener, 2005; Lehtonen, 2017). For taxa sourced from the nursery trade we have used the names under which they were supplied. Genetic analysis is underway to confirm the identity of these.

The Alismataceae test species native to Australia included: *Caldesia oligococca* (F.Muell.) Buchenau, *Damasonium minus* (R.Br.) Buchanau, and *Alisma plantago-aquatica* L. The tropical species *Astonia australiensis* (Aston) S. W. L. Jacobs and *Butomopsis latifolia* (D.Don) Kunth were not tested due to practical constraints associated with their field collection and/or propagation.

Non-native Alismataceae included the ornamental species *Echinodorus cordifolius* L. Griseb. and *Hydrocleys nymphoides* (Humb. & Bonpl. Ex Willd.) Buchenau, and a naturalized *Alisma* species, *A. lanceolatum* With.

Of the three non-Alismataceae species included, *Cycnogeton procerum* (R.Br.) Buchenau (Juncaginaceae) and *Oryza sativa* L. "Rizique" (Poaceae) were included because they are sympatric with *S. platyphylla* in southeastern Australia, and *Eleocharis dulcis* (Burm.f.) Trin. Ex Hensch. was included because it has similar morphological structures (tubers) that might be fed upon by *L. sordidus* larvae.

## Laboratory trials

Design efficiency was limited by the availability of plant material at different times. Nevertheless, each trial was a connected block design with *S. platyphlla* replicated at least twice in nearly all blocks (Table 1). Blocks were associated with the date that adults were introduced to the experimental unit (i.e. adults were introduced to plants or containers in the same block on the same day), as well as general management such as the position of containers or plants on benches. The potted plant trial of *C. procerum* was done outside the blocked design. All trials were no-choice. All container trials were conducted in a controlled environment room (CER) at 23°C, 16 h light. All pot trials were conducted in a quarantine glasshouse with the temperature maintained constantly at around 24°C. The natural light was augmented with florescent lights in the morning and evening to maintain a daylength of 14 h light. Adults used in the trials were from fecund populations, and neonate larvae were approximately one day old. The number of adults or larvae used in each trial is recorded in Table 1.

### *Container trials*

Adults were placed into a ventilated rectangular plastic container with a bouquet of fresh leaf material and dried petioles (as oviposition substrate) from a single test species. Adults were removed after four days, and the petioles were placed in petri dishes lined with moist filter paper with lids secured using parafilm. The petioles were observed daily, and as larvae emerged from the petioles, they were counted (as a measure of oviposition) and removed. In a subsequent container trial *Echinodorus* cf. *cordifolius* and *Oryza sativa* were tested in a similar manner, but oviposition was assessed by number of eggs observed during dissections of the petioles.

### *Potted plant trials*

Each replicate was represented by a potted plant encased in a fine mesh sleeve such that adults that

**Table 1.** Summary of laboratory host specificity testing trials conducted on *Listronotus sordidus.*

| Trial | Experimental Unit | Units per block | Replication | |
|---|---|---|---|---|
| | | | *S. platyphylla* | Other species |
| **Container trials** | | | | |
| First | 10 female and 9 male adults/container | 13–15 | 20 | 3–5 (mostly 5) |
| Subsequent | 5 pairs adults/container | 15 | 10 | 10 |
| **Potted plant trials** | | | | |
| Single generation (6 weeks) | 2 pairs adults/pot | 12 | 15 | 5 |
| Single generation (9 weeks) | 2 pairs adults/pot | 10–12 | 13 | 4–5 (mostly 5) |
| Larval development on *Cycnogeton* | 10 neonate lar-vae/pot | 12 | 6 | 6 |
| Multiple generation (mixed colony) | 2 pairs adults/pot | 5 (2 blocks)<br>10 (2 blocks) | 50 | 46 |
| Multiple generation (Tennessee colony) | 2 pairs adults/pot | 4–6 | 5 | 2–9 |

were added to the replicate or that emerged during the trial were confined to the sleeved plant. The soil surface was kept 10–15 cm above the waterline so that the crowns of the plants were not submerged. During each assessment, we recorded whether the plants were dead or alive.

**Single generation trial.** Adults were introduced to each of the replicates along with dried petioles corresponding to that test species. Pots were randomly placed in tubs and blocks that were set up one week apart. After six weeks, the adults were removed, and about half the pots were destructively sampled to record the number of progeny (sum of larvae, pupae and adults). After nine weeks the remaining pots were destructively sampled, and the number of progeny recorded as before.

**Larval development on *Cycnogeton procerum*.** A modified version of the above procedure was carried out for *C. procerum* because this species is outside the Alismataceae family and, after obtaining the results from the container trial and noting that this species is distantly-related to *S. platyphylla*, it was important to test whether larvae were able to develop to adults on this plant.

Ten neonate larvae were added to each of six replicates and controls over the course of five days, with equal numbers added to each replicate each day. The pots were randomly placed in one of two tubs in the quarantine glasshouse and water was kept at a level that left the plant crowns exposed. After eight weeks the plants were destructively sampled, and the number of progeny recorded as before.

**Multiple generation trial.** Replicates were set up as described under above for the single generation trial, but adults were removed after one week. Adults were sourced from one of two colonies. After eight weeks, the plants were dissected and all progeny (adults, larvae and pupae) were collected. $F_1$ generation adults (and larvae and pupae reared to adults) were placed in rearing containers with food bouquets and dried petioles from the species they were collected from. Immature larvae were collected from the petioles and placed onto potted plants of the test species with approximately ten larvae per plant. Adults were counted and collected as they emerged ($F_2$ generation). As at least one mating pair was reared on a test species, this method was continued for a further generation ($F_3$ generation).

**Field studies**

To compare the fundamental and ecological host ranges of *L. sordidus*, we visited sites across the native range of *S. platyphylla* that had records of Alismataceae species included in our laboratory trials. At each site we collected approximately ten plants of each Alismataceae species present, which included *Echinodorus* spp. and *Sagittaria* spp. Samples were collected from eleven sites in late September 2015 and 2016. *Listronotus sordidus* was identified by dissecting all larvae from individual plants and using molecular methods to categorize them into species (as per the method in Blacket et al., 2015). Approximately half of the samples have been identified, consequently only preliminary results are discussed here.

**Statistical analyses**

Prior to statistical analysis, the number of individuals detected was square-root transformed to minimize the change in residual variation with predicted mean and to increase the plausibility of additivity between block and species effects. Data were analyzed as a general linear model with additive terms for block and species. Predicted species values were calculated after adjusting for blocks, with marginal weighting for blocks, before back transforming to the original scale. Although predicted values were calculated using all containers or plants, the residual mean square used in calculating precision the standard errors of difference (s.e.d.) was obtained from a separate analysis that only included species for which individuals were detected to avoid underestimation of standard errors. A hypothesis test for the effect of species was calculated using the usual F-value statistic for species, after adjusting for blocks, but with the level of significance calculated using a permutation test with 10,000 randomly chosen permutations of the units of analysis. In the container trial, the residual mean square, used for calculating standard errors and used in the permutation test of species, came from a more saturated model that also included an effect of the collection location of *Sagittaria platyphylla*. Dependent on trial, individual containers or individual potted plants were used as the unit of analysis. The analyses were carried out using the MODEL, TERMS, FIT and PREDICT directives, and the RPERMTEST procedure in *GenStat 17* (VSN International, 2014).

## Results and discussion

In the container trials, *Listronotus sordidus* laid eggs on all but one Alismataceae test species as well as the more distantly-related native plant *C. procerum,* but not on the distantly-related aquatic food plants water chestnut (*E. dulcis*) and rice (*O. sativa*) (Table 2). Oviposition measured on several Alismataceae species both within and outside the *Sagittaria* genus was similar to the target weed *S. platyphylla* (within 2 s.e.d.). Oviposition recorded on the one species outside Alismataceae (*C. procerum*) was lower than *S. platyphylla* (>5 s.e.d.).

Similarly, during the single generation potted plant trials there was larval development on all Alismataceae test species, but the number of progeny was much lower than on *S. platyphylla*, except for *S.* cf. *sagittifolia* which was higher (>3 s.e.d.). It is worth noting here that the tabulated values are estimated means from the model, and some species (indicated in bold) were able to support larval development to adult eclosion despite an estimated mean value of zero. Where larval development was observed in Alismataceae species outside the *Sagittaria* genus, it occurred at much lower levels than observed in most *Sagittaria* species. There were fewer resources available for larval development on several Alismataceae test species due to lower plant biomass and, in some cases, plant death during the potted plant trials. It is unclear whether plants died due to reduced water levels or larval damage. Consequently, it is also not known if larval development in these species was reduced due to a lack of host suitability or due to a lack of resources. Due to this uncertainty, these species may still be within the fundamental host range. There was no larval development on the non-Alismataceae species tested: *Cycnogeton procerum* and *Oryza sativa*. These species are likely outside the fundamental host range.

In at least one potted plant trial, adults emerged from all *Sagittaria* species except for *S.* cf. *natans*, and also from the native Alismataceae species *D. minus* and *A. plantago-aquatica* (Table 2). The two native plant species even supported development through to a second generation and *D. minus*, having oviposition and survival rates comparable to the first generation, was able to support the emergence of a third generation (data not presented).

The preliminary results from our field survey in the southern USA indicate detection of *Listronotus sordidus* larvae in several samples of *S. platyphylla* and *S. calycina*, and a single *L. sordidus* larva was dissected from an *Echinodorus* plant. While *S. calycina* is clearly able to support the life cycle of this insect, the presence of *L. sordidus* in an *Echinodorus* field sample occurred despite no oviposition being observed on the ten replicates of this plant species that were examined. However, *E. cordifolius* was found to be within the fundamental host range in trials performed in South Africa (Martin et al., 2018). The weevils have not been released in South Africa, so the ecological host range could not be tested there.

Clearly, the fundamental host range of *L. sordidus* is not restricted to *Sagittaria* but includes other genera within Alismataceae. While resource availability rather than host suitability may have contributed to the lower numbers of progeny in our trials, the same result was found by Martin et al. (2018) for species outside *Sagittaria*.

The results from laboratory host-specificity testing, together with preliminary field observations, confirm that both the fundamental and ecological host range of *L. sordidus* includes congeneric *Sagittaria* species other than the target weed. The fundamental host range includes species from the Alismataceae family that are not *Sagittaria* species, although larval development and adult emergence was low in these species compared to many *Sagittaria* species. Given that the fundamental host range almost always overestimates the ecological host range (Hinz et. al., 2014) we need to be able to evaluate which of these is likely to be utilized as a host. There is progress towards using oviposition and larval development data to quantify the risk of off-target use in the field (e.g., Paynter et. al. 2015). However, the low levels of oviposition and/or larval development observed in our trials cannot solely be used for risk analysis because field data suggests that these are not a true reflection of potential laboratory performance.

**Table 2.** Summary of laboratory trial results for effect of plant taxon on predicted values of offspring emergence. The test plant genera are listed in approximate order of relatedness to *Sagittaria* based on molecular phylogenies (from most to least-closely related according to Chen et al., 2012; Lehtonen, 2017). Numbers presented are square root transformed (back transformed in parentheses). When the back transformed statistical estimate is negative, the back transformed value is automatically taken to be 0.

| Taxa | Container trial | | Potted plant trial<br>Oviposition and larval development | | | |
|---|---|---|---|---|---|---|
| | Larval emergence per container | Eggs laid per container | Single generation trial<br>Total progeny/plant at: | | Multiple generation trial<br>Adults/plant | |
| | | | 6 weeks | 9 weeks | Mixed colony | Tennessee colony |
| **Congeneric species** | | | | | | |
| *Sagittaria platyphylla*[a] | 6.6 (44) | 4.1 (17) | 5.2 (27) | 7.2 (52) | 3.0 (9) | 4.2 (17) |
| *Sagittaria calycina*[a] | 0.7 (1) | - | 1.0 (1) | 1.3 (2) | - | - |
| *Sagittaria latifolia*[b] | 4.7 (22) | - | 2.3 (5) | 1.6 (3) | - | - |
| *Sagittaria* cf. *natans*[b] | 2.3 (5) | - | 0.1 (0) | 0.9 (1) | - | - |
| *Sagittaria* cf. *sagittifolia*[b] | 6.4 (41) | - | 9.2 (84) | 10.7 (114) | - | - |
| **Alismataceae family** | | | | | | |
| *Echinodorus* cf. *cordifolius*[b] | - | 0.2 (0) | - | - | - | - |
| *Caldesia oligococca*[c] | 3.4 (11) | - | 0.4 (0) | -0.2 (0) | 0.1 (0) | 0.2 (0) |
| *Hydrocleys nymphoides*[b] | 3.2 (10) | - | 0.4 (0) | -0.2 (0) | - | - |
| *Damasonium minus*[c] | 4.4 (19) | - | -0.5 (0) | -0.6 (0) | 0.0 (0) | 1.5 (2) |
| *Alisma lanceolatum*[b] | 6.2 (38) | - | - | - | - | 0.0 (0) |
| *Alisma plantago-aquatica*[c] | 7.1 (51) | - | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.6 (0) |
| **Non-Alismataceae species** | | | | | | |
| *Cycnogeton procerum*[c] | 1.9 (4) | - | 0* | - | - | 2–9 |

Plant species: [a] invasive in Australia; [b] ornamental species; [c] native to Australia; [d] food plant. *larval transfer test performed outside the connected block trial; - test not conducted. Values in italics are associated with plants that died and may be underestimates. Values in bold represent test species that could sustain development to adult eclosion, regardless of the value of the estimated mean. # Calculated using residual mean square from Tennessee colony continuation analysis.

We have commenced laboratory testing of these plant species with larval transfer trials to limit the confounding effects of herbivory damage. Molecular identification of the field-collected samples is ongoing to better quantify host suitability in the field as well as genetic barcoding of the test plant species used in Australian and South African trials which may resolve some anomalies before making a final decision whether or not to apply for the release of this weevil in Australia.

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*Poster presentation*

# Will the predicted rise in atmospheric $CO_2$ alter the interaction between *Opuntia stricta* and its biocontrol agent *Dactylopius opuntiae*?

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The biological control of invasive cactus using *Dactylopius* spp. (cochineal) has long been regarded as a success and can be traced back some 200 years. However, research has shown that cactus species respond positively to increased $CO_2$ levels, which may serve to make them more competitive with rising atmospheric $CO_2$. This poses the question as to whether increasing $CO_2$ levels may alter the interaction between cactus and cochineal. To investigate this, *Opuntia stricta* (Haw.) Haw. plants were grown at 250, 400 and 600 ppm $CO_2$ to emulate past, present and future atmospheric $CO_2$ conditions, respectively. Growth parameters and the plants' physiology were measured, along with numbers and densities of the cochineal. The experiment addressed two opposing hypotheses: (1) either that the cactus will grow faster with rises in $CO_2$, despite cochineal herbivory or (2) that the weed will be more palatable and potentially more susceptible to cochineal damage. Outcomes of the experiments were discussed at the symposium.

*Poster presentation*

# Impact of the sap-feeding *Opsius stactogalus* is not influenced by elevated salt concentrations in five different *Tamarix* taxa

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## Abstract

Salt cedar, *Tamarix*, is a genus of many Eurasian shrubby tree species in the family Tamaricaceae that is represented by one native (*Tamarix usneoides* E.Mey.) and two exotic (*Tamarix ramosissima* Ledeb. and *T. chinensis* Lour.) species in South Africa. The exotic *Tamarix* spp. have become invasive in South Africa and are targeted for biological control. *Tamarix* spp. are tolerant to extreme soil salinity, but it is unknown how elevated salt levels in the plant will affect coevolved insect herbivores. This study investigated whether elevated salt concentrations influenced the impact of the leafhopper *Opsius stactogalus* on five different *Tamarix* taxa. The results showed that *T. chinensis* excreted significantly more salt than the other *Tamarix* taxa. The high level of salt in the plants' tissues, however, did not show any significant effect on the feeding performance of *O. stactogalus* and subsequently on plant growth. There are about 25 insects that have evolved to strictly feed and survive on the halophytic *Tamarix* species, and among these are *O. stactogalus* and the *Tamarix* beetle (*Diorhabda* spp). The fact that the feeding performance of *O. stactogalus* across the five *Tamarix* taxa in this study was not significantly affected by salt addition may suggest that the *Tamarix* beetle (*Diorhabda* spp.), which is currently under quarantine investigation in South Africa, will not be influenced by the various salt concertation levels of the two exotic *Tamarix* species or their putative hybrids either.

**Keywords:** *Tamarix*, phytoremediation, *Opsius stactogalus*, electroconductivity, *Diorhabda*

## Introduction

The exotic saltcedar, *Tamarix* spp., native to the Old World (Eurasia) and the northern (e.g., Morocco, Senegal), eastern and southern regions of Africa (Marlin et al., 2017), is listed as one of the 100 most problematic invasive species in the world (Lowe et al., 2000). Its invasion is most pervasive in the USA, Mexico, Argentina, Australia and South Africa, among others (Marlin et al., 2017; Newete et al., 2019). According to the National Environmental Management: Biodiversity Act 2014, the plant is declared as a category "1b" weed in South Africa, necessitating its immediate removal (Henderson 2001; Newete et al., 2019). This phreatophitic shrubby tree is well known for its adaptation to extreme saline soils, which is believed to give *Tamarix* a competitive advantage over many co-existing riparian native species, besides its tolerance to inundation, fire and drought combined with rapid seed germination and dispersion abilities (Brock 1994; Natale et al., 2010). Morphological identification of *Tamarix* species is often confounded by the cryptic hybrids resulting from hybridization between the species in the genus which in the past has led to misclassification of many *Tamarix* species. The advent of advanced molecular analysis techniques has, however, allowed accurate discrimination between *Tamarix* taxa; *Tamarix ramosissima* Ledeb. and *T. chinensis* Lour. have been recognized as the prominent invaders in the USA, South Africa and many other countries in the world and have a marked impact on riparian ecosystems and biodiversity (Zavaleta et al., 2001; Natale et al., 2010; Newete et., 2019). With the indigenous *T. usneoides* E.Mey., a total of three *Tamarix* species are currently known to occur in South Africa (Mayonde et al., 2015; Newete et al., 2019). The proper identification of *Tamarix* species is particularly important for the potential introduction of biocontrol agents.

There is no clear record of when and how the two exotic *Tamarix* species, *T. ramosissima* and *T. chinensis*, were introduced to South Africa, but they were most likely introduced in the early 1900s as ornamental or phytoremediation plants in mining sites (Marlin et al., 2017; Newete et al., 2019). *Tamarix ramosissima* and *T. chinensis* were the two most commonly planted species in mining sites across the country at least until 2005 when strict regulation was enforced to prohibit their further use, promoting the native *T. usneoides* instead. *Tamarix* are capable of removing mining contaminants such as sodium, manganese, potassium, calcium, nitrate, copper, sulphur, aluminium, silica, barium and lithium salts (DiTomaso, 1998). This raises the question as to whether salt might affect the feeding performance of herbivorous insects and thus their effectiveness as potential biocontrol agents. Since no biological control agents have been released yet in South Africa, the cosmopolitan *Tamarix* leafhopper *Opsius stactogalus* Fieber (Hemiptera: Cicadellidae) was used as a surrogate to test this hypothesis.

The taxonomic isolation of *Tamarix* has allowed many insect species to co-evolve with it in its origin of speciation in Asia, and therefore these insects are unlikely to attack other plants in other parts of the world (DeLoach et al., 2000). Kovalev (1995) identified 25 insect species that have coevolved with *Tamarix*, of which the *Tamarix* leafhopper, *Opsius stactogalus*, is found exclusively on *Tamarix* plants (Virla et al., 2010). This cosmopolitan small-sized (0.81–4.5mm) insect is native to Europe and takes about a month to develop from a neonate to an adult through five instars (Harding, 1930). The *Opsius* spp. are known to have 3–4 generations depending on the species and the altitude where they occur (Louden, 2010; Siemion and Stevens, 2015). Although *O. stactogalus* is often found in high numbers, damage caused by the leafhopper is generally considered insignificant (DeLoach et al., 2004; Virla et al., 2010) and, therefore, it is not regarded to be a viable biocontrol agent. High concentrations of salts or metals in plant tissues is known to deter herbivory in some insects (Coleman et al., 2005; Boyd, 2010; Davis et al., 2001; Newete et al., 2014). But such information is not available for the *Tamarix* leafhopper. This study investigated the effect of *O. stactogalus* on *Tamarix* spp. with and without elevated salt concentrations as a surrogate for potential biological control agents.

## Methods and materials

Five *Tamarix* taxa consisting of three pure *Tamarix* species and two hybrids, identified by Mayonde et al. (2015), were propagated from 15 cm cuttings in a greenhouse at the University of the Witwatersrand, Johannesburg (Table 1). Cuttings were first dipped in root growth hormone (Dynaroot Hormone powder Efekto) and rooted in river sand and allowed to grow for three months until the roots and leaves grew well. The saplings were then transferred into 1-liter plastic pots with potting soil and allowed to acclimate for two months before the experiment began. Five to ten plants from each taxon were used as controls (no salt or leafhopper), and ten for each of leafhopper only and leafhopper + salt treatments. Five replicates were used for *T. chinensis* and *T. chinensis* x *T. ramosissima* due to cutting (sapling) mortality before the start of the experiment.

### *Salt treatment and measurement*

Salt water was prepared by adding 480 g of table salt to 16 liters of tap water to make a salt concentration of 3% (w/w). A volume of 400 ml of this salt water was added to each pot (i.e. 180 mmol-1) over four days (100 ml per day) to reduce physiological shock to the plants. Control treatments received only tap water

**Table 1:** The five *Tamarix* taxa, with their respective number of replicates, used in this study. Note: For the "leafhopper + salt" treatment, the plants were first exposed to salt for 21 days before the leafhoppers were added.

| *Tamarix* taxa | Treatments | | | Total Replicates |
|---|---|---|---|---|
| | Control | Leafhopper | Leafhopper + Salt | |
| *T. chinensis* (Tc) | 5 | 5 | 5 | 15 |
| *T. ramosissima* x *T. chinensis* (TrTc) | 5 | 5 | 5 | 15 |
| *T. chinensis* x *T. usneoides* (TcTu) | 10 | 10 | 10 | 30 |
| *T. ramosissima* (Tr) | 10 | 10 | 10 | 30 |
| *T. usneoides* (Tu) | 10 | 10 | 10 | 30 |

(see Table 1). Salt uptake and excretion was recorded from approximately 0.2 g of leaf sample harvested from the leading branch of each *Tamarix* before the inoculation of the *Tamarix* leafhopper on day 21. The harvested leaf biomass was placed in a test tube containing 10 ml deionized water and swirled for 1 minute before measuring salt content of the water using an electro-conductivity meter.

### *Insect inoculation and plant measurement*

A total of ten *Tamarix* leafhoppers collected from a stand of *T. ramosissima* in Germiston, Johannesburg (26°12'37.80"S and 28°2'12.06"E) in October 2016 were inoculated onto the longest branch of each potted plant (5–10 plant replicates per *Tamarix* taxa as indicated in Table 1), and sealed with a small organza net bag. The change in length of the leading branch of each sapling was measured between day 1 and 42.

## Results

### *Electro-conductivity*

Salt excretion of salt-treated plants was significantly higher (> 50%) than salt excretion of controls ($F_{4, 66} = 8.1$, $P = 0.0002$) (Figure 1). *Tamarix chinensis* showed the highest electro-conductivity of all the *Tamarix* taxa tested.

### *Plant growth*

Plants onto which leafhoppers were released showed reduced branch growth compared to control plants for all *Tamarix* taxa (Figure 2). However, this difference was only significant for *T. chinensis*, where leafhopper feeding reduced average percentage increase in branch length by 90.6% compared to that of the control (Figure 2). The control plants showed an average percentage increase in branch length of 3.9% compared to those in the leafhopper treatment (0.2%) ($P$ = 0.006) and leafhopper/salt treatment (0.3%) ($P$ = 0.008). The reduction in plant growth caused by the leafhoppers was not significantly different on *Tamarix* plants with or without elevated salt concentrations.

## Discussion

### *Electro-conductivity*

Heavy metal accumulation in the roots of *Tamarix* plants increases with increasing salinity (Manousaki et al., 2008). This is because salinity improves the availability of metals in sediments and stimulates transport of metals from the roots to the leaves of the plant (Fitzgerald et al., 2003). In this study *T. chinensis* excreted significantly more salt than the other *Tamarix* taxa. However, since this species is not indigenous to South Africa and is a declared invasive species (Marlin at al., 2017), propagation of this plant for phytoremediation should not be promoted in South Africa. Leaves of all the other taxa, including the indigenous *T. usneoides*, also excreted significant amounts of salts.

### *Plant growth*

None of the *Tamarix* taxa tested showed a significant increase in branch length between the leafhopper and leafhopper + salt treatment plants suggesting no effect of salt on leafhopper feeding.

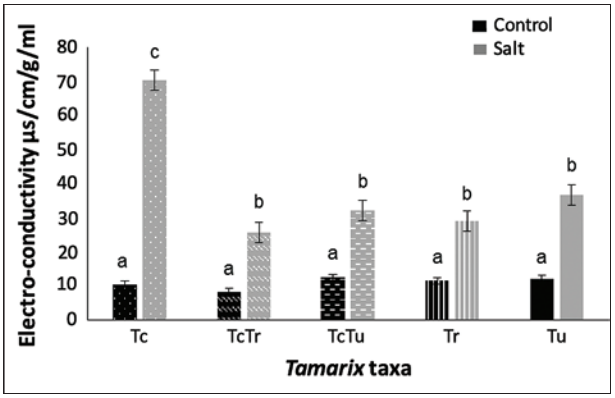


**Figure 1.** The five *Tamarix* taxa, with their respective number of replicates, used in this study. Note: For the "leafhopper + salt" treatment, the plants were first exposed to salt for 21 days before the leafhoppers were added.

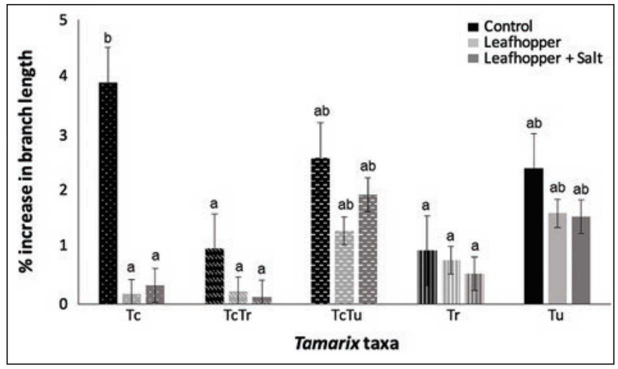


**Figure 2.** Percentage increase in length of the leading branch of *Tamarix* taxa taken before insect inoculation in day 21 and 21 days after inoculation. Means compared by one-way ANOVA; those followed by the same letter(s) across all bars are not significantly different ($P > 0.05$; Fisher's Least Significant difference test). $n = 5$ plants for *T. chinensis* (Tc) and *T. chinensis* x *T. ramosissima* (TcTr); $n = 10$ plants for *T. chinensis* x *T. usneoides* (TcTu), *T. ramosissima* (Tr) and *T. usneoides* (Tu).

The only significant change in plant vigor was seen in *T. chinensis* where the increase in branch length of the control was significantly greater than those in the leafhopper or the leafhopper + salt treated plants. This indicates that the treatments (leafhopper and leafhopper + salt) had a greater impact in decreasing the growth rate of *T. chinensis* as compared to the other taxa. This might be explained by *T. chinensis* excreting the most salt of all the *Tamarix* taxa tested. Energy is expended by *Tamarix* to transport salt from the roots to the salt glands, which results in less energy being available for plant growth (Manousaki et al., 2008).

## Conclusions

In conclusion, leafhopper impacts were not significantly affected by the addition of salt. There are about 25 insects that have evolved to strictly feed and survive on the halophytic *Tamarix* species, and among these are *O. stactogalus* and the *Tamarix* beetle (*Diorhabda* spp). The fact that the feeding performance of *O. stactogalus* across the five *Tamarix* taxa in this study was not significantly affected by salt addition may suggest that the *Tamarix* beetle, which is currently under quarantine investigation in South Africa, will not be influenced by the various salt concentration levels of the two exotic *Tamarix* species or their putative hybrids. Although high levels of salt and heavy metals in different plants are known to reduce or deter insect herbivory (Davis et al., 2001; Coleman et al., 2005; Boyd, 2010; Newete et al., 2014), the insects that feed on *Tamarix* seem to circumvent such physiological stress, since they have evolved and adapted to feed and thrive on strictly halophytic plants (Kovalev 1995).

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*Poster presentation*

# Cochineal and cactus: are new associations biocontrol winners?

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The New World invasive cactus *Opuntia engelmannii* Salm-Dyck ex Englem. is an important invasive weed in Kenya. Biocontrol of *Opuntia* spp. using cochineal insects has an excellent success record, but the underlying reasons are unknown. The winning combination could be either a new association or a long-established relationship between target plant and insect agent. Therefore, matching the cactus biotype to the insect biotype is critical for success. Success against *O. engelmannii* in Africa has been absent because of lack of knowledge on the origin of the plant and its relationship to different biotypes of the cochineal, *Dactylopius opuntiae* (Cockerell). Cochineals and cacti were collected along a coast-to-coast transect in the southern USA from 76 localities in four states. Samples from 10 cochineal populations collected from 10 different *O. engelmannii* geographical lineages were brought into quarantine in South Africa, where they were tested for establishment on cladodes of Kenyan *O. engelmannii*. From these, four “winner” locality cochineal biotypes were placed onto whole plants to assess their efficacy. Morphological matching of USA host plants with the target population of *O. engelmannii* gave mixed results of establishment and in efficacy trials, suggesting either a new association or established relationship could yield a successful agent. Genetic screening of the plants will hopefully shed light on this ambiguity. Nevertheless, the transect collection technique has yielded a strong biocontrol contender to solve the problem in Kenya.

*Poster presentation*

# Establishment of the moth *Hypena opulenta* in Canada: diapause induction and mass-rearing methods to enable biocontrol of *Vincetoxicum* spp.

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*Hypena opulenta* (Christoph) is a multivoltine moth originating from Eastern Europe that was first released in Canada in 2013 for biocontrol of dog strangling vine (DSV). This insect overwinters as a diapausing pupa and is established at early release sites in Ontario. To facilitate mass-rearing and release of this insect, we investigated (1) rearing using DSV-supplemented artificial diets, (2) diapause induction and (3) diapause storage. For rearing, we used a commercial beet-army worm diet, supplemented with four-week-old or ten-week-old dried DSV foliage. We produced viable moths with a mean survivorship of 37% in the 1st generation that increased to >60% in the 2$^{nd}$ generation. Pupal weight of insects produced on plants versus diets was similar. Development times were longer and survivorship was slightly lower for insects reared on older as compared to young foliage diets. For diapause induction, we compared percent diapause of *H. opulenta* on two ages of foliage and at long (16L:8D) and short (12L:12D) photoperiods. Short photoperiod was the dominant factor, inducing 100% diapause, regardless of foliage age. However, at long photoperiod, diapause was induced in 2–4% of individuals. In the field, *Hypena* adults were released in cages for four weeks, starting 14 June, and their development was followed until pupation. No diapause occurred for pupae originating from the first release, whereas 97–100% of pupae diapaused from the subsequent releases. Thus, there is a narrow window for field release to achieve multiple generations of *H. opulenta*. For diapause storage, pupae were stored for one or three months at 4°C without light. After transfer to 22°C, (16L:8D), adult emergence from the one-month cohort occurred sporadically over several months, whereas emergence of the three-month cohort occurred within 28 days, with a peak at 19 days. These results provide a prescription for rearing and stockpiling of pupae during winter and releasing adults early in spring to enhance the likelihood of a 2$^{nd}$ generation in the field.

*Poster presentation*

# Transgenerational effects of host plant quality in biocontrol agents: does offspring environmental matching matter?

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As with various insects, the life-history and feeding preference of phytophagous insects can be influenced by diet quality within and across generations (Boggs, 2009). Indeed, several nutritionally-dependent traits can be conferred to offspring through epigenetic (non-genetic) mechanisms in what is now regarded as transgenerational plasticity or parental effects. By definition, phenotypic plasticity can be regarded as the environmentally-dependent (diet in this case) phenotypic expression of a single genome (Colombo et al., 2014). Such phenotypic plasticity can be adaptive when beneficial phenotypes are expressed, leading to rapid coping mechanism for organisms' rapidly changing environments such as temperature or diet quality. However, phenotypic plasticity can be maladaptive when the expressed phenotypes do not render any benefits for organisms exposed in a novel environment.

For weed biological control programs, where mass-reared insects may be kept for several generations in typically stable laboratory microclimates and optimal diet, transgenerational effects may present challenges when offspring that are released in the field face rapidly changing novel environments, including diet (Hoffmann and Ross, 2018). Hence, some authors have argued that organisms developing under resource-constrained environments (quality and availability) develop plastic responses that favor optimal performance under corresponding conditions for future offspring in what is now regarded as the "environmental matching hypothesis" (EMH) (Scharf et al., 2015; Zizzari et al., 2016). In contrast, some have posited that a "silver spoon" effect exists where parents that developed on good-quality resources (e.g., diets) confer maximum fitness potential on their offspring enabling superior survival, performance and population persistence, even under resource-constrained environments, and has since been termed the "silver spoon hypothesis" (SSH) (Hopwood et al., 2014). Despite the dichotomy of the two hypotheses, both enjoy considerable empirical support (Scharf et al., 2015). Nevertheless, it is very apparent that nutritional quality during ontogeny for both parents and offspring influences fitness and performance, whichever the direction (Hopwood et al., 2014). For weed biological control agents, it is therefore important to understand the consequences of variability of quality among resources as it has direct implications on population persistence, field performance and ultimately the efficacy of biological control programs. Indeed, several arthropod nutrient-dependent traits such as body size and longevity have all been shown to be key in ensuring population persistence through mediation of reproductive success and resistance to climatic stress. From the foregoing, we tested the transgenerational effects of foliar nitrogen variability in the invasive shrub *Tradescantia fluminensis* Vell. (Commelinaceae) on *Neolema abbreviata* Lacordaire (Coleoptera: Chrysomelidae).

*Tradescantia fluminensis* is native to southeastern Brazil and northern Argentina and belongs to a group of succulent monocotyledonous plants known as wandering Jew. Apart from South Africa, it has been declared invasive in Australia and New Zealand, among other countries. Fully grown *T. fluminensis* forms dense undergrowth in the forest, which disrupts seedling recruitment and ultimately forest regeneration

(Standish et al., 2001; Mbande et al., 2018, 2019). Area-wide mechanical control is difficult as it vegetatively propagates with each stem fragment, having the capacity to form new plants through root development at the nodes. Chemical control is also largely unsuitable owing to potential non-target effects in sensitive ecosystems where it grows. This leaves biological control using arthropods and pathogens as the most sustainable and viable strategy to curb *T. fluminensis*.

*Neolema abbreviata* is one of several agents selected for biocontrol of *T. fluminensis*. The beetles lay eggs singly, mostly on new shoot-tips of the plants; a single generation takes more than 120 days under controlled laboratory conditions with optimal ambient conditions and diet.

Using a reciprocal experimental design, we investigated how nitrogen (N) enrichment in parental diets influenced larval and adult feeding damage and mortality, as well as adult eclosion success of $F_1$ and $F_2$ generation offspring. Our results showed that parental diet did not influence larval feeding damage, mortality and eclosion success. Feeding damage among larvae was highest (7.4 ±1 .9 $cm^2$) in the low-nitrogen test plants regardless of generation and parental diet. Offspring diet was more important in determining larval mortality ($F_{1, 224} = 4.78$, $P = 0.029$) where mortality was highest in high-nitrogen plants despite rapid development. The interaction between parental diet and test diet significantly influenced larval feeding ($F_{4, 224} = 3.27$, $P = 0.012$) and mortality ($F_{4, 224} = 4.78$, $P = 0.029$) but not eclosion success. Under the low-nitrogen offspring diet, larvae from nitrogen-rich parental diets performed better in both generations under the poor test diet.

In adults, oviposition preference was largely responsive to test diet ($F_{2, 599} = 7.86$, $P < 0.001$) with medium and nitrogen-rich test plants receiving the most eggs. Feeding was only affected by test diet ($F_{2, 149} = 11.2$, $P < 0.001$). As in larval feeding, feeding by adults increased by almost two-fold in low-N plants compared to medium and high-N plants, regardless of parental diet and generation. Only test diet significantly influenced adult weight ($F_{2, 149} = 5.58$, $P < 0.001$). Feeding on medium and high-N test plants resulted in higher adult weight (0.05 ± 0.2 mg) compared to low-N test plants (0.01 ± 0.02 mg) among adults from all the parental diets and both generations. Adult longevity was also higher in medium and high-N test plants across all parental diets and generations. However, $F_2$ adults from low-N parental diets had even shorter longevity under low test diet suggesting significant influence of parental diet and generation.

Taken together, our results show limited transgenerational plasticity in response to host plant quality among several life-history traits on *N. abbreviata*. The EMH posits that offspring perform best in environments similar to their parents. In our study, the quality of the offspring diet appeared to be more important for the performance of the beetles in both adult and larval stages in $F_1$ and $F_2$ generations. Larval and adult feeding was higher in low-nitrogen plants, previously reported as a mechanism to offset nitrogen deficiency (Nestel et al., 2016).

There were differences in the longevity between $F_1$ and $F_2$ adults among individuals from low-nitrogen parental diets with the $F_2$ generation generally having a shorter lifespan, suggesting a strong parental diet x generation interaction, where prolonged transgenerational exposure to a nitrogen-poor diet brought fitness costs for offspring. This is despite the higher larval mortality that was present in the nitrogen-rich diet. These findings corroborate Tao et al. (2014) where feeding on nitrogen-rich plants increased mortality in the monarch caterpillar *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) following increased expression of defensive cardenolide compounds in the milkweed *Asclepias curassavica* L. It is therefore likely that *T. fluminensis* may resist *N. abbreviata* larvae better under higher nitrogen concentrations.

In conclusion, this study gives insights into how foliar nitrogen may influence plant-insect interactions. Future studies should endeavor to test these findings under field conditions as the results may better explain the population dynamics of biocontrol agents in the field.

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*Poster presentation*

# Do low temperatures and variations in leaf quality of *Chromolaena odorata* play a role in the variable performance of *Pareuchaetes insulata*?

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The invasiveness and negative impacts of the alien scrambling perennial shrub *Chromolaena odorata* (L.) R. King & H. Rob. (Asteraceae) in South Africa resulted in the introduction of two biological control agents, *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Erebidae: Arctiinae) and its congener *P. aurata aurata* (Butler) into South Africa. However, both agents failed to establish, and this consequently resulted in the introduction of a third *Pareuchaetes* species, *P. insulata* (Walker) (from Florida [USA], Cuba and Jamaica) between 2001 and 2009 (Zachariades et al., 2011). Over 1.9 million individuals of different life stages of the moth were released at 30 sites, mainly in subtropical eastern KwaZulu-Natal (KZN) province. Only the Floridian population is thought to have established, at a single site, after 880,000 individuals of this population were released at 21 sites in KZN (Zachariades et al., 2011). Although the moth established and is exerting some control on the target weed, its population level in the field generally remains low. Several factors such as temperature incompatibility, top-down factors (predation and parasitism) or bottom-up factors (foliage quality) may explain the variable performance of this moth (Uyi, 2014).

Despite the fact that the thermal environment of insects and foliar quality of host plants are critical determinants of the fitness of insect herbivores especially those used as weed biological control agents (e.g., Hough-Goldstein et al., 2016; Uyi et al., 2016a), the influence of these factors are not often considered by biocontrol practitioners during pre- and post-release studies on terrestrial weeds. Here, we present results from several laboratory experiments on the effects of low sub-optimal yet sub-lethal temperatures, acclimation temperatures (warm and cold), and variation in foliar quality of host plant on several performance metrics of *P. insulata*.

We found a substantial influence of thermal history (acclimation temperature) on locomotion performance of larvae (e.g., Lachenicht et al., 2010), with the major result being that cooler acclimation temperatures resulted in improved locomotion ability at cooler test temperatures (Figure 1a,b; Uyi et al., 2017). This is interesting given that this is a tropical/sub-tropical specialist herbivore, which originates from stable warm environments in Florida, USA. Low temperatures (below 11°C) significantly affected the locomotor abilities of *P. insulata* larvae (Figure 1a,b). For example, none of the warm-acclimated individuals were able to initiate movement at 6°C, while only 36% of the cold-acclimated individuals dispersed at this temperature, walking for only two seconds of the 30-second exposure time. This is likely to have some serious ecological implications in the field during winter months as the mean absolute minimum temperatures in Sappi Cannonbrae Plantation, Umkomaas (12 months of microclimate temperature data) during winter range between 6.4 and 8.6°C. Older larvae of this multivoltine species are nocturnal, and the

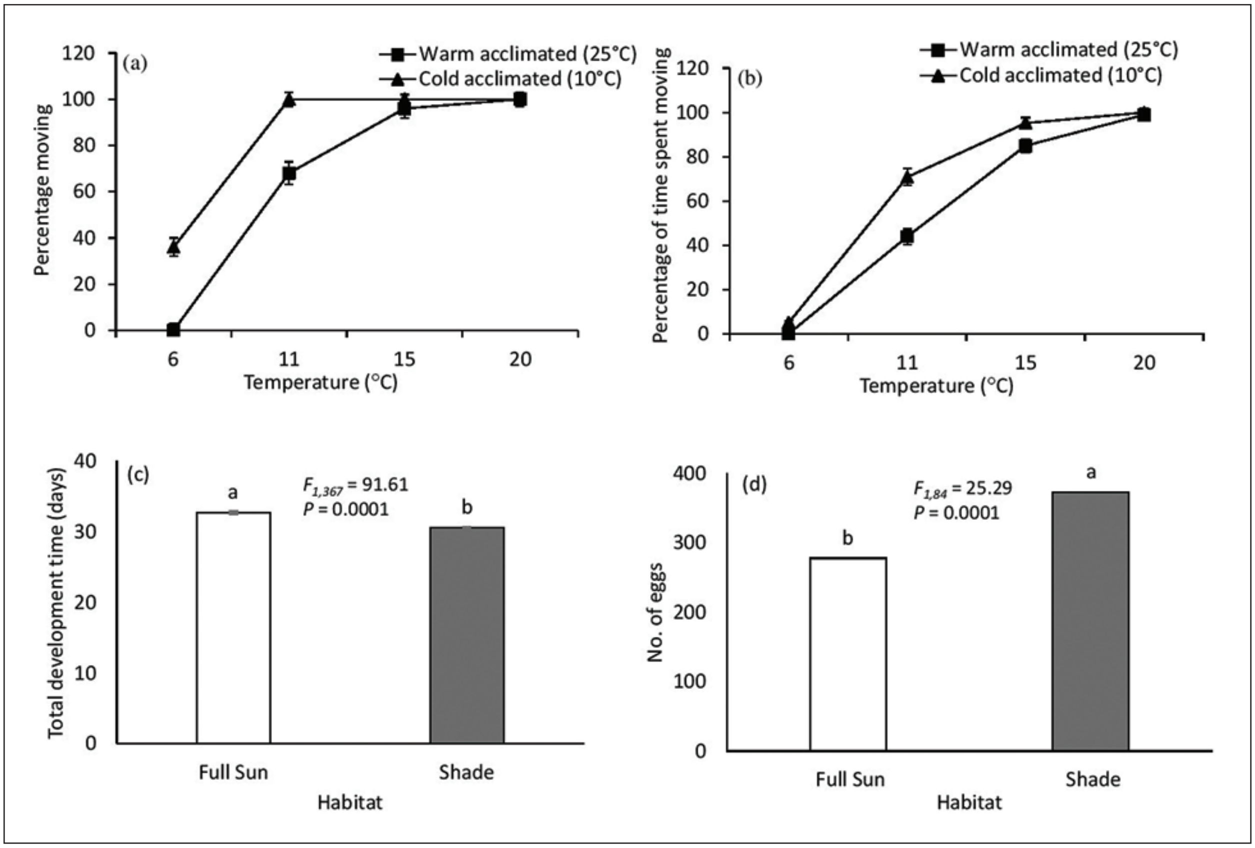


**Figure 1.** Comparative mobility of third instar larvae of *Pareuchaetes insulata* acclimated to warm (reared at 25°C) or cold (at 10°C) for 2 days after consecutive 30-second exposure periods to 6, 11, 15 and 20°C on a temperature-controlled locomotion stage (five individuals were exposed to each temperature and the test was replicated five times). The mean (±SE) percentage of individuals moving (a), and the mean (±SE) percentage of time spent moving (b) for each treatment at each temperature are presented (from Uyi et al., 2017, reprinted with permission). Mean (± SE) total development duration of (c), and number of eggs laid (d) by *Pareuchaetes insulata* reared on *Chromolaena odorata* leaves from two habitat conditions (full sun vs. shade) under constant laboratory conditions at 25°C and L12:D12.

nocturnally feeding stages (instars 3–6) last for an average of 12 days at 25°C in the laboratory (Uyi, 2014). This suggests that *P. insulata* larvae might reduce locomotion and feeding in winter (June and August) when the temperature falls below 11°C, thereby preventing escape from indigenous natural enemies (that are more cold-tolerant) or encouraging larval starvation. Uyi et al. (2017) hypothesized that when larvae are dislodged from the foliage of *C. odorata* plants (either by wind or other factors), the situation may be further exacerbated as attempts to locate their host plant (to either initiate feeding or seek shelter) might prove unsuccessful. All of these factors might lead to an increase in indirect mortality and consequently affect the populations of this biological control agent in field situations. These findings corroborated those of Uyi et al. (2016b) who hypothesized that both direct and indirect negative impacts of low winter temperatures, such as increased mortality, slow development and reduced fecundity may partly explain the low population of *P. insulata* in South Africa.

Our nutritional ecology experiments showed that the leaves of *C. odorata* plants growing in a full-sun environment had increased toughness and lower nitrogen and water content compared with leaves of plants growing in shade. From the viewpoint of herbivore performance, *P. insulata* performed better on *C. odorata* leaves from shaded environments. For example, *P. insulata* developed faster and had larger pupal mass, and adult females laid higher numbers of eggs when reared on shaded foliage compared with full-sun foliage (Figure 1c,d). We suggest that the benefits obtained by *P. insulata* feeding on shaded foliage are associated with reduced toughness and enhanced nitrogen and water contents of leaves. These results demonstrate that light-mediated changes in plant traits and leaf characteristics can affect the developmental and reproductive performance of *P. insulata* in South Africa. The improved performance of *P. insulata* on shaded leaves might have some implications for weed biological control. For instance, the fact that *C. odorata* plants are known to be shade intolerant (Zachariades et al., 2009), suggests that female adults might struggle to locate oviposition sites because of the sparse distribution of the plant in such habitats. Alternatively, when eggs are laid on leaves of plants in full-sun habitats, survival might be negatively affected due to their exposure and that of the resulting early instars to adverse environmental conditions. Also, larvae might suffer fitness costs (expressed as prolonged development time and reduced pupal mass and fecundity) on full-sun plants, and any attempts by larvae to migrate to a more nutritious environment might expose them to predation or other mortality factors. All of these can potentially lead to reduced fitness or a decline in population levels of this insect in the field.

The results of this study and others (e.g., Uyi et al., 2015, 2016b, 2018) not only demonstrate that variations in leaf characteristics can negatively affect the developmental and reproductive performance of *P. insulata*, but also suggest that reduced locomotion at low sub-lethal temperatures may be an important driver of the population dynamics of the biocontrol agent (especially in winter months). This may consequently explain the often low population levels of the moth in South Africa. To conclude, we recommend that weed biological control practitioners conduct thermal tolerance and other temperature-dependent development trials, as well as nutritional ecology trials on biological control agents in the laboratory as part of rigorous pre-release studies to avoid wasting resources on agents that would not be climatically adapted or nutritionally suitable to the new environments of their host plants.

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*Poster presentation*

# The effects of topping and humidity on establishment of the arundo wasp and scale released to control *Arundo donax*

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Arundo (*Arundo donax* L., Poaceae) is a large perennial grass species that is invasive in Texas and California. Besides displacing native vegetation and obstructing flood channels, arundo consumes scarce water resources in dry riparian habitats. Mechanical and chemical control are common methods used to eradicate invasive arundo, but these methods prove ineffective or cost-prohibitive against large infestations. Two biological control agents, the arundo wasp (*Tetramesa romana* [Walker]) and the arundo armored scale (*Rhizaspidiotus donacis* [Leonardi]), have been approved for release against arundo. Seven years after initial release in Texas, the arundo wasp decreased live biomass of arundo shoots by 30–40% and promoted revegetation of native plants. While the wasp is well established in southern Texas, previous establishment trials in northern California were not successful. In 2017, our goal was to release the arundo wasp and arundo armored scale at seven different sites across central and northern California. To investigate if changes in methodology would facilitate wasp establishment, we conducted a large-scale field experiment to examine the effects of topping (cutting to 1 m) the arundo approximately six weeks prior to release on wasp and scale establishment. We expect to find higher wasp and scale presence at topped sites, since topping arundo promotes the growth of side shoots where the wasp prefers to oviposit. We also investigated the effects of humidity on wasp oviposition. This poster presented our methodology, current images of the arundo biocontrol program, and results from the greenhouse humidity experiment.

*Poster presentation*

# Evolutionary ecology at the expanding edge

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As populations spread, increased dispersal can evolve at the expanding edge relative to the core. This, and other evolutionary dynamics at expanding edges, might influence the predictability of range expansion and range limits of biological control agents. *Diorhabda* beetles released for the biological control of *Tamarix* spp. in North America are spreading rapidly southward. We are evaluating dispersal in core and range-edge populations. This poster outlined our research plans and presented preliminary results.

*Poster presentation*

# Searching for host-pathogen compatibility: how cpDNA analysis can aid successful classical biological control of *Impatiens glandulifera*

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Himalayan balsam (*Impatiens glandulifera* Royle), native to the foothills of the Himalayas, is an invasive weed widespread throughout the UK. In 2014, the rust fungus *Puccinia komarovii* var. *glanduliferae* R.A. Tanner, C.A. Ellison, L. Kiss & H.C. Evans, collected from India, was released in the UK following approval from the UK authorities. A strain from Pakistan was subsequently released at selected sites due to the variation in susceptibility of different populations of the weed to the Indian strain. Controlled inoculation studies with the two rust strains have identified weed populations with partial and full resistance to either, and in some cases, both strains. In order to achieve control of the weed throughout the UK with the rust, it is essential to (1) clarify how many genotypes are present in the UK and (2) identify where to survey for new strains in the native range. Therefore, in this study, molecular analyses based on nuclear rDNA-ITS and six chloroplast DNA (cpDNA) sequences were applied. Leaf samples were included from a total of 26 sites in the British Isles and from 8 sites in the native range. In addition, 18 herbarium samples from both the introduced and native range, collected from 1881–1956 were included. The cpDNA sequences showed more variations between leaf samples compared with rDNA-ITS sequences. Hence, phylogenetic analyses focused on the cpDNA data and found that the plant samples separated into three groups. Two groups consisted of samples from both the introduced and native range; however, the third group contained only UK samples. These results suggest that Himalayan balsam in the UK was introduced at least three times from the native range. Based on the cpDNA data, 10 and 15 haplotypes were found in the introduced and native range, respectively, and two haplotypes were found in both regions. The results show where to survey in the native range to have the best chance of collecting new virulent strains for the UK and enhance the impact of classical biological control on this invasive weed.

# Session 6: Regulations for agent release and access to genetic resources

## Session Chair: Philip Weyl

**Keynote**

# Biological control of weeds in the Anthropocene: why has introducing new agents become so challenging?

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## Abstract

The *Anthropocene* was proposed in 2016 for the epoch dating from the beginning of significant human impact on the Earth's geology and ecosystems. Human activities have caused mass extinctions of plant and animal species, polluted the oceans and modified the atmosphere, among other lasting impacts such as the rampant spread of invasive alien species. The importation and release of invertebrates and microorganisms to control invasive alien plants has been a cornerstone to manage these threats since the beginning of this epoch. However, an unintended consequence of the Anthropocene has been humanity's increased concern for the preservation of biodiversity and the equitable sharing of its benefits. Tighter regulations and impediments to accessing biocontrol genetic resources are the result. The weed biocontrol community can take measures to overcome these hurdles.

**Keywords:** biological control, regulation, access and benefit-sharing

## Introduction

Biological control of weeds has a long history. Beginning in 1836, 551 biocontrol agents targeting 224 weeds have been released in 130 countries (Winston et al., 2014). There are many success stories, whereby 65.7% of the weeds targeted for biological control experienced some level of control (Schwarzländer et al., 2018). Some of these successes have been dramatic. For example, water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae), in Africa where the release of *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) led to the dramatic decline of this invasive alien weed and saved the livelihoods of 30 million people (Collis, 2000). Prickly pear cactus, *Opuntia* spp. (Cactaceae), in Australia is another example where introduction of the cactus moth *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) and the mealybug *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) resulted in spectacular control (Julien, 2006; Page and Lacey, 2006). The benefits provided to society by weed biological control programs have been significant over the years. So, why has introducing new weed biocontrol agents become so challenging?

The answer may lie in what has become known as the Anthropocene (Carrington, 2016). To define a new geological epoch, a signal must be found that occurs globally and will be incorporated into deposits in the future geological record. First proposed in 2009, the Anthropocene is marked by human signals dating from 1950, including: radioactive elements from nuclear bomb tests, unburned carbon spheres emitted by power stations, plastic pollution, aluminum and concrete particles, high levels of nitrogen and phosphate in soils. Associated biological phenomena include the rapid spread of Invasive Alien Species (IAS) across the world and the potential for the sixth mass extinction of species in the 700 million-year history of complex life on earth.

Unintended effects of the rapid spread of IAS include the effects of chemical pesticide residues on human health from their widespread use, leading to de-registration of existing products and fewer new products being developed. Hence, *the need for biological control solutions has never been greater!* The extinction of biodiversity has led to an unprecedented awareness of threatened and endangered species and destruction of natural environments on which these species depend, thus *risks to biodiversity becomes central to decision-making*. Furthermore, the race to exploit biodiversity is on, raising concerns about bio piracy and a nation's sovereign rights, resulting in *access to genetic resources becoming limited*. These issues have resulted in unintended consequences for biological control.

## Unintended consequences to biological control

The need to address IAS that threaten food security and productivity have been the main drivers of weed biological control programs since its inception. However, humanity's increasing concern for the preservation of biodiversity, and the equitable sharing of its benefits, have resulted in tighter regulations and impediments to accessing biocontrol genetic resources in the last 20 or so years. Each of these "hurdles" has slowed the pace of biological control introductions and increased the need for additional resources, already challenging to obtain, for implementing biological control against invasive alien weeds.

## Regulation of biological control

Currently, releases of biocontrol agents are regulated internationally, regionally, and by country. While international and regional oversight has come into play more recently, individual countries have regulated the importation and release of biocontrol agents for decades. Regulatory approval can only be given by the country in which the biocontrol agent is released.

### *National legislation*

A survey of national legislation shows a wide variation in the types of Acts under which biocontrol of weeds is regulated (Table 1). Only Australia has legislation specific to biological control. Others, for example, Canada, Kenya, Mexico and the USA regulate biological control organisms under plant protection legislation. Hunt et al. (2008) provide a review of legislation overseeing biological control in North America, Europe and the island nations of Australia and New Zealand. In some cases there may be additional country-specific regulations that must be fulfilled, such as the National Environmental Policy Act and the Endangered Species Act in the USA. Even within a country there may be state or provincial legislation that is also invoked (e.g., Argentina). Many countries have no legislation in place for biological control organisms; some fall back on legislation suited for registration of synthetic pesticides.

### *International standards*

The International Plant Protection Convention (IPPC) of 1951, is a multilateral treaty deposited with the Food and Agriculture Organization (FAO) of the United Nations (FAO, 2019). The IPPC "… aims to secure coordinated, effective action to prevent and to control the introduction and spread of pests of plants and plant products." To facilitate coordinated and effective action, the IIPC has developed 28 International Standards for Phytosanitary Measures (ISPMs) relating to plant protection. Among these, ISPM 3: 1996 (2005) provides general guidelines for risk management related to the export, shipment, import and release of biological control agents and other beneficial organisms (IPPC, 2019). While not legally binding, ISPM 3 is a tool to guide regulators and users on the requirements that should be addressed to ensure safe use of biological control.

### *Regional standards (where borders meet)*

Under the auspices of the IPPC, 10 Regional Plant Protection Organizations are recognized and some have developed Regional Standards for Phytosanitary Measures (RSPMs) for biological control (Table 2). Each country within a region is represented by a National Plant Protection Organization.

**Table 1.** Legislation for select countries under which biological control of weeds is regulated.

| Country | Legislative Act (year implemented) | Reference |
|---|---|---|
| Australia | Biological Control Act (1984) | https://www.legislation.gov.au/Details/C2008C00315 |
| Brazil | Pesticide Law (2000) | http://allierbrasil.com.br/english/services_brazilian.htm |
| Canada | Plant Protection Act (1990) | https://laws-lois.justice.gc.ca/eng/acts/p-14.8/ |
| England | Wildlife and Countryside Act (1980) | https://www.legislation.gov.uk/ukpga/1981/69 |
| Kenya | Plant Protection Act (2012) | http://extwprlegs1.fao.org/docs/pdf/ken18403.pdf |
| Mexico | Plant Health Act of the Mexican States (1980) | http://www.diputados.gob.mx/LeyesBiblio/regley/Reg_LSFEUM_MSV_orig_18ene80.pdf |
| New Zealand | Hazardous Substances and New Organisms Act (1996) | http://www.legislation.govt.nz/act/public/1996/0030/93.0/DLM381222.html |
| South Africa | Agricultural Pests Act 36 (1983) | https://www.nda.agric.za/docs/NPPOZA/Agricultural%20Pests%20Act.pdf |
| Switzerland | Ordinance on Handling Organ-isms in the Environment (2008) | https://www.admin.ch/opc/en/classified-compilation/20062651/index.html |
| The Netherlands | Flora and Fauna Act (1998) | https://www.ecolex.org/details/legislation/act-containing-rules-relative-to-the-protection-of-wild-plant-and-animal-species-flora-and-fauna-act-lex-faoc017422/ |
| United States | Plant Protection Act (2000) | https://www.law.cornell.edu/uscode/text/7/chapter-104 |

**Table 2.** Regional plant protection organizations recognized under the International Plant Protection Convention and Regional Standards for Phytosanitary Measures (RSPM) for biological control (IPPC, 2019).

| Regional plant protection organization | RSPM |
|---|---|
| Asia and Pacific Plant Protection Commission (APPPC) | |
| Caribbean Agricultural Health and Food Safety Agency (CAHFSA) | |
| Comunidad Andina (CAN) | |
| Comite de Sanidad Vegetal del Cono Sur (COSAVE) | RSPP 4 (2016) |
| European and Mediterranean Plant Protection Organization (EPPO) | PM6 (2014) |
| Inter-African Phytosanitary Council (IAPSC) | |
| Near East Plant Protection Organization (NEPPO) | |
| North American Plant Protection Organization (NAPPO) | RSPM 7, RSPM 12 (2015) |
| Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) | |
| Pacific Plant Protection Organization (PPPO) | |

To facilitate review of submissions for release of biological control agents among countries Comité de Sanidad Vegetal (COSAVE), European and Mediterranean Plant Protection Organization (EPPO), and North American Plant Protection Organization (NAPPO) have developed regional standards outlining the information required for regulatory review. In the case of NAPPO, the three member countries (Canada, the USA and Mexico) have agreed that the requirements outlined in RSPM 7 *Guidelines for Petition for First Release of Non-indigenous Phytophagous or Phytopathogenic*

*Biological Control Agents* are the minimum information that must be provided for review by regulatory authorities in each country. This common approach ensures that each member country has a say in whether a weed biological control agent is approved in a neighboring country. As noted above, each country may have additional requirements that must be met. In the EPPO region, Standard PM 6/2 (3) *Import and release of non-indigenous biological control agents* gives guidelines for the import, release and required application procedures for non-indigenous biological control agents (EPPO, 2014). These guidelines are considered as recommendations and there is no obligation to use these, reflecting the challenges of trying to bring many views of the 51 member countries together.

**Actions to improve the regulatory environment**

In response to tightened regulatory requirements, the weed biological control community has conducted reviews to emphasize the benefits of biological control. For example, the safety and successes have been documented, showing that >99% of 512 agents released have had no known significant adverse effects on non-target plants (Suckling and Sforza, 2014). A global review of the risks-benefits-costs of weed biological control concluded that a common approach that is open and objective is key to successful weed biological control (Sheppard et al., 2003). Surveys to understand public perception showed that there is a general positive attitude towards biological control (McNeil et al., 2010). There are five publics that need to be considered, each with different communication needs: clients (land managers and farmers); funders (interested in managing invasive species); regulators; scientific allies (public-interest ecologists interested in pest-management alternatives, e.g., Weed Science Society of America); and contrarians (interest groups who are philosophically opposed to biological control) (Warner et al., 2009). Strategies to educate these various groups on the benefits and risks will garner support for weed biological control.

Actions to engage regulators have also been undertaken to make the issues known (e.g., ISAC, 2015). Best practices are being developed, for example a standardized risk assessment (Paynter et al., 2015), and data are being generated to demonstrate what non-target impact really is (e.g., Catton et al., 2015). These latter efforts contribute to science-based decision-making by regulators.

Together these actions promote biological control as a valuable control strategy. As Heimpel and Cock (2018) conclude, the paradigm is shifting to where **benefits and risks** are evaluated.

**Convention on Biodiversity and Access and Benefit-Sharing**

Access to biological control genetic resources has become challenging because of implementation of the Convention on Biological Diversity (CBD). The CBD is a legally binding international treaty (CBD, 2019a) and it has three main objectives:

1. the conservation of biological diversity;
2. the sustainable use of its components;
3. the fair and equitable sharing of benefits arising from genetic resources.

The CBD is one of the most significant treaties in human history as it concerns the diversity of life on earth (Prathapan and Rajan, 2011). However, methods for implementation and enforcement of the CBD were not outlined, and participating countries were left to determine how to comply with the agreement using whatever available resources they have.

The Nagoya Protocol (NP) is the instrument through which the 3rd objective of the CBD is to be implemented (CBD, 2019b). It is intended to contribute to the conservation and sustainable use of biodiversity and is an agreement between the parties to the CBD as to how access and benefit-sharing of genetic resources will be handled in the future and includes all of biodiversity. Each country that has ratified, acceded to, accepted or approved the Nagoya Protocol must prepare legislation and regulations. Key components are that indigenous people, local communities involved and traditional knowledge associated with genetic resources must be considered when genetic resources are accessed, and to access genetic resources prior informed consent (PIC) of the providing party (country of origin or other party that has acquired the genetic resources in accordance with the CBD) is required unless otherwise determined by that party.

The NP instructs that legislation must include:

1. requirements for acquiring PIC must: provide legal certainty, be transparent, inform on how to apply for PIC (including involvement of indigenous and local communities where relevant);
2. clear and written decisions by a competent national authority;
3. a permit or equivalent of the decision to grant PIC and of the establishment of mutually agreed terms (MAT);
4. clear rules and procedures for establishing MAT.

To facilitate access to genetic resources, Article 13 *National Focal Points and Competent National Authorities* states that each party to the NP is required to designate a **national focal point** on ABS whose role is to make information available for applicants seeking access to genetic resources, and designate **competent national authorities** who would "… *be responsible for granting access or, as applicable, issuing written evidence that access requirements have been met and be responsible for advising on applicable procedures and requirements for obtaining prior informed consent and entering into mutually agreed terms.*" Internationally the Nagoya Protocol establishes "*An Access and Benefit-sharing Clearing-House*" via the CBD, that will provide information relevant to the implementation of the protocol.

Of particular relevance to biological control is Article 8 "Special Considerations" of the Nagoya Protocol which states:

"*In the development and implementation of its access and benefit-sharing legislation or regulatory requirements, each Party shall:*

*(a) Create conditions to promote and encourage research which contributes to the conservation and sustainable use of biological diversity, particularly in developing countries, including through simplified measures on access for non-commercial research purposes, taking into account the need to address a change of intent for such research;*

*(b) Pay due regard to cases of present or imminent emergencies that threaten or damage human, animal or plant health, as determined nationally or internationally. Parties may take into consideration the need for expeditious access to genetic resources and expeditious fair and equitable sharing of benefits arising out of the use of such genetic resources, including access to affordable treatments by those in need, especially in developing countries;*

*(c) Consider the importance of genetic resources for food and agriculture and their special role for food security.*"

## Implications for Biological Control

There are three ways that national legislation and regulations could affect biological control.

1. Free use and exchange of biological control genetic resources:
   a. up to now, well facilitated through the principal of free use and exchange of genetic resources;
   b. future access to natural enemies for new IAS restricted or prevented if "public good" not considered.
2. Perception that all biological control genetic resources are used for commercial purposes:
   a. confused with the commercial aspect of bio-pesticides which generate profits;
   b. most projects are non-commercial and introduce agents to permanently suppress an IAS;
   c. benefits are global, poorer countries have shared the benefits;
   d. commercialization for a very few biological control genetic resources, benefit sharing may then apply.
3. Response time for implementing biological control:
   a. depending on the legislation and regulations biological control will be impeded or facilitated;
   b. if considered non-commercial, simplified measures should facilitate use of biological control in emergencies and for the needs of sustainable food and agriculture;
   c. if considered commercial, some countries may inadvertently make use of biological control unnecessarily difficult or even impossible;
   d. additional bureaucratic procedures will slow efforts, already dealing with tight regulatory procedures and limited funding, to find biological control solutions to IAS problems.

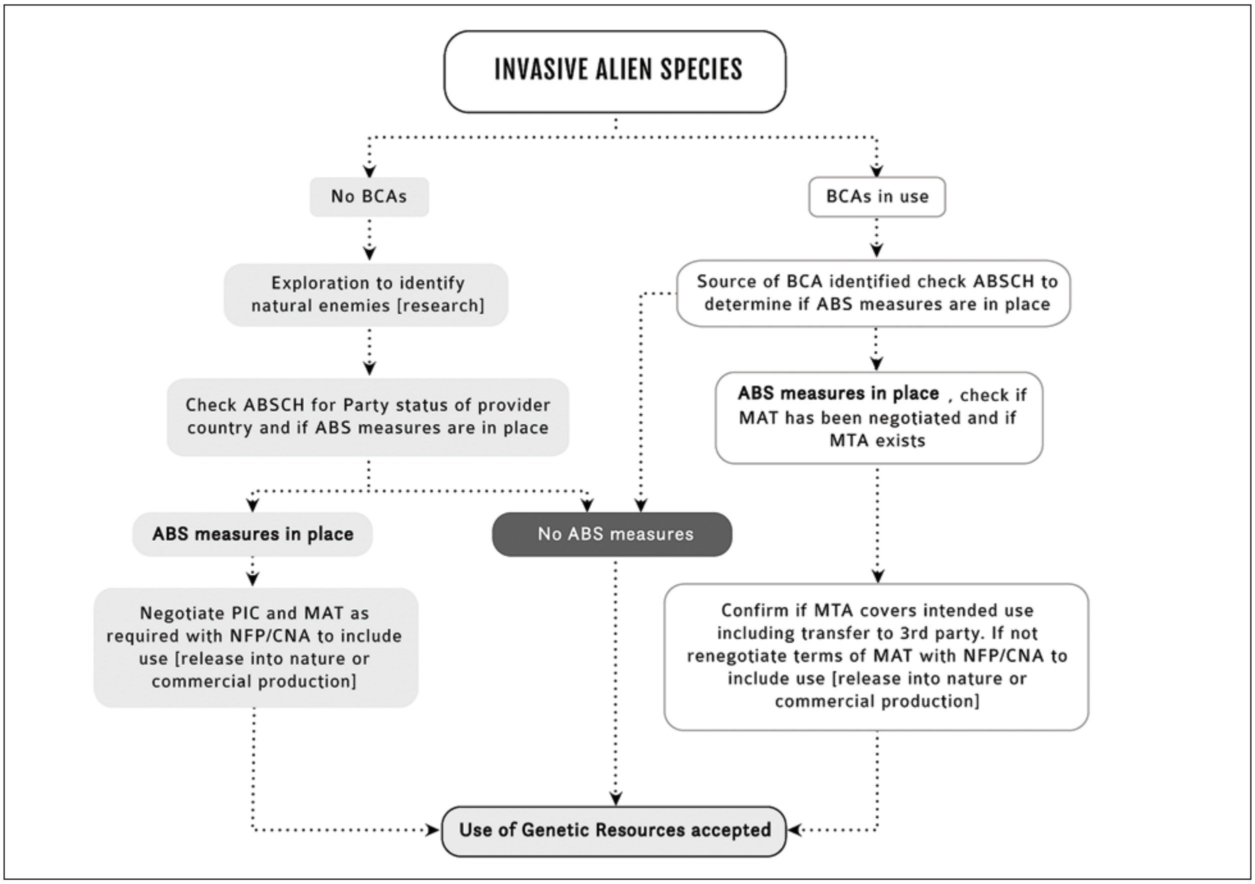


**Figure 1.** Generalized procedures for access to invertebrate biological control genetic resources. (ABSCH = Access and Benefit-Sharing Clearing House; CAN = Competent National Authority; MAT= Mutually Agreed Terms; MTA = Material Transfer Agreement; NFP = National Focal Point; PIC = Prior Informed Consent)

## Actions to improve access to biological control genetic resources

It is essential that the biological control community make the case that biological control provides global benefits and that access to biological control genetic resources is key to providing these. To this end, the International Organization for Biological Control (IOBC) produced a background study paper, *The use and exchange of biological control agents for food and agriculture,* for the FAO Commission on Genetic Resources for Food and Agriculture (Cock et al., 2009). The IOBC Global Commission strongly recommended that biological control agents should be considered as a special case with respect to access and benefit sharing (ABS) under the CBD and that access to biological control genetic resources should be facilitated (Cock et al., 2010). The International Union for the Conservation of Nature-SSG Invasive Species Technical Group was commissioned to prepare a technical report for the CBD on how biological control is implemented for control of IAS (ISSG, 2018).

Understanding access and benefit-sharing policy of provider countries is key to sorting through the process (see Silvestri et al., 2019, these Proceedings). Because there will be differences among countries, it will be necessary for the biological control community to document the process required for each provider and to make this information available to other parties interested in accessing the same or other biological control genetic resources. Generalized procedures for accessing biological control genetic resources are presented in Figure 1.

Demonstrating due diligence in complying with ABS requirements is another action that will help to improve access to biological control genetic resources. For example, CABI has developed a policy for clients and best practices for staff to ensure that all activities relating to the acquisition of weed biological control agents comply with requirements of partner countries that have ABS legislation and regulations in place (Smith et al., 2018; Hinz et al., 2019, these Proceedings). The IOBC has also produced a best practice guide to assist the biological control community to access biological control genetic resources (Mason et al., 2018).

## Moving forward (some suggestions)

Increased regulatory oversight and introduction of access and benefit-sharing measures are but two of the challenges facing weed biological control (Barratt et al., 2018). It will be important to understand the applicable Legislative Acts and regulatory process(es) in the country/region where the project is implemented and to work with regulatory authorities to ensure that decisions are science-based and benefits as well as risks are considered.

Presenting weed biological control in a biodiversity context can help to garner support, for example, stating how biological control contributes to the CBD Aichi Biodiversity Targets, particularly *Strategic Goal B: Reduce the direct pressures on biodiversity and promote sustainable use*, Target 9, "By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment."

The relevance of biological control to food and agriculture must also be emphasized. In particular noting that classical biological control: is a research-based activity that requires access to genetic resources; does not generate large monetary returns; utilizes organisms that cannot be patented; main beneficiaries are the farmers who have their [weed] problems reduced without necessarily actively using BC agents, which by spreading and reproducing naturally contribute to the public good; reduces crop losses [from weeds], leading to improved food security and improved livelihoods, and farmers in all parts of the world have benefited; benefits the public at large from reduced use of pesticides, and hence less pesticide residues in food.

Building trust is a basis for acceptance and support for biological control. Public education involves transparency and helps to get buy-in for biological control projects. Invasive species councils are valuable venues for information on the impact of invasive weeds and for building cross-border co-operation.

On a global level, demonstrating the benefits of biological control to providers of genetic resources is needed. Biological control projects not only benefit recipients, they benefit the providers of the genetic resources through: improved local knowledge of flora and fauna; training the new generation of biological control scientists; and contributing to building local infrastructure. Furthermore, the provider of the genetic resources contributes to helping solve problems as global citizens.

Weed biological control researchers are already global leaders in developing best practices for the safe and responsible use of biological control organisms (e.g., DiTomaso et al., 2016). This leadership has been a model, particularly for arthropod biological control as the issues faced are common to both.

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*Oral presentation*

# Horehound biocontrol: a case study in public consultation

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White horehound, *Marrubium vulgare* L., is a perennial herb in the mint family, Lamiaceae. It is native to temperate Europe, northern Africa, and southwestern and central Asia (Ohtera et al., 2013; Rodriguez Villanueva and Martin Esteban, 2016), and has become naturalized in Australia, Japan, North and South America and New Zealand (NZ) (Weiss and Sagliocco, 2012). In Australia, horehound has become a weed primarily on conservation land in Mallee habitats in the State of Victoria. A biocontrol program was initiated there in the 1990s, leading to the introduction of two agents: the horehound plume moth *Wheeleria spilodactylus* (Curtis) (Lepidoptera: Pterophoridae) and the horehound clearwing moth *Chamaesphecia mysiniformis* Rambur (Lepidoptera: Sesiidae) (Weiss and Sagliocco, 2012).

In NZ, horehound has become a weed in dryland sheep farming in the eastern parts of the South Island, especially in alfalfa (lucerne, *Medicago sativa* L.) crops. Alfalfa is recommended in these dryland farming conditions, yet is becoming uneconomical due to horehound invasion (Groenteman, 2018). Chemical and mechanical control are ineffective and/or impractical in most of the hill- and high-country farming situations. A farmers' group was formed in late 2016 to replicate in NZ the program for biocontrol of horehound undertaken in Australia. Manaaki Whenua-Landcare Research (MWLR) was approached to be the research provider.

Following notification in the media about the intent to pursue biocontrol for horehound, MWLR was contacted by a concerned medical herbalist who harvests this plant in the wild. Horehound is valued as a treatment for an array of medical conditions, especially for soothing respiratory symptoms (summarized in Groenteman et al., 2017). A dialogue developed with the concerned herbalist, during which he informed MWLR that other herbalists would soon follow suit. The following weeks brought a steady stream of letters of opposition from medical herbalists. A repetitive pattern in the letters soon emerged and raised the suspicion that MWLR was under a coordinated opposition campaign. Responding to this campaign placed a financial burden on the organization since the project was not externally funded at that time. Opposition to biocontrol in NZ is rare, especially such an organized campaign. As well as the potential to affect the horehound project, this group may oppose future weed biocontrol programs in NZ in instances where the target weed is harvested for medicinal uses.

We discovered that two organizations were behind the campaign: the New Zealand Association of Medical Herbalists and the Herb Federation of New Zealand. We decided to approach the opposition proactively, collectively and early, on their home grounds. Our aim was to divert the conversation from individuals to organizations and their representatives, and to identify what the underlying concerns really were. We wanted to promote constructive participation in the public consultation process. One feature of the regulatory system in NZ is the commitment to pre-consultation, and it is not surprising/unusual for MWLR to receive and respond to concerns prior to the formal decision-making process.

We negotiated an opportunity to address the Association and the Federation at their annual conference. We used this opportunity to explain how the regulatory process works, and how medical herbalists, as a group, could participate in the process constructively. It was important to highlight to the opposition groups that the regulatory process in NZ is an enabling process, and that an approval to introduce a new organism will be granted unless it is not appropriate for that approval to be granted. Aspects considered by the Environmental Protection Authority (EPA) were highlighted. These are risks, costs and benefits to the five domains of environment; human health; market economy; society and communities; and indigenous culture, traditions and rights. The opposition groups were encouraged to ensure these domains were addressed in any formal submissions in order to provide the EPA with meaningful information to support their decision-making.

Ongoing consultation with the opposition groups ensued, and relevant reports and draft documents were supplied proactively to assist their preparations for formal consultation. The draft application to the EPA was provided to these groups to assess whether they perceived their concerns as being represented and addressed adequately in the application. The opposition groups were notified that the application was being submitted and that the formal consultation period was imminent.

Actions by the opposition groups in the months leading up to the application included a newspaper article and a submission made to the Prime Minister's office, opposing the introduction of biocontrol agents.

The application to the EPA to approve the introduction of the two biocontrol agents for horehound was submitted in May 2018, and a six-week period of formal public consultation followed. Upon conclusion of the formal consultation period, only one submission opposing the application was received, from an herbal medicine company, and no submissions were made by the Federation and Association representing medical herbalists. The submission made to the Prime Minister's office only weeks earlier could not be considered in the EPA process because it was not submitted as part of the formal consultation, despite proactive encouragement for the opposition to do so. In addition to the one opposing submission, there were 39 submissions in support of the introduction of biocontrol agents, from individuals and groups spanning farmers, farm industry bodies, conservation groups, regional authorities and central government departments.

A public hearing was held in August 2018, and the herbal medicine company who submitted the opposition chose to present via video link. Five submitters in favor of the proposal also presented at the hearing, three in person and two via video link. The EPA decision-making committee's line of questioning aimed to ascertain the potential for growth in the value of horehound to the herbal medicine sector, as well as to explore how open farmers and medical herbalists were to developing arrangements for access and harvest of horehound on affected farmland.

Approval for the release of both biocontrol agents in NZ was granted in September 2018. As of November 2018 the agents have been imported to NZ but not yet released from containment.

Despite the regulatory process having concluded, communication with the opposition groups continues. It can be anticipated that medical herbalists will emerge in opposition to future weed biocontrol projects targeting plant species that are harvested in the wild for medicinal purposes. It is therefore important to engage with this group so that their concerns can be addressed, and win-win solutions found where possible. It is also an opportunity to reflect on what did or didn't work well in the pre-consultation process.

On the one hand, the pre-consultation process achieved the goals of eliminating the stream of opposition letters and providing valuable information about the concerns held by the opposition groups, thus allowing us time to incorporate and respond to these concerns in the formal application. It also may well have served to limit the number of opposing submissions received as part of the formal consultation. On the other hand, the opposition groups conveyed that they felt they were up against a bigger and better-resourced force, and that their position was not well catered for by the regulatory process. It is possible that a sense of disillusionment with the regulatory process and a perception that the process is biased to benefit stronger economic forces was the driver behind the negligible involvement of the opposition groups in the formal consultation.

While little opposition makes an individual case for introducing biocontrol agents simpler in the short term, lack of participation by identified opposition groups is a symptom that the regulatory process may not be fulfilling its role optimally. One way in which the regulator (the EPA) can encourage participation is by supporting identified opposition groups in their preparation of submissions, in a similar manner as they support applicants, by ensuring the information provided explains the criteria used by the decision-making committee in coming to its conclusions, and advising submitters how they can provide input that addresses those criteria.

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*Oral presentation*

# Regulations for access to genetic resources and exportation of weed biocontrol agents in Argentina

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During the last hundred years, plants from Argentina (e.g., water hyacinth and alligator weed) have been accidentally or deliberately transported to other countries where they became invasive weeds. In 1962, the Agriculture Research Service (ARS) of the United States Department of Agriculture established the South American Biological Control Laboratory (SABCL) in Argentina to search and evaluate water weed biocontrol agents. For 50 years, the SABCL contributed to the search and study of 250 natural enemies to control 44 pests in the USA, Africa, Australasia and Europe. In 2003, a more demanding regulatory frame, derived from the Convention on Biological Diversity, was implemented in Argentina. In 2009, the issuing of exportation permits by the Ministry of Environment and Sustainable Development was interrupted, affecting the SABCL's mission. To resume operations, in 2011, the Argentine government and the ARS negotiated the transformation of the SABCL into the FuEDEI, Fundación para el Estudio de Especies Invasivas. Between 2012 and 2015, FuEDEI exported 14 natural enemies to the USA, Australia, South Africa and Europe. As of 2015, export permits from most Argentine provincial authorities became very hard to obtain. To continue with the exportation of beneficial organisms, FuEDEI acted as liaison to obtain permits from the regulatory agencies of Uruguay, Brazil and Paraguay. At present, there are a few institutions in Argentina conducting weed biocontrol research projects in collaboration with researchers in the USA, New Zealand, Australia, South Africa and Europe. These institutions have joined forces to organize weed biocontrol courses, workshops and online surveys, and to submit weed biocontrol grant proposals to Argentine government funding agencies. We expect these initiatives to raise public visibility of weed biocontrol, revert the ban on organism exchange between Argentina and other countries, and promote research opportunities in Argentina.

*Oral presentation*

# Regulatory oversight of non-native invertebrate biological control agents in Europe, using England as an example

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Until recently, invertebrate biological control agents (IBCAs) were unregulated in Europe, under the assumption that they had little or no impact on the environment. However, towards the end of the 20th century, perceptions were beginning to change and there was an increasing concern that IBCAs may cause harm to the environment and to non-target organisms. The harlequin ladybird, *Harmonia axyridis* (Pallas), which was released into France in the 1990s without assessment, is a case in point. Although beneficial in killing aphid pests, the harlequin ladybird harbors a number of negative traits, such as feeding on non-target organisms. If a risk assessment had been carried out, it is highly likely that its release would not have been allowed. In 1996, the International Organization for Biological and Integrated Control (IOBC) and the European Plant Protection Organization (EPPO) established a panel, which provided guidance on the import and release of IBCAs. This was followed by CHIBCA and the EU funded REBECA project, which reviewed previous recommendations and guidelines and engaged with industry, regulators and scientists to produce coordinated guidelines for Europe.

The Department for Environment, Food and Rural Affairs (Defra) has used these guidelines to establish a regulatory framework for England. Licenses are granted for invertebrate non-native species to be used as classical biological control agents, including those used to control weeds. For new species that have not previously been released in England, a risk assessment is required to ensure the species are safe and fit for purpose. While some other European countries have a similar regulatory framework, there are a number that differ, making it more difficult to release weed biocontrol agents across Europe. There are also some countries that do not have any regulation at all, leaving countries with regulation susceptible to incursion from risky IBCAs. A more harmonized system across Europe is therefore recommended.

*Oral presentation*

# Access and benefit-sharing, the Nagoya Protocol and its implementation in Switzerland

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The Nagoya Protocol on Access and Benefit-sharing, which was negotiated within the framework of the Convention on Biological Diversity, regulates access to genetic resources and the fair and equitable sharing of benefits arising from their utilization. It therefore supports the implementation of the third objective of the Convention on Biological Diversity and contributes to the preservation of biodiversity and the sustainable use of its components. Moreover, the protocol is intended to strengthen the degree of legal security in the use of genetic resources and related traditional knowledge, which is an essential prerequisite for research and development in companies and science.

Switzerland ratified the Nagoya Protocol on 11 July 2014. Its implementation through amendments in the Natural and Cultural Heritage Protection Act (NCHA) came into force for Switzerland on 12 October 2014. It introduced a due diligence requirement to ensure that users of a genetic resource abide by the domestic regulations of the provider country and share the benefits arising from its utilization in a fair and equitable way. Compliance with the due diligence requirement has to be notified to the Swiss national authority before market authorization or commercialization of a product developed on the basis of utilized genetic resources. These provisions of the NCHA were further detailed in the Nagoya Ordinance, which came into force on 1 February 2016. In addition, the ordinance provides the possibility for recognition of best practices and collections, and it regulates the access to genetic resources in Switzerland. At large, these measures provide an adequate framework to ensure legal security when accessing genetic resources as well as the fair and equitable sharing of benefits arising from the utilization of genetic resources.

*Oral presentation*

# The Nagoya Protocol: implications for classical biological control of invasive plant species

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## Abstract

The Nagoya Protocol is a supplementary agreement to the Convention on Biological Diversity (CBD) with the aim to provide a legal framework for the *Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization* (ABS). The Protocol is impacting our work when searching for, collecting and studying natural enemies in their native range as potential biological control agents for invasive weeds. Some countries, for example most of Europe, are not restricting access to their genetic resources. However, in other countries the situation has proven more complicated. Presented herein is an overview of the challenges CABI has encountered and the measures that have been implemented to overcome these. We emphasize the importance to exercise due diligence when it comes to ABS to guarantee that classical biological control remains a viable tool for invasive plant management.

**Keywords:** Access and Benefit Sharing (ABS), Best Practices, Convention on Biological Diversity (CBD), India, Turkey

## Introduction

The Convention on Biological Diversity (CBD), which entered into force on 29 December 1993, had as one of its objectives the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. The Nagoya Protocol is a supplementary agreement to the CBD with the aim to provide a legal framework and transparent conditions for the *Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization* (ABS). The Protocol entered into force on 12 October 2014 and, as of 16 July 2019, there are 117 countries party to it. The necessity for such a framework was born out of the increasing prospecting by commercial companies from industrialized countries to find bio-active compounds, typically in biodiversity-rich but low-income countries without any or adequate sharing of resulting benefits. Although CABI fully supports the underlying reasoning for such a framework, we were concerned about the potential negative consequences, especially with respect to the exchange of natural enemies for use in classical biological control, which prompted respective recommendations to be made (Cock et al. 2010).

In the following, we give an overview of the steps CABI took to react to the Nagoya Protocol, our experiences thus far in different countries with special emphasize on India and Turkey, and an outline of remaining challenges and potential solutions.

## CABI's Best Practice

CABI is both a provider and a user of genetic resources and often acts as an intermediary between provider and user countries. As a user, CABI accesses and collects genetic resources (natural enemies) to develop biological control solutions for basic and applied research, for diagnosis and identification of pests and diseases, and to conserve and store genetic resources in our microbial collection as reference material and for later use or, potentially, to transfer to third parties. In 2016, CABI developed a policy and, subsequently, Best Practice for Access and Benefit Sharing Compliance as guidance for staff and as a basis to seek approval from its member countries on its actions regarding ABS (https://www.cabi.org/news-and-media/2018/conserving-and-using-genetic-resources-as-part-of-cabi-s-commitment-to-the-nagoya-protocol/). A designated person, Dr. David Smith, was appointed to act as the point of contact for ABS issues within the organization. Each CABI Center assigned an ABS Champion, and all staff were informed about the implications of the Nagoya Protocol and CABI's Best Practice. Projects in which genetic resources are accessed are captured in a central database with details on the provider country, the type of material collected, location, date, use etc. In addition, any contact with National Focal Points (NFP) or Competent National Authorities (CNA) is documented centrally. We have also developed a template Material Transfer Agreement (MTA), which is being adapted on a case by case basis and which now accompanies all our shipments of biocontrol agents to third parties. Thus far, CABI has not established a template for mutually agreed terms (MAT) or prior informed consent (PIC) as country requirements differ.

## Our experiences thus far

Overall, CABI has contacted the NFPs and/or CNAs in 30 countries thus far. Most European countries are not restricting access to their genetic resources. However, some countries, such as France, have introduced ABS legislation that distinguishes between commercial and non-commercial use of genetic resources. A relatively simple declaration including the sharing of non-monetary benefits needs to be filled out and sent to the CNA for non-commercial use, while an authorization is necessary in the case of commercial use (https://absch.cbd.int/countries/FR). Following the definition of "utilization of genetic resources," only the analyses on the "genetic and/or biochemical composition of genetic resources" falls under the scope of the French national legislation, not the study of the biology, host range and impact of a potential agent. Other European countries, such as Serbia, are currently still preparing legislation.

For countries outside of Europe, the situation is usually more complicated. Some NFP's did not respond at all, were hard to reach or there was a high turnover of the people responsible, making it difficult to obtain definite answers. In general, it proved to be advantageous to have local collaborators support negotiations with the NFP or CNA. However, some of our local collaborators were hesitant to contact the NFP or did not think it was necessary. In some projects where pressure was high to make progress, collaborative agreements between institutions were sometimes advocated by local collaborators as the path of least resistance. However, CABI has a global reputation to uphold and therefore a duty to engage with official protocols, regardless of how protracted and bureaucratic they may be. Some NFP's had requirements that were difficult or even impossible to meet (e.g., identifying material in-country despite a lack of respective taxonomic expertise or leaving a set of voucher specimens in-country when there were no facilities available to accept them). For countries that have only signed the CBD and have no NFP or CNA (e.g., Georgia, Greece, Russia), we try to exercise due diligence by working as much as possible with local collaborators with which we share monetary and/or non-monetary benefits.

CABI was able to sign a collaborative agreement for specific projects with India (details given below), and our collaborators in Argentina (FuEDEI) were able to obtain collection and export permits from the Ministry of Environment in Paraguay. We have also submitted our Best Practice to national authorities of 12 countries for recognition, but there has been little precedent set, and few countries have processes in place for this. Thus far, Ghana has signed a Memorandum of Understanding (MoU) with CABI that contains most points of our Best Practice, and we are in discussion with the Swiss CNA to get our Best Practice officially recognized. The following sections provide more detailed accounts of our experiences in two countries, India and Turkey.

## India

India was one of the first countries to adopt comprehensive legislation to regulate access to their genetic resources. The National Biodiversity Authority (NBA) was established in 2003 by the central government to implement India's Biological Diversity Act (2002). The NBA is a statutory body which facilitates, regulates and advises the central government on issues of conservation, sustainable use of biological resources and access and benefit sharing from these resources. Furthermore, the NBA delivers its mandate through a structure made up of the authority, secretariat, state biodiversity boards, local biodiversity management committees and expert committees. It was a response to what was perceived as exploitative use of India's biodiversity by bio-prospectors and was largely triggered by the CBD and discussions on ABS. The intent was that all matters relating to access by foreign individuals, institutions or companies, or transfer of results of research to any foreigner would be dealt with by the NBA.

There are two options for the export of biological material from India that are sanctioned by the NBA: (1) apply directly to the NBA for a fee, filling out the relevant forms available online either as a foreign organization or as an Indian researcher wanting to send the biological material to a researcher abroad (http://nbaindia.org/content/26/59/1/forms.html). This option requires prior knowledge of the scientific name, provenance and number of specimens to be exported as well as central government approval of an MTA. There is a condition that 0.1% of the monetary gain (if any) should be shared with the NBA as per the "Access and Benefit Sharing" guidelines. For a prospective biocontrol project in its scoping phase, this prescriptive approach is not feasible.

The alternate option (2) is to have a collaborative project with a research organization in India, with an objective on bilateral exchange of biological material. Here the collaborative project should be approved by the authority under whom the organization is working. In this option, there is no need to obtain an approval from the NBA; however, it is nonetheless vital for the information on any approved collaborative project to be conveyed to the NBA, by the competent authority, in the prerequisite format, with copies of approved collaborative agreements/ MoUs/workplans attached, and accompanying notifications of exploratory activities and any novel species collected.

CABI has had a long and successful association with India; India is a CABI member country and in 1998, CABI established an overarching MoU for collaboration with the Indian Council of Agricultural Research (ICAR) through its national bureaus. This umbrella agreement, and subsequent bureau-specific MoUs, facilitated close research partnerships to be implemented through joint workplans. One significant area of collaboration has been on biological control; CABI's Commonwealth Institute of Biological Control (CIBC), Indian Station, was established in 1957, and marked the beginning of organized and systematic biological control research in India. Many successful weed biocontrol releases have been made, e.g., *Neochetina bruchi* Hustache and *N. eichhorniae* Warner for the control of water hyacinth in the 1980s, but it has been over a decade since the last introduction. Recently the Indian Council of Agricultural Research organized the First International Conference on Biological Control in Bengaluru, India and invited bureaucrats and senior officials to participate and reflect on the merits of biocontrol as well as draw attention to the challenges faced by modern-day practitioners.

Despite CABI's long and positive historical relationship with ICAR, biocontrol project activities and the associated exchange of genetic resources came under critical review with the appointment of several new senior managerial staff. The institutional bureaucratic and administrative processes required to receive clearance and endorsement from the relevant government ministries and the Department of Agricultural Research and Education (DARE, the government's nodal agency for international cooperation in the area of agricultural research and education) resulted in a three-year hiatus in the export of organisms from India.

In late 2017, after many iterations, a comprehensive MoU concerning scientific and technical cooperation between the ICAR and CABI was signed. A year later, a three-year collaborative workplan (2018–2020) for three biocontrol initiatives using natural enemies from India was approved by DARE. Individual collaborative workplans with the relevant ICAR institutes had to be outlined for each project. While these projects can now resume, albeit subject to the necessary and potentially lengthy protocols of export facilitation, the sustainability and applicability of these research collaborations for small biocontrol initiatives with limited funding is questionable. The growing expectation for international collaborations, particularly those involving the export of biological material, to have substantial financial contributions, mutual training opportunities through exchange visits, as well as in-country research components are unfortunately often incompatible with the modest realities of biocontrol funding.

In summary, the Indian system is burdened by entrenched bureaucratic procedures, to which Indian national researchers are also subjected, that lead to inevitable delays and constraints given the seasonality of biocontrol agent prospection. CABI's international presence and historical reputation has undoubtedly been advantageous, and personal relationships cannot be underestimated when it comes to championing individual causes. Fundamentally, however, a respect and adherence to the country's legislation is key to maintaining diplomatic, long-term relations. Recent discussions with the NBA have been positive and may herald a more pragmatic and easily reproducible pathway for mutual exchange of natural enemies in the future. CABI visited the NBA in 2018 and was told that there is now a four-step process starting with an online application (http://nbaindia.org/), consultation with relevant states (that is carried out by the NBA), the setting up of an agreement (i.e., Mutually Agreed Terms) with no monetary benefits to share until a commercial product arises, and the final step of monitoring and reporting requirements. CABI has yet to test this process.

## Turkey

The CABI Center in Switzerland has been collaborating with Turkish researchers and institutions since the 1990s on various biocontrol projects. Collaboration was based on MoUs and specific research agreements, including funding, but also non-monetary benefits such as hosting of visiting scientists, joint publications, training etc. At that time, the export of material was facilitated by the fact that the Dean of Ege University, one of our main collaborators, knew the Minister of Agriculture personally. However, from approximately 2012 onward, several entomologists (non-CABI staff) were arrested and fined because they did not have the proper permits. Even for Turkish scientists it became necessary to obtain collection permits prior to any field work. Permits for foreign scientists required at least three months, needed to indicate the exact timing and location and be in the Turkish language. Since this was not very practical for surveys, which are very weather dependent, we started concentrating our surveys in surrounding countries where access was easier to obtain (e.g., Armenia, Georgia). In parallel, we continued our efforts to work in Turkey, for instance by trying to approach the Turkish authorities at the diplomatic/political level via the Foreign Agricultural Service through the USDA ARS, but this was not successful. Although Turkey is not a party to the Nagoya Protocol, it does have an NFP, whom we contacted multiple times (because the person responsible repeatedly changed) directly or via our local collaborator. In June 2016, we received the information that collected material cannot be removed from the country and that all activities (including identification) must be done in Turkey. We therefore stopped all of our activities in Turkey in relation to classical biological weed control. Similar to India, the irony is that Turkey has profited from biocontrol in the past by releasing parasitoids against insect pests in citrus plantations (Erkiliç and Demirbaş 2007) and has recently imported and released the parasitoid *Torymus sinensis* Kamijo from Italy for control of the invasive Oriental chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (K. Ipekdal, pers. comm.). Current information on the website of the Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policies, Turkey (TAGEM), says that "In order to send samples abroad for studies that cannot be conducted in Turkey, a permission from TAGEM is needed." This does seem to leave a door open for export of material, but CABI has not yet tried this potential option.

## Challenges and recommendations

The implementation of the Nagoya Protocol currently poses numerous challenges to the discipline of classical biocontrol, especially in relation to countries outside of Europe. Where legislation exists, it is often convoluted and/or complicated and comes with a heavy administrative burden. Sometimes requirements are very difficult or impossible to fulfill, since they were put in place by staff without any biological or science background. In addition, the NFPs and CNAs responsible for the CBD and the Nagoya Protocol are typically situated in the ministry of the environment or related fields, while previously the responsibility for export permits was typically situated in the ministry of agriculture. This, combined with a frequent change in staff, does not make the task of obtaining correct information any easier. In some countries, different administrative levels (local, provincial, national), which may differ in their requirements and/or their understanding, are involved in the decision process (see Silvestri et al., 2019, these Proceedings). Finally, we believe that some difficulties are politically motivated and are thus hard to overcome. For the above reasons, timelines to obtain permission to access genetic resources can be very long (several months to years), which can delay project work considerably if surveys can only be conducted at specific times of the year. In addition, many smaller organizations (but even universities) are lacking the personnel and resources to fight their way through the administrative jungle. This harbors the danger that people might decide to ignore Nagoya altogether or find a way around it.

Another difficulty is that many countries are still trying to decide whether they should become party to the Nagoya Protocol. Of those that are party, many are still drafting legislation (only 61 of 116 parties have legislation or administrative measures listed on the Access and Benefit-Sharing Clearing-House [ABSCH] website; https://absch.cbd.int/). This is the case for China where the Ministry of Ecology and Environment (MEE) has been tasked with leading the development of ABS legislation. However, this process will take at least one to two years. For this interim period, it was recommended to CABI to establish a collaborative research contract with its main national partner (the Institute of Plant Protection under the Chinese Academy of Agricultural Sciences), which—after some additional steps—should allow for the export of material (F. Zhang, pers. comm.). CABI has yet to try this process.

Another strategy is to choose countries for surveys where legislation already exists, access is easier or that are not a party to the Nagoya Protocol. However, this will lead to the isolation of precisely the countries that were meant to be protected by the establishment of the Nagoya Protocol (Deplazes-Zemp et al. 2018). For microbial biocontrol it may be feasible to use samples from collections that were acquired prior to 12 October 2014.

Our experience has shown that it is always advisable to work with local collaborators for whom it is usually easier and more effective to contact or meet the NFP and/or CNA, and which might be better informed about recent decisions and developments. Good, long-term personal relationships within the country in question are certainly advantageous and should be fostered, e.g., through truly collaborative projects, joint meetings and publications, the exchange of scientists and students etc.—basically all features of the non-monetary benefits that are commonly practiced within non-commercial biocontrol initiatives (Cock et al. 2010; Mason et al. 2017). Countries also need to be made aware that they could profit (or may have already) from the free exchange of genetic resources to control invasive pests and diseases, and that biocontrol is not a one-way road.

We also recommend following existing best practices (e.g., Mason et al. 2017; Smith et al. 2018), or developing one's own to follow, to guarantee a certain standard in accessing genetic resources. This should facilitate avoiding bad practices and the building of trust with provider countries. Example best practices can be found on https://absch.cbd.int/. In the meantime, the country profiles on the ABSCH website (https://absch.cbd.int/countries) have become a reliable and very useful information resource for checking current country requirements. The website also allows registered users to set up personalized email alerts when new country records are published.

In conclusion, ABS and the Nagoya Protocol are here to stay. We therefore need to be prepared, proactive and patient and exercise due diligence to guarantee that classical biological control remains a viable tool for invasive plant management. Importantly, we need to find mechanisms to share experiences to facilitate the implementation of practical and implemented national processes that meet scientific and societal needs while delivering the fair and equitable sharing of benefits that the CBD and Nagoya Protocol were designed to deliver.

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# Session 7: Social and economic assessments of biological control

## Session Chair: Marion Seier

Keynote

# Quantifying the economic and social benefits of weed biological control

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This paper considers the information required to quantify the benefits of invasive alien plant management, and reviews studies that have evaluated the benefits of biological control. Estimating these benefits requires answers to a number of questions, including (1) what is the magnitude of the impacts of invasive species on the ecosystem? (a biophysical question); (2) what is the value of these impacts in monetary terms? (an economic question); (3) what consequences do these impacts have for the people affected by them? (a social question); (4) how effective is control in reducing these impacts? (a management question); (5) how can we deal with cases where the target weed is both harmful and beneficial? (a complex social-economic question); and (6) what returns on investment can be expected from biological control? (an economic question).

There have been remarkably few studies that have assessed the actual impacts of alien plant invasions on biodiversity and ecosystem services. Those that have been done confirm that in many cases invasion reduces biodiversity and the flow of ecosystem services such as harvested products from natural vegetation, livestock production from rangelands and water yield from catchment areas (e.g., van Wilgen et al., 2008).

In order to be able to compare a range of different biophysical impacts in the same ecosystem with each other, it is necessary to express these impacts in common monetary terms. There have been several studies that have evaluated ecosystem services in monetary terms by, for example, establishing the market value of harvested goods, the value of livestock production or the value of water from catchment areas. The economic impact of invasive alien plants on these values can then be estimated from the reduction in value of the flow of ecosystem services (e.g., De Lange and van Wilgen, 2010).

The way in which these impacts affect people is often assumed to simply be equivalent to the economic impact. However, the impacts of invasive alien plants are often borne by poor people living in rural environments, and these impacts have seldom been adequately documented. Some recent studies have used questionnaire surveys to estimate the economic impacts of invasions on rural households (e.g., Shackleton et al., 2017).

Whether or not these impacts can be offset by implementing control measures will depend on how effective the control measures are. While mechanical and chemical control can work at smaller scales, they are generally unsustainable, and ineffective over large areas. A recent 10-year study from South Africa (Henderson and Wilson, 2017) showed that, on average, individual alien plant species increased in range by 50%, and ongoing mechanical and chemical control had no detectable effect on range. Biological control, on the other hand, significantly slowed or reversed the spread of 33 alien plant species (Henderson and Wilson, 2017). It is becoming increasingly clear that biological control is the only long-term effective method for reducing the impacts of alien plant invasions.

Returns on investment from biological control are usually expressed in the literature in terms of benefit:cost ratios. The benefit side of these ratios arises from the restoration (or prevention of the loss of) ecosystem services impacted by alien plant invasions. The cost side is estimated from the cost of locating, screening, releasing and monitoring the biological control agents. Case studies on alien plant species in South

Africa, Europe, Australia and on island ecosystems have reported returns on investment from biological control that ranged from 3.5:1 to >3,000:1 (e.g., Page and Lacey, 2006; van Wilgen and De Lange, 2011). In other words, where it works, weed biological control can deliver exceptional returns on investment.

Biological control becomes controversial in cases where the target invasive alien plant is simultaneously useful and harmful. In such cases, a careful evaluation of the costs and benefits of the target plant can be used to inform a decision on whether or not to release a biological control agent. Such a release would be justified if the value of the harm caused was greater than the value of the benefit realized. In Australia, the rangeland weed *Echium plantagineum* L. (salvation Jane or Paterson's curse) reduced livestock carrying capacity but was useful for honey production. An economic study demonstrated that the detrimental effects of the weed outweighed benefits by 10:1 (Page and Lacey, 2006), which paved the way for the release of biological control agents. In South Africa, the Australian tree *Acacia mearnsii* De Wild. (black wattle) has considerable economic value for wood and bark products, but it invades natural ecosystems where it depletes water resources, reduces grazing capacity, and impacts negatively on biodiversity. A study by De Wit et al. (2001) found that a "do nothing" scenario (with no attempts being made to control the spread of black wattle beyond the limits of plantations) was not sustainable, as the benefit:cost ratio was around 0.4. A control option that combined physical clearing and seed-attacking biological control with the continuation of the commercial growing activities led to a positive benefit:cost ratio. This study paved the way for the release of non-lethal biological control agents and substantially improved the prospects for controlling invasive populations (Moran and Hoffmann, 2012).

Despite very attractive returns on investment, governments around the world continue to regard the practice as risky and avoid making decisions to release biological control agents. This approach is counter-productive because it overemphasizes potential problems and ignores or underestimates the benefits of weed biological control, it offers no viable alternatives, and it overlooks the inherent risks of a decision not to use biological control (van Wilgen et al., 2013). In fact, the protection of un-invaded or partial restoration of invaded ecosystems can be achieved safely, at low cost and sustainably through the informed and responsible application of biological control. The challenge will be to effectively communicate this message to stakeholders in the future.

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*Oral presentation*

# Economics of weed biocontrol in New Zealand: a lot can be done with minimal data, but even more with good data!

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Recent economic analyses of several weed biocontrol programs in New Zealand show strongly positive ongoing returns. For example, the largely successful biocontrol of ragwort saves the New Zealand dairy sector NZD 44 million per year in weed control costs, with a benefit:cost ratio of 14:1. To put this in perspective, New Zealand spends only a few million dollars each year in total on operational and research costs for weed biocontrol. These analyses of past programs generally rely on substantial assumptions, e.g., of agricultural costs and losses from weeds or on the fortuitous discovery of early data. In agriculture, it is often possible to estimate likely control costs and productivity losses from displacement of pasture by weeds. However, estimating benefit:cost ratios for environmental weeds is more challenging because (1) conservation benefits are very difficult to monetize and (2) control costs are seldom measured and/or seldom recorded. For both agricultural and environmental weeds, it was common for the memory of the severity and costs of weed problems to be rapidly forgotten once biological control had been achieved. However, enough programs could be analyzed to provide useful data to support continued investment in weed biocontrol. Two further standout results were (1) some early, and unusually detailed, economic data on the partial biocontrol of alligator weed in New Zealand showed that even a mere 8% suppression of the weed resulted in benefits worth NZD 0.5 million per year with a cost:benefit ratio of over 100:1, and (2) an unscientific and unjustifiable decision to not use the ragwort flea beetle (an agent short-listed in New Zealand's pioneering ragwort biocontrol program in the 1930s) resulted in an astounding present-value net loss of NZD 8.6 billion to the NZ dairy sector.

*Oral presentation*

# Economic analysis demonstrates that ecosystem service benefits of water hyacinth management greatly exceed research and control costs

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It often takes years for biological control agents to become established and produce obvious results. During the initial phase of biological control activity, impacts to the target plant may be so gradual (and even unnoticeable) that the collection of significant amounts of data is necessary to document impacts. Historically, biological control researchers have focused on the scientific approaches to expediting the release and establishment of control agents to reduce the targeted invasive species. However, data on social and economic implications of classical biological control programs are important for justifying research and development costs and investments in programs. Unfortunately, at present, these data are often not examined or obtained. Water hyacinth (*Eichhornia crassipes* [Mart.] Solms) has been a significant invasive aquatic weed in the USA since the late 1800s. A unique, 38-year (1975–2013) dataset of water hyacinth coverage in Louisiana has allowed for the examination of environmental and economic benefits derived from long-term chemical and biological control management. A seasonal logistic population model was fitted to survey data and estimated, in a no-action scenario, that peak plant cover would be 76% higher without growth suppression from biological control. The economic analysis generated a 34:1 benefit to cost ratio based on data for anglers, waterfowl hunters, boating-dependent businesses and water treatment facilities in Louisiana. This represents USD 4.45 billion in ecosystem goods and services for the state derived from a USD 124 million investment in control over the 38-year period. Our study showcases the critical need to coordinate collection of social and economic data early in biological control research studies to support a comparison of outcomes decades in the future.

*Oral presentation*

# Socio-economic impact of water hyacinth on riparian communities of the Wouri River Basin (Douala, Cameroon)

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Since publication of the first record of *Eichhornia crassipes* (Mart.) Solms in Cameroon in 1997, it has become highly invasive in the Wouri River Basin impacting riparian communities. Between June and September 2014, a socio-economic survey using participatory and qualitative methods was undertaken by 32 women and 68 men from 25 villages to assess the impact of water hyacinth. The survey revealed that water hyacinth was perceived to cause severe damage and was a significant threat to activities along the Wouri Basin, which include fishing, sand-extraction and water transportation. Based on the average monthly income before and after water hyacinth invasion, the yearly economic loss perceived to be due to water hyacinth was estimated at USD 2,028, USD 498, and USD 1,800 per household involved in fishing, sand-extraction and transportation activities, respectively. This translates to a reduction in income of approximately 75% for fishing, 24% for sand extraction and 75% for water transportation. The impacts, in terms of loss in biodiversity, included a decrease in the quantity of fish and the disappearance of plants of medicinal value such as *Ageratum conyzoides* L. Cameroon employs manual clearing of water hyacinth; however, respondents indicated they would consider other control methods, provided they do not have any negative impacts.

*Oral presentation*

# Do good insects go bad? A tale of two cactus biological control agents

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Biological control of cacti has a complicated history. The dramatic reduction of invasive *Opuntia* (prickly pear) species by *Cactoblastis cactorum* (Berg) was a stunning success in many parts of the world. This program launched the tactic of weed biological control and overshadows the payoff from all other weed control programs. Not as well known, but equally successful, is another biological control agent from Argentina—the *Hypogeococcus* mealybug controlling invasive *Harrisia* and other columnar-type cacti. However, both of these programs have become blemished by the movement of the biological control agents into areas where they are attacking native cacti species. These beneficial agents have now become pests and are interfering with environmental function. But the stories of the two biological control agents diverge relative to their avenue of introduction into new areas of non-targets, their culpability in the act of destroying native species, and the potential management options studied to limit their habitat destruction. Scientists brought *C. cactorum* into the Caribbean for biological control of cactus, but the insects' spread to North America was caused by human commerce. Impacts to native *Opuntia* in the Caribbean are important, but the anticipated destruction to *Opuntia* species in the western USA and Mexico represents a potential breakdown of desert ecosystems and rural Mexican subsistence farming. On the other hand, movement of the *Hypogeococcus* mealybug into the Caribbean had an unknown avenue and was unrelated to a biological control program. In fact, the mealybug in Puerto Rico is a cryptic species of the biological control mealybug, likely coming from Brazil, not Argentina. Management tactics developed for *C. cactorum* include the release of sterile insects, pheromone disruption (mating and larval following) and biological control. Current *Hypogeococcus* management hopes rest on biological control. Although biological control practitioners can take pride in past control of invasive cacti, the discipline has a responsibility to respond to agents that become pests.

*Oral presentation*

# *Nassella trichotoma*: modeling the costs and benefits of proposed classical biocontrol in New Zealand

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*Nassella trichotoma* (Nees) Hack. & Arechav. (nassella tussock) infests over 524,000 ha of pastoral land in the Canterbury and Marlborough regions of New Zealand's South Island and occurs also in the Auckland, Hawke's Bay and Northland regions of the North Island. Management is costly at NZD 1.71 per plant grubbed, and is required annually in perpetuity to keep populations at their current low level and to prevent the significant economic losses that would accrue in the absence of control (NZD 417 million for Canterbury alone) (Lamoureaux et al., 2015). Classical biological control could provide a more cost-effective and sustainable solution. Previous work identified three fungal pathogens of nassella tussock in Argentina, the plant's native range (Anderson et al., 2010). Here we model the costs and benefits of a hypothetical biocontrol program against the weed to determine if it would be economically worthwhile.

The net discounted present value criterion approves of an investment so long as the net present value (NPV) of the investment is greater than zero, i.e. if

$$NPV = \sum_{t=1}^{n} \frac{B_t - C_t}{(1+i)^t} > 0$$

where *B* and *C* are the benefits and costs respectively in year *t*, and *i* is the discount rate to be applied to the analysis. We have used $i = 0.08$ (8%) suggested by the NZ Treasury, but arguably rates lower than this are justifiable for investments such as classical weed biological control (Lamoureaux et al., 2015). Lower rates have the effect of increasing the NPV thereby favoring the proposed investment and implying that the analysis here is conservative.

The benefits of the proposed biological control program, $B_t$, were taken to be the savings made in the annual grubbing program in the Hurunui district of north Canterbury. These savings were calculated as the product of (a) the proportional reduction in the population density of nassella tussock, assumed to increase logistically over time, $A_t$,

$$A_t = \frac{A_0 + A_{max}}{A_0 + \ (A_{Max} - A_0)e^{-rt}}$$

where $A_0$ and $A_{max}$ define the initial and maximum proportional reductions due to the biocontrol program, *r* is the rate at which $A_{max}$ will be approached over time *t* (years after the beginning of the program), and (b) the cost of the annual grubbing program in north Canterbury, calculated to be NZD 4,466,284/year. This grubbing cost was based on a cost of NZD 1.71 per plant grubbed, 7.73 plants grubbed per ha over 313,140 ha plus a grubbing program management cost of NZD 0.38 per ha over 864,646 ha (Lamoureaux et al., 2015). The 7.73 plants grubbed per ha was calculated as 0.34 x 22.7; respectively, the mean grubbing rate in Hurunui pastures (Verkaaik et al., 2006) and the pre-grubbing population density (22.7 plants/ha) required to give the stable post-grubbing mean population of 15 plants/ha as determined from a long-term population monitoring study (Bourdôt and Saville, 2016).

Three values for $A_{max}$ were selected for the analyses (0.47, 0.65 and 0.76) representing 47, 65 and 76% reductions in the population density (and hence plants to be grubbed) of nassella tussock. These population reductions were projected by the nassella tussock population model NASSIM (Lamoureaux et al., 2015) given an average annual grubbing rate of 34% (Verkaaik et al., 2006) plus a biocontrol agent-mediated reduction in seeding of 10% along with reductions in plant growth rate of either 5, 10 or 15%. These reductions were considered to be achievable since they are at the lower end of expected impacts of plant pathogens that might be introduced as biocontrol agents for nassella tussock (Barton and Anderson, 2016).

The total cost of a biocontrol program for nassella tussock is estimated to be between NZD 0.92 million and NZD 2.5 million (Barton & Anderson, 2016). In a comprehensive review of historical weed biocontrol programs in New Zealand, it was shown that the total cost of a single-agent program was, on average, less than NZD 1.0 million, and for a three-agent program, less than NZD 2.0 million (Paynter et al., 2015). To ensure a conservative cost-benefit analysis, we have chosen a cost at the higher end of these estimates.

The cost of the biocontrol program, $C_t$, has two components. The first is the cost of the research and development phase. This was set at NZD 2 million. This cost is assumed to be incurred over the first five years of the program at NZD 400,000/year. The second component, the cost of post-release monitoring of the program, was set at NZD 100,000 and assumed to be incurred over the four years following release of the agent(s) at NZD 25,000/year.

A range of rates, *r*, at which the program's maximum population reduction effect, $A_{max}$, would be approached were tested for each of the three population reduction scenarios. The rates corresponded to 10, 20, 30, 40, 50 and 60 years for the program to attain 90% of its maximum effect ($T_{90}$).The analyses were made in Excel with a 100-year time frame.

The NPV for each of the three modeled scenarios declined with declining *r* (i.e. increasing $T_{90}$), falling below zero only when $T_{90}$ exceeded 50 to 60 years (Figure 1). This result indicates that a plant pathogen-based classical biological control program for nassella tussock in the Hurunui District of North Canterbury is likely to be economically worthwhile so long as the agent (or agents) are effective and that they become distributed throughout the Hurunui District within 50 to 60 years following their release.

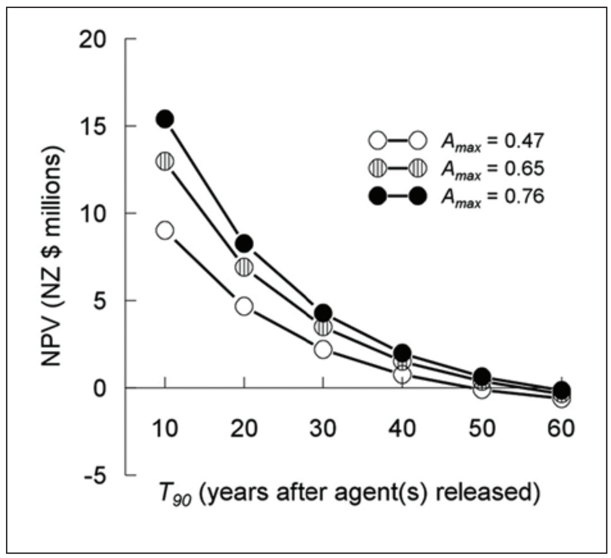


**Figure 1.** Net present value (NPV) (NZD millions) plotted against time (years) for the hypothetical biocontrol agent(s) released against Nassella trichotoma in the Hurunui District of North Canterbury, New Zealand, to achieve 90% of maximum potential impact ($T_{90}$). The three modelled maximum population reduction scenarios ($A_{max}$ = 0.47, 0.65 and 0.76) are illustrated. Initial impact $A_0$ = 0.001 and discount rate $i$ = 0.08.

A framework developed earlier to predict how amenable different weeds are likely to be to biocontrol predicts nassella tussock to be an intermediate target (Paynter et al., 2012). This means that a 50% reduction in percentage cover of the weed or its biomass could be expected, a level of control similar to the 47% reduction in population density scenario ($A_{max}$ = 0.47) modeled here.

There are a number of fungal options to be explored should a biocontrol program for nassella tussock be pursued: Corticiaceae; *Fusarium* sp.; *Dinemasporium* sp.; *Zinzipegasa argentinensis* (Speg.) Nag Raj (Barton & Anderson 2016). A combination of these natural enemies could be most effective since severe disease outbreaks observed in nassella tussock in Argentina and Australia typically involve a *Fusarium* species along with several other pathogens. Many *Fusarium* species occur in New

Zealand, and any that cause disease in nassella tussock could be exploited in combination with introduced specialist nassella tussock pathogens or insects. Surveys in Argentina may reveal other candidates, including insects that damage the plant or its seeds allowing endemic pathogens to gain entry.

## Acknowledgments

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*Oral presentation*

# Projecting the economic benefits of biological control of common ragweed in Europe

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Invasive alien species not only cause major environmental damage but also enormous economic costs as they impact human well-being. Yet, detailed assessments of their negative effects on human health and the potential benefits of implementing sustainable management are understudied. We present an interdisciplinary approach to quantify the effects of the allergenic plant *Ambrosia artemisiifolia* L. on public health in Europe. Moreover, we analyzed the projected impact of the recently established ragweed leaf beetle *Ophraella communa* Le Sage, which is used as a biological control agent in China, on the number of patients and healthcare costs in Europe. We first estimated the impact of the ragweed leaf beetle on ragweed pollen production in northern Italy where the beetle was first detected in 2013. By spatially modeling aerobiological, medical and ecological data, and relating seasonal total ragweed pollen counts to the number of persons requiring medical care, we projected the impact of *O. communa* to those regions in Europe that are climatically suitable for high population densities of the biological control agent. We argue that such interdisciplinary studies are essential to inform invasive alien species policy both at the national and continental level and to achieve coordinated and cost-efficient management actions.

*Poster presentation*

# Ghosts of the mongoose: perceptions of classical biological control in the 21st century

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Public perceptions and beliefs about classical weed and insect biological control appear to fluctuate between full acceptance of the practice and deep mistrust. These opinions are heard regularly by biocontrol researchers and practitioners, but there have been very few studies that investigated where these ideas come from or how best to address misinformation. We are implementing surveys of the general public, Washington State Master Gardeners and county pest management programs to help determine the origin of these perceptions and compare attitudes towards classical biocontrol with other control methods, such as chemical control. Information on survey respondents' educational background, experience with and understanding of invasive species, and their level of knowledge about classical biocontrol agent testing, approval, safety and expectations are being assessed. In addition, we hope to ascertain whether the risks and benefits of classical biocontrol are viewed differently when the pest or potential non-target is an insect or a plant. This poster presented preliminary survey results and described future steps in this project. Ultimately, this survey should identify options to improve public communication about classical biological control, which may potentially influence support of classical biocontrol projects by policy makers and regulatory agencies.

# Session 8: Opportunities and constraints for classical weed biocontrol in developed countries

## Session Chair: Hariet L. Hinz

**Keynote**

# Opportunities and constraints for classical biocontrol of invasive plants in Europe

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In Europe, invasive alien species (IAS) and their negative impacts on biodiversity and economy are recognized by scientists, managers and European authorities who recently adopted legislation to prevent and manage their introduction and spread (EU Regulation no 1143/2014). Despite the long history of successful biocontrol against invasive alien plants/weeds (IAP) worldwide, the considerable economic benefits, the rare significant non-target impacts (Suckling and Sforza, 2014), and the long list of potential targets with biocontrol agents (BCA) previously selected (Sheppard et al., 2006), the first four BCA against IAP were introduced in Europe only in the last decade (Shaw et al., 2018). The first was *Aphalara itadori* Shinji (psyllid) against *Fallopia japonica* (Houtt.) Ronse Decr. in 2010 in the United Kingdom; the second, *Puccinia komarovii* var. *glanduliferae* R.A. Tanner, C.A. Ellison, L. Kiss & H.C. Evans against *Impatiens glandulifera* Royle in 2014, also in the United Kingdom; the third (and first in continental Europe), was *Trichilogaster acaciaelongifoliae* (Froggatt) against *Acacia longifolia* (Andrews) Willd. in 2015 in Portugal; and the fourth, *Aculus crassulae* Knihinicki & Petanović (Acari: Eriophyidae) against *Crassula helmsii* (Kirk) Cockayne in 2018, again in the United Kingdom. In contrast, at least 176 species of exotic arthropod BCA have been released in Europe against 59 agricultural pests (Gerber and Schaffner, 2016). Why is Europe still so conservative when it comes to classical biocontrol of IAP, especially considering that such a conservative approach does not seem to be applied when targeting plant pests? Several authors have recently explored the challenges and constraints of biocontrol worldwide and in Europe (Barratt et al., 2018; Schwarzländer et al., 2018; Shaw et al., 2018). Here, we resume some of the key ideas, add a few others and use the case of *T. acaciaelongifoliae* introduced in Portugal to illustrate our points.

New introductions of BCA face constraints in scientific, bureaucratic and regulatory terms, but also deal with poor engagement and communication with stakeholders and citizens in general. Among the main constraints/challenges for classical biocontrol of IAP in Europe we can include (1) a risk-averse society, which fears the potential non-target effects on useful plants; (2) a missing history of BCA introductions against IAP in Europe, increasing the "fear of the unknown"; (3) a negative perception of classical biocontrol (Shaw et al., 2019, these Proceedings), but also a lack of interest and awareness, by citizens, researchers, practitioners, politicians, etc., emphasizing the need to better communicate the risks and benefits of classical biocontrol of IAP; (4) IAS are not yet widely perceived as a serious threat in Europe, but this is (slowly) changing, not only with the increasing impacts and number of species, but also as a result of the new EU IAS dedicated regulation; and finally (5) in most European countries legislation is either completely missing or regulations are very restrictive in relation to the use of classical biocontrol and, in general, there is a lack of harmonization.

Despite these constraints, there are also opportunities for classical biocontrol of IAP in Europe: (1) a risk-averse society and strict regulations have pushed for innovative responses to the challenges by improving the safety and success of classical biocontrol, not only in Europe but potentially worldwide; (2) the four recently introduced BCA open the door to a new paradigm; (3) The EU Regulation on IAS emphasizes the need for IAS management and specifically refers to biological actions, and also the reports on management measures for some listed species include classical biocontrol; (4) potential BCA for several IAP in Europe have already been selected elsewhere (Sheppard et al., 2006), which may ease the way; (5) although this is not the way forward, several unintentional introductions of potential BCA have occurred (e.g., for *Ambrosia artemisiifolia* L.; Shaw et al., 2018), and their presence offers opportunities for research on their safety and effectiveness; (6) several IAP (e.g., *Hakea* spp., *Acacia* spp., etc.) that may be targets for biocontrol are phylogenetically distant from the European flora, making it less challenging to find safe BCA for such targets; (7) Europe has been the origin of BCA for other regions, with several biocontrol facilities conducting foreign exploration (CABI, USDA, CSIRO), which provides for a wealth of biocontrol expertise; (8) there is a need for alternatives to chemical control (e.g., controversy over glyphosate) and other types of control, which have proven to be unsustainable; and finally, (9) in July 2018, the Subsidiary Body on Scientific, Technical and Technological Advice, of the Convention on Biological Diversity, "Requests the Executive Secretary to continue collaboration with the IUCN, its ISSG and relevant international organizations to report on the use of BCA against IAS …".

Considering the introduction of *T. acaciaelongifoliae* in Portugal (Marchante et al., 2017), the main constraints/challenges throughout the process were related to (1) the long legal and bureaucratic process taking more than 12 years to get a permit for the introduction of a BCA that had been previously selected and successfully used in South Africa; (2) the lack of awareness or distrust of citizens and regulators, that, nevertheless, declined throughout the process; (3) the lack of an agency or research facility dedicated to classical biocontrol; (4) risk-averse and poorly-informed regulators, as well as citizens, researchers, politicians, etc. (nevertheless the permit to introduce two new BCA [*Melanterius* spp.] for specificity testing was much faster, and there is a new plant protection regulation in place which is clearer); and (5) the introduction of a BCA from the southern hemisphere resulted in asynchrony between the BCA and the host-plant phenology, delaying establishment.

Despite these constraints, we also see opportunities that arose: (1) long-term collaboration with colleagues in South Africa has made it possible to share both knowledge and the possibility of new BCAs; (2) the first BCA introduction has changed the perception of biocontrol throughout the country; (3) we were able to raise public awareness about biocontrol, and to promote engagement and support among IAP managers, politicians and citizens, and our perception is that lack of awareness, mistrust and misinformation have declined; (4) the collaboration with pest biocontrol practitioners from the forest industry has enabled sharing of expertise and facilities; (5) to respond to criticisms and challenges, we developed innovative research, such as: (a) ecological networks to assess indirect effects of BCA, which helped to improve the predictive ability of direct and indirect non-target effects and that have the potential to improve both the safety and success of biocontrol (López-Núñez et al., 2017); (b) remote sensing and machine learning to assess the establishment and impact of BCA (de Sá et al., 2018); and (c) modeling to understand and predict establishment and impact of BCA, e.g., using dynamic models.

In conclusion, despite the constraints and challenges, there are also opportunities, and the future for classical biocontrol of IAPs in Europe is bright. There is, however, a need to further raise awareness and information about biocontrol of IAP among European citizens and stakeholders and to improve communication on their risks and benefits.

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*Oral presentation*

# The classical weed biocontrol imperative: managing through the invasion curve in the Western United States

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The USA has a history of successful weed biocontrol programs and a process in place to evaluate and regulate weed biocontrol agents. Recent regulatory impediments are being addressed, and new weed biocontrol agents are being petitioned and permitted. Weed biocontrol information is becoming more readily available in technical manuals and on credible websites, and the important role classical biocontrol can play in landscape invasive plant management is being discussed in academic journals. In the Western USA, many states have identified weed biocontrol coordinators to increase implementation of weed biocontrol in large weed infestations. Conceptual models like the invasion curve and the enemy release hypothesis are being used to facilitate conversations about where and why weed biocontrol makes sense. Federal, state and private land managers and weed control authorities are forming cooperative weed management areas and ensuring weed biocontrol plays an appropriate role in landscape-integrated pest management strategies. While progress is occurring, the pace of weed biocontrol development and implementation is constrained by a number of factors. Comprehensive legislation on the treatment of invasive species has never been enacted in the USA, and no single law directs coordination among the dozens of federal agencies with invasive species responsibilities. Funding for weed biocontrol research and development is not secure, and many weed biocontrol researchers are not being replaced when they retire. Media in the USA pays little attention to the problem of widespread invasive plants, and media coverage of weed biocontrol historically highlights concerns over success. In spite of constraints at the highest levels, state and local efforts continue to highlight the demand for existing weed biocontrol agents and the need for continued classical biocontrol research and development. In the western USA, state and local entities are weed biocontrol's best proponents.

*Oral presentation*

# Charismatic microfauna: using *Lilioceris cheni* to increase public perception and acceptance of the biological control of weeds

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The Intergovernmental Panel on Climate Change (IPCC) has expressed concern about the state of the environment (IPCC, 2014). Declining biodiversity and ecosystem productivity are particularly worrisome to scientists (Hooper et al., 2012; Isbell et al., 2013). Invasive species are one factor that has been shown to reduce biodiversity and that can negatively impact ecosystem services (Charles and Dukes, 2007; Pyšek et al., 2012). According to the United Nations Educational, Scientific and Cultural Organization (UNESCO) (1975), education is critical to developing a world population that has the knowledge, skills and attitudes to solve current and future environmental issues.

Florida's (USA) climate, tourism and growing population make it particularly at risk for invasion by exotic species. Biological control is one of the best tools to reduce the damage done by widespread invasive species, especially when compared to the high cost of application and potential non-target impacts of other management strategies (e.g., pesticides) (VanDriesche et al., 2008). However, neither the negative impacts of invasive species nor the safety and effectiveness of classical biological control are well known to the general public. Increasing public knowledge about these environmental topics is an important step in the fight against invasive species.

We designed an outreach and education program that is centered on the charismatic biological control agent *Lilioceris cheni* Gressitt & Kimoto (Coleoptera: Chrysomelidae) and its target, the invasive air potato (*Dioscorea bulbifera* L.), an easily recognizable, climbing vine in the southeastern USA. Many landowners struggle to control this invasive vine, and *L. cheni* can reduce vine cover and propagule production significantly (Rayamajhi et al., 2019). This education program was paired with a mass-rearing and release effort and was conducted at extension offices and community events throughout the state of Florida. The campaign provided *L. cheni* to interested stakeholders for release and provided educational activities for both adults and children. Educational activities included games for children, such as "pin the insect on the host plant" to demonstrate host specificity and biological control agent tic-tac-toe. Educational displays for adults included live invasive plants and their associated biological control agents, brochures, diagrams and photographs illustrating agent efficacy and host specificity.

The program has been highly successful with nearly 57,000 *L. cheni* beetles reared and provided to stakeholders for release on public and private lands in 2017 and 2018. The program also significantly increased the general knowledge (on a self-assessed 10-point scale) of invasive species of the people surveyed by an average of 227% and general knowledge about biological control by an average of 401%. Perception of the safety of biological control of those surveyed increased by an average of 434% and perceived effectiveness of biological control increased by 344%. The combination of a charismatic and effective natural enemy along with an educational program has significantly increased the knowledge about invasive plant species and the knowledge and acceptance of biological control as a safe and effective control method.

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*Oral presentation*

# Field release of a rust fungus for the biological control of Himalayan balsam in the UK: constraints to success

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In 2014, the rust fungus *Puccinia komarovii* var. *glandulifera* R.A. Tanner, C.A. Ellison, L. Kiss & H.C. Evans (Pucciniales) was released in the UK. It was the first fungal agent to be released into Europe for the biological control of an invasive weed. The target, *Impatiens glandulifera* Royle (Balsaminaceae), known as Himalayan balsam (Figure 1a), is native to the Himalayan foothills from Pakistan to Nepal. It is a prolific invader of predominantly riparian habitats but is also found in damp woodlands and disturbed areas in temperate parts of Europe, New Zealand and North America. In its invasive range, Himalayan balsam forms dense monocultures which can negatively affect whole ecosystems, displacing native vegetation (Hulme and Bremner, 2006) and reducing biodiversity of above-ground invertebrate populations (Tanner et al., 2013) and mycorrhizal fungi in the soil (Pattison et al., 2016). Furthermore, as an annual species, plants die back in the autumn leaving river banks exposed, increasing the potential for erosion and increasing the risk of flooding as plant material is incorporated into watercourses (Greenwood and Kuhn, 2014).

The biological control program against Himalayan balsam was funded by the UK government and began with natural enemy surveys in the native range in 2006. Surveys revealed a suite of potential biocontrol agents; however, the rust fungus *Puccinia komarovii* var. *glanduliferae* was prioritized for further testing due to its prevalence and high level of damage observed in the field (Tanner et al., 2014). Further evaluation of this fungus found it to be macrocyclic (all five potential spore stages present in the life cycle), autoecious (all spore stages occur on one host) and highly host-specific (74 plant species were screened). In addition, experiments to determine the infection parameters of the pathogen found it to be well suited to the UK climate (Tanner et al., 2015).

In 2015, the first phase of rust releases began, and a strain from India was released at 20 sites across the UK by planting out rust-infected plants into infestations of the weed, allowing for natural rust spread (Figure 1b). This release strategy was not without difficulties; inoculation of plants had to be strategically planned to ensure plants were producing urediniospores at the correct time, particularly when the climatic conditions were suitable, and transporting plants 1.5 m in height to sites across the UK proved to be impractical. Release sites were carefully selected to ensure stability (sites not at risk of flooding) and high levels of humidity to enhance the likelihood of the rust establishing. However, initial infection in the field was lower than anticipated at a number of sites; uredinia on leaves were smaller and elicited plant defense responses (e.g., anthocyanin production around the pustules) when compared to infected plants observed in the native range and during quarantine assessments (Varia et al., 2016). Further analysis of field data (temperature and humidity recording) determined that these differences could not be explained by unfavorable environmental conditions or a sub-optimal release strategy alone. Subsequent inoculation experiments of Himalayan balsam populations collected from all 20 sites conducted under controlled conditions at CABI revealed significant variation in the susceptibility to the rust, with some populations being completely resistant. Fortuitously, a previously-collected strain of the rust from Pakistan had been stored in liquid nitrogen, and after confirmation of the host-range, was approved by the UK government for release from quarantine in January 2017 (Varia et al., 2016; Shaw et al., 2018). Inoculation studies conducted under controlled conditions found that the strain from Pakistan could infect many, but not all, of the populations which were resistant to the Indian strain.

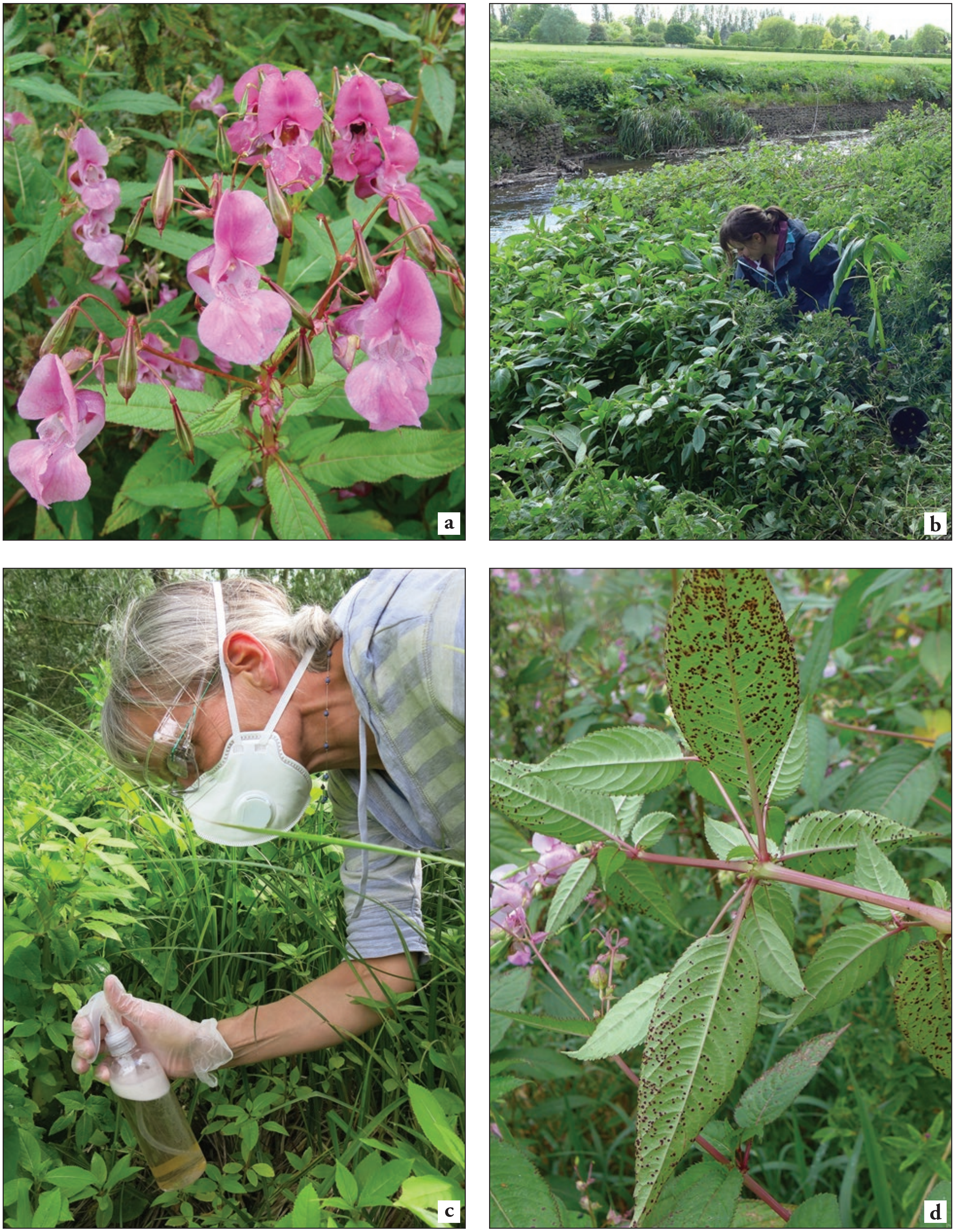


**Figure 1.** Himalayan balsam rust release strategy (a) the target—a Himalayan balsam plant; (b) planting out of rust-infected plants at field release sites; (c) release of urediniospores in an aqueous spray; (d) Himalayan balsam plant heavily infected with urediniospores of the rust.

In 2017 a second phase of rust releases was initiated integrating local action groups (LAGs) from across the UK. This was necessary due to a decrease in government funding which had to be offset by a cost-recovery approach to rust implementation. In addition, a new release strategy was developed whereby the rust urediniospores were applied to plants in an aqueous spray (Figure 1c); this was made possible by the issuing of a permit by the UK Chemicals Regulation Division. Detailed instructions, release equipment and one-to-one training in the rust release and monitoring protocols was provided to LAGs by CABI staff, enabling the rust to be released three times over the growing season (June, July and August) and in greater numbers at each site. Urediniospores are harvested from inoculated plants in the greenhouse throughout the year and stored in liquid nitrogen until required, with no decrease in spore viability. In addition, the application of the rust spores in solution in a hand-held sprayer, targeting the lower leaf surface, allows for more precise application and greater coverage of Himalayan balsam plants in a stand.

Since the two rust strains are able to infect a different range of genotypes of Himalayan balsam, it is important to assess the plants at each release site for their compatibility with the two strains so that the more effective strain can be released (or so no release is made at all, if the plants are resistant to both strains). In addition, there are considerable costs associated with producing the rust, therefore a mixed inoculum released at all sites would not be cost effective. The results of the establishment and spread of the rust are recorded by the trained LAG personnel and the data sent to CABI for collation and further analysis. This phase has recently been expanded under a European Commission-funded project "RAPID LIFE" where funding is supplied to LAGs to implement rust releases and monitoring across England.

The release of the rust through LAGs has been a learning process, e.g., collaborators need clear, unambiguous instruction. However, results of these field releases have been very encouraging with high urediniospore infection recorded at most release sites (Figure 1d). Nevertheless, natural spread of the rust is still localized, and the rust has not yet reached sufficient levels to trigger an epiphytotic (epidemic). The rust has successfully overwintered at a limited number of sites in the UK, and experiments are currently underway to determine why it has not persisted at others. Expectation management has been an important part of public engagement; i.e. the rust is not a "quick fix solution." Furthermore, the presence of weed genotypes not susceptible to either rust strain in the UK requires additional strains to be collected from the native range.

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*Oral presentation*

# Modeling the biocontrol of an invasive tree by a bud-galling wasp, *Trichilogaster acaciaelongifoliae*

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*Acacia longifolia* (Andrews) Willd. is one of the most widespread invasive plants in coastal areas of Portugal, mainly because of its ability to produce a high amount of long-lasting seeds that accumulate in the soil, germinating after disturbance and resulting in rapid reinvasion of the areas. After conducting host-specificity tests and many years of licenses and bureaucracy, the Australian bud-galling wasp, *Trichilogaster acaciaelongifoliae* (Froggatt), was introduced as a biocontrol agent for *A. longifolia* in late 2015. The wasp is highly host-specific, exclusively attacking *A. longifolia*. It is univoltine, and most of the annual life cycle is spent as eggs, larvae and pupae within the developing galls. The adults are small (3 mm), short-lived (2–3 days) and parthenogenic. The females lay their eggs in the flower buds of *A. longifolia* (and also vegetative buds) which develop into galls instead of pods. This wasp has been used as a biocontrol agent in South Africa for more than 30 years with great success. In the short term it reduces the annual seed production, which in turn results in fewer seeds for dispersal; in the long term it results in a reduction of re-invasion after control operations, fire or other disturbances. We created a dynamic model to simulate the establishment and population growth of this biocontrol agent and its impacts on the seed production of *A. longifolia* over time. The model was created using STELLA 10.0.5 and was divided in three interconnected sub-models: the climatic scenario, the *Acacia* model and the *Trichilogaster* model. The model featured an intuitive interface to promote its use by non-experts in the management of invaded areas. Parameters were obtained from a literature review, field records and expert knowledge. Results are presented, showing a slow initial exponential growth followed by stabilization with natural fluctuations between years, resulting from meteorological variations. Advantages of this approach for biocontrol research and for management of invaded areas are discussed.

*Oral presentation*

# Landowners and researchers in partnership to ensure the success of biological control in South Africa

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For over a century, researchers have safely implemented biological control to manage invasive alien plants which impact South Africa's rich biodiversity and agricultural assets. Research in this field has been funded by various government departments, including: Agriculture, Water Affairs and Environmental Affairs.

With the establishment of the Centre for Biological Control, a consortium of four universities involved in biological control, it is an appropriate time to expand biological control efforts and to develop a diversity of funding sources. In order to achieve both of these, the research community needs to work in closer partnership with the users of biological control: landowners, livestock farmers and rural communities.

In South Africa, there are two organizations that lead biological control research: the Agricultural Research Council's Plant Protection Research Institute and the Centre for Biological Control. Both these entities undertake a variety of communication activities to promote biological control. Neither organization overtly aims to build a partnership between researchers and the users of our research. We hypothesize that a stronger partnership will result in an improved understanding of our work, increased use of biological control and ultimately the partnership may provide funds from a diversity of sources.

In order to assess the effect of communications efforts and success of the partnership, we need to understand the baseline acceptance of biological control among the target audience. The danger of undertaking an assessment of this nature is that the target audience may end up being or feeling like they are the subject of the research and not partners in the management of invasive plant species (as described in Figure 1).

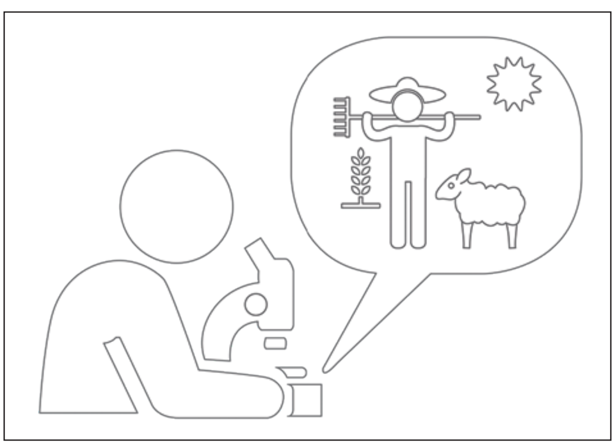

**Figure 1:** Assessing audience perceptions can lead the audience to feel they are the subject of the research rather than a partner in a biological control program.

**Figure 2:** A graphic depiction of the "virtuous cycle" we hope to establish through a partnership between biological control researchers and the users of biological control: landowners, farmers and rural communities.

Therefore it was, and is, important to emphasize that the users of biological control are not the subject of the research but are partners in how to make more and better use of biological control. Ultimately, we want a partnership that ensures a virtuous cycle of information and ideas coming from farmer to researcher and implementer of biological control and back to farmer (illustrated in Figure 2). The research is not about "studying what the farmer wants," it is about how the "researcher becomes more part of the farmer's reality" and developing a sustainable partnership between all the stakeholders with a joint mission to use biological control more effectively.

Cognizant of this danger, our first task would be to ascertain the levels of potential users' knowledge of the threat of invasive species and the use of biological control to manage target plants. We propose the use of the Net Promoter Score model to ascertain "appetite" and additional questions to ascertain levels of knowledge. The Net Promoter Score is derived from a simple research question "On a scale from 1 to 10, how likely are you to recommend the use of biological control to a family member, neighboring farmer, fellow farmers and friends?" We hypothesize that the current Net Promoter Score would be around -20 and that we should work on shifting it over a period of 3–5 years to +20. This is calculated by deducting the number of respondents who are considered "detractors" from the number of respondents considered to be "promoters" of biological control (Table 1.). Net Promoter Score does not need to be done in isolation of other research questions. Using it would simply give us a solid benchmark to measure success.

**Table 1:** Describes the hypothesis of current Net Promoter Score for biological control among the stakeholders in the commercial farming community of South Africa.

| Respondent Group | Description of respondents | Action required | Respondents |
|---|---|---|---|
| Promoters (score 9 to 10) | This is the group who would be supportive of using biological control agents | This is the group of people who would be your agents to grow the project. We need to win them over as quick adapters and showcase them | 10% |
| Passives (Score 7 to 8) | There is probably a large group who have no interest and or knowledge of biological control agents | The task on hand is to encourage them to become promoters | 60% |
| Detractors (Score 0 to 6) | Those who can afford to use pesticides, which they know are effective. This group is change resistant. | This is the group that will do most damage to the initiative. Education of the Detractor group is also of paramount importance to reduce damage they may cause to promotion of use of biological control. A successful program will encourage Detractors to become at least Passives if not Promoters. | 30% |
| **Net promoter score = % Promoters – % Detractors** | | | 10%-30% = **-20%** |

In order to assess the Net Promoter Score of any audience, you need access to this audience. While my coauthor has access to over 70,000 respondents per month, shoppers at a leading retailer, the biological control fraternity does not have this level of access to its target audience.

How should biological control researchers interact with farmers who are likely to be impacted by invasive plant species and therefore interested in biological control? South Africa has an established network of agricultural extension officers across all nine provinces who deal with a range of issues from livestock to crops. Unfortunately, at present, extension officers do not deal solely with invasive species or biological control as their work encompasses a much broader range of content than a single focus on these topics.

So, if not through the extension officers, then how do we contact farmers directly? There are between 30,000 and 35,000 commercial farmers in South Africa who manage approximately 45 million hectares of agricultural land, 37% of South Africa's land surface. Agri-SA, the South African farmer's union, has access to 28,000 farmers (80%) through its provincial and commodity affiliates. An obvious access route to farmers would be through Agri-SA; thus, in 2018, the Centre for Biological Control joined Agri-SA as a corporate member to gain access to Agri-SA members.

Simpson and Calitz (2014) undertook a survey of South African farmers' access to information. They found that 55% accessed the internet from home and 40% spent between one and two hours a day on the internet predominantly for email, banking and business purposes but also for sports scores and social media. In addition, this survey revealed that farmers have embraced mobile technology with 57% of users accessing internet through mobile phones, with Samsung and Blackberry (remember them) having 31% and 29% usage respectively. Agri-SA has a social media presence of more than 21,000 Facebook and 10,000 Twitter followers.

So in good faith, believing that Agri-SA would open access to 28,000 farmers who were potentially tech-savvy, we prepared a very broad-brush introduction to invasive plants and a survey of six questions. This was sent out in Agri-SA's newsletter. The results were disappointing to say the least. We received two responses in the first three weeks and in the end received a total of six completed questionnaires.

So why was the response so poor? There are a multiplicity of factors that could be the cause:

1. Incorrect medium was used
2. Message was not attractive
3. Audience saturated with questionnaires
4. Audience saturated with news feeds
5. Cactus and waterweeds not their current problems but lantana and prosopis may be of more interest
6. More important current issues: expropriation without compensation, farm murders, drought and the pest fall army worm

For us to promote biological control to communities across South Africa, we need to be accessing greater numbers of farmers more efficiently. So it was back to the drawing board. Our relationship with Agri-SA allows for us to contact the provincial and commodity groupings directly. We can liaise with the Natural Resources committees which are made up of farmers committed to addressing particular issues in each province. Of the nine provinces, we have meetings set up with two, and over the next year (2019) will meet with the remaining seven.

Our first meeting was with Agri-Noord Kaap (Northern Cape), and after a long day's discussion about water rights, polluted water, fracking for shale gas, and the Square Kilometer Array telescope, we finally came to discussions around biological control of cactus, waterweeds and prosopis. We presented an overview of biological control, its history, its safety and its success. Even after this detailed presentation, the primary question from the audience members was, "What happens when the insect has eaten all of the target plant, won't it spread to other plants?" We still have a lot of work to do! However, the key lesson from this meeting was that we need the farmers to identify the subjects in which they are interested. While cactus invasion was an issue for them, it was prosopis that is their primary interest.

So what have we learned so far, and what direction should we be going?

1. We have an excellent product that we are currently giving away free of charge. We really should be able to get customers to request this product.
2. There are farmers that would like to be partners in invasive species management and the use of biological control.
3. These farmers are connected through a variety of electronic platforms which we can access through farmers' networks. We need to work closely with these entities, not to create our own platform but to partner on theirs.
4. These farmers have a huge number of issues to address and we need to make sure that we attract their attention.

In order to build the engagement in electronic communication we will need to have a greater presence on the ground to start. We need to step out of the laboratory and into the farmers' meetings and onto their mobile devices. These engagements should be in the appropriate forums, for example the Natural Resource Management or Council meetings of the provincial bodies of Agri-SA, and then the regional meetings of farmers at which they have requested information on invasive species and biological control and where they can give their undivided attention to our input and the biological control community can hear the needs of the landowners, farmers and rural communities.

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*Oral presentation*

# Perceptions of weed biocontrol in Europe: what's going on?

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It has long been apparent that the adoption of weed biocontrol in Europe has been very slow in arriving, which is in stark contrast to the wide-scale use of predatory and parasitic arthropods against crop pests in the very same countries. The latter has taken place often with little or no safety testing in advance of release. There appears to be a lack of awareness among the general public and their elected representatives of the issue of invasive species themselves, let alone of the procedures and safety of classical weed biocontrol. People are becoming more aware of invasive species in Europe, not least because of the recently enacted European Regulation, but also because of the media's increased interest in the subject. A previous exploratory survey of plant ecologists, carried out by the authors and followed by a discussion forum, revealed that some appeared to be willing to let their hearts rule their heads when considering classical weed biocontrol, showing risk-averse attitudes. In order to find out more about the attitudes towards biocontrol in Europe, a survey was designed and distributed to representative groups of professionals using various channels. The data are used to give an insight into perception of the discipline among stakeholders and influencers, and suggestions are made on what actions and advocacy would be useful to change any deep-seated but potentially unfounded fears.

*Poster presentation*

# Evaluating the potential for the biological control of floating pennywort (*Hydrocotyle ranunculoides*) in the UK

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*Hydrocotyle ranunculoides* L.f. (Araliaceae, formerly Apiaceae) is fast becoming a contender for the title of worst aquatic weed in the UK. Forming dense, interwoven mats on water bodies, the costs linked to its management and impact on tourism are estimated to exceed £25 million per year in Great Britain. The plant continues to expand its range in the UK and Europe, contributing to the degradation of important wetland ecosystems and outcompeting native species. It is one of 23 plant species listed in the EU IAS regulation. *Listonotus elongatus* (Hustache) was identified as having potential in the 1980s and is the most common and damaging herbivore on the plant in the Argentine native range. Adults feed on the leaves and lay eggs inside the petioles, where the developing larvae continue to mine before completing their development in the submerged stolons. The impact can be devastating in the field and has been linked to the periodical collapse of local patches of the plant in Argentina. A biological control program began in 2011, funded by the Department for Environment, Food and Rural Affairs (Defra). Despite an embargo on export from Argentina throughout much of the project, a total of 62 species have been assessed in laboratory host-specificity trials, with the test plant list going through a number of iterations (now consisting of 70 species). In the native range, no sign of weevil activity has been recorded on any species surveyed, including congeneric *Hydrocotyle* species. In the lab, however, two species (*Hydrocotyle vulgaris* L. and *Apium* [*Helosciadium*] *repens* [Jacq.] Lag.) are within the fundamental host range of the weevil and can sustain development to adult. The number of eggs and larvae were significantly higher in the target host, however, and the experimental setup used to test the non-targets was highly artificial and precautionary. In 2017, a pest risk assessment was submitted to the UK regulators. Further research is being undertaken to address the feedback received before the peer consultation and release application processes can be resumed.

*Poster presentation*

# *Ludwigia*: a prime target for biocontrol in Europe

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*Ludwigia peploides* (Kunth) P.H.Raven and *L. grandiflora* (Michaux) Greuter & Burdet are highly invasive aquatic weeds native to North, Central and South America, but which are now well established in parts of Europe. Able to double in biomass in 2–3 weeks, these plants can quickly clog water bodies, release allelopathic chemicals, outcompete native species, reduce water quality and damage fragile aquatic environments. Vast amounts of money have been spent trying to control *Ludwigia* species in Europe, but with little success in preventing the impact and spread of these weeds. In the UK where few, small populations of *L. peploides* have been identified, the weed is subject to an eradication program in an attempt to prevent the invasion becoming as problematic as it is in France, where chemical control measures are not permitted and mechanical removal results in fragmentation of the plant which can exacerbate infestations. It is clear that large-scale control using traditional methods is expensive and ineffective and that a more sustainable solution is needed. Classical biological control is a safe and effective alternative method of control with an excellent track record against invasive aquatic weeds. This poster highlighted the potential for biocontrol of *Ludwigia* species with reference to promising natural enemies already identified in the native range. Host range studies tailored to Europe are now required to determine the safety and potential of these agents among others from the plant's area of origin. The stage is set for biological control to finally bring *Ludwigia* under control.

*Poster presentation*

# A "mitey" solution for Australian swamp stonecrop in the UK

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*Crassula helmsii* (Kirk) Cockayne (Crassulaceae), commonly known as Australian swamp stonecrop, is an aquatic weed introduced to the UK from Australia as an ornamental pond plant in the early 1900s. Since then, it has become widespread throughout much of the UK and is spreading in parts of Western Europe, including France, Belgium and the Netherlands. Australian swamp stonecrop is an invader of still and slow-moving water bodies, and its ability to tolerate extreme environmental conditions and produce different growth forms depending on water depth enables it to outgrow less competitive plants (Dean, 2015), alter plant species composition (Smith and Buckley, 2015) and reduce the availability of bare ground for feeding by wading birds (Dean, 2015) in these sensitive habitats. Dense infestations can also obstruct leisure activities and affect water industry processes. The control of *C. helmsii* is challenging, with mechanical control exacerbating the spread of viable plant fragments and restrictions on the use of chemicals near water bodies limiting herbicide use. To help reach ecological targets set out in the European Union Water Framework Directive, the UK government provided funds in 2011 to initiate a biological control program for *C. helmsii*. This legislation was introduced to commit EU member states to achieve good ecological status for all water bodies.

Following extensive natural enemy surveys in Australia, the mite *Aculus crassulae* Knihinicki & Petanović (Acari: Eriophyidae), which is new to science (Knihinicki et al., 2018), was prioritized for further study as a potential biological control agent for *C. helmsii*. Feeding by this species causes the development of "big bud" galls in emergent and terrestrial *C. helmsii* plants (Figure 1), resulting in a significant reduction in growth. Host-range testing of the mite was carried out against selected non-target plant species of European relevance in no-choice feeding and development tests. Of the 40 species tested, non-target feeding and oviposition occurred on one species of the same genus, but no further development occurred. Studies

**Figure 1.** *Crassula helmsii* stem with big bud galls (white arrows) infested by *Aculus crassulae.*

investigating the impact of temperature on the development and survival of the mite were also carried out, demonstrating its potential to survive and establish under UK environmental conditions. These studies were compiled in a dossier, using the EPPO (European and Mediterranean Plant Protection Organization) Pest Risk Analysis (PRA) template, which was submitted to UK regulators for the assessment of *A. crassulae* for release as a biological control agent of *C. helmsii* in 2017. The PRA was assessed by the UK government, was sent for external review by the Advisory Committee on Releases to the Environment (ACRE), underwent public consultation and was reviewed by the EU Standing Committee on Plant Health (SCOPH). Following approval by these reviewers, the PRA was formally approved by the UK government in summer 2018, and permission was granted to release the mite from quarantine. The first field releases took place in September 2018 at three sites in England, which were selected based on the availability of persistent populations of terrestrial and emergent *C. helmsii* growth forms and on ecoclimatic variability. These sites will be monitored closely for mite infection and overwintering, with further releases at these sites and at additional sites planned in 2019. These releases mark the first biological control agent released against Australian swamp stonecrop and the fourth released against a weed in the EU.

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*Poster presentation*

# Outbreaks of *Nipponaclerda biwakoensis* (Hemiptera: Aclerdidae) in Louisiana: implications for biological control of *Phragmites australis*

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Since 2016, die-offs of *Phragmites australis* (Cav.) Trin. ex Steud. have been reported over thousands of hectares at the Mississippi River Delta, Louisiana, USA. Die-offs are characterized by reduced plant height, thin stems, and lower plant density, eventually resulting in death of the plant. Upon close examination, die-offs were associated with outbreaks of an Asian scale, *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae). Crawlers emerge from females and settle in tight spaces between the leaf sheath and the culm. In Louisiana, infestations of *N. biwakoensis* increased from 30% in the spring to 98% in the fall of 2017; they reached an average of 380 scales per stem, suggesting that this scale has adaptations for rapid dispersal and population increase. In its native range, *N. biwakoensis* was found in association primarily with *P. australis* but also with *Agropyron* sp. and *Juncus* sp. The presence of native and non-native lineages of *P. australis* in Louisiana offered a unique opportunity to study the host specificity of *N. biwakoensis*. The objectives of this study were to (1) evaluate the physiological host range under no-choice conditions and (2) determine the ecological host range in the field. Test plants were selected based on their phylogenetic proximity to *P. australis* and economic and ecological importance. Under no-choice conditions, *P. australis* stems infested with scales were placed adjacent to test plants, and crawlers were allowed to colonize the plant material. One month after exposure, the presence of nymphs was recorded. Under field conditions, grass species located at outbreak sites were inspected, and the presence of the scale was recorded. Preliminary results revealed that all four lineages of *P. australis* present in Louisiana can sustain populations of *N. biwakoensis*. This poster discussed implications of the host range of the scale in the context to non-target risks.

# Session 9: Post-release monitoring and evaluation

## Session Chair: Lincoln Smith

**Keynote**

# Agent monitoring and evaluation: a New Zealand perspective

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Weed biocontrol research should be in its heyday as case studies reported over many decades are now sufficiently numerous to enable powerful studies that test a range of hypotheses. In New Zealand, a major thrust of weed biocontrol research over the last two decades has been aimed at making biocontrol more cost-effective without compromising environmental safety. Nationwide surveys of parasitism and predation of agents have resulted in novel approaches to prioritizing agents that are less likely to be negatively impacted by biotic interference. Ongoing nationwide surveys of the field host range and non-target impacts of agents have enabled the development of a quantitative approach to interpreting host-specificity testing results, enabling the release of agents that would otherwise have been rejected. A review of biocontrol impact and plant traits has indicated which target plants are likely to be most susceptible to biocontrol. These studies have indicated that future research should be directed towards the evolutionary aspects of agent-plant interactions.

*Oral presentation*

# Biological control of weeds: a summary of introductions, rates of establishment and estimates of success

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The foremost reference that comprehensively reports on biological control introductions against weeds, "Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds," has been updated and now includes all deliberate releases made through 2012. It includes data on 1555 intentional releases of 468 biological control agent species used against 175 species of target weeds in 48 plant families in 90 countries. For 55 (31.4%) of the target weed species, only one biocontrol agent was introduced. The largest number of agent species (44) was introduced for the biological control of *Lantana camara* L. (Verbenaceae). Overall, biocontrol agent releases peaked in the 1990s, followed by continuous decline caused by a more risk-averse environment, tighter regulations and/or a decrease in funding. Three insect orders (Coleoptera, Lepidoptera and Diptera) comprised about 80% of all biocontrol agent species released and releases made. Of the 468 biocontrol agent species introduced, 332 (70.9%) established in at least one instance. Of the 313 species for which impact could be categorized, 172 (55.0%) caused medium, variable or heavy levels of damage. Hemiptera, Coleoptera and fungal pathogens caused highest impact. Of all releases made through 2012, 982 (63.2%) led to establishment. Of the 940 releases for which impact could be categorized, again approximately half (503 or 53.5%) caused medium, variable or heavy impact, and almost a quarter caused heavy impact (225 or 23.9%). Across all countries and regions, 65.7% of the weeds targeted for biological control experienced some level of control. Additional information about source and receiver countries/areas and impact by agent family was presented.

*Oral presentation*

# A post-release assessment tool and its implementation in the USA

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Post-release monitoring of biological control is a crucial component to determine biocontrol agent establishment and the impact on the target invasive plant and subsequent plant community response. Commonly, the initial releases of a biological control agent involve a specialized monitoring protocol conducted by the implementation entity responsible for the initial releases. As biological control agents become established at multiple locations, the time-consuming monitoring protocol developed for that specific biological control agent and personnel to conduct the monitoring become scarce. As such, a group of biological control practitioners from Idaho, USA developed a regional, multi-system, interagency post-release assessment program—the "Standard Impact Monitoring Protocol" (SIMP). SIMP was developed to be citizen-science-friendly and statistically sound with regard to data analysis. SIMP is used to document the change in vegetation cover, target weed density and biological control agent abundance over time. This provides biological control implementation specialists and land managers with a tool to assess the relative impact of the biological control agent and the corresponding change in vegetation after a biological control agent release. Beginning in 2017, a smart phone application was created to collect SIMP data and georeferenced pictures of the monitoring sites. This approach aims to eliminate hard copy data sheets and reliance on old technology that requires significant post-process editing. This, in turn, will make SIMP more user-friendly and accessible to anyone with a smart phone. Included in this presentation was a brief analysis of trends after collecting SIMP data for eleven years for Canada thistle (*Cirsium arvense* [L.] Scop.), Dalmatian toadflax (*Linaria dalmatica* [L.] Mill.), leafy spurge (*Euphorbia esula* [L.]), and spotted knapweed (*Centaurea stoebe* [L.] sens. lat.).

*Oral presentation*

# Identifying factors influencing establishment discrepancies of a bud-feeding weevil, *Dicomada rufa*: a case study from South Africa

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*Hakea sericea* Shrad. & J.C.Wendl. (Proteaceae) (silky hakea) is an Australian native which invades and threatens the rich biodiversity of the endemic Fynbos vegetation in South Africa. Following the release of the bud-feeding weevil *Dicomada rufa* Blackburn (Coleoptera) in 2006, establishment has only been successful at a third of the original release sites, all within a particular region. Ecophysiology, abundance and host-agent mismatch are three hypotheses invoked to explain these discrepancies. Rainfall patterns across the distribution of *H. sericea* vary considerably, and environmental conditions (e.g., temperature and humidity) can influence an insect species' distribution range. Thermal tolerance data show that climatic mismatch is not limiting establishment of *D. rufa*, but that desiccation resistance may be important. Studies on whether host "biotypes" play a significant role in the success or failure of the insect have compared trees from three separate regions to determine whether genetic and phenotypic differences (especially chemical composition of plant volatiles produced by the trees) exist between populations. Overall, these investigations are providing insights into the viability of *D. rufa* as a biocontrol agent and providing an explanation for the current establishment discrepancies.

*Oral presentation*

# Ten years of post-release evaluation on *Myriophyllum aquaticum*, what have we learned?

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The biological control of parrot's feather, *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae), was initiated in South Africa in 1994 with the release of the leaf-feeding chrysomelid, *Lysathia* sp. In 2008, a post-release evaluation program was initiated comprising annual surveys of 56 parrot's feather sites across South Africa. After six years of surveys the weed had disappeared at 12 survey sites; after ten years the weed was absent from the majority of sites and only remained weedy at two sites. Field observations from these sites suggested that the beetle may not be as effective in seasonal ponds where at certain times of the year the weed grows on the banks under water-stressed conditions. A laboratory study showed that when given the opportunity, the females chose to oviposit on healthy plants as opposed to water-stressed ones. Therefore, when given the choice, adult females would rather disperse to another locality where plants are growing under better conditions. The success of this biological control program can be ascribed to (1) the fact that only female plants are present in South Africa so there is no viable seed, (2) a very efficient mass-rearing program that has resulted in large numbers of healthy insects being released and (3) the agent is an excellent disperser. Parrot's feather is now considered to be under complete biological control; it may persist in seasonal pans, but there is still no need for additional control agents to be considered.

*Oral presentation*

# Suppression of seed production, a basic long-term strategy in weed biocontrol, as exemplified by a midge on *Acacia mearnsii* in South Africa

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The efficacy of weed biocontrol agents that suppress or prevent seed production continues to be perceived with skepticism. However, long-term observations and careful measurements of what has been accomplished are positive. This is illustrated by the results achieved using a flower-galling midge, *Dasineura rubiformis* Kolesik, which inhibits pod (and hence seed) production by the Australian invasive tree *Acacia mearnsii* De Wild. in South Africa. High fecundity and long-lived soil seed banks have made *A. mearnsii* an extremely successful invader, and it is prominent and problematic in the wetter parts of the country. Since dispersal is the primary target for managing invasion pathways, limiting propagule pressure is essential to the management of *A. mearnsii* and many other weeds. *Dasineura rubiformis* has become astonishingly prolific on *A. mearnsii* trees in the winter rainfall regions, and detailed measurements over the last 12 years have shown that up to 99% reduction in annual seed production can be achieved. While sizable seed banks remain under existing *A. mearnsii* stands, further seed accumulation has virtually ceased. Modeling indicates that these extreme levels of seed suppression will, over time, decrease population growth rates and limit the spread and invasiveness of the target plants. Thus, this case demonstrates an important generality: the use of biocontrol agents that directly or indirectly suppress seed production should be seen as an integral part of any long-term management strategy against invasive plants.

*Oral presentation*

# Fungal biological control of the invasive plant mistflower (*Ageratina riparia*) facilitates recovery of native vegetation

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It is often assumed, but rarely demonstrated, that a decline in the abundance and competitive performance of invasive plants following successful biological control facilitates the regeneration of native vegetation. This presentation examined native vegetation community responses to biological control of the invasive plant mistflower, *Ageratina riparia* (Regel) R. M. King & H. Rob. (Asteraceae), with the white-smut fungus *Entyloma ageratinae* Barreto & Evans. Native vegetation (i.e. species richness, community composition and percent cover of vascular plants) was surveyed in 24 permanent 1 m x 10 m belt transects at seven sites within wet sclerophyll forest in New South Wales and southeast Queensland. Surveys were conducted in areas of dense mistflower infestation (>70% cover) prior to or soon after the fungus colonized each site in 2011, and subsequently in 2012 and 2017. By 2017, there was a significant 5-fold reduction in mistflower abundance as a result of disease pressure through time. This, in turn, was associated with a 4-fold increase in native plant species richness, from an average of 5 native species per transect in invaded areas in 2011 to an average of more than 20 native species in 2017. The magnitude of such positive indirect effects of biological control on native plant richness was similar in New South Wales and Queensland, although the community composition varied between regions. The reduction of mistflower abundance following biological control, and flow-on increases in native species richness, are likely to lead to benefits for ecosystem structure and function.

*Oral presentation*

# Assessing herbivore biocontrol impact on common ragweed, combining field experiments and population models

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The decline of populations is an important aim of classical weed biological control, but predictive and post-release data on this target impact remain scarce. Using a case study of invasive common ragweed (*Ambrosia artemisiifolia* L.), we herein present how a combination of experimentation and population models can advance this knowledge.

The North American *A. artemisiifolia* is an annual with a long-lasting soil seed bank, adversely affecting human health and crop production worldwide. Traditional control methods (herbicides, mechanical) are insufficient to reduce population sizes and are inapplicable in many habitat types. Biological control by the North American leaf beetle *Ophraella communa* Le Sage is considered successful in China and Australia, but measures on the population-level impacts of this biocontrol agent are lacking.

We used the recent accidental introduction of *O. communa* in Europe to experimentally manipulate its presence in natural populations of *A. artemisiifolia* during three consecutive years. We assessed the impact on vital rates of *A. artemisiifolia* and used these results to construct population models. Projections of our models show that the beetle was able to reduce population growth of *A. artemisiifolia* populations in each year, but the magnitude of this impact exhibited strong temporal variation. Life table response experiments identified that effects on different vital rates were responsible for the reduction in different years. Although reduction in growth and fecundity is generally assumed most effective for fast-growing short-lived species, we found that reduction in survival was most important in two years.

In this presentation, we discussed implications for the potential further use of *O. communa* as a biocontrol agent of *A. artemisiifolia* in Europe, also in integrated management. Linking our results to aerial records of *A. artemisiifolia* pollen and species distribution models for the target and the agent, we also presented potential corresponding benefits for human health.

*Oral presentation*

# Beyond the target: developing the right metrics and measurements to determine success

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Biological weed control aims to reduce or prevent negative impacts attributed to plants introduced outside their native ranges without their suite of specialized natural enemies. We typically assume that introduced species have negative impacts and that negative impacts increase as abundance or dominance of introduced plant species increases. However, these assumptions usually lack theoretical or empirical support in sophisticated analyses or experiments. Consequently, we cannot simply assume that reductions in invasive plant abundance, biomass or cover through release of biocontrol agents will automatically benefit native species. Fortunately, post-release assessments are slowly becoming more common, a welcome development. However, are we measuring the right "stuff," and are we using appropriate metrics to assess success? I argue that because invasive plant management is typically justified because of negative ecosystem impacts, follow-up investigations need to do more than assess biological success (i.e. determining if biocontrol agents are able to affect host plant abundance and population dynamics after release). We instead need to measure outcomes of biocontrol agent releases on species, or processes, that were assumed to be negatively affected by the target weed in the first place. In conservation biological control, these impacts of an introduced plant can affect native, rare or endangered species or habitats. Depending on the target weed species and its particular assumed or documented impacts, post-release assessments then need to focus on measuring the demographic responses of native species or changes in ecosystem processes. The benefits of using such novel post-release monitoring schemes are enormous as we can assess benefits to native species through biocontrol. A further benefit is the ability to assess whether introduced plants were the driving force in ecosystem deterioration, or just responding to other stressors, such as overgrazing or following invasions by other introduced species.

*Oral presentation*

# Could hybridization between agent biotypes increase biological control efficacy?

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Hybridization is often invoked to explain the success of invasive species as it can increase the fitness of individuals (heterosis), create novel genotypes with traits enabling colonization of new habitats, and increase genetic variation that may speed up adaptation to novel biotic and abiotic conditions. These mechanisms could also increase the effectiveness of biological control by enabling faster build-up of agent populations and by facilitating their colonization of and adaptation to novel environments. However, it is typically unknown whether distinct biotypes or hybrids are more likely to establish when genetically distinct populations are released, whether hybridization between those occurs and whether hybrids provide better control. Using molecular tools, we assessed the extent of natural hybridization between two biotypes, a Swiss and Italian, of the ragwort flea beetle *Longitarsus jacobaeae* (Waterhouse), a biological control agent of the invasive weed J*acobaea vulgaris* Gaertn. We found that at high-elevation sites in northwestern Montana, almost half ($n$ = 7) of the 15 sampled populations contained hybrid individuals. We subsequently evaluated the impact of biological control on six sites with the pure Swiss biotype and three sites where hybrid beetles were present in a two-year exclusion experiment. Biological control by either Swiss or hybrid beetles increased mortality of the target by 44–52% and reduced plant fecundity by 50–67%, respectively. Interestingly, the impact of biological control and beetle densities tended to be higher at sites with hybrids present. These results suggest that hybridization of ragwort flea beetles at high-elevation sites might improve biological control and that agent hybridization could benefit biological control.

*Oral presentation*

# Do shared parasitoids represent apparent competition or biotic resistance? Evidence from the waterhyacinth system in Florida

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Biological control insects exert top-down control on their target plant but also interact with the native community. Biotic resistance may thwart establishment, but indirect interactions may also arise, e.g., apparent competition via shared parasitoids. Waterhyacinth (*Eichhornia crassipes* [Mart.] Solms [Pontederiaceae]) is perhaps the world's most invasive weed; it affects commerce, ecology and human health. *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) was introduced in 2010 to Florida from Argentina to control waterhyacinth. *Megamelus scutellaris* overlaps in aquatic habitats with a native leafhopper, *M. davisi* Van Duzee, which is attacked by the native egg parasitoid *Kalopolynema ema* (Schauff & Grissell) (Hymenoptera: Mymaridae). *Kalopolynema ema* also successfully utilizes *M. scutellaris*. To gauge the spatial interactions between *M. scutellaris, K. ema* and *M. davisi*, we built 1m$^2$ plots of waterhyacinth with *M. scutellaris* within ponds containing *M. davisi*, its host plant *Nuphar lutea* (L.) Sibth. & Sm., and *K. ema*. The abundance of *K. ema* was then measured at 1, 5, 10, and 20 m from waterhyacinth for 11 months. Additionally, a mesocosm experiment was constructed with the following treatments: no herbivores (host plants only), *M. davisi* only, *M. scutellaris* only, *M. davisi* + *K. ema*, *M. scutellaris* + *K. ema* and *M. davisi* + *M. scutellaris* + *K. ema*. Waterhyacinth ramets and *N. lutea* leaves were isolated at two separate times for leafhopper and *K. ema* emergence to measure parasitism. Plant biomass was sampled at the end of the study. We found no significant relationship between the distance from waterhyacinth and *M. scutellaris* and the incidence of parasitism in *M. davisi.* Parasitism levels were not affected by the presence of either herbivore, but *M. scutellaris* showed signs of biotic resistance in the presence of *K. ema*. We observed no producer-level effects in waterhyacinth or *N. lutea*. No evidence of apparent competition was detected, rather *K. ema* has the potential to negatively affect *M. scutellaris* and its impact.

*Oral presentation*

# Using drones in pre- and post-release monitoring and evaluation of the biocontrol of *Cylindropuntia* spp. in Australia

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Unmanned Aerial Vehicles (UAVs or drones) have become an increasingly useful and cost-effective technological tool for capturing data in agricultural and environmental research. In Australia, they are increasingly used for agricultural property inspections (checking fences, water troughs, stock activity etc.), assessing pasture/crop health and vigor (using vegetation indices such as Normalized Difference Vegetation Index), invasive animal detection (using thermal infrared radiation), and more recently in detecting invasive weed species (using multi-spectral reflectance). *Cylindroputnia* spp. are Cactaceae from the Americas. They have become weeds of arid and semi-arid regions of mainland Australia, with eight species currently recorded as naturalized. A biocontrol program was initiated in Australia in 1925 for the control of *C. imbricata* (Haw.) F.M. Knuth through the introduction of a cochineal, *Dactylopius tomentosus* (Lamark). More recently, different lineages of *D. tomentosus* (which have specific impacts on the different *Cylindropuntia* spp.) have been imported, tested and approved for release. Two *Cylindropuntia* species, *C. fulgida* var. *mamillata* (A. Schott ex Engelm.) Backeb.(boxing glove cactus) and *C. pallida* (Rose) F.M. Knuth (Hudson pear) were considered in this study. For *C. fulgida* var. *mamillata*, a UAV (equipped with a 12MP sensor) was used to acquire Red Green Blue (RGB) aerial imagery of a long-term monitoring site to visualize *D. tomentosus* "cholla" crawler dispersal over time, as well as its overall impact on the weed. For *C. pallida,* a fix-winged UAV fitted with multi-spectral and RGB sensors was used to assist in mapping the species in areas of its invaded range, while the impact of *D. tomentosus* "californica var. parkeri" on target weed vigor was assessed using a multi-rotor UAV (also fitted with a multi-spectral and RGB sensor). In this presentation, the results of these studies were discussed with particular reference to drones and their future use in monitoring and evaluating weed biocontrol research projects.

*Poster presentation*

# Post-release assessment of classical biological control of *Cirsium arvense* in the Western United States

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Classical biological control of weeds has been used for more than 50 years to control Canada thistle (*Cirsium arvense* [L.] Scop., Asteraceae) in the USA. However, there are few studies that have assessed the efficacy of the two main biological control agents, the stem-gall fly *Urophora cardui* (L.) (Diptera: Tephritidae) and the stem-mining weevil *Hadroplontus litura* (Fabricius) (Coleoptera: Curculionidae) in the field. We set up permanent study transects, following the standardized impact monitoring protocol (SIMP), at 94 *C. arvense* infestations in the states of Idaho ($n$ = 59), Utah ($n$ = 8), North Dakota ($n$ = 4), and South Dakota ($n$ = 23) to monitor weed populations and biological control agent abundance between 2008 and 2014. Transects were set up in groups of four randomly assigned treatments: (1) release of *U. cardui*, (2) release of *H. litura*, (3) release of both insects and (4) no insect releases. For our analysis, we analyzed whether biological control agent abundance, vegetation cover composition and *C. arvense* density could explain changes in *C. arvense* stem density. While *U. cardui* and *H. litura* were widely established, their abundances varied greatly among sites/years and were generally low. *Cirsium arvense* stem density during the previous year negatively affected the change in Canada thistle stem density, likely due to intraspecific competition. Changes in *C. arvense* stem density were negatively affected by weevil mining but only during years or at sites that were subject to relatively high summer temperatures. We found no evidence for an effect of *U. cardui* or local vegetation community on *C. arvense* population dynamics. Overall, the two biological control agents had small to no impact on *C. arvense* abundance or persistence in our study area in the Western United States.

*Poster presentation*

# Release and monitoring of a biocontrol agent in an actively-managed environment

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Waterhyacinth (*Eichhornia crassipes* [Mart.] Solms) is an invasive, free-floating aquatic plant found in Florida. It can grow in dense mats and entirely cover areas of open water, altering the ecosystem, reducing navigability and obstructing water control structures, thus necessitating intensive herbicidal management. Biocontrol agents released in the 1970s have helped reduce waterhyacinth biomass and seed production, but not coverage. In 2010, the waterhyacinth planthopper *Megamelus scutellaris* Berg (MS) was released as an additional biocontrol agent of waterhyacinth and has since established wild populations. As part of the Comprehensive Everglades Restoration Project, the objective was to release and disperse this biocontrol agent as quickly and efficiently as possible. An experiment on release methods to determine best practices indicated that releasing adults only and releasing egg-laden plants only were equally as effective in establishing MS populations large enough to reduce plant biomass. Small-scale dispersal studies supported this, showing that females tend to remain in the area and oviposit up to one week post-release, thereby creating egg-laden plants at the release site. While MS populations have established, they are persisting at low densities. At the landscape level, we have been able to track their movements between release sites and control locations using both direct and indirect survey methods. At all sites, waterhyacinth plants were visually inspected for MS adults and nymphs. Sample plants were also collected and monitored for nymph emergence. This indirect approach has been useful in detecting MS at low densities where the direct method has failed. This species has dispersed >6 km from release sites in two years. It can also better integrate with herbicidal management of waterhyacinth compared to its predecessors as all life stages feed externally. When an herbicide begins to affect a host plant, the insects readily move elsewhere in the same mat and are capable of moving at least 15 m between patches of herbicide-treated waterhyacinth within two months.

*Poster presentation*

# Establishment and early impact of the florivorous weevil *Anthonomus santacruzi* on *Solanum mauritianum* in South Africa

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*Anthonomus santacruzi* Hustache (Curculionidae) was released in South Africa in 2009 to reduce fruiting and seed dispersal by *Solanum mauritianum* Scop. (Solanaceae). The weevil was released throughout KwaZulu-Natal (KZN) province, with widespread establishment in its warmer coastal regions. Limited releases in other provinces delivered mixed results, with no establishment in high-altitude areas. Post-release monitoring in KZN was initiated to determine whether *A. santacruzi* populations (1) display seasonal abundance, (2) are influenced by floral availability, (3) negatively affect the weed's fruit production and (4) are negatively affected by climate. Weevil abundance recorded over 12 months varied seasonally across coastal and inland sites, with consistent peaks in autumn and declines during winter. Weevil numbers were significantly positively correlated with floral availability in the previous month. However, <10% of available flower buds were infested monthly by weevil larvae. Also, significant negative correlations between weevil numbers and fruit set recorded two months later may be due to ripe fruit abscission, rather than feeding damage. Sampling of *S. mauritianum* infestations across an altitudinal gradient in KZN indicated that despite significantly higher amounts of floral material on plants at the higher-altitude inland sites, weevil numbers were significantly higher at the lower-altitude coastal sites. There was a significant negative relationship between weevil numbers and altitude and significant positive relationships between weevil numbers and both temperature and humidity. Substantially higher weevil numbers are needed throughout the year to achieve meaningful impact, although this may increase with time. Given the climatic constraints, future releases should focus on coastal regions and lower-altitude inland regions (below 1,000 m above sea level).

*Poster presentation*

# How do climatic conditions affect the establishment of *Catorhintha schaffneri* on *Pereskia aculeata* in South Africa?

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*Pereskia aculeata* Mill. (Cactaceae) is an invasive creeping cactus which has a negative impact on indigenous biodiversity in South Africa. Experimental releases of a new biological control agent for *P. aculeata*, the stem-wilting bug *Catorhintha schaffneri* Brailovsky & García (Hemiptera: Coreidae), were performed at 16 sites across South Africa with a range of climatic conditions. Surveys to assess insect establishment over a two-year period demonstrated that *C. schaffneri* had established at only two of the 16 release sites. The thermal tolerance and humidity requirements of *C. schaffneri* were subsequently investigated under laboratory conditions, and these results were compared to the climatic conditions at the release sites in South Africa and the source of the agent population in southern Brazil in an attempt to understand the low rate of establishment. Thermal tolerance was investigated by developing a degree-day model and determining the critical and lethal limits of *C. schaffneri,* while the lethal humidity levels for the adult and egg stages were also calculated. From these results, the low initial establishment rate of *C. schaffneri* can be attributed to a mismatch in climatic conditions between the native distribution and some parts of the introduced range in South Africa, namely lower temperatures within the lower-lying areas of the invaded range in the Western Cape and inland in the Eastern Cape, as well as low moisture levels in the higher-lying areas in KwaZulu-Natal. The low moisture conditions are uncharacteristic of high lying areas of KwaZulu-Natal due to a severe drought over the time of this study, and therefore establishment is likely to improve in this area in the future. Post-release research like this can help increase the effectiveness of biological control programs by prioritizing release sites which are climatically favorable.

*Poster presentation*

# Folivory impact of the biocontrol beetle *Cassida rubiginosa* on population growth of *Cirsium arvense*

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The folivorous beetle, *Cassida rubiginosa* Müller, was released in New Zealand in 2007 as a biocontrol agent against *Cirsium arvense* (L.). Scop. (Californian, Canada, creeping thistle). The impact of the beetle was assessed in a population of *C. arvense* over two years, from spring 2015 to spring 2017. Experimental plots (1-m diameter) were isolated within the population by removing the *C. arvense* shoots in a 2-m perimeter area surrounding the plots with a broadleaf selective herbicide. This allowed for thistle shoot population density and spread to be measured from year to year, following treatment with different levels of folivory. The folivory treatments were imposed by applying 0, 5, 10 or 20 larvae per shoot within the experimental plots in early spring. Shoot growth and development were measured throughout the growing season, along with estimates of % folivore damage. In the year following treatment (spring 2016), shoot population density was significantly reduced by 28 and 75% in the 10- and 20-larvae per shoot treatments, respectively. However, following the second year of treatment (spring 2017), there were no significant changes in shoot population density due to the beetle. Thistle shoot spread (the number of shoots entering the perimeter zone of the treated area) was reduced in both years where 10 or 20 larvae per shoot were applied. Five larvae per shoot had no effect on shoot population density or spread. The percentage of shoots reaching a reproductive stage (flowering or seeding) was negatively correlated with increasing % folivore damage. This poster presented these data in conjunction with observations and measurements from several release sites where *C. rubiginosa* folivory has ranged from trace amounts to nearly all shoots in a population completely defoliated.

*Poster presentation*

# Establishment and post-release evaluation of *Lilioceris cheni* (Coleoptera: Chrysomelidae), a biological control agent of air potato in Louisiana

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Air potato, *Dioscorea bulbifera* L. (Dioscoreaceae), is a perennial vine that has invaded many urban and natural ecosystems in the southern USA, including Florida and Louisiana. This vine is native to Asia and Africa, and it was originally introduced into the USA as an ornamental plant in the 1800s. The specialist leaf beetle *Lilioceris cheni* Gressitt & Kimoto has been released as a biological control agent of air potato in Florida since 2011. Several federal, state and local agencies are working together for mass rearing, field releases and monitoring of *L. cheni* in Florida. Field studies have shown reduction of the air potato vine growth and reproduction in various locations throughout the state, followed by recovery of native vegetation. *Lilioceris cheni* was originally collected in Nepal (Nepalese biotype), and a second biotype was found in China (Chinese biotype). Previous studies suggest that the Nepalese biotype may be better adapted to colder temperatures; therefore, this biotype was selected for release in Louisiana in 2016. During 2016 and 2017, 2020 beetles were released at 14 sites in central and south Louisiana. All release sites were monitored during the fall of 2017. Field transects 100 m long were used in each field site, and ten 1 $m^2$ quadrants were placed randomly for measurements of (1) air potato plant cover, (2) leaf beetle feeding damage and (3) number of leaf beetles. Results showed that *L. cheni* has successfully survived the winter at all release sites in Louisiana. Damage by the beetle resulted in early plant senescence and reduction in plant cover leading to the recovery of understory vegetation at some sites. The early establishment and impact of *L. cheni* on air potato vines in Louisiana looks promising, and field evaluations will be continued to better assess the outcomes of this biological control program.

*Poster presentation*

# Can *Lilioceris cheni* suppress the climbing growth and bulbil production potential of its invasive host, *Dioscorea bulbifera*?

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Air potato, *Dioscorea bulbifera* L., a major invasive weed of Afro-Asian origin, has infested, spread and smothered plant communities in various natural and man-made ecosystems of the southeastern USA. In heavily infested areas, this weed has displaced native plants and endangered biodiversity. The ability of *D. bulbifera* to grow rapidly, trellis over supporting vegetation and produce large quantities of vegetative propagules (bulbils) have been identified as the invasive attributes that contribute to its invading potential. Herbivorous insects imported from a weed's native habitat may suppress these invasive attributes in adventive ranges. Herein, we tested this hypothesis by deploying *Lilioceris cheni* Gressitt & Kimoto, a foliage-feeding beetle from Nepal and China. To accomplish this objective, we established beetle-restricted (insecticide control) and unrestricted (beetle inoculated) experiments in five *D. bulbifera* infested sites. At each site we documented vine coverage (% of 5x3 m plot), feeding damage (% of total vine cover) and *L. cheni* population densities at six-week intervals, as well as bulbil densities and overall plant species richness on an annual basis from 2012–2016. Our findings indicate that feeding damage from *L. cheni* (1) reduced vine coverage and trellising on native vegetation (2) decreased bulbil density and biomass and (3) increased plant species richness in beetle-unrestricted versus beetle-restricted treatments. Despite significant differences in bulbil density and biomass and overall species richness, both treatments manifested similar trends over time. Similarities between treatments have been attributed to the feeding damage by spilled over *L. cheni* populations that swarmed into the restricted treatment plots from unrestricted treatment areas experiencing unseasonal vine mortality due to extensive feeding damage. Our data clearly demonstrate the ability of *L. cheni* to suppress invasive attributes of *D. bulbifera* in its adventive range in the southeastern USA.

*Poster presentation*

# The biological control of tutsan (*Hypericum androsaemum*) in New Zealand

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Tutsan (*Hypericum androsaemum* L.) is native to parts of Europe and Asia, northwest Africa and Caucasia. It was first recorded in New Zealand in 1870 and was believed to have been introduced as an ornamental garden plant. A biological control program against tutsan was begun in 2007. This study found two potential biocontrol agents, a foliage-feeding Chrysomelid beetle (*Chrysolina abchasica* [Weise]; Coleoptera: Chrysomelidae) and a leaf-, stem- and seed-feeding moth (*Lathronympha strigana* [Fabricius]; Lepidoptera: Tortricidae, Olethreutinae) in Georgia, Eurasia. Host testing showed the moth to be specific to tutsan while the beetle showed some attack on two New Zealand native species of *Hypericum*; however, the risk score (Paynter et al., 2015, Biological Control 80: 133–142) was very low, and approval to release both agents was given in 2016. Field releases of both agents have been made at several sites in central North Island of New Zealand plus at Lincoln, Canterbury, South Island. To date the beetle has been recovered from the Lincoln site only. This poster reported the host-testing results, rearing methods, field releases and post-release monitoring of both agents.

*Poster presentation*

# Do augmentative releases of the pompom rust fungus *Puccinia eupatorii* optimally impact pompom weed in South Africa?

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Despite its wide distribution throughout its invaded range in South Africa, naturally occurring field infections of the pompom rust fungus, *Puccinia eupatorii* Dietel, have had little or no effect on pompom weed. The aim of the project was to determine the optimal inoculation time and the effect of inoculation month on disease incidence, leaf severity and proportion of leaves infected. Plant mortality, flower head production and intraspecific competition were investigated. Three sites were selected in the Gauteng, Mpumalanga and Limpopo provinces. Two blocks, consisting of three rows of six 0.5 x 1 m plots each, were set up per site. Each plot was inoculated during a different month (November–March) and each row included repeat-inoculated and naturally-infected (control) plots. Each plot was inoculated with 20 g of a 1:200 mixture of *P. eupatorii* urediniospores in talc. Plant density was recorded in each plot prior to each inoculation. Disease incidence and severity were measured every four to six weeks after the first pustules emerged. All other measurements were taken when the plants started to die back. Over all parameters tested, significant differences were observed between the years and sites assessed. There was a significant difference between inoculation times at the first assessment date for disease incidence. Thereafter, the inoculation month had no effect. Disease severity (measured as the percentage of leaves infected) varied between year, site and assessment date, with inoculation month having little or no significant effect. Disease severity classes varied with inoculation month having either no or significant effect. The repeat inoculated plots differed significantly in the number of flower heads and invasive alien plants within 20 $m^2$ (intraspecific competition) compared to the plots inoculated monthly and natural infection. The effects of inoculation month were the same or similar on pompom weed as natural infections of *P. eupatorii*.

*Poster presentation*

# Biological control of *Cirsium arvense* using *Puccinia punctiformis* as a statewide program in Colorado

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The pathogen *Puccinia punctiformis* (Strauss) Röhl. is an obligate systemic rust fungus on Canada thistle, *Cirsium arvense* (L.) Scop. (Asteraceae). The fungus enters the root system in the late summer and fall when teliospores that form on senescing leaves land and germinate on the rosettes that form late in the season. Once in the root system, the fungus spreads and eventually kills the plant. The ability of this biological control agent to kill Canada thistle makes it highly promising as a control agent. The rust was suggested as a biological control pathogen as far back as 1893; however, only recently has the necessary research been conducted, by Dana Berner and his colleagues, to utilize the rust to control *C. arvense*. The first statewide biological control program using *P. punctiformis* in the USA was implemented in Colorado in 2014. Permanent monitoring transects ($n$ = 115) were established at *C. arvense* infestations in 57 of the 64 counties across Colorado. In the fall, each transect received approximately 22 grams of inoculum consisting of dried ground teliospore-bearing leaves. *Cirsium arvense* stem density and occurrence of systemically infected shoots were recorded annually from 2014 to 2017. We found *P. punctiformis* could be readily established at previously uninfected sites and was likely a causal factor in subsequent patch decline. At some of our study sites, *C. arvense* stem densities declined by more than 90%. We also noted, at sites where *P. punctiformis* was present before our treatment, infection had caused 50% decline in *C. arvense* stem density and continued to reduce patch density over the course of our study.

*Poster presentation*

# The biological control of *Azolla filiculoides* in South Africa: the final curtain

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*Azolla filiculoides* Lam. is a floating aquatic fern that was introduced to South Africa in 1948 and by 1990 had infested over 300 water bodies and impacted water utilization and aquatic biodiversity. The frond-feeding weevil, *Stenoplemus rufinasus* Gyllenhal, was released against this weed in 1997. The weevil was released at 112 sites throughout South Africa and rapidly dispersed to all sites of *A. filiculoides*. Since 1997, annual quantitative post-release evaluation has been carried out at 105 sites . These surveys revealed that the weevil caused a dramatic reduction in the populations of the weed, with local extinctions occurring at 100 of the sites within a year. Over the last 20 years, the weed has only reoccurred at five of the original sites. These re-infestations did not reach the levels recorded prior to 1997 and were brought under control by the weevil. *Azolla filiculoides* no longer poses a threat to aquatic ecosystems in South Africa and is considered to be under complete control. Following this sustained suppression of the weed, an application was made in 2018 to the environmental authorities to de-list *Azolla filiculoides* as an invasive species in South Africa.

*Poster presentation*

# Leafy spurge (*Euphorbia esula*) control and soil seed bank composition 19 years after release of *Aphthona* spp. biological control agents

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Leafy spurge (*Euphorbia esula* L.) was considered a prime candidate for biological control in the USA in the 1980s because the deep-rooted perennial weed had invaded thousands of hectares of land in a variety of habitats such as grasslands, woodlands, riparian areas and waterways. This invasive weed outcompeted native species and had become a near monoculture in rangelands and wildlands within the northern Great Plains region of North America.

*Aphthona nigriscutis* Foudras and *A. lacertosa* Rosenhauer were introduced into the USA in the late-1980s for biological control of leafy spurge. Significant reductions of leafy spurge root biomass often occurred within two to three years of release, but recovery of native species was slow. The purpose of this study was to evaluate changes in the leafy spurge density and soil seed bank composition 19 years following the initiation of a leafy spurge biological control program with *Aphthona* spp.

The study was established in 1999 in the Little Missouri National Grasslands (LMNG) within the Badlands region in southwestern North Dakota where the landscape is rolling with many gullies, ravines and prominent rugged buttes located. The study was conducted over a 520 ha area on two ecological sites within the LMNG: valley (loamy overflow) and ridgelines (loamy). There were 12 sampling sites established in each ecological site (24 total). Criteria used to select the plots within each site were leafy spurge density (stems per $m^2$) with four levels within each ecological site (uninfested [0], light [45-90], moderate [80-130], heavy [180-220]) and with lack of *Aphthona* spp. within 2 km.

Each sampling site was 255 $m^2$ in size and separated into eight equal transects radiating clockwise from a center stake at 45° angles. A mixture of 3,000 *Aphthona lacertosa*/*A. czwalinae* (Weise) and 3,000 *A. nigriscutis* were released at each site for leafy spurge control in June and July 1999. Leafy spurge stem density was determined by counting the number of stems in four 0.25 $m^2$ quadrats at 1-m intervals along the transects on the cardinal directions. Soil cores were collected at 1-m intervals along five randomly selected transects with a 10-cm diameter golf-cup cutter to a depth of 5 cm. Seed bank analysis was conducted by seed germination methods outlined by Ter Heerdt et al. (1996). Samples were washed to remove debris and unwanted material. The resultant soil slurry was poured onto a soil/sand base in greenhouse trays and were grown in greenhouse conditions between 20 and 28°C for 24 weeks.

Seedlings were identified, removed and then grouped into one of seven categories including major invasives, high- and low-seral forbs, high- and low-seral grasses, hydric/mesic species, and unknown species. There were three replicates of each leafy spurge category per soil type (24 sites total). Data were analyzed as a completely random design utilizing the General Linear Models procedure of SAS.

*Aphthona* spp. reduced leafy spurge stem density to less than 5% of the original stand within five years of release and maintained greater than 90% control in both loamy overflow and loamy sites for 19 years (Figure 1). Leafy spurge stem density was reduced from as much as 224 stems per $m^2$ in high density overflow sites (Cline et al., 2008) to less than 10 per $m^2$ . This reduction was maintained from 2004 to 2014 with no additional control inputs. Such high-level sustained reduction of leafy spurge would not be possible with herbicides due to both cost constraints and environmental impacts (Lym, 1998).

Similarly, leafy spurge seed in the soil seed bank decreased from nearly 67% in 1999 to 2% in 2014 in

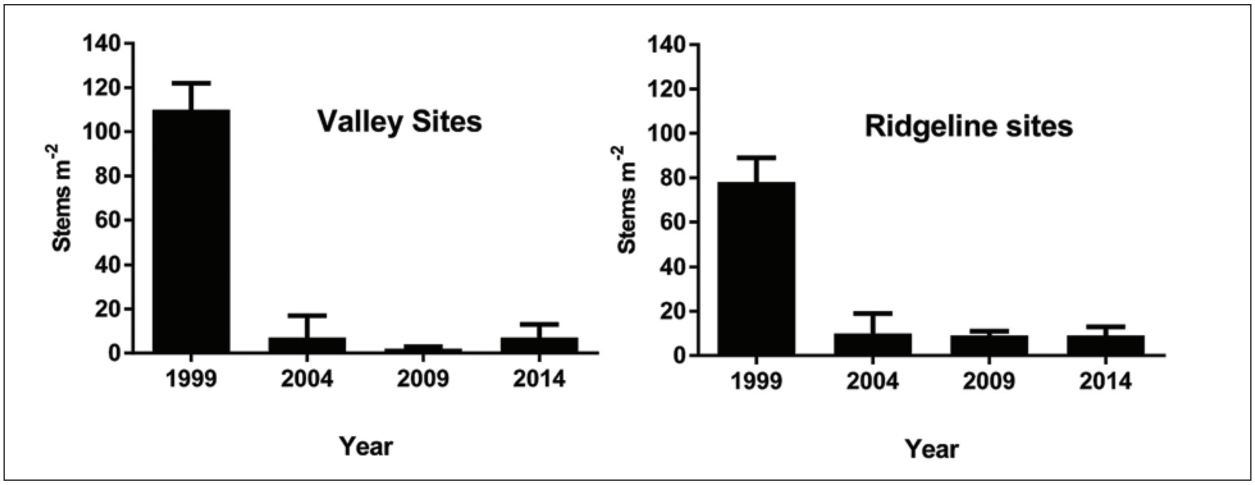


**Figure 1.** Change in leafy spurge stem density following release of *Aphthona* spp. biological control agents in 1999. Suppression was reevaluated thereafter every 5 years until 2014 and again in 2018 in valley and ridgeline sites in the Little Missouri National Grasslands in southwestern North Dakota.

the valley sites and from 70% to 6% in the ridgeline sites (data not shown, see: Thilmony and Lym, 2017). The total number of native species (grasses and forbs) increased from 32 to 45 in valley sites and from 31 to 65 in the ridgeline sites 15 years after *Aphthona* were released. In general, an increase in desirable species abundance in the seed bank indicated a trend towards a more natural and higher quality plant community. However, less desirable forb species doubled in both ecological sites during the 19-year study.

Reduction of leafy spurge infestations made resources that had been used by the invasive weed available for enhanced growth of other individuals already within the community. Species richness doubled in both sites from 1999 to 2014. Based on these and other similar studies, the ability of native species to re-establish once an invasive species is reduced likely will depend on site history, conditions, abundance of desirable natives in the area and propagules in the seed bank. Reseeding of native species following the release of flea beetles could help to restore the native community by allowing a more rapid reintroduction of desirable species into the seed bank and inhibiting invasion by non-desirable species such as Kentucky bluegrass (*Poa pratensis* L.).

Biological control of leafy spurge with *Aphthona* spp. has successfully managed infestations in the LMNG in southwestern North Dakota for the past 19 years. The flea beetles reduced leafy spurge in areas of high infestation levels with as much vigor and success as areas with low levels of infestation, in addition to dispersing to and establishing at sites where flea beetles were never released. Once the *Aphthona* spp. established, leafy spurge infestations were successfully managed without additional costs or use of other control methods such as herbicides. The successful control of leafy spurge with *Aphthona* spp. flea beetles in the LMNG for the last 19 years could not have been achieved by any other single control method. *Aphthona* spp. were both effective and an economically-feasible option for leafy spurge control across large areas and in a variety of terrain in this and similar studies.

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*Poster presentation*

# Analyses of citizen-based monitoring of spotted knapweed biological control over nine years in Idaho, USA

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Spotted knapweed (*Centaurea stoebe* [L.] sens. lat.) has been a target for biological control in North America since the 1960s. Among several biocontrol agents, the root-mining weevil *Cyphocleonus achates* (Fåhraeus) was first released in the USA in 1988, and the almost morphologically identical seed-feeding weevils *Larinus minutus* Gyllenhal and *L. obtusus* Gyllenhal were first released in the USA in 1991 and 1992, respectively. Since 2007, a citizen-based monitoring program in Idaho, USA has supplemented data collection from 39 field sites to help evaluate the impact of *C. achates* and *Larinus* species on spotted knapweed abundance and assess changes in the surrounding plant community. Here, we analyzed trends in spotted knapweed, root-mining and seed-feeding weevils, and the plant community abundance following biocontrol agent releases at the regional and site level across Idaho. Over the nine-year period between 2007 and 2016, the stem density of *C. stoebe* decreased by 25%, while the number of *C. stoebe* plants decreased by 19%. At the same time, the population of the root-mining weevil *C. achates* showed nearly a 54-fold increase, whereas the populations of the seed-feeding weevils *L. minutus* and *L. obtusus* declined by 90%. This poster discussed implications of the differing trends in root-mining and seed-feeding biological control agent abundance for the management of *C. stoebe*.

*Poster presentation*

# Attempts to establish *Dichrorampha odorata* on *Chromolaena odorata* in South Africa

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The biotype of *Chromolaena odorata* (L.) R.M.King & H.Rob. (Asteraceae) invading southern Africa ("SA biotype") differs from that invading other parts of the Old World. The origin of the SA biotype remained unknown for a decade after the biocontrol program was initiated in South Africa in 1988, resulting in problems of compatibility between candidate biocontrol agents collected from other forms of *C. odorata* and the SA biotype. Since 1997, genetic and morphological studies have provided strong evidence that the SA biotype originates in the Greater Antilles, most probably Jamaica (Zachariades et al., 2011; Paterson and Zachariades, 2013; Shao et al., 2018).

*Dichrorampha odorata* J.W. Brown & Zachariades (Lepidoptera: Tortricidae) moths lay flimsy scale-like eggs singly on the upper leaf surface, close to actively growing vegetative shoot tips of *C. odorata*. Larvae tunnel into the shoot tips for about 20 mm, killing the tip. Prior to pupation, the larva emerges from the shoot tip and pupates in a leaf roll close by (Brown & Zachariades, 2007).

The moth was considered to be a desirable agent due to the type of damage it inflicts, because *C. odorata* achieves competitiveness through the rapid growth of its terminal shoots. A culture of *D. odorata* was collected in Jamaica and imported into South African quarantine in 2005. The insect proved easy to rear in the laboratory, was fairly prolific with a short (six-week) life cycle, and was highly host specific (Dube et al., 2017). A laboratory impact trial conducted over eight months indicated that the insect affected some measured growth parameters negatively, and that a 50% shoot-infestation rate often caused as much damage as 100% (N. Dube, unpubl.).

In 2013, permission was granted for the release of *D. odorata* as a biocontrol agent by the South African authorities. To date, over 20,000 insects (92% as pupae) have been released at 17 sites in the three provinces of South Africa in which *C. odorata* is most widespread (KwaZulu-Natal, Mpumalanga, Limpopo), and releases are ongoing. Although there was some persistence at several of the sites for some months after releases were terminated, the moth disappeared thereafter and there are apparently no established sites. There are a number of possible reasons for the lack of establishment (Table 1). Some of these have been controlled or tested for. It appears likely that field temperatures and humidities may play an important role in poor establishment of *D. odorata*. A thermal biology trial currently being completed (S.B. Nqayi, unpubl.) indicates poor mating and/or slow development at the lower temperatures frequently encountered in the field in South Africa, although the pre-release cooling regime applied (Table 1) widened thermal tolerances. In addition, high mortality of the flimsy eggs has been noted at times and possibly corresponds to low humidity, also quite frequently encountered in the field in South Africa. In addition, high levels of field predation of larvae and pupae have been observed.

**Table 1.** Possible reasons for lack of field-establishment of *Dichrorampha odorata* on *Chromolaena odorata* in South Africa.

| Possible problem | Potential solution | Status |
|---|---|---|
| Poor release techniques. | Test success of these in terms of adult eclosion (when pupae released). | High adult eclosion rates from release containers. |
| Compatibility between insect and host-plant. | Collect insect from genetically similar plant population. | Done (collected from Jamaica). |
| High levels of larval and pupal predation observed at release sites. | Increase number and duration of releases per site. | Done as far as possible (e.g., almost 7,000 insects released at one site on 28 occasions over >1 year). |
| Biology of insect (Allée effects etc.). Lepidoptera often have complex mating biology and are difficult to establish. | Release into field cages (reduce dispersal out and predation/ parasitism in). | Done to some extent (three sites). Plant pest build-up was a problem. |
| Poor matching of climate between Jamaica and South Africa (Robertson et al., 2008). Eggs appear vulnerable to low humidity; laboratory culture declined at lower temperatures; field population appeared to perform better under hot, humid conditions. | Conduct releases in warm areas with high humidity. | Done as far as possible. Sites along KZN coast, wetter areas. Currently releasing at site with high moisture. |
| | Raise insects at lower laboratory temperatures. | Done to small extent. Have to balance reducing temperature with maintaining culture. |
| | Apply cooling regime to insects before release. | Being applied to pupae: 15/25°C for 22:2 hour regime for 7 days. Temperature reduced to 10°C for 2 hours prior to release (J. Terblanche, pers. comm.). |
| | Test using laboratory thermal trials. | Current studies. |
| | Test using laboratory humidity trials. | Not yet done. |
| | Obtain fresh culture from more climatically similar area (e.g., Cuba, high-altitude areas of Jamaica). | Not yet done. |
| Culture has become laboratory-adapted since 2005. | Obtain fresh culture from Jamaica, release quickly. | Not yet done. |

In conclusion, *D. odorata* is host specific and fairly damaging to its host plant *C. odorata*, and the insect has additional traits that make it desirable as a biocontrol agent. However, attempts to establish it in South Africa appear to have failed thus far. The most likely explanation is poor climatic matching, but predation may also play a role.

## Acknowledgments

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*Poster presentation*

# Management of invasive *Acacia longifolia* and *A. pycnantha* in South Africa using biological control: progress thus far

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*Acacia* (Leguminosae: Mimosoideae) ranks among the worst invasive tree genera in the world (Impson et al., 2011). In South Africa, *Acacia longifolia* (Andrews) Willd. and *A. pycnantha* Benth., both of which are native to Australia (Boucher and Stirton, 1978a,b), were historically regarded as being among the worst alien invaders (Boucher and Stirton, 1978b; Dennill and Donnelly, 1991). Since the 1970s, the utilization of biological control as a sustainable and long-term management tool for these alien tree weed species has been the primary focus of research (Dennill and Donnelly, 1991; Impson et al., 2011). The univoltine and parthogenetic bud-galling wasp *Trichilogaster acaciaelongifoliae* (Frogatt) (Dennill, 1988; Dennill and Donnelly, 1991) became the first biocontrol agent to be used against an alien acacia in the country when it was released in 1982 and 1983 (Dennill and Donnelly, 1991). Another species, *T. signiventris* (Girault), was released on *A. pycnantha* in 1987. Two seed-feeding weevils, *Melanterius ventralis* Lea and *M. maculatus* Lea, were subsequently released on *A. longifolia* in 1985 (Dennill and Donnelly, 1991) and *A. pycnantha* in 2005 (Impson et al., 2011) to supplement the two pioneering bud-galling wasps.

Both *Trichilogaster* species, as well as both *Melanterius* species, have similar biologies (Dennill and Donnelly, 1991; Impson et al., 2011). The wasp species lay eggs in buds that are destined to become inflorescences or branches, thereby producing galls instead (Dennill and Donnelly, 1991; Impson et al., 2011). Galling of the reproductive buds completely precludes the development of the would-be inflorescence, thus reducing the reproductive potential of the weed (Dennill and Donnelly, 1991; Dennill and Gordon, 1991; Impson et al., 2011). Galling of vegetative buds severely suppresses shoot growth, and eventually causes phyllode abscission and shoot die-back on infested branches (Dennill, 1988; Dennill and Gordon, 1990; Impson et al., 2011). Seed reduction by *Trichilogaster* is estimated at 85–100% (Dennill, 1988; Dennill and Gordon, 1990; Dennill and Donnelly, 1991). The adults and larvae of the weevil species feed on the green, developing seeds of their hosts and, despite reductions in pod production due to the activity of the bud-galling wasps, their efficacy is not affected, as they efficiently locate pods even at low densities (Dennill and Donnelly, 1991). The levels of seed damage attributable to the weevils are estimated to be approximately 57–95 % depending on the site (Dennill and Donnelly, 1991; Impson et al., 2011).

The galling and seed-feeding insect agents used for biocontrol of these acacias only curtail their capacity to reproduce (Dennill and Donnelly, 1991). This compromise ensures that management efforts do not interfere with the beneficial and economically exploitable attributes of these alien tree species (Impson et al., 2011). However, the apparent inadequacy to affect immediate control and reduction in parent plant densities has created perceptions that biological control has been unsuccessful (Impson et al., 2011). This post-release evaluation study (2013–2018) aimed to assess the progress of biocontrol thus far in the management of these alien acacias. It was hypothesized that though seemingly persistent in riparian habitats and on mountain slopes, as well as the substantial regeneration of infestations observed after fire events, these species are no longer a significant threat due to the long-term presence of biological control, which has significantly curtailed their ability to reproduce and, consequently, any further encroachment and range expansion. Historical data shows that in the case of *A. longifolia*, average annual seed rain was in the region of 7,646 seeds/m$^2$ (Milton, 1980), with soil seed bank densities of up to 45,800 seeds/m$^2$ (Holmes et al., 1987). Current data shows variability both between the six sites investigated and across years (2013–2018) with an average annual seed production of 235 seeds/m$^2$ (inter-site and season range 5–753 seeds/m$^2$). Soil seed banks averaged 18 and 500 seeds/m$^2$ in mountain and riparian sites, respectively, indicating a significant reduction in seed production of existing infestations due to biocontrol. While no historical data could be found for *A. pycnantha*, current data shows that seed rain also varies between sites and across years averaging 1,655 seeds/m$^2$ (inter-site and season range 31–8 161 seeds/m$^2$). *Acacia pycnantha* current soil seed bank densities averaged 271 seeds/m$^2$ to 2,645 seeds/m$^2$ (inter-site range). The results from the current assessments conducted on *A. longifolia*, when compared with historical data, show that the seed-reducing biological control agents are curtailing reproduction and playing a prominent role in containing the encroachment of *A. longifolia*.

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*Poster presentation*

# Current assessment of weed biological control projects and their agents in California

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The intentional use of a live organism for the biological control of a weed in North America began in California in 1940 with the use of a native scale insect collected from mainland populations and released onto native *Opuntia* spp. on Santa Cruz Island, 40 km offshore. Since then, 77 organisms have been released on 39 weed species in California. Perennial species make up more than half of the weed targets; the remainder was evenly divided between annual and biennial species. About half of weed targets (47%) infested rangelands, 25% infested natural areas, 15% occurred in managed areas of production agriculture, and 13% occurred in aquatic and riparian habitats. Published literature, government and university reports and unpublished quarantine records were examined, and a complete list of weed species subject to biological control releases and their agents was compiled. The list of weed targets consists of two kinds of projects: transfer experiments ($n$ = 11) where previously-released agents from related weed hosts were released on a new host, and classical biological control projects ($n$ = 28) where agents were obtained from the target weed's area of origin.

An assessment of the level of control for the older classical biological control projects ($n$ = 19), where sufficient time has elapsed, suggests that 42% of weed targets were completely or partially successful. Agent establishment rate was 82%, an amount comparable with projects in Australia, South Africa and New Zealand, but a control success rate of less than 50% is substantially lower than the other regions. An assessment of the ability of each established agent to control its host plant suggests that 54% of agents provide some level of control, but only 24% of established agents are considered effective, a rate similar to that reported in other project reviews. The climate of California is Mediterranean with cool, wet winters and hot, dry summers. Areas with a Mediterranean climate are rare worldwide. An examination of weed biological control projects in California provides a unique opportunity to examine project success in an unusual climate.

*Poster presentation*

# Development of a predicted suitable habitat model for biocontrol systems in Montana

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A major hurdle in a biological control of weeds program is the introduction and establishment of viable populations of biological control agents in a region, locality or site. Site selection is one of the most important choices we can make at the beginning of a biological control program. The majority of information we have on best-suited sites for successful weed management with biocontrol are based on anecdotal evidence. Most people assume that if the target weed is present, the agent can be released, but many other factors can negatively impact success. It is often difficult to identify and quantify reasons why biological control agents fail to establish. Factors such as soil, slope, aspect, shade or moisture could potentially all have an influence on successful establishment. The development of a predicted suitable habitat model allows us to determine the ideal site characteristics associated with successful establishment. This model helps to increase the efficacy of biocontrol by guiding land managers' releases of the insects to sites where they will most likely survive, establish and assist in the management of the target weed species. In the long term, this model will also increase the ecological and economic return for Montana. When insects are released in suitable habitat, they are more likely to reduce the weed infestation and build up their populations to the point that they can be collected and moved to new locations, or they will disperse themselves to nearby infestations. When managed properly, established populations of biocontrol agents are a renewable resource that can be utilized for cost-effective control of their target weed.

*Poster presentation*

# A tool to support learning about the success of biological control agent introductions

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The early stages of biological control agent introduction programs are often characterized by uncertainty as to the optimal conditions for establishment. Even when establishment succeeds, the spread of introduced agents across the landscape can be slow, and their beneficial impacts delayed, if release conditions are suboptimal. Experimental field releases provide valuable information about the conditions that favor establishment, and are especially useful when biological control agents are abundant or easy to rear and establishment is detectable within one or a few years. However, for many agents there is a long time frame needed to detect establishment. When biological control agents have a low rate of detection, short-term studies could underestimate the rate of establishment success. Understanding the key drivers underpinning establishment success can be achieved through capturing existing data into a quantitative process model, allowing managers to explore the likelihood of establishment in different contexts. In our case study, we present a process model, in this case a Bayesian network, for the introduction of gorse soft shoot moth *Agonopterix umbellana* (Fabricius) to Australia. We outline the development of the Bayesian network, and demonstrate its advantages and potential utility using typical introduction scenarios. We propose methods to elicit and evaluate competing models. Finally, we assess the suitability of Bayesian networks as part of an adaptive management approach to biological control agent introduction.

*Poster presentation*

# Impact of *Pareuchaetes insulata* (Lepidoptera: Erebidae) on *Chromolaena odorata* (Asteraceae) in South Africa: a case study on secondary metabolites

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The biotype of the shrub *Chromolaena odorata* (L.) R.M.King & H.Rob. invading South Africa is dissimilar from that invading other parts of the world, and has its origin traced to the Caribbean Islands. Biological control of this plant in South Africa was initiated in the 1980s. An erebid moth with defoliating larvae, *Pareuchaetes insulata* (Walker), was established at one site in southern KwaZulu-Natal province in the early 2000s and has spread northwards into Mpumalanga province and the neighboring countries of Swaziland and Mozambique, but has not yet reached Limpopo province. We studied plant response to *P. insulata* herbivory by determining secondary metabolites as primary plant defense at three sites: the original release site, where the moth has been present for 13 years; a site in Mpumalanga where it has been present since 2016 and a site in Limpopo which it has not yet reached. Thin-layer chromatography indicated presence of pyrrolizidine alkaloids in the roots of *C. odorata* in all sites and gas chromatography-mass spectrometry (GC-MS) confirmed the presence of intermidine and rinderine in one of the sites, also recorded in the Asian/West African biotype. Further GC-MS analysis revealed the presence of indolizine, a notorious secondary compound incriminated with allelopathic properties in plants. Although the Asian/West African biotype is genetically dissimilar to the southern African biotype, the two seem to possess analogous chemical compounds. A shift of allocation of resources to growth-enhancing invasiveness of alien plants in the introductory country means preservation of defense resources for an appropriate time.

*Poster presentation*

# The influence of low-density populations and Allee effects on biocontrol agents

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Successful establishment of a newly introduced biocontrol agent is one of the most critical phases in any weed biocontrol project, and despite intense efforts, there is a high fail rate in establishment success. During this phase, population dynamics of agents are strongly influenced by stochastic processes and Allee effects, both of which may lead to extinction of low-density populations. Allee effects occur when a decrease in the fitness of individuals is generated by a decrease of either their density or their numbers. In many cases initial populations of biocontrol agents are relatively small, due to constraints such as rearing difficulties and limited resources, and these small populations may be subjected to Allee effects and fail to establish and spread. The Allee effect has strong theoretical underpinnings, but robust empirical evidence that Allee effects operate in introduced populations is scarce. We studied the release of *Neoloma ogloblini* (Monros), a chrysomelid leaf-feeding beetle, introduced into New Zealand to control *Tradescantia fluminensis* Vell., a shade-tolerant ground-smothering plant. This provided an opportunity to study how initial population size influences establishment success and the occurrence of Allee effects. At the beginning of the 2017/2018 austral summer, we manipulated initial sizes of populations of the beetle and followed population growth and development of life stages over a five-month period. Greater establishment occurred at release sites with higher initial population size, suggesting that the beetle populations are influenced by an Allee effect. Observations and additional experiments suggest a strong role for a predator-driven Allee effect. As the releases of biocontrol agents are essentially planned biological invasions, this project contributes to defining the role of Allee effects in the establishment phase of not only biocontrol agents but also invasive pest species. Allee effects may, therefore, be of critical importance to understanding why some insect species more readily establish and spread than others.

*Poster presentation*

# The impact of pre-release testing for pathogens in weed biocontrol agents: evidence from New Zealand

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Pre-release screening of weed biocontrol agents for pathogens became mandatory in New Zealand in 1984. Agents released prior to this date may have hosted undetected pathogens. Field populations of these agents today might be expected to exhibit a greater pathogen incidence, impacting their success. Conversely, it might be predicted agents released since mandatory pathogen screening will be more successful due to the presence of fewer pathogens. Twenty-five invertebrate biocontrol agents from eight target pest plant species across New Zealand were screened for microorganisms. Agents were representative of releases made before and after mandatory pathogen screening began. Pathogen occurrence was low in both these groups, suggesting unscreened agents were likely pathogen-free on release. Numbers of micro-organisms found varied significantly between species collected and regions of collection. Presence of pathogens did not appear to impede the success of some biocontrol agents.

*Poster presentation*

# A decade in biocontrol monitoring and release: a South African case study

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The Natural Resource Management programs (NRM) of the Department of Environmental Affairs (DEA) has funded research into biological control programs over the past two decades. The biocontrol implementation program was initiated in earnest in the year 2000 when the first implementation staff were employed by the DEA. In the beginning the implementers reared, released and monitored the biocontrol agents themselves. The program has increased over the years and developed into a more sustainable strategy where research, mass rearing, releases and large-scale monitoring is possible. The proposed emphasis should be on a "cradle to grave" strategy where the research and implementation of a biocontrol agent is seen to function as a unit and not as separate entities. Research goals and successes should be fed back into implementation and feedback on corrective actions given. Research priorities should be identified with implementation input and monitoring programs identified for established agents.

*Poster presentation*

# Suppression of the invasive aquatic weed *Hydrilla verticillata* by the "Asian hydrilla moth" *Parapoynx diminutalis* in South Africa

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A classical biological control program for the invasive aquatic weed, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), was initiated in South Africa following the discovery of extensive mats in one of the largest reservoirs in the country in 2006. However, in mid-2008, high densities of a leaf-cutting insect, *Parapoynx diminutalis* Snellen (Lepidoptera: Crambidae) were discovered on the *H. verticillata* infestation, causing high levels of defoliation and die-back of the weed. The demand for classical biological control agents for release against *H. verticillata* was therefore brought into question. In order to quantify the impact of the moth and to assess the long-term threat of *H. verticillata* to South African water bodies, a monitoring program was initiated in Jozini Dam and the Phongolo River in 2013 and 2016, respectively. Although *P. diminutalis* did not demonstrate a preference for *H. verticillata* over native aquatic plants, moth populations followed a cyclical pattern of abundance, with rapid population increases coinciding with increases in the abundance of *H. verticillata*. High damage levels to *H. verticillata* were associated with high densities of immature *P. diminutalis*, which consistently led to population crashes of *H. verticillata*. The ability of *P. diminutalis* to peak rapidly following the temporary recovery of *H. verticillata* populations has led to a dramatic reduction in the size and extent of the *H. verticillata* infestations. *Parapoynx diminutalis* appears to have great potential to contribute to the management of *H. verticillata* in South Africa; however, the moth's oligophagous habits will likely preclude its intentional introduction into water bodies with *H. verticillata* where it does not already occur.

*Poster presentation*

# Evaluating establishment and impact of four biological control agents on *Parthenium hysterophorus* in South Africa

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*Parthenium hysterophorus* L. (Asteraceae: Heliantheae; parthenium) is a serious terrestrial invader globally, negatively impacting crop and animal production, biodiversity conservation and human and animal health in Australia, Asia and Africa. Biological control is crucial to curb its aggressive spread and impact. Following success in Australia, biological control on parthenium began in South Africa in 2003. Technology was transferred subsequently to East Africa through an international collaborative program. In South Africa, the winter rust fungus *Puccinia abrupta* var. *partheniicola* (H.S. Jacks.) Parmelee is present, although not deliberately introduced. The summer rust fungus *Puccinia xanthii* var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero (Pucciniales: Pucciniaceae) and three insect agents (defoliating *Zygogramma bicolorata* Pallister [Coleoptera: Chrysomelidae], stem-boring *Listronotus setosipennis* [Hustache] [Coleoptera: Curculionidae] and seed-feeding *Smicronyx lutulentus* Dietz [Coleoptera: Curculionidae]) were evaluated and released in South Africa from 2010, 2013 and 2015 onwards, respectively. More than 35,000 *L. setosipennis*, 50,000 *Z. bicolorata* and 35,000 *S. lutulentus* have been released in densely-invaded regions, with ongoing efforts. All four agents have established, despite intervening drought conditions. *Puccinia xanthii* has dispersed beyond 50 km in some cases, largely unaided. *Listronotus* established readily at more than 50% of release sites but has dispersed slowly, while *Z. bicolorata* establishment is limited. *Smicronyx* is well established at a few sites. Evaluation of agent impact is underway, assessing the dynamics of parthenium, other vegetation, and four biocontrol agents using chemical (insecticide and fungicide) exclusion in a replicated four-treatment field study. Although the established agents have exerted some level of control in South Africa, additional agents are required to improve the extent of control. Therefore, the root-crown borer *Carmenta* sp. nr. *ithacae* (Lepidoptera: Sesiidae) and the stem-galler *Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae) are being investigated in quarantine. Although host-range complications arose during laboratory testing of *E. strenuana*, it is desirable to further investigate this agent due to its considerable impact. Efforts to expand and intensify biological control efforts in Africa are essential to manage this weed.

*Poster presentation*

# An overview of a success story of biological control of *Opuntia stricta* (balas) in Jezan, Saudi Arabia

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In 2006, farmers in the Jezan area and the Asir region complained about the invasion of *Opuntia stricta* (Haw.) Haw., known locally as balas. The Ministry of Environment, Water and Agriculture (MEWA) initiated a mission between October 2009 and 2013. The recommendations were that biological control offered the best and most cost-effective solution, in view of previous successes in South Africa and Australia. An application for the release from quarantine of the biocontrol agent *Dactylopius opuntiae* (Cockerell) (biotype "stricta"), commonly known as cochineal, was proposed. The biocontrol agent was transferred to the Jezan area under quarantine conditions, and a number of experiments were conducted. Mass-rearing of the cochineal was adapted to Saudi Arabia conditions. Some of these experiments focused on host choice and macro- and micro-environmental adaptations. In addition, training was provided to the Jezan facility personnel on many aspects such as evaluation, release processes and rearing of the cochineal agent. In 2010, releases of cactus leaves infested with *D. opuntiae* were started. The releases were done manually by placing the leaves on plants of *O. stricta* and allowing the cochineal to move naturally, causing the death of the invasive plants. The project was highly successful, and it can be stated that "balas" is now under control and no longer a problem or a threat. Emphasis will now shift to mass-rearing the cochineal for releases on regrowth of plants that are free of insects. This may continue for a few years. A local grass, *Cenchrus ciliaris* L., will be used to protect the soil against possible erosion. The grass will, at the same time, increase the grazing value of previously-invaded areas. An awareness and publicity campaign is planned to inform the farmers of the progress and the availability of cochineals to control regrowth.

# Session 10: Integrated weed management and restoration

**Session Chair: Heinz Müller-Schärer**

## Keynote

# Integration of biocontrol and ecohydrological assessment in restoration of riparian ecosystems invaded by *Tamarix*

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The tamarisk leaf beetle *Diorhabda* spp. (Coleoptera: Chrysomelidae) was introduced almost 20 years ago to suppress invasive *Tamarix* spp. (Tamaricaceae) in riparian ecosystems of western North America. The biocontrol program has resulted in water conservation, reduced fire risk, new food resources for insectivorous wildlife and modest recovery of some native (and non-native) vegetation. As the most widespread agent, *Diorhabda carinulata* (Desbrochers), evolved to colonize southward from release areas, the potential risk of *Tamarix* defoliation to the endangered southwestern willow flycatcher (*Empidonax traillii extimus* A. R. Phillips) led to legal actions by wildlife agencies concerned that a perceived lack of riparian rehabilitation would further endanger the bird, thus causing USDA-APHIS to terminate this successful program. In response, we have developed a strategic approach to promoting restoration of native cottonwood-willow habitat to mitigate the anticipated decline in *Tamarix* cover. Ecohydrological assessment evaluates the potential for riparian recovery, and targets restoration actions based on characterizing the ecological and hydrological factors that can facilitate sustainable recovery of this iconic habitat type. The approach has been applied in the Virgin and Gila watersheds of the southwestern USA. While the results are not immediate, the process leading to riparian restoration has brought specialists from both sides of the debate together in search of resolution via collaboration and, if successful, may allow re-initiation of the *Tamarix* biocontrol program attendant with habitat enhancement for wildlife species of conservation concern.

*Oral presentation*

# Anticipating secondary invasions: cautionary tales for ecosystem restoration

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Classical biological control and other forms of invasive plant management are undertaken because an invasive species is threatening a valued ecosystem service. Control has, however, often been treated in isolation of ecosystem restoration rather than as an integral part of it. Yet invasive plants that are the target of biocontrol can exert priority effects over other non-native plants, effectively keeping them out of an ecosystem. If a target weed has been exerting priority effects, its removal could facilitate secondary invasions (= invaders that are either facilitated or suppressed by a primary invader). As part of an effort to anticipate when secondary invasions will interfere with management objectives, we evaluated the outcome of invader removals in three Hawaiian forests spanning a habitat harshness gradient from an arid dry forest to a wet rainforest. In the former, removal of the dominant invader did not lead to secondary invasions because the environment is so harsh that few persistent invaders are present, and system response is slow enough to actively manage secondary invaders that appear. Thus, knocking out the invader is an effective tactic for restoration. Invader removal in the wet forest, by contrast, led to a high diversity of secondary invaders that scrambled for light, with wide variation in outcome among locations where the primary invaders were removed. Many of these secondary responders did not persist but some did, supporting the importance of local variation in propagule supply of all species to predicting the outcome of weed control. Primary weed removal in an intermediate-rainfall forest led to persistent secondary invaders that hijacked succession and created new substantial ecological "problems." We concluded this presentation by placing these examples into the larger context of removal experiments from around the globe and emphasized the importance of viewing species removals, or the development of biocontrol agents, within an ecosystem context.

*Oral presentation*

# Integration of mechanical topping methods to accelerate biological control of *Arundo donax*

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*Arundo donax* L., known as giant reed or carrizo cane, is native to Mediterranean Europe. Genetic studies of *A. donax* indicate it was introduced into the Rio Grande Basin of Texas from Spain. *Arundo donax* has historically dominated riparian habitats in the Rio Grande Basin where it competes for scarce water resources, lowers riparian biodiversity, reduces visibility for law enforcement and facilitates the invasion of cattle fever ticks from Mexico. Two agents, the stem-galling wasp *Tetramesa romana* Walker and the rhizome-feeding scale *Rhizaspidiotus donacis* (Leonardi), are widely established and are having significant impacts in Texas and Mexico. Releases of a third agent, the leaf-miner *Lasioptera donacis* Coutin, are in progress. Thus far, we have documented a 32% decline in above-ground biomass of *A. donax* and return of native vegetation along the Rio Grande. Economically, the reduction in carrizo cane biomass is estimated to save 6,000 acre-feet of irrigation water per year, and worth USD 4.4 million. To accelerate the decline of *A. donax*, we have integrated biological and mechanical methods. Topping (cutting) of *A. donax* at 1 m is a method to improve visibility for law enforcement and accelerate the decline of this invasive weed. Topping increases the production of side shoots that are preferred oviposition sites for the arundo wasp, *T romana.* In a study from January 2017 to February 2018 at the U.S. Fish and Wildlife Lower Rio Grande Valley National Wildlife Refuge, we found that topping increased solar penetration and significantly increased the diversity and abundance of native plant species as compared to un-topped controls. Integration of mechanical and biological control methods accelerates the decline of *A. donax* and restoration of the native riparian plant community. Methods for large-scale implementation of these methods using tractors with boom hedgers have been developed and transferred to the U.S. Border Patrol and USDA Cattle Fever Tick Eradication Program for use on the Rio Grande.

*Oral presentation*

# Restoring sites invaded by *Persicaria perfoliata*, mile-a-minute weed, by integrating management techniques

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Weed biological control programs in natural areas often strive to restore native plant communities to some pre-invasion level. Biological control alone may sometimes achieve this goal, but it may also be necessary to integrate biological control with other weed management techniques. Restoration challenges increase if reduction of the target weed results in replacement by another, the "invasive treadmill effect." *Persicaria perfoliata* (L.) H. Gross (Polygonaceae), mile-a-minute weed, is an aggressive annual vine from Asia that has invaded the eastern United States. In some sites, *P. perfoliata* has been successfully controlled by the biological control weevil *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), but it has been replaced by other invasive weeds. We integrated releases of the weevil with a single application of a pre-emergent herbicide and plantings of a native forb and tree in a split-plot experiment. Mile-a-minute seedling counts were significantly lower in the herbicide plots after one-time treatment in 2009, but also in three annual counts from 2010–2012. After two years, native plant cover was greater than 80% in the integrated treatment plots. In 2015, mile-a-minute seedling numbers did not differ between the herbicide and no-herbicide plots and were low across all sites. Six years post-treatment one site does not need additional management, mile-a-minute cover has increased at a second site, and a third site has very high cover of the replacement weed Japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus (Poaceae). Integrating management techniques reduced the abundance of the target weed, promoted recruitment of additional native plant species not included in the experimental plantings, and at least temporarily prevented dominance by other invasive plants compared to non-planted and unsprayed control plots. Sites will vary from each other and over time, and adaptive management strategies will be required to achieve restoration goals.

*Oral presentation*

# Postmortem impacts of *Melaleuca quinquenervia* on the Everglades landscape

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A six-year field study in the western Everglades region of Florida examined the role of woody debris from *Melaleuca quinquenervia* (Cav.) S. T. Blake on invertebrate richness, abundance and diversity, as well as mass loss, resource quality and nutrient fluxes and pools. Designated samples of decomposing coarse woody debris (CWD) and fine woody debris (FWD) from *M. quinquenervia* and a native tree *Pinus elliottii* Engelm., were removed from the field every six months to determine mass loss, percent moisture, nutrient content, and invertebrate richness, abundance and diversity. All invertebrates were identified first to order and then further separated using morphologically-based recognizable taxonomic units. A total of 61,985 invertebrates from 18 orders were recovered from 520 pieces of debris. Although both tree species supported similar diversities of invertebrates, taxon richness and abundance were greater in the native *P. elliottii* compared with the exotic *M. quinquenervia*. Richness and diversity measures were influenced primarily by debris size and age and were inversely related to C:N ratios. Mass loss of *P. elliottii* CWD biomass averaged 62.6% after six years while *M. quinquenervia* averaged 39.6%. Moisture levels and C:N ratios were consistently higher with *P. elliottii*, and invertebrate diversity was inversely related to the C:N ratios of both tree species. Overall, based on abundance, CWD of *M. quinquernervia* provided a lower quality resource for decomposing communities of invertebrates which has implications for food webs in areas where the tree has been killed and left to rot. The rate of decomposition will inform integrated weed management approaches that combine biological, chemical and cultural methods.

*Oral presentation*

# Integrated control of water hyacinth in peri-urban environments, linking science to society

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Water hyacinth (*Eichhornia crassipes* [Mart.] Solms) is one of the worst aquatic weeds worldwide. In Argentina, in spite of being in its native range, there are two registered cases of invasion outside its natural distribution area. In the first one, in the dam "Los Sauces" of La Rioja province, a biological control program was successfully implemented using the weevil *Neochetina bruchi* Hustache. This was one of the first cases where biological control was used against this weed in the world. Secondly, an invasion of *E. crassipes* was recorded in Laguna del Ojo, San Vicente, Buenos Aires province. There, the weed coverage went from 1–2 ha to more than 25 ha in two years, affecting the navigability, fishing and weekend tourism, which are activities of great importance for the local community due to its proximity to Buenos Aires city. The objective of this work was to conduct an integrated biological and mechanical control plan for this aquatic weed, in collaboration with social and political stakeholders that make use of the lake. In the short period from 2015 to 2017, the positive effects of this management strategy began to be evident. This was reflected in the decrease of biomass and volume of *E. crassipes* by approximately 60%. The control was coupled with the removal of plants mechanically, clearing the water body almost entirely. By initially using biological control, there is an assumed economic saving of 40–60% for the municipality. Currently, periodic monitoring is maintained, and an interaction with the local community has been established. An agreement has been made with schools, through the government's department of education, to carry out mass-rearing of the insects *Megamelus scutellaris* Berg and *N. bruchi*, with active participation of the students, for subsequent releases in the lake. This is the first weed biological control work in South America which actively integrates a local community.

*Oral presentation*

# The future of *Solanum mauritianum* biocontrol in South Africa: prospects, problems and promise

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*Solanum mauritianum* Scop. (Solanaceae) (Figure 1a) remains one of South Africa's most widespread and long-standing invasive trees (Olckers, 2011), occupying more than 80,000 ha of land. Invasions of *S. mauritianum* typically result in dense monospecific stands which shade-out and outcompete surrounding flora. These invasions are particularly troublesome in riparian, agricultural and commercial forestry settings, where the tree reduces water availability, harbors crop pests and interferes with the growth of saplings (Olckers, 2009; Cowie et al., 2018). Given the numerous threats posed by *S. mauritianum*, South Africa initiated several control measures against the weed. Chemical and mechanical controls were initially employed against the weed but were deemed economically unfeasible and unsustainable, and control efforts were quickly focused on biocontrol (Witkowski and Garner, 2008; Olckers, 2011).

Despite biocontrol providing the only long-term solution to managing *S. mauritianum* in South Africa, the program suffered a troubled history since its inception in 1984 (Olckers, 2009). Temporary suspensions, host-specificity concerns, a high rejection rate of candidate agents and a lack of post-release funding/research all contributed to the South African government abandoning the program by 2003. Although the program was abandoned, research continued in an unofficial capacity at two South African universities (University of KwaZulu-Natal and the University of the Witwatersrand). Extensive research efforts have culminated in the eventual release and establishment of two agents, namely the sap-sucking lace-bug *Gargaphia decoris* Drake (Hemiptera: Tingidae) in 1999, and the flower bud-feeding weevil *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) in 2008 (Figure 1b,c). Unfortunately, both of these agents have failed to impact

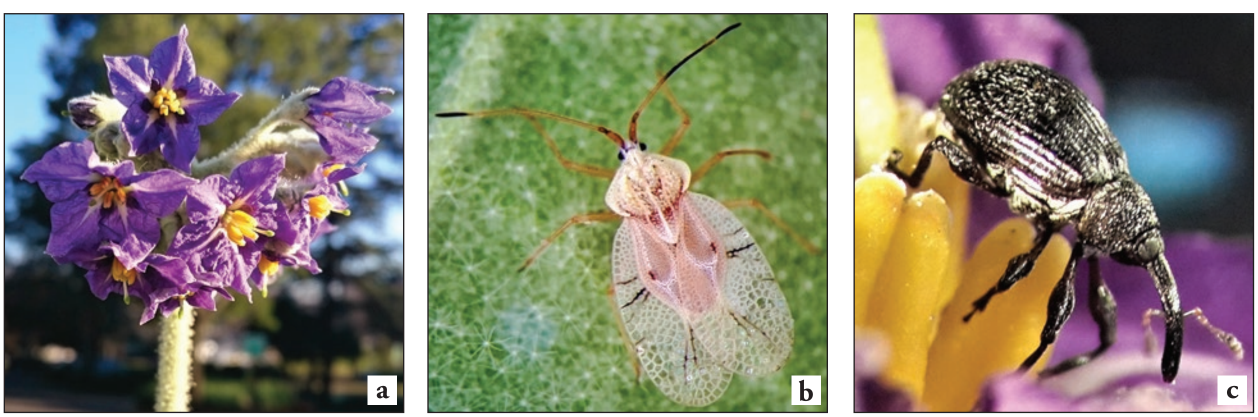


**Figure 1.** *Solanum mauritianum* and its biological control agents: (a) characteristic flowers of *S. mauritanum*; (b) the sap-sucking lace-bug *Gargaphia decoris*; (c) the flower bud-feeding weevil *Anthonomus santacruzi.*

*S. mauritianum* infestations, and as such were largely abandoned given their poor performance to date. However, ongoing post-release studies, carried out by the University of the Witwatersrand and the University of KwaZulu-Natal, have managed to renew interest and investment into the biocontrol of *S. mauritianum* (Cowie et al., 2018). It is hoped that the revival of the *S. mauritianum* biocontrol program in South Africa will be aimed at three avenues of research and implementation: (1) understanding and optimizing the current agents, (2) investigating new damaging candidates and (3) integrating management to contain and reduce the spread of *S. mauritianum*.

In terms of understanding and improving our current agents, various studies have investigated the potential constraints. The lace-bug *G. decoris* maintains the ability to be extensively damaging, under both shaded and full-sun conditions, suggesting that its outbreaks are constrained by biotic factors, such as predation (formicids) or plant quality (Cowie et al., 2016a). Potential mitigations to the negative effects of predation may be attained by changes to the lace-bug's release strategies. Fewer, larger releases (approximately 1,000) into semi-shaded areas have shown the highest likelihood of establishment (Patrick and Olckers, 2014). Furthermore, trees upon which *G. decoris* releases are made should be protected from predators (e.g., formicids) using long-lasting sticky residues, which are applied to the main stem. In contrast, the weevil *A. santacruzi* was found to be climatically constrained, particularly within high altitude areas (>1,000 m above sea level) with low winter temperatures (<4°C) and relative humidity (<50%) (Cowie et al., 2016b; Singh and Olckers, 2017). This has severely limited the weevil's effective range in South Africa. However, in the warmer coastal regions of KwaZulu-Natal, where *A. santacruzi* populations have established, the weevil may be reducing the fruit/seed set of *S. mauritianum* by up to 70%, as well as promoting the detrimental self-pollination (inbreeding) of the tree (Cowie et al., 2017; English and Olckers, 2018).

Although historical difficulties halted research, recent insights have renewed investment in biocontrol, which has seen international collaboration between South Africa and New Zealand. It is hoped that this collaboration will see new agents imported from Uruguay into South Africa in 2019 for host-specificity testing. Numerous potential candidate agents exist (Table 1), with the most promising of these including the stem-boring weevil *Conotrachelus squalidus* Boheman (Coleoptera: Curculionidae) and the stem-galling weevil *Collabismus notulatus* Boheman (Coleoptera: Curculionidae) (Olckers, 2009, 2011; Cowie et al., 2018). Additionally, researchers may want to import the seemingly hardier congeneric flower bud-feeding weevil *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) to offer seed reduction within the cooler inland areas of the country (Singh and Olckers, 2017).

The revival of *S. mauritianum* biocontrol efforts is highly commended and indeed may offer South Africa a promising future. However, host-specificity concerns surrounding native and cultivated Solanaceae is likely to increase research time and make attaining permission to release new agents more challenging. Nonetheless, the country is also still faced with the detriments of an ongoing *S. mauritianum* invasion, which needs to be addressed. In the interim, integrated guidelines have been proposed, incorporating the current biological, mechanical and chemical controls in a region-specific context (Cowie et al., 2018). Dividing the country into priority-based management zones is likely to aid in containing and reducing the spread of *S. mauritianum* in the affected areas while the crucial biocontrol research is conducted. This research advocates for the revival of *S. mauritianum* biocontrol in South Africa and discusses the potential prospects and problems for future research over the next decade.

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**Table 1.** Shortlist of candidate agents to be considered for revived biocontrol efforts against *Solanum mauritianum* in South Africa (adapted from Cowie et al., 2018).

| Species | Order: Family | Damage type | Status | Concern(s) |
|---|---|---|---|---|
| **Priority candidates** | | | | |
| *Collabismus notulatus* | Coleoptera: Curculionidae | Shoot-galler | Shelved | Untested (Agent biology) |
| *Conotrachelus squalidus* | Coleoptera: Curculionidae | Stem-borer | Shelved | Untested (Culturing issues) |
| **Reconsideration (promising)** | | | | |
| *Acallepitrix* sp. | Coleoptera: Chrysomelidae | Leaf-miner | Rejected | Host-specificity |
| *Acrolepia xylophragma* | Lepidoptera: Acrolepiidae | Leaf-miner | Rejected | Host-specificity |
| *Adesmus hemispilus* | Coleoptera: Cerambycidae | Stem-borer | Shelved | Untested (Agent behavior) |
| *Anthonomus morticinus* | Coleoptera: Curculionidae | Flower bud-feeder | Shelved | Competition / Hybridization |
| **Reconsideration (lesser priority)** | | | | |
| *Aponychus schultzi* | Acari: Tetranychidae | Leaf-sucker | Shelved | Untested (Potential crop pest) |
| *Platyphora* spp. | Coleoptera: Chrysomelidae | Leaf-feeder | Rejected | Host-specificity/Establishment |
| **Released** | | | | |
| *Anthonomus santacruzi* | Coleoptera: Curculionidae | Flower bud-feeder | Released | Established (Localized) |
| *Gargaphia decoris* | Hemiptera: Tingidae | Leaf-sucker | Released | Established (Inconsistent damage) |

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*Poster presentation*

# Restoration after rust: how are native communities responding to Himalayan balsam biocontrol, and can we improve their recovery?

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The invasive weed *Impatiens glandulifera* Royle (Himalayan balsam) was first introduced as an ornamental plant to the UK from the Himalayas in 1839. It is now the most common non-native plant species along the river systems of England and Wales and is a growing problem in other parts of Europe and North America. *Impatiens glandulifera* forms dense stands on riverbanks, reducing native plant diversity, outcompeting them for pollinators, and altering native soil fungi and invertebrate communities. In 2006, CABI launched a biological control program for *I. glandulifera* and identified a rust fungus, *Puccinia komarovii* var. *glanduliferae* R.A. Tanner, C.A. Ellison, L. Kiss & H.C. Evans, which was successfully screened and approved for release in the UK in 2014—the first release of its kind in the European Union. Field trials show the rust fungus has spread within release sites and has overwintered in the soil, so that seedlings in subsequent years have become infected. This poster presented research that builds on the biocontrol program and investigates the potential direct and indirect responses of native plant, microbial and invertebrate communities to the rust as it becomes established. Furthermore, with invaded soils having a depleted fungal community, we investigate the potential to augment the soil mycorrhizal community to promote the restoration of these fragile riparian habitats.

*Poster presentation*

# New insights and prospects into *Parthenium hysterophorus* biocontrol from South Africa

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The noxious shrub *Parthenium hysterophorus* L. (Asteraceae) is an invasive weed of global significance. Biocontrol efforts in South Africa have seen four agents released, the most promising of which is the leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), first released in 2013. Leaf-feeding by *Z. bicolorata* is highly damaging, reducing and slowing growth, flowering and seed set of *P. hysterophorus*. More interestingly, *Z. bicolorata* herbivory was found to metabolically impair *P. hysterophorus* leaves by promoting the spread and ingress of potentially deleterious microbes, further enhancing damage. Despite damaging outbreaks having already been recorded, plant nutrient quality may be an underlying constraint for *Z. bicolorata* populations in the field, with poorer-quality plants resulting in slower larval development, smaller adult beetles and consequently less fecund females. Prolonged development of *Z. bicolorata* was shown to leave eggs and larvae susceptible to predation and desiccation. Given that *P. hysterophorus* biocontrol has yet to reach its full potential in South Africa, integration with other management strategies (such as fire) has been suggested. The feasibility of integrating prescribed fires and biocontrol is under investigation by examining the thermal tolerances of the soil-dwelling stages of each of the three insect agents (*Zygogramma bicolorata*, *Listronotus setosipennis* [Hustache] and *Smicronyx lutulentus* Dietz) to the passage of fire. This will determine if prescribed burning will help or hinder long-term biocontrol efforts. Overall, recent biocontrol efforts against *P. hysterophorus* in South Africa have shown promise, advocating that future biocontrol research must be sustained and integrated where possible.

*Poster presentation*

# Endophytes associated with the invasive weed medusahead (*Taeniatherum caput-medusae*)

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Medusahead is an invasive weed in the Great Basin region of the western USA that originated in the northern Mediterranean basin of Europe. Microbial endophytes play an important role in a plant's ability to adapt to abiotic and biotic stresses. Currently, there is little knowledge on how endophytes enhance or alter the phenotype of invasive weeds. The goal of this study was to compare the endophytes of medusahead in the native range to endophytes of medusahead in the introduced range. This poster presented results of culture-dependent and culture-independent approaches.

# Workshops and Panel Discussion

## Biological control of grasses

**Organized by:** John Goolsby[1], Iain Paterson[2], Massimo Cristofaro[3]

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Grasses are traditionally considered poor targets for classical biological control but recent successes, particularly with *Arundo donax* L. in southern Texas, suggest that this traditional view should be changed. The challenges and opportunities for grass biological control were discussed, and the participants generally agreed that the prospects for controlling a number of very damaging invasive alien grasses are good. There are a number of new projects against grass targets, and although these projects are in the early stages, some promising natural enemies have been discovered. Many of the natural enemies of grasses have also been found to be highly host specific, even at the intraspecific level, so including genetic analyses of both the target plants and agents is likely to be important. If realistic goals are set, many invasive grass species could be successfully controlled using biological control. In the end, it was concluded that grass targets face similar challenges as any other targets and should be treated as such.

## Arts and science of native range explorations

**Organized by:** Kunjithapatham Dhileepan[1], Matt Purcell[2]

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Among the discussions with the participants of the workshop, we touched on (1) the Nagoya Protocol and the utilization of material transfer agreements (MTA) for the shipping/import of potential biological control agents (USA and CABI). Regulations vary widely between countries and no consensus was reached on how the protocol would account for moving biological control agents from one country to another which were originally accessed from elsewhere (native range); (2) the creation of databases for both points of contact in different countries/regions as well as taxonomists for the identification of potential agents collected was suggested (possibly the IOBC could host and maintain such a database); (3) genetic techniques, which are becoming more important in biological surveys but need to be considered in combination with behavioral and population genetic assessments to reduce the chances of overlooking cryptic species.

# Implications of weed biotypic variation for biocontrol programs using fungal pathogens

**Organized by:** Kate Pollard[1], Marion Seier[1], Carol Ellison[1]

[1]*CABI, Egham, United Kingdom*

The workshop "Implications of weed biotypic variation for biocontrol programs using fungal pathogens" organized by Kate Pollard and held on Monday evening stimulated a lively discussion around the susceptibility of different UK Himalayan balsam biotypes to the biocontrol agent *Puccinia komarovii* var. *glanduliferae* R.A. Tanner, C.A. Ellison, L. Kiss & H.C. Evans, as well as the variability of invasive *Rubus* spp. in Australia. Ideas discussed were to pool different biotypes of the biocontrol agent as mixed inoculum to select the most virulent from inoculated plants and using trap gardens in the native range with biotypes of the target weed from the introduced range. The need to conduct thorough molecular studies to better understand invasion histories prior to surveys was highlighted, as well as that specificity issues linked to the biotypic variation of target weeds are by no means restricted to fungal pathogens as biocontrol agents, but also need to be considered regarding insects and mites.

# Taking biological control to our communities

**Organized by:** Kim Weaver[1], Philip Ivey[1]

[1]*Centre for Biological Control, Rhodes University, Department of Zoology and Entomology, Grahamstown, South Africa*

The Centre for Biological Control invited delegates of the XV International Symposium to participate in a survey entitled "Taking biological control to our communities." Delegates were asked to participate and share information of their experiences from their respective institutions and countries. Responses were received from 62 delegates from 14 countries and 36 different institutions. The questionnaire requested information on: ways in which respondents conduct public awareness and outreach, their funding diversity and if they are involved in mass-rearing of biological control agents.

The Convention on Biological Diversity stipulates that the public needs to be engaged in the management of invasive alien species (UNEP Report 2004). Novoa et al. (2017) adds that the more the public knows about the threats of invasive species and their impact on humans and the environment, the greater the support for the management of these species. Many different tools and approaches were proposed to raise awareness around environmental threats. Among them, respondents claimed to use public talks most frequently, followed by posters, pamphlets and then popular articles. Some researchers use information stands and field demonstrations, contribute to books, journals and newsletters and share information through social media as this becomes more popular. Biological control researchers shared what guided their awareness-raising initiatives, and what stood out above the rest was that researchers wanted people to know about invasive species, biological control and their research institutions. For us to ensure that our research is effectively implemented, we need to share what is known. Five of the 36 organizations that had respondents participate in the questionnaire have an allocated budget for communication and outreach, and a third of the organizations have some sort of communication strategy in place.

As part of the survey, respondents were asked to comment on their funding and where it came from. Many of the researchers claimed to have state funding in varying amounts. Additional sources of funding are also used for biological control research; these include funds for collaborative work from other institutions, such as agricultural institutions, universities, community groups or landowners. Funding for biological control research, as well as the implementation thereof, is important for managing the control of invasive plants. This includes mass-rearing of agents for augmented releases in affected areas. Around 60% of the researchers who responded to the survey have mass-rearing activities in their countries.

As part of the workshop, participants were asked to develop a plan to promote biological control at an event where a new biological control agent would be released. These ideas and plans will be collated. With the workshop participants, we will put together a manuscript of our experiences in building public awareness around invasive species and biological control.

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# The Nagoya Protocol and its implications for classical weed biological control

**Organized by:** Luciana Silvestri[1]*, Alejandro Sosa[2,3], Fernando McKay[2], Marcelo Diniz Vitorino[4], Martin Hill[5], Costas Zachariades[6,7], Stephen Hight[8]

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## Abstract

The international agreement on Access and Benefit-Sharing (ABS) establishes that genetic resources shall be accessed only upon the existence of prior informed consent of the country that provides those resources and that benefits arising from their utilization shall be shared between the users of the resources and the provider. The ABS mechanism was first introduced in the Convention on Biological Diversity and was further developed by the Nagoya Protocol. ABS national legislations or regulatory requirements have been considered a challenging obstacle to classical weed biological control (CWBC) as added bureaucracy has proved an impediment for efficient and effective implementation of CWBC programs across the world. In this paper we briefly draw on the experiences of Argentina, Brazil and South Africa, three source countries of biological control agents, to discuss how national ABS legislation can negatively impact the exchange of biodiversity resources. We conclude it is important to raise awareness among policy makers on the key role CWBC could play in different sectors and to persuade them to develop tailored ABS legal frameworks that adequately suit CWBC research and practice.

**Keywords:** Nagoya Protocol, classical weed biological control, Argentina, Brazil, South Africa

## Introduction

Classical Weed Biological Control (CWBC) involves the deliberate release of specialist natural enemies from the invasive species' native range. Usually, biological control agents are collected in and imported from a different country where the agents and target coevolved. As such, CWBC relies on the ability to effectively and efficiently access biological resources of many countries. In this context, concerns have been raised that CWBC research, primarily a non-commercial activity, could be negatively impacted if providing countries adopt and implement legislation or regulatory requirements that restrict or complicate users' ability to collect and export biological control agents (Smith et al., 2018).

Within this framework, two international agreements—the Convention on Biological Diversity (CBD), which entered into force on 29 December 1993, and the Nagoya Protocol (NP), a supplementary instrument to the Convention, which became effective on 12 October 2014—are considered a reason of concern for CWBC practitioners. They create a global legally binding regime on access to genetic resources and benefit-sharing which, despite its noble objectives, i.e., promotion of biodiversity conservation and of fair international economic relations between developing and developed countries, has the potential to adversely impact the effective and efficient exchange of biodiversity between countries.

The CBD and the NP empower parties (countries that have ratified the agreement) to regulate access to their genetic resources in view of the sovereign rights States exert over their natural resources (articles 15.1 CBD and 6.1 NP). If a party decides to adopt access measures, then users seeking access to a countries' genetic resources are required to obtain prior informed consent for that access, unless otherwise determined by the party (articles 15.5 CBD and 6.1 NP). In addition, monetary and non-monetary benefits arising from the utilization of genetic resources are to be shared in a fair and equitable manner upon mutually agreed terms between the user and the providing country (articles 15.4 CBD and 5.1 NP).

To ease access to genetic resources for non-commercial research purposes, the CBD obliges parties to strive for facilitated access to genetic resources for environmentally sound uses and avoid constraints that run counter to the objectives of the CBD (article 15.2 CBD). Likewise, the NP requires parties to consider simplified measures on access for non-commercial research purposes when developing and implementing national legislation or regulatory requirements (article 8.a NP). Furthermore, to set criteria on how ABS national legislation or regulatory requirements should be implemented, the NP establishes that parties provide for legal certainty, clarity and transparency, and fair and non-arbitrary rules and procedures. The entire procedure must end in a written decision that shall be granted by a competent national authority, in a cost-effective manner, and within a reasonable period of time (article 6.3 NP).

Unfortunately, despite good intentions, legal requirements and criteria set in the CBD and the NP, countries have rushed to adopt national ABS legal frameworks without undertaking a process of comprehensive and strategic planning (Glowka, 2000). Consequently, there are several examples where ABS national regimes have negatively impacted CWBC in particular, and non-commercial research on genetic resources in general (Cock et al., 2010; Neumann et al., 2018; Prathapan et al., 2018; Smith et al., 2018).

As of 16 July 2019, 117 countries have become parties to the NP, and most of them have already adopted or are planning to adopt/update new ABS national legislation. This paper is a review of how national ABS legal frameworks can impact the effective and efficient exchange of genetic resources by drawing on the experience of three significant players in the field of CWBC: Argentina, Brazil and South Africa.

## The case of Argentina, Brazil and South Africa

### *Argentina*

During the last century, a number of native plants (e.g., water hyacinth, *Eichhornia crassipes* [Mart.] Solms, and alligator weed *Alternanthera philoxeroides* [Mart.] Griseb.) were accidentally or deliberately transported from Argentina into other countries where they became invasive weeds. In Argentina, legislation regarding the collection and movement of living organisms was in place since the 1960s (Coutinot et al., 2013). However, it was not until 2007, thirteen years after Argentina ratified the CBD, when specific ABS regulations were adopted for the first time at the federal level through Administrative Decision Nº 1659 of the Secretary of Environment and Sustainable Development (SESD). Three years later, the SESD decided to derogate the previous regulations and pass new regulations just before the NP was adopted: Administrative Decision Nº 226 of 2010. Consequently, the Decision does not include the new developments undertaken under Nagoya.

Decision Nº 226 covers access to genetic resources only when there is an intention to export them later. As such, the access and utilization of Argentine genetic resources within the country by national scientific institutions is not regulated, at least at the federal level. If biological control agents are to be exported from Argentina, a permit authorizing the access to genetic resources and subsequent export must be obtained from the SESD. This permission will only be granted if the applicant presents evidence that a prior inform consent was conceded by the relevant provincial authority and that mutually agreed terms were established.

In addition to the federal regulation, eight provinces out of the existing 23 (namely, Neuquén, San Luis, Jujuy, Formosa, Santa Cruz, Entre Ríos, Tierra del Fuego and Misiones) have regulated access to their biodiversity under a different extent of ABS issues. The local legislation applies to any access to genetic resources occurring within their own jurisdictions, no matter the intention (commercial/non-commercial), the person (national or foreigner), and where the resources will be utilized (in or outside the country). ABS provincial legal frameworks greatly differ from one province to another.

The most important challenge the Argentine ABS legal framework faces is the absence of a strong and complete ABS national system that applies uniformly to all the provinces. As a consequence, the disparity between provincial ABS regulations is overwhelming and confusing for researchers trying to gain access to biological control agents. Secondly, the ABS national and provincial legal frameworks are neither strategic nor efficient; for example, they do not establish simplified measures for non-commercial research of genetic resources. Consequently, collection and export of biological control agents go through the same lengthy and bureaucratic administrative procedures as a bioprospecting commercial project. Finally, users that want to access and export genetic resources for CWCB must comply with ABS-specific legislation and also satisfy several other regulations, e.g., interprovincial transport of biological material, phytosanitary aspects and collection and research permits, all of which frequently overlap in contradictory terms relative to jurisdiction, paper filling requirements and authority. The lack of coordination between all these regulations has created more inefficiencies and confusion among researchers. As a consequence of the above-mentioned problems, since 2010 when Decision Nº 226 was adopted, it has become increasingly difficult to access genetic resources in Argentina and to transfer them abroad for CWBC purposes.

In recognition of the above-mentioned challenges, the SESD has undertaken a national project under the auspice of the Global Environmental Facility and the United Nations Development Program with the aim to contribute to the effective national implementation of the NP in Argentina. Within its framework, the SESD has prepared a legislative draft proposal that establishes minimum and uniform ABS national legal standards. The proposal, however, has been contested by the local scientific community claiming it does not provide simplified measures for non-commercial access and utilization of genetic resources.

Recently, access to biodiversity for CWBC appears to be improving in Argentina. In 2018, the Foundation for the Study of Invasive Species (Fundación para el Estudio de Especies Invasivas [EI]), an Argentine scientific research institute based in Buenos Aires, obtained an access and export permit for the use of the water primrose thrips, *Liothrips ludwigi* Samar (Thysanoptera), in an unprecedented rapid time; it only took 3 months. Thrips insects will be exported to the Western Regional Research Center of the US Department of Agriculture, California, USA to determine this insect's potential to control invasive water primrose (*Ludwigia* spp.). Although the reasons for the rapid acceptance of the permit request are unclear, successful cases like this one will hopefully become more common in the future.

### *Brazil*

Brazil is a Party to the CBD, and a signatory of the NP. It is expected to shortly become a party to the NP as necessary legal steps towards ratification have been taken. Brazil has had a complex ABS system in place since 2001 when Provisional Administrative Measure (Medida Provisória) Nº 2186 was adopted. The regulation was recently updated to cover the new developments included in the NP. The cornerstones of the current legal system are Law Nº 13123 of 2015 and related regulatory Federal Decree Nº 8772 of 2016.

The Law creates the Genetic Heritage Management Council (Conselho de Gestão do Patrimônio Genético [CGEN]) and the National System of Genetic Heritage Management and Associated Traditional Knowledge (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado [SisGen]) as the main institutional structure upon which the regulation operates. The main achievement of the system is that prior informed consent is no longer required to access Brazilian genetic resources. Instead, the Law mandates that any access to Brazilian genetic heritage shall be electronically notified in the SisGen database. In addition, those exporting material containing Brazilian genetic resources with the purpose to access them or to provide any service based on them must register these activities at SisGen. Consequently, activities such as the collection and the shipment of biological control agents for CWBC must be recorded at SisGen. If access to Brazilian genetic resources involves the development of a commercial product, then benefits arising from their utilization shall be shared between the manufacturer of the finished product and the provider upon the base of mutually agreed terms.

Although the collection, research and potential shipment of CWBC agents are registered at SisGen, the Law does not actually regulate the collection of biological materials. Collections of biodiversity must be registered in several other electronic systems, including the System for Authorization and Information about Biodiversity (Sistema de Autorização e Informação em Biodiversidade [SISBio]) from the Institute Chico Mendes, the Information System on Brazilian Biodiversity (SIBBr) that systematizes the registration of biological collections, and the endangered species CITES and Non-CITES System from the Brazilian Institute of Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis [IBAMA]).

The most outstanding challenge faced by the new legal regulations is that SisGen has created redundancies and added additional layers of bureaucracy to an already complex system (Alves et al., 2018).

The new legal framework is expected to impact CWBC in both positive and negative ways. One benefit of Law Nº 13120 is the advancement of special considerations for scientific research, though the process is still somewhat cumbersome. Another benefit is that the new regulations have been constructed in a manner as to not hinder the continuation of ongoing research projects that are based on the access and utilization of genetic resources.

On the negative side of the new biodiversity legislation, CWBC research projects involving the collection and export of Brazilian biological control agents will not only have to be recorded at SisGen, but possibly at other functioning systems. This would entail more time and workload, a situation that would likely impact CWBC-related activities. Even more, researchers will still have to sign a Material Transfer Agreement, prepare a shipment guide for all samples to be exported and request a scientific expedition authorization to collect material from the field when a foreign researcher is involved. Processing time for permits will take at least eight to ten months and require a significant paper work investment to obtain personal data from all participants, their signatures, translation of all documentation into Portuguese and documentation of specific legal counseling. The complicated process requires involvement of a Brazilian scientist to negotiate the requirements and gain access and input information into SisGen and other systems.

An example that represents the above-mentioned problems was a CWBC project to export two biological control agents to the University of Florida in the United States: the pit gall-maker *Calophya terebinthifolii* Burckhardt & Basset (Hemiptera: Calophyidae) and the sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae). It took one month to gather all necessary information for registration at SisGen. Then forms and documents had to be sent to the University of Florida for translation and signature, a task that took more than two months. Once back in Brazil, applications were sent to different governmental agencies that approved the scientific expedition (three months) and granted the export permit (two months). Finally, it took another week to update and finish completing required information at SisGen. A total of eight months was required to gain permission to collect and export the two CWBC agents. Any additional collection and export request will likely require another eight-month period.

### *South Africa*

ABS is not a new concept in South Africa as the country already had a well-developed legal framework in place before the NP was even negotiated. The first ABS national legal provisions were adopted in 2004 within the framework of the National Environmental Management Biodiversity Act (Act Nº 10 of 2004) (NEMBA or Biodiversity Act) as an attempt to reduce biodiversity loss. Later, in 2008, the Bioprospecting, Access and Benefit Sharing (BABS) Regulations were adopted and further developed the system. Both legal instruments are administered by the Department of Environmental Affairs (DEA), which also hosts the national focal point for the implementation of the CBD and Nagoya.

The objectives of the Act are the conservation of biological diversity, sustainable utilization of indigenous biological resources, and the fair and equitable sharing of benefits among stakeholders arising from bioprospecting involving indigenous biological resources. Furthermore, the Act seeks to give effect to the ratified international agreements relating to biodiversity, which are binding in South Africa, such as the CBD and the NP.

Chapter six (6) of the Biodiversity Act regulates bioprospecting involving indigenous biological resources, the export thereof for any kind of research, and provides for fair and equitable sharing by stakeholders in benefits arising from indigenous biological resources and their associated traditional knowledge. It is worth noting the Act goes beyond the material scope of the CBD and the NP; it does not regulate genetic resources as such, but "indigenous biological resources," a broader category that includes, according to the Act, any living or dead animal or plant or other organism of an indigenous species, any derivative thereof or any genetic material thereof, among others.

Some activities undertaken in the field of CWBC may fall within the scope of the current legislation. First, if a user of indigenous biological resources pursues non-commercial research, then he/she may need a collection and/or research permit from the competent provincial authority. If resources are to be researched within South Africa, and the research does not pursue any commercial or industrial purposes, then there is no need of a bioprospecting permit. However, if indigenous biological resources are to be exported later, no matter what the intention is, an export permit must be obtained when the material does not come from an ex situ collection. This permit must be obtained from provincial authorities in terms of the current legislation. If the resources to be exported come from an ex situ collection, in order to export them, an exchange agreement must be concluded with the provider (the local scientific institution), and the provincial competent authority has to be notified.

The main challenges that the South African legislation faces are that the current legal framework does not focus on the concept of genetic resources, but rather regulates access and utilization of indigenous biological resources, a broader concept. These two different categories, which pivot in the first case on the use of the unique genetic characteristics of an organism and, in the other, on the utilization of biological resources as bulk material, are identically regulated in the Act.

Another challenge refers to increasing bureaucracy and the significant workload it demands to get all permits needed (for collection/research first, and for export later) and the need for authorization from the owner of the resources.

Since NEMBA and related BABS Regulations were adopted before the NP entered into force, legislation is currently going through amendment. Even though specific changes are not yet known, it is expected DEA will conduct consultations with the main stakeholders involved in ABS practice, including the CWBC community.

## Conclusions

A brief review of the experiences of Argentina, Brazil and South Africa as source countries of biological control agents seems to indicate that their respective national ABS legal frameworks have the potential to obstruct the research and practice of CWBC. Detected impacts include difficulties in collecting biological material and accessing genetic resources contained within them, limitations to access genetic resources that are held in ex situ collections and inefficiencies in the transfer and/or exchange of genetic resources between international collaborating partners, among others.

The reasons that explain difficulties for collecting, accessing, exporting and utilizing genetic resources relate to the absence of separate and simplified measures for access to genetic resources for non-commercial research. In addition, overlapping, uncoordinated and bureaucratic regulations entail excessive administrative burdens and/or high transaction costs that prevent the undertaking or continuation of collaborative research projects. Finally, another law-related reason for concern is found in expanding material scopes which cover absolutely every aspect of the use of biodiversity and over-complicate activities that do not truly imply an access to genetic resources. Worse than that, authorities competent for the implementation of ABS legislation waste energy and resources controlling activities that should not be included in an ABS regime while real opportunities for promoting benefits from the utilization of genetic diversity are lost.

We conclude that policy makers in Argentina, Brazil and South Africa should acknowledge the key role biological control agents play in controlling invasive weeds and thus, should take appropriate actions towards promoting the effective and efficient exchange of biological resources.

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# Alien invasive plants: do we need to control them and, if yes, how?

## Panel Discussion

Although biological control of weeds has been practiced for over 100 years, it is still a neglected tool in managing alien invasive weeds in Europe. Taking advantage of the fact that the Symposium took place in Switzerland, a panel discussion was organized, inviting five participants with different backgrounds and viewpoints on the subject:

1. Nicola Schönenberger (*Consultant at INNOVABRIDGE Foundation, Switzerland*)
2. Elizabete Marchante (*University of Coimbra, Centre for Functional Ecology, Portugal*)
3. Christoph Küffer (*ETH Zürich, Department of Environmental Systems Science, Switzerland*)
4. Heinz Müller Schärer (*University of Fribourg, Department of Biology, Switzerland*)
5. Richard Shaw (*CABI, United Kingdom*)

Sarah Pearson Perret from Pro Natura led the discussion, starting by asking the panel members about the current situation on invasive plants in their respective countries from their perspectives, the challenges that they perceive and why they have not given up yet. Finally, she asked "since biological control of weeds is so successful, why is the method not readily taken up in Europe?"

**Some of the take home messages from the discussion were:**

1. Invasive plants can be a big problem, also in Europe;
2. Invasive plants are only one factor besides others threatening biodiversity;
3. Communication and education is vital, but is a 2-way process, not just scientists disseminating expert information. We need to listen and take interests and values of the relevant stakeholders serious. Values are subjective and often more important than objective information to people;
4. To increase visibility and buy in at the right level, a "champion" biological control target is needed that everybody agrees should be controlled; and a success story is desperately needed;
5. Biocontrol is an opportunity because it does not aim to eradicate the target plant, but finding a new equilibrium. However, biocontrol needs to be better communicated. At the moment the message "alien vs. alien" is confusing and counterintuitive to the general public;
6. Overall, the prospects for biological control of invasive plants in Europe are positive.

# Author Index

## S



## T



## U



## V



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## Y



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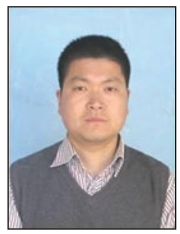

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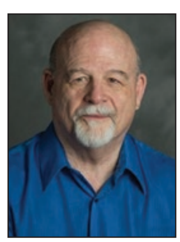

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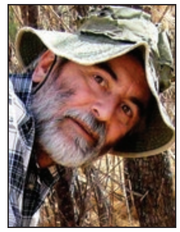

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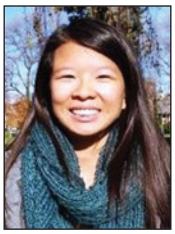

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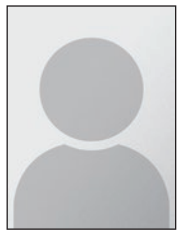

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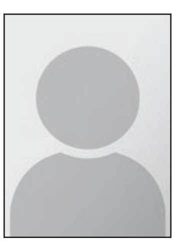

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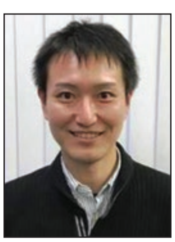

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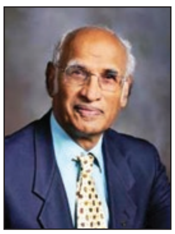

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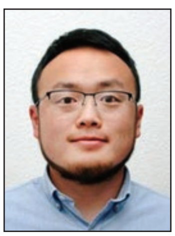

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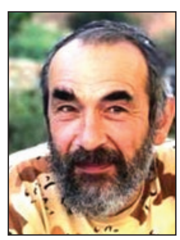

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