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Abstracts of talks and posters
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 will be 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 will focus on pathogen agents and provide 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 will be 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.
Session 1-O1 - Target and agent selection

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

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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; Brassicaceae) has been used as a dye source for at least eight centuries in Eurasia, was introduced to 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 (Hₛ) 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 (Fₛₛ) 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.
Do host races exist in the Sagittaria fruit-feeding weevil?

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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 paper looks specifically at *Listronotus appendiculatus* (Bohem.) (Curculionidae), a fruit-feeding weevil under consideration for release into Australia and South Africa.
Session 1-O3 - Target and agent selection

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

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Acacia auriculiformis, 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 prioritising 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.
In recent years, eriophyid mites are gaining popularity in weed biological control based on a number of assumptions, including high host specificity, short life cycles and high potential impact. Eriophyid mites are among the smallest plant feeders (100-200µm). These tiny herbivores are characterised by having an intimate relationship with their hosts, about 80% of currently known species are reportedly associated in nature with a single host. 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. Specific challenges commonly encountered include: 1) Extremely high level of specificity on specific genotypes of a host; 2) Lack of host specificity under experimental conditions; 3) Measuring impact under experimental conditions; 4) Identifying potential effects of predation on vagrant vs. gall formers; 5) Recognising possible abiotic limiting factors e.g. soil characteristics and climate; 6) Understanding basic life history traits, such as, overwintering requirements, dispersal capabilities and the relationship with annual vs. perennial host plants. In this presentation we will discuss these challenges, and use specific examples of how best we have managed to address these challenges. We will attempt to pull together what we have learnt over the years and identify potential knowledge gaps and future research questions to give eriophyid mites a viable future in classical weed biological control.
Giant rat’s tail grasses (*Sporobolus pyramidalis* and *S. natalensis*) (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 labour-intensive, and thus biological control is being pursued. This study aimed to characterise 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 characterised 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.
Session 1-O6 - Target and agent selection

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

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Biological control is the most economically viable management option for prickly acacia (*Vachellia nilotica* subsp. *indica*), a serious weed of grazing areas in 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, focussing 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 which occur in the invasive subspecies *indica*. In Ethiopia, several prospective biological control agents were recorded. These were a gall thrip (*Acaciathrips ebneri*) inducing shoot-tip rosette galls, a gall midge (*Lopesia niloticae*) inducing leaf rachis galls, and three morphologically distinct eriophyd gall mites (*Aceria* spp.): type-1 which forms red, spherical leaflet galls; type-2 which forms creamy-white fluted leaflet galls; and type-3 which deforms leaflets, rachides and shoot-tips. In Senegal, in addition to the gall thrips, an eriophyd gall mite (*Aceria* sp. type-3) and a tephritid fly (*Notomma mutilum*) inducing stem-galls have been identified as prospective biological control agents. This is the first time gall-inducing agents associated with *V. nilotica* subspecies with moniliform fruits, including the subspecies *indica*, have been collected. Based on damage potential, field host range and climate match, the gall thrip from Ethiopia was imported into quarantine in Brisbane, Australia and host specificity tests are in progress. The type-3 eriophyd 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 importation and host specificity testing of the gall-inducing tephritid fly from Senegal.
Medusahead (*Taeniatherum caput-medusae*) (Poaceae) is an annual grass from the Mediterranean region, that is an invasive noxious weed in many western US states. At the beginning of 2000s, a biological control program was started by USDA, combining the screening of different subspecies, populations, and biotype-s of the target weed with the presence and identification of natural enemies to be used as perspective biological control agents. During an exploration carried out in 2014, a new species of eriophyid mite, *Aculodes altamurgiensis* has been 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 australis*, *Avena sativa*, *Triticum durum* and *T. aestivum*), 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 5 different biotypes (2 Italian and 3 from US) 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.
**Session 1-O8 - Target and agent selection**

**Using field host range testing to prioritise 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 prioritised for further studies according to their damage inflicted, abundance, distribution and other successful examples of biological control agent taxa on Solanaceae elsewhere. The prioritised 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. australi* and one economically important species, *L. barbarum*. Subsequent open-field choice and no-choice tests were set up at three different climates (50m, 25km & 35km 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 prioritise agents before their introduction into quarantine facilities.
Session 1-O9 - Target and agent selection

Integrating results from host range, efficacy and fitness trials to prioritise biotypes of Dactylopius tomentosus

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A biological control program targeting eight invasive Cylindropuntia species naturalised in Australia began in 2009. Twenty biotypes of Dactylopius tomentosus have been imported from Mexico, South Africa and the US into an Australian quarantine facility to evaluate their potential as biocontrol agents. These biotypes showed significant variability in the level of impact on the naturalised 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 have a maximum score of 20. Whereas, 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. However, two field observations 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 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.
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, *Calophya terebinthifolii* Burckhardt & Basset and *Calophya lutea* Burckhardt (Hemiptera: Calophyidae), and the 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 the release of biological control agents and to determine the host specificity of *C. terebinthifolii* and *C. lutea*. The herbivory impact on plant growth and reproduction was collected from insecticide protected and unprotected trees every 3 months for 3 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. Results from the field study showed that fruit production and plant growth were not different between treatments. In the host-specificity study, the selected insect species oviposited only on five non-target species in the Anacardiaceae family. Oviposition was greater on BP when compared with non-target species. Gall development and adult emergence occurred only on BP. These results provide justification for the introduction of host-specific biological control agents into Florida.
Session 1-O11 - Target and agent selection

*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* and *V. nigrum*; 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, 1 or 2 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 two 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.
The first instance of biological control of an invasive weed, *Opuntia monacantha* (Cactaceae) took place in India, a developing country, in 1795, with the serendipitous introduction of *Dactylopius ceylonicus* under the colonial influence. A century later, biological control of weeds became a major activity in developed countries, starting with the introduction of natural enemies of *Lantana camara* in Hawaii in 1902 and cactus in Australia in 1912. Since then, most of the work on weeds of temperate regions has been carried out in Australia, Canada, New Zealand, South Africa, and U.S.A., while proportionately less on tropical weeds. Although biological control of weeds is gaining momentum in developed countries, it lagged behind in developing countries. Developing countries lack financial and human resources as well as awareness by local authorities on the safety and efficacy of biocontrol agents. Most activities on the biological control of weeds in developing countries took place sporadically with external support that too limited the technology transfer from developed countries. Opportunities for successful biological control of weeds in developing countries are plenty. Some of the weeds that could readily be targeted for which technologies are available are *Chromolaena odorata, Eichhornia crassipes, Lantana camara, Mimosa diplotricha, Mikania micrantha, Mimosa pigra, Parthenium hysterophorus, Prosopis spp., and Salvinia molesta*. Constraints are mostly limited financial, institutional, and human resources, and sometimes competition between inter-institutes and disciplines.
Session 2-O1 - Opportunities and constraints for classical weed biocontrol in developing countries

Weed biocontrol in India - Opportunities and constraints

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With more than 250 invasive alien plants identified in India, Chromolaena odorata, Eichhornia crassipes, Lantana camara, Leucaena leucocephala, Mikania micrantha, Mimosa diplotricha, Parthenium hysterophorus and Prosopis juliflora 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 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, 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 amongst 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. Utilising experience from elsewhere, India has enormous opportunities for weed management using biocontrol: a more suitable pathotype of Puccinia spagazzinii for M. micrantha; Heteropsylla spinulosa for M. diplotricha and Neohydronomus affinis for Pistia stratiotes form excellent examples of these opportunities. Developing an efficient framework will assist the advancement of weed management through biocontrol in India.
Prospects of classical biological control of weeds in Pakistan: Challenges and opportunities

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Invasive alien weeds are recognised as a global threat to natural and agro-ecosystems. The list of alien invasive weeds of Pakistan is long, and some of them have become a serious threat to agricultural production, biodiversity and human and animal health. The worst affected environments are the forest ecosystems where some of these invasive species have transformed the native vegetation by altering native species composition and diversity. \textit{Lantana camara}, \textit{Prosopis juliflora}, \textit{Broussonetia papyrifera}, \textit{Euclayptus} spp. \textit{Leucaena leucocephala} are the worst woody invaders while \textit{Parthenium hysterophorus}, \textit{Orobanche aegyptiaca}, \textit{Conyza bonariensis}, \textit{C. ambigua} and \textit{Soliva} spp. are problematic herbaceous invasive species. Water weeds like \textit{Salvinia molesta}, \textit{Pistia stratiotes}, \textit{Alternanthera philoxeroides} and \textit{Eichhornia crassipes} have been established in wetlands of Sindh and Punjab. CABI- Pakistan has a long history and contributed to global efforts of biological control of weeds in different parts of the world. Paradoxically, there is not a single example of deliberate introductions of biological control agents against any weed in Pakistan. This is partly due to policy barriers and a lack of awareness of the problems invasive plant species can cause. CABI – Pakistan with the help of key stakeholders has recently started a programme on invasive species which is focused on management of \textit{Parthenium hysterophorus} as a pilot. This program includes awareness campaigns and the testing and ultimate release of suitable biological control agents. Classical biological control is a promising weed control strategy and developing countries such as Pakistan should harness its benefits. However, before the initiation of this program, some policy gaps and barriers need to be addressed.
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*), 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* and a stem-boring weevil, *Listronotus setosipennis* 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, 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.
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* for *Lantana camara* 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* provides complete control of *Sida acuta* and *S. rhombifolia*. Control of two water weeds *Eichhornia crassipes* by *Neochetina bruchi* and *N. eichhorniae* and *Pistia stratioties* by *Neohydonomus affinis* has also been fairly good in most areas. Anecdotal evidence suggests the rust *Puccinia spegazzinii*, released in 2012, is having an impact on *Mikania micrantha*. More agents are needed to help manage others weeds, both existing and new targets. A new 5 year project, aimed at key pasture weeds affecting the beef industry, will begin in 2018, funded by New Zealand’s Ministry for Foreign Affairs and Trade. This project will seek agents for three novel targets: *Solanum torvum*, *Urena lobata* and *Senna tora*. Known agents available in Australia will also be released against *L. camara*, *Parthenium hysterophorus*, and possibly *Mimosa diplotricha*. Agents will also be released against two environmental weeds: *Dolichandra unguis-cati* and *Spathodea campanulata*. Lessons learnt 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.
Session 2-O5 - Opportunities and constraints for classical weed biocontrol in developing countries

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 benefitting 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*, is problematic in both South Africa and Namibia and has recently been targeted in Australia using a new lineage of the cochineal, *Dactylopius tomentosus* ‘californica var. parkeri’. Similarly, the creeping cactus, *Pereskia aculeata*, is problematic in Australia and a new agent, the stem-wilte, *Catorhintha schaffneri*, 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* ‘stricta’ for *Opuntia stricta*; *Dactylopius tomentosus* ‘imbricata’ for *Cylindropuntia imbricata*; 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.
Session 3-O0 - Bioherbicides

Navigating the bioherbicide trail to reach the goal of wide spread use

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Conceptually, bioherbicides should be a desirable weed management solution that aids in reducing the ecological footprint of synthetic herbicides and lowers the risk of hazards to health. Despite these potential benefits, navigating the path to achieve the wide spread use of bioherbicides in agriculture, horticulture, and forestry situations has been circuitous with limited successes. The science behind historical products such as Collego® and DeVine® demonstrated that these products were efficacious when applied under the right environmental conditions, yet the wide-spread and long-term use of these products was limited due to not meeting certain commercialization needs such as only creating a niche product by targeting a single weed with high host specificity and requiring infrequent product applications. No matter how good the science maybe, there must be a commercial incentive by the public to use and the industry to produce. To reach the goal of wide spread bioherbicide use, scientists must assess how to better integrate the developmental science with commercial needs. With a focus on mycoherbicides, this paper will explore several examples to show which aspects of the scientific evaluation most readily support the commercialization demands of industry, so we can turn more potential bioherbicides into actual bioherbicides.
**Session 3-O1 - Bioherbicides**

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

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*Mycosphaerella polygoni-cuspidati* (Mycosphaerellaceae, Ascomycota) is a fungal pathogen of Japanese knotweed (*Fallopia japonica*) in its native Japanese range, where it is widespread and causes a damaging leaf spot. The pathogen has undergone extensive evaluation as a potential classical agent for control of Japanese knotweed which is a major invasive plant in its introduced European and North American ranges. However, the ability of *M. polygoni-cuspidati* to infect and produce its first life cycle stage, fertile spermogonia with spermatia, on two UK and one North American native plant species raised the question as to whether the pathogen could also complete its life cycle on these non-target plants. This question is still unanswered, which currently precludes the fungus from use as a classical agent. Nevertheless the unique biology of *M. polygoni-cuspidati* – producing herbicide-like symptoms – lends itself to the potential development as a mycoherbicide. The *Mycosphaerella* leaf-spot lacks an asexual conidial stage 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 (requires two complementary mating types to complete its life cycle and develop sexual structures) with ascospores as its only infective and dispersal propagule. By using a single mating type isolate, the fungus would thus be prevented from reproduction, spread and persistence in the field which would allow for targeted application against invasive Japanese knotweed stands, especially in urban situations. This concept has been protected through UK and International patent applications held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK. This presentation will report on the current “Proof of Concept” research for such a proposed mycoherbicide and discuss the road ahead.
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. Change in the amount of certain microorganisms due to different environmental factors can change the interaction from neutral or beneficial interaction 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 endophytes 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 analysed, 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 Wapsherer (1974). Therefore the method has to be reviewed for biological weed control.
Session 3-O3 - Bioherbicides

Requirement of mycotoxin TeA-activated singlet oxygen signalling 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*, *A. longipes* and *A. tenuissima*. 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 signalling 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 signalling is also involved during the development of disease caused by *A. alternata*. That 1O2 signalling 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 signalling is to promote disease development.
Session 3-O4 - Bioherbicides

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

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The toxigenic potential of the fungus, *Stagonospora cirsii*, a possible biocontrol agent against *Sonchus arvensis*, was evaluated. For this purpose, 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 were determined. Maximal YEM from the culture filtrates (ca. 300 mg/L) was obtained from the 2-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 2-week culture on pearl barley. The highest level of phytotoxic (on leaf segments of *Sonchus arvensis*) and antimicrobial activity (against *Bacillus subtilis*) was from a dichloromethane (DCM) extract from the 3-week culture grown on modified Czapek medium (CM). Maximal cytotoxic activity (on U251 cell line) was demonstrated using an ethyl acetate extract from a 3-week culture grown on liquid YMG medium. Esterase activity inhibition was maximal for the 2-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 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.
Setting the mood: Initiating herbivory response increases impact of fungal pathogens on Canada thistle

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*Cirsium arvense*, 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*, or CT-rust, systemically infects and kills only *C. arvense*. However, CT-rust rarely reaches epidemic proportions in natural populations. The objective of this study was to determine if manipulating plant defence 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 week 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 10 weeks post incubation and then harvested for above and belowground biomass. All inoculated plants exhibited symptoms by week 10. 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 defence 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.
Isolate differences in *Sclerotinia sclerotiorum* applied as a bioherbicide to *Cirsium arvense*

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*Sclerotinia sclerotiorum* has been proposed for use in a bioherbicide formulation to control *Cirsium arvense* populations in pastures in New Zealand. Our research has investigated whether there is a difference between isolate S36 (the currently favoured 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*. 
Session 3-O7 - Bioherbicides

Persistence and effectiveness of Sarritor [Sclerotinia minor (IMI 344141)] in the southeastern U.S.

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Experiments were conducted to evaluate the efficacy of Sarritor on broadleaf weeds common to turfgrass in North Carolina, USA (35.790899, -78.699707) 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 8 weeks following Sarritor treatment. Additionally, in the fall experiments, control of field madder (Sherardia arvensis) 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.
Session 3-O8 - Bioherbicides

Development of a granular bioherbicide using Sclerotium rolfsii for biocontrol of broad leaf weeds

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Sclerotium rolfsii, isolated from a destructive stem rot of diseased goldenrod plants (Solidago canadensis), has been attempted to be developed into a mycoherbicide for biocontrol of broad leaf weeds and sedges. The effectiveness and specificity tests showed that the mycoherbicide could control most of the broad leaf weeds and some sedges in fields, turfgrass land and invaded habitats. The mass production of 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 broad leaf weeds and sedges in paddy and corn fields, turfgrass and invaded habitats.
Session 4-O0 – Novel methods to determine efficacy and environmental safety of agents

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

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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 interactions are important. Knowledge of these interactions can direct the selection of potential agents and assist in the prioritization of species in test plant list. Several topics will be integrated 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 will be highlighted that demonstrate examples of these interactions that could be widely adopted by researchers.
Session 4-O1 - Novel methods to determine efficacy and environmental safety of agents

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 improving our understanding of the mechanisms underlying acceptance or rejection of critical test plant species. The weevil *Ceutorhynchus cardariae* is being considered as a biological control agent against the invasive *Brassicaceae Lepidium draba*. 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.
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*, to olfactory and visual cues emitted from *Cynoglossum officinale*, a rangeland weed in North America, and eight confamilial, native North American non-target plants including four threatened and endangered (T&E) in the US. *Mogulones borraginis* reacted indifferently to *Andersonglossum occidentale*, 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.
The role of thistle phylogeny on long-range and short-range host selection behaviour of the biocontrol agent *Cassida rubiginosa*

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The oligophagous thistle tortoise beetle, *Cassida rubiginosa*, was introduced to New Zealand to control the noxious weed *Cirsium arvense*. 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 behaviour. 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.
Session 4-O4 - Novel methods to determine efficacy and environmental safety of agents

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 (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: (i) studies of the realized host-range of the midge in Australia; (ii) the phylogenetic relatedness of D. pilifera to a clade of its congeners; and (iii) 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.
The search for generalisations in the face of complex species-environment interactions is particularly important for minimising the cost of managing populations of species. We tested whether we could generalise, at various nested scales, the species-level demography of the widely invasive plant species *Parkinsonia aculeata* (Fabaceae), and whether these generalisations 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 1000 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*. 
Session 4-O6 - Novel methods to determine efficacy and environmental safety of agents

Demographic modelling 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 programmes 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*, a chrysomelid beetle native to North America is considered a successful BCA of *A. artemisiifolia* in China. The species was first recorded in Europe in 2013 in northern Italy, from where it has started spreading to neighbouring 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 approx. 8°C), generation time ranged from 28-73 days, resulting in 1-4 generations per year. Adult mortality and pre-oviposition period was 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 modelling 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 programmes more predictive.
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* (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* and *E. vollonoides*, 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 modelling scheme to optimize selection of model background and other parameter settings. Projected distribution of *E. cisplatenisis* 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. cisplatenisis* and *E. vollonoides* in Australia, and the establishment data gathered as part of post-release monitoring will be used to test these model projections.
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* (rust fungus) and *Chondrilla juncea* (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 have previously been 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 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.
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*) and cochineal insects (*Dactylopius* spp.) have colonised 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* biotypes collected from 10 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.
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: i) What is the diversity of members of the community, and what is the specificity of the herbivores feeding on the plant? ii) Is there a top-down control of herbivores by natural enemies? iii) Are there differences in network structure between geographical areas? Our study targets the sowthistle, *Sonchus oleraceus*, 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 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.
Session 4-11 - Novel methods to determine efficacy and environmental safety of agents

Insect thermal tolerance - 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 modelling, 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 the biocontrol literature. This is surprising, as metabolic changes have significant effects on feeding behaviour, an important component of agent efficacy. Preliminary investigations into metabolic rates over a range of temperatures in a field-established South African biocontrol agent, *Neochetina eichhorniae* (Coleoptera: Curculionidae), of water hyacinth (*Eichhornia crassipes*), have illustrated that metabolic rates can differ between populations, despite a lack of 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 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 at determining how much this metabolic rate can be forcibly adapted to produce more effective agents for release elsewhere.
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. Ground breaking new genetic approaches like RNAi and gene-drive open new possibilities for tackling intractable weeds species and resistant genotypes. In this paper I will explore 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?
Session 5-O0 – Making classical biological control more predictive: moving from ecological to evolutionary processes

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

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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* into Europe, which needed an urgent decision on how to respond to this unforeseen arrival of an oligophagous insect and potential effective biological control agent of common ragweed, *Ambrosia artemisiifolia*. We immediately reacted to this event by our newly formed COST-SMARTER consortium composed of specialists in weed and invasive species management, ecology, aerobiology, allergology and economics. Firstly, we will summarize our findings on the beetle’s potential benefits, ranging from its impact on ragweed performance, demography, spread, aerial pollen concentrations up to reducing health cost. We will further present our results on the risks of the beetle for non-host plants in Europe. So far, we detected no impact on sunflower, probably because the window of vulnerability of this crop does not coincide with high beetle densities at the end of the growing season. The same holds true for taxonomically closely related ornamental and endangered native plant species. Secondly and in view of improving predictions for future long-term benefits and risks of this potential biological control program, we initiated a novel experimental evolutionary approach to assess the beetle’s potential to select for resistant/tolerant ragweed populations, as well as the beetle’s potential for evolutionary adaptation to novel biotic (host plants) and abiotic (colder temperatures) conditions, using next generation sequencing and bioassay approaches. We will also briefly discuss other ongoing evolutionary studies from China and Japan using the *Ambrosia-Ophraella* biological control program. This is the first attempt to rigorously and simultaneously assessing the evolvability of a biological control agent and its target weed.
Session 5-O1 - Making classical biological control more predictive: moving from ecological to evolutionary processes

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. I will show evidence from work in my laboratory using Tribolium castaneum 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.
Session 5-O2 - Making classical biological control more predictive: moving from ecological to evolutionary processes

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* (a benefit) but also led to acquisition of a non-target host *Senecio triangularis* (a potential risk). The strength of interactions between 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 are essential for evaluating both the benefits and risks of biological control.
Invasive alien plants together with their natural enemies from their native range used as biocontrol agents are an ideal study system to address questions of whether and how fast organisms adapt to changing environments. Climate change is likely to impose further selection on invasive plant populations in interactions with the biocontrol process. In 2016, we started a study into the evolvability of a European plant invader, *Ambrosia artemisiifolia*, 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*, and a warming treatment (+3°C) in a two-by-two experimental design with five replicates. To test for evolutionary changes of this selection experiment, pooled samples from each of the 20 experimental populations will be analysed over four years (a) for their genetic composition using next-generation sequencing (pool-seq) and (b) in various bio-assays. By now, we collected seeds from the first (F1) and second (F2) generation from all four field treatments and grew them alongside their parents in growth chambers under two experimental temperature conditions, in the quarantine and greenhouse to assess beetle performance and plant competitive ability. We found high variation in trait means and plastic responses to temperature treatments in performance variates among F0 mother plants indicating high initial standing genetic variation. We will present the top differentiated SNPs (0.1%) among two generations from pool-seq analyses. We already found that F1 plants from elevated temperature perform differently in phenology, growth and plasticity in the field plots compared to plants from the ambient temperature plots. Finally, I will present results of quarantine preference and performance studies with *O. communa* on F2 plants from field populations, and of the competitive ability of F2 plants from greenhouse studies. These studies will improve forecasting of the efficiency of biocontrol and spread of invasive alien plants in a changing world.
Session 5-O4 - Making classical biological control more predictive: moving from ecological to evolutionary processes

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 United States through next-gen 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.
Session 5-O5 - Making classical biological control more predictive: moving from ecological to evolutionary processes

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. With the introduced tamarisk beetle species complex (Diorhabda spp.) as a case study, I will show 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 from Uzbekistan, D. carinulata from China and Kazakhstan, D. elongata from two sites in Greece, and D. sublineata 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. I discuss the 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. I present evidence of serial bottlenecking at the edge of range expansion, differential establishment across source populations, extensive admixture and hybridization across source populations, and show that proportions of ancestry do not necessarily reflect introduction history. Then I present our cost-effective de novo assembly of D. carinulata, the first weed biocontrol agent reference genome, and illustrate its potential as a resource for weed biological control to identify genomic changes and the genomic architecture of rapid evolution in ecologically relevant traits.
Admixtures of Chinese and Nepalese Lilioceris species: helpful or harmful for U.S. 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 and its congener L. egena. 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, F1 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 F2 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 F1 or F2 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.
Anticipating cryptic species and determining their host associations in weed biological control

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The presence of unrecognised 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 unrecognised 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 recognised 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 harbour unrecognised 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 unrecognised 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 signalling 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 will 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.
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*, 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 voltnism, sex ratio, fecundity, and adult emergence patterns of the biotypes. Females from populations also were 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 ca. 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, voltnism, and other biological traits of importance to the species’ efficacious use in biological control.
Session 5-O9 - Making classical biological control more predictive: moving from ecological to evolutionary processes

*Rhinusa pilosa*: a case study of environmental bottleneck

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The shoot gall weevil, *Rhinusa pilosa* (Mecinini, Curculionidae), was first released in Canada in 2014 for biological control of *Linaria vulgaris* (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*, 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 mtDNA 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 yellow toadflax.
Session 5-O10 - Making classical biological control more predictive: moving from ecological to evolutionary processes

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 programme. Demonstrating host specificity of biocontrol agents is crucial for Australia, which harbours 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 determining 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 prioritised for more intensive host-range assessments.
Session 5-O11 - Making classical biological control more predictive: moving from ecological to evolutionary processes

Application of DNA barcoding to compare the fundamental and ecological host ranges of a proposed biocontrol agent for *Sagittaria platyphylla*

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Natural enemy surveys give us an indication of the ecological host range of candidate biocontrol agents, the range of plants that are utilised in the field. Adult insects are usually easily found on the outside of the plants and have morphological features that make species identification clear in many cases. However, adults can be collected from a range of plant species that don't necessarily represent the range of plants on which they can develop but might instead be simply a place to rest. By contrast, larval and pupal stages collected on or within plants provide a better indication that the plant species is a host. But identifying immature stages to species level can be very difficult, and rearing them to adult stage is not always possible during foreign exploration surveys. We encountered this issue when undertaking natural enemy surveys for the aquatic weed delta arrowhead, *Sagittaria platyphylla* (Engelm.) J.G. Sm. (Alismataceae) in its native range of the southern USA. More specifically, we wanted to determine the ecological host range of a crown-boring weevil, *Listronotus sordidus* (Gyllenhal) by distinguishing its immature stages from any of the other 24 species of weevils reported from *Sagittaria* species in the USA. We did this by collecting larval and/or pupal samples from within the tissues of a range of Alismataceae plant species across the native range. To identify the larvae and pupae to species level, we used molecular methods to overcome morphological similarities. In addition to this, molecular phylogenies can be easily constructed to compare the evolutionary relationships between *Listronotus* species and their Alismataceae hosts. We compared the ecological host range of *L. sordidus* from field studies with host-specificity testing results in the laboratory to better understand how the fundamental host range relates to the ecological host range.
Biological control of weeds in the Anthropocene: Why has introducing new agents become so challenging?

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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 micro-organisms 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.
Horehound biocontrol - a case study in public consultation

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White horehound, *Marrubium vulgare*, is a perennial herb in the mint family. It has become a weed especially in dryland sheep farming in the eastern parts of the South Island of New Zealand. A farmers’ group formed in late 2016 to replicate in New Zealand the programme 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 for use in his products. A dialogue developed, during which the herbalist informed us that others would soon follow suit, and the following weeks brought a steady stream of letters of opposition from medical herbalists. Opposition to biocontrol in New Zealand is rare, especially such an organised campaign. As well as the potential to affect the horehound project, this group may oppose future weed biocontrol programmes in New Zealand where the target weed is harvested for medicinal uses. In this presentation we will explain how we are using best practice and lessons learnt in the past to manage this situation and achieve the best possible outcomes for all.
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, on-line surveys, and submitting 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.
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*, which was released into France in the 1990s without assessment, is a case in point; although beneficial in killing aphid pests, the harlequin ladybird harbours 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 Organisation for Biological and Integrated Control (IOBC) and the European Plant Protection Organisation (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. Licences 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 harmonised system across Europe is therefore recommended.
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 negotiate 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 utilisation. 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 has 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 October 12, 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 utilisation in a fair and equitable way. Compliance with the due diligence requirement has to be notified to the Swiss national authority before market authorisation or commercialisation of a product developed on the basis of utilised genetic resources. These provisions of the NCHA were further detailed in the Nagoya Ordinance, which came into force on February 1st 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 utilisation of genetic resources.
The Nagoya Protocol: implications for classical biological control of invasive plant species

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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 entered into force on 12 October 2014 and as of 21 March 2018, is party to 104 countries. “Utilisation of genetic resources” is defined as “to conduct research and development on the genetic and/or biochemical composition of genetic resources”. This therefore impacts 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. Despite some regional efforts each country is likely to implement the Nagoya Protocol in different ways thus complicating legal access and use of genetic resources. Our presentation aims to give an overview of the issues arising for classical biological control through the Nagoya Protocol and the recent steps CABI took to try and find pragmatic solutions. We emphasize the growing importance to exercise due diligence when it comes to ABS to guarantee that classical biological control remains a viable tool for invasive plant management.
Session 7-O0 - Social and economic assessments of biological control

Quantifying the economic and social benefits of 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 evaluate the benefits of biological control. Estimating these benefits requires (1) quantification of the impacts of invasive species on the ecosystem; (2) a conversion of these impacts to monetary values; (3) consequences for the people affected by them; (4) an assessment of the effectiveness of control in reducing these impacts; and (5) a comparison of the costs of control to the value of avoided harmful impacts. Standardised schemes for assessing the environmental and social impacts of invasive species have recently been developed, but there are not yet many examples of where these have been applied. Their effective application is hampered by a dearth of studies on the impacts of invasive alien species, but the situation is changing, and several studies that assess returns on investment from biological control, while still rare, are emerging. Examples from South Africa suggest that invasive alien species generate impacts of hundreds of millions of US$ per year, and growing as invasive alien plants continue to be introduced, become invasive, and spread. Case studies on alien plant species in South Africa, Europe, Australia and on island ecosystems suggest that returns on investment from biological control range from 3.5:1 to >3000:1. 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 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. It is becoming increasingly clear that biological control is the only long-term effective method, and that where it works, it can deliver remarkable returns on investment.
Recent economic analyses of several weed biocontrol programmes in New Zealand show strongly positive on-going returns. For example, the largely successful biocontrol of ragwort saves the New Zealand dairy sector NZ$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 programmes 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: i) conservation benefits are very difficult to monetarise, and; ii) 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 programmes could be analysed to provide useful data to support continued investment in weed biocontrol. Two further standout results were: i) 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 NZ$0.5 million benefits per year with a cost:benefit ratio of over 100:1 and; ii) an unscientifically unjustifiable decision to not use the ragwort flea beetle, an agent short-listed in New Zealand’s pioneering ragwort biocontrol programme in the 1930’s, resulted in an astounding net present value loss of NZ$8.6 billion to the NZ dairy sector.
Session 7-O2 - Social and economic assessments of biological control

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 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 R&D costs and investments in programs, even though presently the data are often not examined or obtained. Water hyacinth (*Eichhornia crassipes* (Mart.) Solms) has been a significant invasive aquatic weed in the United States since the late 1800s. A unique, 38-year (1975-2013) data set 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, 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 US$4.45 Billion in ecosystem goods and services for the state derived from a US$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.
Session 7-O3 - Social and economic assessments of biological control

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* 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 US$2028, US$498, and US$1800 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*. Cameroon employs manual clearing of water hyacinth, however, respondents indicated they would consider other control methods, provided they do not have any negative impacts.
Biological control of cacti has a complicated history. The dramatic reduction of invasive Opuntia (prickly pear) species by Cactoblastis cactorum 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.
Nassella trichotoma (nassella tussock), an invasive weed of grasslands in New Zealand, is being considered as a target for classical biological control. We conducted a cost benefit analysis for a hypothetical fungal agent released against the metapopulation of the weed in the Hurunui District of North Canterbury using the net discounted present value (NPV) criterion with a 100-year time frame and a discount rate of 8%. The costs of the programme were assumed to occur in the first nine years (NZ$0.4m y⁻¹ in years 1-5 for research plus NZ$0.025m y⁻¹ in years 6-9 for post-release monitoring). The benefits, taken to be the savings made in the current annual control programme, were accrued from year 6 and were calculated as the product of the proportional reduction in the population size of the metapopulation expected from the agent and the cost of the district’s current control programme (NZ$4.5 m y⁻¹). The rate at which the agent’s maximum impact is approached was given by a logistic function. Under this model, the slower-acting the agent is, the greater the maximum impact must be for NPV > 0.0. Maximum expected agent-induced reductions in the current metapopulation of 47, 65 and 76% were estimated using a matrix population model for the weed with 10% reduction in per capita seed production and either 5, 10 or 15% reduction in plant growth. These agent-induced effects were taken from relevant literature as representative of those to be expected. The NPV was greater than zero for all three scenarios so long as the agent achieved 90% of its potential impact within approximately 50 years from release. The analysis indicates that a plant pathogen-based classical biological control programme for nassella tussock in New Zealand is likely to be economically worthwhile.
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* on public health in Europe. Moreover, we analysed the projected impact of the recently established ragweed leaf beetle *Ophraella commun*na, 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 modelling 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. commun*na 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.
Opportunities and constraints for classical biocontrol of invasive plants in Europe

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In Europe, invasive alien species 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. However, despite the long history of successful biocontrol against weeds worldwide, the considerable economic benefits, the rare non-target impacts, and the long list of potential targets with biocontrol agents, the first biocontrol agents against weeds were introduced in Europe only in the last decade. On the contrary, many biocontrol agents have been introduced for the control of plant pests. Why is Europe still so conservative in what concerns classical biocontrol of invasive plants? Constraints and opportunities will be discussed and illustrated with several case studies. New introductions face constraints in scientific, bureaucratic and regulatory terms, but also poor engagement and communication with several stakeholders and even citizens in general; a recent survey about biocontrol of invasive plants puts some clarity into this issue. In spite of these constraints, there are also opportunities. Europe has been the origin of biocontrol agents for other regions, with several biocontrol facilities established in its territory, and accumulating valuable know-how that can be used also at home. The three biocontrol agents introduced in the last decade open the doors to a new paradigm. The process leading to the introduction of *Trichilogaster acaciaelongifoliae*, the first biocontrol agent introduced into continental Europe, will be addressed as well as the other biocontrol agents first released in the UK and new agents under study. Several unintentional introductions have been reported, and although this is not the way to go, they are here and may be an opportunity. The need to engage and raise awareness amongst European citizens and stakeholders will be also addressed.
Session 8-O1 - Opportunities and constraints for classical weed biocontrol in developed countries

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

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The United States 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 U.S. 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 U.S., 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 U.S. 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 U.S., state and local entities are weed biocontrol’s best proponents.
Invasive species are one of the biggest threats to global biodiversity. Florida’s climate, tourism, and growing population make it particularly at risk for invasive species. Biological control is one of the best tools to reduce the damage done by widespread invasive plant species. However, neither the negative impacts of invasive species nor the safety and effectiveness of biological control are well known to the public. Increasing the public’s awareness of the negative impacts of invasive species and the safety and effectiveness of biological control is an important step in the fight against invasives. We designed an outreach and education program that is centered on the charismatic biological control agent *Lilioceris cheni* (Coleoptera: Chrysomelidae) and its target, the invasive air potato (*Dioscorea bulbifera*). Air potato is an easily recognizable, climbing vine in the southeastern United States, which many landowners struggle to control. 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 *Lilioceris cheni* to interested landowners and managers for release and provided educational activities for both adults and children. The campaign has been highly successful and has increased the general knowledge 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%.
In 2014 the rust fungus, *Puccinia komarovi var. glandulifera* (Pucciniales), was the first fungal agent to be released into Europe for the biological control of an invasive weed. The target, *Impatiens glandulifera* (Balsaminaceae), Himalayan balsam, is a prolific invader of riparian habitats in Europe and North America. In 2015, a strain of the rust from India was released at 20 sites across the UK, by planting out infected plants into infestations of the weed, allowing for natural rust spread. However, infection in the field was lower than anticipated at a number of sites. The observed reduction in size and number of uredinia, when compared to infected plants in the native range and during quarantine assessments, could not be explained by unfavourable environmental conditions nor sub-optimal release strategy alone. Subsequent inoculation experiments conducted under controlled conditions, revealed significant variation in the susceptibility of Himalayan balsam populations 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 for release from quarantine. Inoculation studies found the strain from Pakistan could infect many, but not all, of the populations which were resistant to the Indian strain. In advance of the 2017 rust releases, the plant genotype present at each potential release site was assessed so the optimum rust strain could be used. In addition, the issuing of a Permit by the UK Chemicals Regulation Division, has allowed aqueous spray application of the rust spores. Results of these field releases have been more encouraging, with high spore infection as most release sites; although natural spread of the rust is still localised. However, the presence of weed genotypes not susceptible to either rust strain in the UK requires additional strains to be identified in the native range.
Session 8-O4 - Opportunities and constraints for classical weed biocontrol in developed countries

Modelling the biocontrol of an invasive tree by a bud-galling wasp, Trichilogastra acaciaelongifolii

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Acacia longifolia 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 re-invasion of the areas. After conducting host-specificity tests and many years of licences and bureaucracy, the Australian bud-galling wasp, Trichilogastra acaciaelongifolii, was introduced as a biocontrol agent for Acacia 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 and in the long term 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 Trichilogastra model. The model was featured with 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.
Landowners and researchers in partnership to ensure the success of biological control in South Africa

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For future biological control research to flourish in South Africa, the research community needs to work in partnership with the users of this technology, namely: landowners, farmers and rural communities. To date the ‘users’ of biological control have had a limited role in determining the research direction and have been passive recipients of the biological control agents. We will developed a partnership model that ensures a virtuous cycle of information, ideas and actions from farmer to researcher to implementer of biological control and back to farmer. The research is not about “studying what the farmer wants”, it is about how the “researcher becomes more part of the farmer’s reality”. It will:

1. Liaise with leaders in agribusiness, farming media and rural communities to get support for the partnership approach;
2. Ascertain the levels of knowledge amongst the ‘users’ of invasive species and biological control. We will use the Net Promoter Score (NPS) model to ascertain “appetite” and additional questions to ascertain levels of knowledge. The NPS 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 fellow farmers and friends?” In applying this research methodology we will be able to track the success of the project initiatives over an extended period of time. We hypothesize that the current NPS would be around -20 and that we should work on shifting it over a period of 3 to 5 years to +20.
3. Work with partners to foster the appropriate use of biological control to manage invasive species on their land;
4. Seek commitment of partners through a ‘call to action’ to: report localities of target invasive species, assess presence or absence of biological agents and request biological control agents for their properties;
5. Measure the effectiveness of the partnership on an ongoing basis.
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 amongst 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, 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 amongst stakeholders and influencers and suggestions are made on what actions and advocacy would be useful to change any deep seated but potentially unfounded fears.
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 prioritising 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.
The foremost document 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* (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 will be presented.
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 is 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.) ssp. *micranthos* (Gugler) Hayek).
Session 9-O3 - Post-release monitoring and evaluation

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 a 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.
Session 9-O4 - Post-release monitoring and evaluation

10 years of post-release evaluation on *Myriophyllum aquaticum*, what have we learnt?

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The biological control of parrot’s feather, *Myriophyllum aquaticum* (Haloragaceae), was initiated in South Africa in 1994 with the release of the leaf-feeding chrysomelid, *Lysathia* sp. In 2008, a post-release evaluation programme was initiated comprising annual surveys of 56 parrot’s feather sites across South Africa. After 6 years of surveys the weed had disappeared at 12 survey sites, after 10 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 programme can be ascribed to: (i) the fact that only female plants are present in South Africa so there is no viable seed; (ii) a very efficient mass-rearing programme has resulted in large numbers of healthy insects being released; and (iii) 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.
Session 9-O5 - Post-release monitoring and evaluation

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 scepticism. 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*, which inhibits pod- and hence seed-production by the Australian invasive tree, *Acacia mearnsii*, 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. Whilst sizeable seed-banks remain under existing *A. mearnsii* stands, further seed accumulation has virtually ceased. Modelling 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.
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 paper examines native vegetation community responses to biological control of the invasive plant mistflower *Ageratina riparia* (Asteraceae) with the white-smut fungus *Entyloma ageratinae*. 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 south-east 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.
Assessing herbivore biocontrol impact on common ragweed combining field experiments and population models

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The decline of pest populations is an important aim of classical weed biological control, but predictive and post-release data on this objective remain scarce. Using a case study of invasive common ragweed (*Ambrosia artemisiifolia*), we 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* 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. We discuss implications for the potential further use of *O. communa* as a biocontrol agent of *A. artemisiifolia* in Europe and integrated management of this weed. Linking our results to aerial records of *A. artemisiifolia* pollen and species distribution models for the target and the agent, we also present potential corresponding benefits for human health.
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., are biocontrol agents 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.
Could hybridization between agent biotypes increase biological control efficacy?

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 it can 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*, a biological control agent of the invasive weed, *Jacobaea vulgaris*. We found that at high elevation sites in Northwest Montana almost half (n=7) of the 15 sampled populations contained hybrid individuals. Subsequently, we evaluated the impact of biological control on six sites where pure Swiss, 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.
Session 9-O10 - Post-release monitoring and evaluation

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 (Pontederiaceae)) is perhaps the world’s most invasive weed: it affects commerce, ecology and human health. Megamelus scutellaris (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, which is attacked by a native egg parasitoid, Kalopolynema ema (Hymenoptera: Mymaridae). Kalopolynema ema also successfully utilizes M. scutellaris. To gauge the spatial interactions between M. scutellaris, K. ema and M. davisi, we built 1m² plots of waterhyacinth with M. scutellaris within ponds containing M. davisi, its host plant Nuphar lutea, and K. ema. The abundance of K. ema was then measured at 1, 5, 10, and 20m 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 shows 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.
Session 9-O11 - Post-release monitoring and evaluation

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 vigour (using vegetation indices such as Normalised Difference Vegetation Index), invasive animal detection (using thermal infrared radiation), and more recently in detecting invasive weed species (using multi-spectral reflectance). Cylindropuntia 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 naturalised. A biocontrol program was initiated in Australia in 1925 for the control of C. imbricata, through the introduction of a cochineal, Dactylopius tomentosus. 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 (boxing glove cactus) and C. pallida (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 visualise 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 vigour was assessed using a multi-rotor UAV (also fitted with a multi-spectral and RGB sensor). The results of these studies will be discussed with particular reference to drones and their future use in monitoring and evaluating weed biocontrol research projects.
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*, evolved to colonize southward from release areas, the potential risk of *Tamarix* defoliation to the endangered southwestern willow flycatcher (*Empidonax traillii extimus*) 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 U.S., and 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.
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 conclude by placing these examples into the larger context of removal experiments from around the globe and emphasize the importance of viewing species removals, or the development of biocontrol agents, within an ecosystem context.
Integration of mechanical topping methods to accelerate biological control of *Arundo donax*

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*Arundo donax*, 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 these habitats 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* and the rhizome-feeding scale, *Rhizaspidiotes donacis* are widely established and are having significant impacts in Texas and Mexico. Releases of a third agent, *Lasioptera donacis*, a leaf miner 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 US$4.4 million. To accelerate the decline of *A. donax* we have integrated biological and mechanical methods. Topping (cutting) of *A. donax* at 1 meter 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 untouched 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.
Session 10-O3 - Integrated Weed Management and restoration

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.
Session 10-O4 - Integrated Weed Management and restoration

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* 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*, 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 6 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. quinquenervia* 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.
Integrated control of water hyacinth in peri-urban environments, linking science to society

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Water hyacinth (\textit{Eichhornia crassipes}) 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', La Rioja province, a biological control programme was successfully implemented using the weevil, \textit{Neochetina bruchi}. This was one of the first cases where biological control was used against this weed in the world. Secondly, an invasion of \textit{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, 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 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 massive rearing of the insects \textit{Megamelus scutellaris} and \textit{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.
The future of *Solanum mauritianum* biocontrol in South Africa: prospects, problems and promise

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*Solanum mauritianum* Scop. (Solanaceae) remains one of South Africa’s most widespread and long-standing invasive trees. Despite biocontrol providing the only long-term solution to managing *S. mauritianum*, the South African programme has suffered a troubled history. Extensive research efforts have culminated in the eventual release and establishment of only two agents, namely the sap-sucking lace-bug *Gargaphia decoris* Drake (Hemiptera: Tingidae) in 1999, and the flowerbud-feeding weevil *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) in 2008. Although these agents have largely been abandoned given their poor performance to date, recent post-release studies suggest that they may be deserving renewed investment and attention. The lace-bug *G. decoris* maintains the ability to be extensively damaging, under both shaded and full-sun conditions, suggesting that outbreaks are likely constrained by biotic factors, such as predation or plant quality. Whereas the weevil *A. santacruzi*, was found to be climatically constrained, particularly by low winter temperatures (<4 °C) and humidity (<50%). However, *A. santacruzi* showcased the potential to reduce fruit/seed production by ~70% as well as promote the detrimental self-pollination of *S. mauritianum*. Although historical difficulties halted research, recent insights have revived interest in biocontrol, which will soon see international collaboration to attain new additional agents, in South Africa and New Zealand. In the interim, integrated guidelines are proposed, incorporating biocontrol, to reduce the spread of *S. mauritianum* in heavily affected areas of South Africa. This research advocates for the revival of *S. mauritianum* biocontrol programme in South Africa and discusses the potential problems and prospects for future research.
Abstracts for poster presentations

Session 1-P1 - Target and agent selection

Exploration for effective biocontrol agents of the climbing fern *Lygodium microphyllum*: progress, problems, potential

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*Lygodium microphyllum* 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 and is considered to have become naturalised by 1965. It is one of the worst weeds in Florida and is listed on the US 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 the native plant diversity. Biological control is considered to be the best solution for controlling this weed. ABCL has found only about 40 herbivore species in 10 countries over 20 years of research. The majority of these species are generalists, leaving only 18 candidates that have so far required any assessment as potential biocontrol agents. The history of biocontrol efforts on *L. microphyllum* is summarised, explaining some problems associated with finding specific and impactful agents. An integrated approach of genetics and field research can maximise the chances of successful, sustainable control; the need to examine possible cryptic species or population level differences through genetics and comparative testing is discussed.
Session 1-P2 - Target and agent selection

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 US, 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* is widespread and damaging but its impact on hydrilla in the field is limited and more agents are required, especially on monoecious hydrilla which is spreading into the cooler regions of the U.S. 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 genetically to be a match to US monoecious populations. A leaf-mining *Hydrellia* sp. genotype, possibly highly specific and adapted to the US monoecious form, has been collected from these plants and targeted for further evaluation.
Session 1-P3 - Target and agent selection

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 (Cecidomyiidae) and the bud-gall former, Tephritis formosa (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, have been 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.
Session 1-P4 - Target and agent selection

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

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In assessing the fitness of more than one species as potential biological control agents for the management of pest populations, a detailed knowledge of the insect species is essential. Despite the success of biological control agents against weeds in some areas, they have been less successful in others. This has been ascribed to several abiotic and biotic factors including the local climate, nutrient status that affect plant quality and interaction between species. The invasive aquatic weed, *Eichhornia crassipes* Mart (Solms-Laubach) (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. 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*. Here, I present the outcomes of laboratory experiments investigating the impact of some abiotic and biotic factors on the fitness traits of two geographic species of *E. catarinensis*. The most advantageous biocontrol agent would have the ability to cope with temperature changes while taking into account host quality, and have no negative interaction with other species or sub-species present in the field after release.
Session 1-P5 - Target and agent selection

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

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Ailanthus altissima, 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 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 using mechanical and chemical treatments which provide only short-term control, however, this usually exacerbates the problem. One potential agent for Ailanthus, the eriophyid mite Aculus mosoniensis (Acari: Eriophyoidea), has been already recorded in six European States: Hungary, Italy, Serbia, Austria, Croatia, Macedonia and Greece. Beside 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. In this work we are providing some life history data and we are describing the results of some impact bioassays carried out in field and laboratory conditions, comparing the effects of classic biological control alone and in combination with other management approaches.
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* 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, as a potential biocontrol agent. *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 begin feeding on new leaves. The time taken for larvae to develop to pupation and adult emergence in the field is around 2 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*, a NZ native stem borer and began rearing first instar larvae extracted from excised stems. After cf. 30 days the weight of larvae from excised stems averaged 0.003gms whereas larvae reared on artificial diet for the same time averaged 0.07gms in weight. After cf. 60 days larvae reared on diet were 0.16gms and appeared to be inactive, non-feeding and pre-pupal. A further 120 days in over-wintering conditions larvae were placed in warm conditions and long day-length where pupation occurred and new adults emerged.
Session 1-P7 - Target and agent selection

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

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Sagina procumbens (Caryophyllaceae), procumbent pearwort, 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 co-evolved 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, Mycosphaerella saginae, Puccinia arenariae, Puccinia saginae (synonym of P. arenariae) and Macrophoma sagina. 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. arenariae 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.
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 odour. 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. Here, we summarize the relevant information from a biological control perspective based on literature review and present the results of the first field surveys carried out in the native range.
Session 1-P9 - Target and agent selection

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*) 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 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*, 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* sub-genera *Esula*, *Athymalus*, *Chaemsyce* and *Euphorbia*, as well as selected species from the Picrodendraceae and Phyllanthaceae families. Results obtained so far on the host-specificity will be 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.
Session 1-P10 - Target and agent selection

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

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Guineagrass Megathyrysus maximus/infestus is a very serious alien weed invading rangelands, agroecosystems, urban landscapes and natural areas of South Texas. A biological control program 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, 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.
Session 1-P11 - Target and agent selection

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, and 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 programme 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.
Session 1-P12 - Target and agent selection

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 (\textit{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 U.S, 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 U.S. 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 U.S. 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.
Is being a specific and damaging insect enough to be considered as good candidate for the biological control of water hyacinth?

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Cuernavaca longula is a phloem-feeding insect associated with the aquatic invasive weed Eichhornia crassipes, 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 to maintain a colony of C. longula under laboratory conditions, due to failure 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 a year in two different localities with the aim of studying the reproductive biology of C. longula and its phenological relation with abiotic and biotic parameters, and morphological and physiological aspects of the insects were also considered. Interestingly, this species as many dictyopharids present 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. In spite of many females collected in autumn, almost all of them were immature. The largest number of mature females seemed to be associated with low leaf and pseudopetiole toughness, may be associated to the ovisposition. The pattern found through the year could indicate that the species have one, may be two generations a year, even in tropical areas. In addition thermal tolerance studies helped us to understand and modelling its distribution and phenological patterns found in the field. In spite of mating was still not ever observed in nature, these results would allow us to improve and detect rearing features necessarily to have a reproductive laboratory colony.
Cogongrass (Imperata cylindrica) is a diploid C4 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, pine plantations, and thrives in poor soil conditions such as ditch banks, roadside and railroad rights-of-way as well as reclaimed phosphate-mining areas. Control of cogongrass relies primarily on mowing and herbicide applications. The Indonesian gall midge Orseolia javanica Kieffer and 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) was compared. Preliminary results indicate that gall production and development of O. javanica on the two Florida clones of cogongrass were 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.
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) 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 focusses on a weevil species in the genus Bagous, two species of Diptera, and, since 2016, on the white smut Doassansia niesslii, 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 Columbia, 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.
Calotrope, *Calotropis procera* (Aiton) W.T. Aiton (Apocynaceae), a serious weed of rangelands in northern Australia, is a target weed approved for biological control in Australia. Surveys in its native range yielded several agents, among them, a pre-dispersal seed feeding fruit fly *Dacus persicus* Hendel (Diptera: Tephritidae) and a weevil, *Paramecops farinosus* Schoenherr (Coleoptera: Curculionidae) have been identified as prospective biological control agents. 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 start declining in autumn (November). The duration of life cycle of *D. persicus* (egg to adult) was 42.2 ± 0.38 days (mean ± se) with a range of 38 to 50 days. The average life span of an adult fly was 16.4 ± 0.68 days and this agent can potentially undergo 2-3 generation in field. The average duration of the life cycle of *P. farinosus* (egg to adult) was 59.9 ±0.82 days (mean ± se) with a range of 53 to 67 days. The Aak fruit fly larvae destroyed (100%) all immature seeds and internal tissue of infested pods of the host plant. In contrast, the weevil population built slowly during spring and early summer (March to May) and peaked at late summer (August). Like the fruit fly, the larvae of the Aak weevil also destroyed (100%) all immature seeds of infested pods. The field host specificity, high reproductive capacity 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.
The plant popularly known in Brazil as Buva - *Conyza bonariensis* is native to the South America. Although considered as 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 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. In each site, in addition to the natural enemies; herbarium sample of the different *Conyza* species and DNA samples (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. Up to 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* (Diptera: Tephritidae). Another potential agent for the control of flaxleaf fleabane found is a beetle from the family Mordellidae that is consistently present in the roots and stems in about 90% of the plants of *Conyza bonariensis* sampled.
Session 1-P18- Target and agent selection

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, in Rothschild), 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.
Spotted knapweed, *Centaurea maculosa*, is a native species to Asia and eastern Europe, consisting of two varieties or subspecies, *Centaurea stoebe* - *stoebe* and *micranthos*. The species 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. Up to date, several biocontrol agents have been introduced, but in general, they have not been shown to be efficient against *C. maculosa*. The fruitfly *Acinia corniculata* (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 *C. jacea*. It was hypothesized that its larvae develop in flowerheads, like other species of fruitflies. 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 flowerheads. The fact that the attacked shoots remain smaller and no longer produce flowers, or are completely destroyed, indicate that *A. corniculata* could be an additional, effective biocontrol agent for *C. maculosa*. 
Session 1-P20 - Target and agent selection

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

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Passiflora foetida (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) 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.
Flowering rush (*Butomus umbellatus*) was introduced from temperate 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 negative impacts. The semi-aquatic weevil *Bagous nodulosus* is currently one of our most promising candidates for the biological control of *Butomus umbellatus*. Its larvae feed in the leaves and rhizomes of flowering rush. Although it was reported to be rare in the field, we have collected it at over 20 sites so far. In our initial rearing trials, we covered potted plants of flowering rush with gauze bags (mesh width 1mm) 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. Similar to weevils on terrestrial weeds, we assumed that larvae of *B. nodulosus* do not change plants once females have inserted an egg into the plant tissue. However, in two impact experiments, 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 in our artificial pond, where flowering rush plants were not infested, but left uncovered. This revealed that larvae of *B. nodulosus* must be able to swim and to actively move from plant to plant even at a later larval stage. This new knowledge also has implications for the methodology of host-specificity tests. So far, we have only conducted sequential no-choice oviposition tests with the weevil, but we will now also conduct larval host choice tests.
Garlic mustard (*Alliaria petiolata*) is an invasive biennial plant and in North America, poses a threat to native herbaceous and woody plants in the forest understory. *Ceutorhynchus scrobicollis* 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 US, 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 western US native Brassicaceae species to ensure compliance with the Endangered Species Act through 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.
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*. 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 programmes 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 programme aims to include biological control programmes against the invasive trees *Robinia pseudoacacia* and *Gleditsia triacanthos*, and biological control feasibility studies into: *Pyracantha angustifolia*, *Populus alba*, *Populus canescens*, *Salix fragilis*, *Salix babylonica*, *Rosa rubiginosa*, *Cotoneaster* spp. (*C. franchetii* and *C. pannosus*), *Acer* spp. (*A. negundo*, *A. buergerianum*), *Fraxinus* spp. (*F. americana*, *F. angustifolia*, *F. pennsylvanica/velutina*).
The rust fungus *Puccinia rapipes*: a potential biological control agent of African boxthorn (*Lycium ferocissimum*) in Australia

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Suitability of the rust fungus *Puccinia rapipes* as a biological control agent for African boxthorn (*Lycium ferocissimum*) is being assessed. African boxthorn is a major environmental and agricultural weed in regional Australia and a Weed of National Significance (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*) and exotic goji berry (*L. barbarum*) 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 characterisation 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.
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*, is a plant native to Eurasia that has become an increasing weed problem in pastures, riparian areas and forest margins across Canada and the northern USA. The plume moth *Platyptilia ochrodactyla* is currently being studied as a potential biological control agent for *T. vulgare*. 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* and *T. huronense*. 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 all 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 protected from attack by *P. ochrodactyla* or, if *P. ochrodactyla* become reproductively active before any *T. vulgare* are flowering, may accept non-target species that would not be accepted if flowering *T. vulgare* were present. To further evaluate the risk of non-target attack by *P. ochrodactyla* we therefore exposed non-target species next to a natural population of *T. vulgare* and *P. ochrodactyla* and recorded the number of larvae found on *T. vulgare* and on the non-target plants. The results of this open-field test will be presented in conjunction with observations on the phenology of *P. ochrodactyla* and of the target and non-target species.
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* (Poaceae) is a widespread and highly invasive weed in South Africa (S.A.) that negatively impacts native biodiversity and the country’s scarce water resources. Several genetically-distinct genotypes of a stem-galling wasp, *Tetramesa romana* (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 U.S.A., was found to be present on *A. donax* infestations throughout the country. Molecular studies were undertaken to investigate the genetic variation present in the S.A. *T. romana* population across the distribution of the species. Additionally, we investigated genetic variability within the S.A. *A. donax* population and compared samples to native and introduced populations from Mediterranean Europe and the U.S.A., respectively. In total, 135 *T. romana* wasps collected from all nine provinces in S.A. 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 S.A., the U.S.A., Spain, France, Greece and Mexico. South African *A. donax* populations were found to be genetically similar to *A. donax* from Spain and the U.S.A. and S.A. samples shared the same haplotypes as samples from Spain, the U.S.A. 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 S.A. Further, the highly successful Granada *T. romana* genotype used for biocontrol of *A. donax* in the U.S.A. is a promising candidate agent for S.A., since these countries share genetically similar host-plant genotypes.
Session 1-P27 - Target and agent selection

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

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Abstract

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 prevailing on different field crops in the region. Sixteen of these were observed to suppress the growth of weeds under field conditions; thus, could be potential biological control agents of host weed species. These sixteen fungi were the most common species observed in the region. These commonly observed fungi species were; *Cercospora centaurea*, *C. sorokinii*, *Coleosporium datiscae*, *Erysiphe convolvuli*, *Passalora ferruginea*, *Puccinia xanthii*, *P. aristolochiae*, *P. bromina*, *P. calcitrapae* DC. var. *calcitrapae*, *P. montana*, *P. malvacearum*, *P. phragmitis*, *Pyrenophora chaetomoides*, *Uromyces glycyrrhizae*, *U. acetosae* and *U. vesicatorius*. The respective host weed species of these fungi were; *Centaurea behen*, *Convolvulus arvensis*, *Datisca cannabina*, *C. arvensis*, *Artemisia vulgaris*, *Xanthium strumarium*, *Aristolochia bottae*, *Bromus sterilis*, *Echinops orientalis*, *Serratula cerinthifolia*, *Centaurea balsamita*, *Malva sp.*, *Phragmites australis*, *Avena sterilis*, *Glycyrrhiza glabra*, *Rumex crispus* and *Leontice leontopetalum* 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.
Session 2-P1 - Opportunities and constraints for classical weed biocontrol in developing countries

Embarking on classical biological weed control in Brazil - the rust fungus, Maravalia cryptostegiae versus Cryptostegia madagascariensis

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Following prolonged efforts in project development, lobbying and liaison with key stakeholders, a classical biocontrol (CBC) programme for Cryptostegia madagascariensis (Madagascan rubber-vine, devil’s claw, unha-do-diabo) in the semi-arid north-east region of Brazil has commenced. The invasive vine is threatening the unique Caatinga ecosystem, including natural stands of the iconic Carnaúba palm (Copernicia prunifera), source of the Carnaúba wax. A prioritization tool to select target weeds for CBC in Brazil had previously identified C. madagascariensis as the top candidate out of more than 100 non-native invasive weeds. The proposed CBC programme involves the assessment of the rust fungus Maravalia cryptostegiae associated with the invasive weed in its native Madagascan range. This rust has already been used successfully to control another Madagascan species of rubber-vine (Cryptostegia grandiflora) in Australia. Funding for the programme has been secured from the Government of Ceará through the Development Agency of Ceará (ADECE) and the Ceará Syndicate of Carnaúba Wax Refiners (SINDCARNAUBA). Outside Brazil, the initiative is supported with funding from the family company SC Johnson in the USA. The collaborative project will be undertaken by scientists from several Brazilian Universities, the Associação Caatinga and CABI. As first steps, a monitoring protocol will be devised to capture baseline data of the current invasion at selected sites in the State of Ceará and a list of non-target species relevant to Brazil is being compiled for host-range testing of the rust in the UK. Field surveys to be undertaken in western Madagascar aim to collect M. cryptostegiae isolates across the native range of C. madagascariensis to identify the best match for the invasive biotype in Brazil, using molecular tools, and the climatic conditions. If successful this pioneering project could pave the way for future management of invasive alien weeds in Brazil using the CBC approach.
A weed biocontrol programme for the Cook Islands: progress report

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Much of the Cook Islands’ natural habitats and agricultural land are threatened by invasive weeds. A five-year weed biocontrol programme for the Cook Islands started in November 2013, funded by the Ministry of Foreign Affairs and Trade of New Zealand. In this progress report, we describe how agents have subsequently been released against four weed species using agents already developed in other countries: Noogoora burr, *Xanthium pungens* = *X. strumarium* (Asteraceae); grand balloon vine, *Cardiospermum grandiflorum* (Sapindaceae); mile-a-minute vine, *Mikania micrantha* (Asteraceae) and strawberry guava, *Psidium cattleianum* (Myrtaceae). In addition, ‘novel’ programmes have been developed, resulting in agents also being released against red passionfruit, *Passiflora rubra* (Passifloraceae) and African tulip tree, *Spathodea campanulata* (Bignoniaceae). Preliminary information on the establishment, impact and field specificity of these agents is presented. In addition, we describe research that has been conducted on *Merremia peltata*, (Convolvulaceae), using DNA technologies to verify where it is native and where it has been introduced, and to determine whether it should be a target for biocontrol in the Cook Islands.
Preliminary study on the intrinsic mechanism of appressorium formation in *Exserohilum monoceras*

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*Exserohilum monoceras* (Drechsler) Leonard and Suggs is a phytopathogenic fungus on barnyardgrass (*Echinochloa crus-galli* L.), 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 could show 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 on the relieve contents of alkanes and aldehydes between the compounds of barnyardgrass and rice epicuticular wax.
Session 2-P4 - Opportunities and constraints for classical weed biocontrol in developing countries

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. 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 (2000 NTU), medium (850 NTU) low (160 NTU). Three larvae were inoculated into each of these plants. Plant growth parameters were measured weekly for three months, while adult emergence was observed and recorded from the 56th day of the experiment. 70% of adult weevils emerged from the low turbidity treatment compared to 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.
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 an option. The successful biocontrol programme against invasive *Tamarix* in the USA, using several species 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*, 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 between our *Tamarix* spp., and currently we are using HRS to map the extent of their distribution. 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* 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.
Host specificity of the stem-boring weevil, *Listronotus setosipennis* (Hustache)

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The annual weed, parthenium (*Parthenium hysterophorus*), causes crop yield loss, invades pastures, and is a threat to biodiversity in Ethiopia and other eastern African countries. The stem-boring weevil *Listronotus setosipennis* is one of the biocontrol agents released in Australia and South Africa to manage parthenium. Host-range evaluation of *Listronotus setosipennis* was conducted in a quarantine facility on thirty-nine non-target plant species mainly 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-P1 - Bioherbicides

Could fungi stop Buddleia in its tracks?

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*Buddleja davidii* (Buddleia, butterfly bush) is a large perennial, woody shrub or small tree native to China and Japan, imported into the UK in the 1880s as an ornamental. Cultivars of the shrub are popular with horticulturalists due to their colourful, showy flowers which are attractive to butterflies. The species can grow up to 2m 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 colonise derelict land and are known to out-compete 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, 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 2-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*, 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.
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.) 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 treat and untreated. The 0.1, 0.5, 1, 3 and 5 g/20mL (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.
Session 3-P3 - Bioherbicides

**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* (Tree of haven) 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, 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.
Session 3-P4 - Bioherbicides

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 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 an ophiobolin-producing fungus, *Biopolaris eleusines* (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, BeIPP1, 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 supply more clues about target enzymes that might enhance the production of ophiobolin A for practical application.
Session 3-P5 - Bioherbicides

**Biological Control of *Fusarium avenaceum* 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* is a pathogenic fungus that causes disease in *Avena fatua* L. Our study aimed to optimise 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 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 16.3mg/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, structure elucidation of secondary metabolites and culture conditions in the production of bioherbicidal substances. Our results provide 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.
Session 3-P6 - Bioherbicides

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

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European blackberry (*Rubus fruticosus 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. *Phytophthora* 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* and *P. pseudocryptogea*, 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.
Session 3-P7 - Bioherbicides

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

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Goldenrod (*Solidago canadensis*) 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 in spite 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*.
Session 3-P8 - Bioherbicides

Optimization of fermentation process of \( \text{(Serratia marcescens)} \) 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, \( \text{Serratia marcescens} \) Ha1 from marine microorganisms, was used as the experimental material. In this study, the fermentation medium and environmental conditions for \( \text{S. marcescens} \) 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, \( \text{CaCl}_2 \) 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 \( \text{Digitaria sanguinalis}, \text{Amaranthus retroflexus} \) and \( \text{Pharbitis nil} \) was better than the sterilized fermentation broth reducing the fresh mass of these weeds 62, 77, and 47%, respectively. (3) granule application to foliage was superior to that of soil treatment giving 92, 91, and 74% reduction in \( \text{D. sanguinalis}, \text{A. retroflexus} \), and \( \text{A. theophrasti} \), respectively. By contrast, the inhibition of these weeds by soil-applied granules was 90, 89 and 70%, respectively.
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 favourably 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* from diseased leaves of dayflower and found that the metabolites from this strain inhibited seed germination, restrained plant growth and caused necrotic spots on the leaves of dayflower (*Commelina communis*). 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.
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 what makes them as generalist/specialist, for example, 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 target weed species. In this presentation, we highlight 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.
Session 4-P2 - Novel methods to determine efficacy and environmental safety of agents

A biogeographical comparison of herbivory on Phragmites australis

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Biological control of the European subspecies of Phragmites australis that has invaded across much of North America is promising. As a result, a petition is underway to approve field release within the United States of two extremely host-specific stem-mining noctuid moths: Archanara geminipuncta and A. neurica. 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, and 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.
Risks and decisions: is *Leptinotarsa texana* suitable for biological control of silverleaf nightshade in Australia?

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The leaf beetle *Leptinotarsa texana* was introduced to South Africa from the USA to control silverleaf nightshade *Solanum elaeagnifolium*. 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* 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 (potato *S. tuberosum* and eggplant). *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 utilising 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.
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 United States. 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. Settling by the weevils on *C. officinale* and the alternative hosts in these stands will be reported and 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 will be discussed.
Session 4-P5 - Novel methods to determine efficacy and environmental safety of agents

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 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., *C. amabile* Stapf & J. R. Drumm, an ornamental; 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 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. The finding shows how ecological host ranges can be maintained and calls into question the reliance on the fundamental host range when assessing the environmental safety of biological control candidates.
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* is a prospective biological control agent for Russian thistle, *Salsoa tragus*. 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 nontarget 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 nontarget plants, the population was reduced by 52 to 86%. The results indicate that mites more readily disperse from nontarget plants, but that many remain even after a 43 hour period under laboratory conditions.
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 subsp. micranthos) is a short-lived perennial that is widespread in North America although not as common in New York State. Meadow knapweed (C. x moncktonii) 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 3 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). Data will be presented on model parameters derived to date.
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 1950s, continued in Florida between 1980s-1990s and since 2004 to present. The defoliating sawfly, Heteroperreyia hubrichi 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 native range in Northeastern Argentina were made between 2016/17. A total of 399 H. hubrichi larvae were found by checking 815 S. terebinthifolia, 86 S. lentiscifolius, 34 S. longifolius, 200 S. molle, 86 S. weinmannifolius, 40 Astronium balansae, 30 Lithrea molleoides (Anacardiaceae) and 39 Zanthoxylum rhoifolium (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 P. integerrima. 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.
Biological control of the European subspecies of *Phragmites australis* that has invaded across much of North America is promising. As a result, a petition is underway to approve field release within the United States of two extremely host-specific stem-mining noctuid moths: *Archanara geminipuncta* and *A. neurica*. However, before introducing biocontrol agents to a novel range, we must first understand how herbivores attack and impact *Phragmites* within both their native and introduced range. 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 based either on the presence of the herbivore itself or the type of damage inflicted (> 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 on plant fitness, we can better determine where releases of *Archanara* will have the highest efficacy with the lowest risk of impacting co-occurring native *Phragmites*. 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.
Schinus terebinthifolia (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, Oxydia vesulia, Hymenomima nr. memor, and Prochoerodes onustaria - 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.
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*, 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*, 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.
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), and *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.
Session 4-P13 - Novel methods to determine efficacy and environmental safety of agents

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*), and a univoltine biological control weevil, *Ceutorhynchus scrobicollis*. 
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* and *L. peploides* subsp. *monteviensis* 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 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* as the most promising candidate. In host specificity tests, *L. ludwigi* was highly specific for *L. g. hexapetala and L. p. monteviensis*, and was able to persist on both species completing their development. Both of these species coexist as invasive plants in the US, France, England, Belgium, Italy and 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. Potential biological control insect agents when confined to the laboratory for host specificity testing (either in the native range or quarantine) 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. monteviensis*. The interaction between crossed generations was not significant suggesting that except on a specific combination, maternal effects did not influence the results.
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* 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* 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* has been recorded on *I. tinctoria* in two other localities, Italy and Germany, respectively. 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.
Session 4-P16 - Novel methods to determine efficacy and environmental safety of agents

Examining pre-alightment host-selection of 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., an Eurasian mustard that is invasive in the western USA. Oviposition and developmental tests of 114 test plant species, indicate *C. peyerimhoffi* to have 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 response 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*. 
A novel approach to host-specificity testing for non-target plant species restricted to highly specialized soil types

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, i.e. *S. inflatus* (S. Watson) Greene; *S. flavescens* Hook.; *S. anceps* (Payson) Hoover; and the threatened and endangered *S. albidus* Greene ssp. *albidus*, when propagated in their native serpentine soils versus the standardized horticulture soils. In addition, we also measured the performance of *C. cardariae* on *L. draba* propagated in both soil media as control. We discuss 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.
Session 4-P18 - Novel methods to determine efficacy and environmental safety of agents

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, is a perennial vine native to Eurasia. It was introduced into North America in the 18th century. It out-competes native North American forbs and grasses and is considered a noxious weed in agricultural fields. Melanagromyza albocilia is a small agromyzid fly which 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 prove to be challenging. Several test plants were accepted for oviposition, but larval development in captivity is unreliable. Rearing in the lab has failed so far and the process to obtain 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, while 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 will be presented. We will discuss the option to determine the host range of M. albocilia using open-field tests as an alternative to caged development tests.
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 congeneric species. The European species is the beetle, *Chrysochus asclepiadeus*, which was initially considered for biocontrol of swallow-worts, *Vincetoxicum* spp. The two congeneric species are *C. auratus*, which feeds on dogbane in eastern NA and *C. cobaltinus*, 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 congenericers may better define the risks of release of the European species.
Geographic and genetic variability of disease resistance in flowering rush in the U.S.

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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 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 in the U.S. 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 US 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.
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 (CTmin) compared to the population from the coldest establishment site, suggesting that cold hardening of agents could be conducted before release to improve their cold tolerance and increase their chances of establishment, allowing adaptation to colder climates in the field to occur. Increasing establishment of the most effective agents will decrease the number of agent species needed in a biological control programme, thus encouraging a more parsimonious approach to biological control.
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* 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. Either that the cactus will grow faster with rises in CO$_2$, despite cochineal herbivory. Or, that the weed will be more palatable and potentially more susceptible to cochineal damage. Outcomes of the experiments will be discussed at the symposium.
The salt cedar, *Tamarisk* is one of the many euro-asian shrubby tree species in the family Tamaricaceae, represented by one native (*Tamarix usneoides* E. Mey. Ex Bunge) and two exotic (*Tamarix ramosissima* Lebed. and *T. chinensis* Lour.) species in South Africa. The first record of the exotic *Tamarix* in the country is believed to be from the early 1900s, when they were introduced for erosion control on mine dumps. *Tamarix* are tolerant to extreme soil salinity, which is attributed to their salt excretion abilities through their leaf salt glands. It is however unknown how such high salt levels on the leaves affect insect herbivory. This study investigated the efficiency of the *Tamarix* species and their hybrids in salt uptake and excretion and their impacts on the sap feeding *Tamarix* leafhopper (*Opsius stactogalus*). Ten potted plants of *T. usneoides*, *T. ramosissima* and *T. chinensis* and the hybrids *T. chinensis* x *T. ramosissima* and *T. chinensis* x *T. usneoides* were exposed to salt at a concentration of 3% (w/w) (180mM) for three weeks, after which leafhoppers were added to all but the control plants, and allowed to feed for three weeks. Measurements of chlorophyll content, plant vigour, stomatal conductance, chlorophyll fluorescence, water pressure, and electroconductivity were taken from the two phases of the experiment. The results showed that *T. chinensis*, excreted significantly more salt than all the other *Tamarix* taxa. It also showed that the high level of salt uptake by plant tissues had no significant effect on the herbivory of *O. stactogalus* or plant growth. The findings of this leafhopper-plant-salt interaction study could therefore suggest that the potential biocontrol agent, the *Tamarix* beetle (*Diorhabda* spp), which is currently under quarantine investigation in South Africa, may not be affected by the high level of salt concentrations in the *Tamarix* plant tissues, owing also to the fact that it is a co-evolved and a host specific feeder of *Tamarix* species.
Session 5-P3 - Making classical biological control more predictive: moving from ecological to evolutionary processes

Cochineal and cactus: are new associations biocontrol winners?

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The New World invasive cactus *Opuntia engelmannii* is an important invasive weed in Kenya. Biocontrol of *Opuntias* 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*. 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.
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* 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 4-week-old or 10-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 2nd 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 4 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 1 or 3 months at 4°C without light. After transfer to 22°C, (16L:8D), adult emergence from the 1-month cohort occurred sporadically over several months, whereas emergence of the 3-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 2nd generation in the field.
Session 5-P5 - Making classical biological control more predictive: moving from ecological to evolutionary processes

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

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As the global population continues to rise, alterations in soil and water nutrient loads due to anthropogenic activities such as agriculture and industrial waste disposal present major challenges to the environment. Nitrogen (N), a key element supporting life on earth, is one of the major elements with the most altered natural cycles resulting in significant nutrient gradients in both terrestrial and aquatic ecosystems. Differential host-plant quality is one major consequence of such elevated N levels resulting in changes in herbivore-plant interactions due to acute and chronic responses in various traits. For insect herbivorous biocontrol agents, transgenerational effects of host plant quality remain relatively underexplored despite the potential insights they may give on their population dynamics under global change. We evaluated how N enrichment in parental diets of Neolema abbreviata (Larcordaire) (Coleoptera: Chrysomelidae) may impact life history and performance of F₁ and F₂ offspring in reciprocal experiments on Tradescantia fluminensis Vell. We found limited transgenerational effects of foliar nitrogen variability among life-history traits in both larvae and adults. Larval weight, mortality, feeding damage, pupal weight and days to pupation were all not responsive to the main effects of parental diet. There was however a significant parental diet x test interaction with offspring from parents under high N plants generally performing better under low N test plants in the case of larval weight gain and pupal final weights. Adult traits including oviposition preference, feeding damage and longevity did not respond to the effects of parental diet nor its interaction with test diet as was the case in the larval stage. Our results show conflicting responses to parental diet between larvae and adults of the same generation among an insect species with both actively feeding larval and adult life-stages. These results may have implications on the mass-rearing and field performance of N. abbreviata along variable nutrient gradients.
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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Despite the fact that the thermal environment of insects and foliar quality of host plants are critical determinants of the fitness of insect herbivores, 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 and acclimation temperatures (warm and cold), and variation in foliar quality of host plant on several performance metrics of *Pareuchaetes insulata* (Lepidoptera: Erebidae), a specialist multivoltine moth introduced into South Africa for the biological control of *Chromolaena odorata* (Asteraceae). Our results showed that temperatures below 11°C (which is not uncommon during winter months) significantly reduced the locomotion abilities of the third instars. 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 7% of the 30-second exposure time. Our nutritional ecology experiments showed that the leaves of *C. odorata* plants growing in full sun had increased toughness, lower nitrogen and water content compared with leaves growing in shade. From the viewpoint of herbivore performance, *P. insulata* had prolonged development, lower pupal mass, decreased growth rate, and lower fecundity when reared on full-sun foliage compared with shaded foliage. These results 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 observed low population levels of the moth because of possible reduced feeding by larvae at low temperatures during night-time.
Session 5-P7 - Making classical biological control more predictive: moving from ecological to evolutionary processes

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*) and the arundo armored scale (*Rhizaspidiotus donacis*), 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 1m) 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. We here present our methodology, current images of the arundo biocontrol program, and results from the greenhouse humidity experiment.
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. Diorhaba 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 outlines our research plans, and we present preliminary results.
Himalayan balsam (Impatiens glandulifera), native to the foothills of the Himalayas, is an invasive weed widespread throughout the UK. In 2014, a rust fungus, Puccinia komarovi var. glanduliferae, 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: i) clarify how many genotypes are present in the UK and ii) 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 7-P1 - Social and economic assessments of biological control

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 presents preliminary survey results and describes 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.
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 waterbodies, 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* 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 programme began in 2011, funded by the Department for Environment, Food and Rural Affairs (Defra), and 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 set up 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.
Session 8-P2 - Opportunities and constraints for classical weed biocontrol in developed countries

Ludwigia - a prime target for biocontrol in Europe

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Ludwigia peploides and L. grandiflora 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 programme 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 will highlight 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 amongst others from the plant’s area of origin. The stage is set for biological control to finally bring Ludwigia under control.
Session 8-P3 - Opportunities and constraints for classical weed biocontrol in developed countries

A „mitey“ solution for Australian swamp stonecrop in the UK

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*Crassula helmsii* (Crassulaceae) commonly known as Australian swamp stonecrop, is an aquatic weed which was introduced to Western Europe in the early 1900s as a pond plant. Its ability to tolerate extreme environmental conditions and grow in dense mats means it can outgrow less competitive plants in sensitive aquatic habitats and obstruct leisure activities. Following natural enemy surveys in Australia, *Aculus crassulae* (Acari: Eriophyidae) was prioritised for further study and was shown to be highly host specific and damaging to the host plant. The mite was tested against 40 non-target species in no choice feeding and development tests with non-target feeding and oviposition but no further development occurring on one species. Studies investigating the impact of temperature on the development and survival of the mite were also carried out to demonstrate its potential to survive and establish under UK environmental conditions. In 2017, a Pest Risk Analysis (PRA) was submitted to the UK regulators for the assessment of *A. crassulae* for release as a biological control agent of *C. helmsii*. The PRA will be assessed by the UK government, devolved governments, the Advisory Committee on Releases to the Environment (ACRE) public consultation and the EU standing committee on plant health (SCOPH). If the PRA is accepted by the aforementioned groups, ministerial approval will be sought. If approved, it will be the first biological control agent released against this species and the fourth released against a weed in the EU.
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* 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* (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 2017, and 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: (a) evaluate the physiological host range under no-choice conditions, and (b) 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*. Implications of the host range of the scale will be discussed in the context to non-target risks.
Session 9-P1 - Post-release monitoring and evaluation

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., Asteraceae) in the United States. 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* (F.) (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 setup in groups of four randomly assigned treatments: 1) release of *U. cardui*, 2) release of *H. litura*, 3) release of both insects, or 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.
Waterhyacinth (*Eichhornia crassipes*) 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 wateryacinth planthopper, *Megamelus scutellaris* (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.
Session 9-P3 - Post-release monitoring and evaluation

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* Scopoli (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: (i) display seasonal abundance; (ii) are influenced by floral availability; (iii) negatively affect the weed’s fruit production and; (iv) 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 1000 m a.s.l.).
How do climatic conditions affect the establishment of *Catorhintha schaffneri* on *Pereskia aculeata* in South Africa?

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*Pereskia aculeata* Miller (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 and García (Hemiptera; Coreidae), were performed at sixteen sites with a range of climatic conditions, across South Africa. Surveys to assess insect establishment over a two-year period demonstrated that *C. schaffneri* had established at only two of the sixteen releases sites. The thermal tolerance and humidity requirements of *C. schaffneri* were thus 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 future. Post-release research like this can help increase the effectiveness of biological control programmes by prioritising release sites which are climatically favourable.
Folivory impact of the biocontrol beetle *Cassida rubiginosa* on population growth of *Cirsium arvense*

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The folivorous beetle, *Cassida rubiginosa*, was released in New Zealand in 2007 as a biocontrol agent against *Cirsium arvense* (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-metre 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 data will be presented 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.
Session 9-P6 - Post-release monitoring and evaluation

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 southern United States including Florida and Louisiana. This vine is native to Asia and Africa, and it was originally introduced into USA as an ornamental plant in the 1800s. The specialist leaf beetle *Lilioceris cheni* Gressitt and 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 m² quadrants were placed randomly for measurements of 1) air potato plant cover, 2) leaf beetle feeding damage, and 3) number 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.
**Session 9-P7 - Post-release monitoring and evaluation**

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

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Air potato, Dioscorea bulbifera, a major invasive weed of Afro-Asian origin, has infested, spread, and smothered plant communities in various natural and manmade ecosystems of southeastern United States. 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 habitats may suppress these invasive attributes in adventive ranges. Herein, we tested this hypothesis by deploying Lilioceris cheni, 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 6-wk intervals, and 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 southeastern United States.
Session 9-P8 - Post-release monitoring and evaluation

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

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Tutsan (*Hypericum androsaemum* Linnaeus 1753) is native to parts of Europe and Asia, north-west Africa and Caucasus. It was first recorded in New Zealand in 1870 and was believed to have been introduced as an ornamental garden plant. A biological control programme against tutsan was begun in 2007. This study found two potential biocontrol agents, a foliage-feeding Chrysomelid beetle (*Chrysolina abchasica*) Weise 1892 (Coleoptera: Chrysomelidae) and a leaf-, stem- and seed-feeding moth (*Lathronympha strigana*) (Tortricidae, Olethreutinae) (Fabricius 1775) 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. Here we report the host testing results, rearing methods, field releases and post release monitoring of both agents.
Session 9-P9 - Post-release monitoring and evaluation

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*, 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 1m plots each were setup 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 4 to 6 weeks after the first pustules emerged. All other measurements were taken when the plants started to dieback. 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 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 m² (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*. 
Session 9-P10 - Post-release monitoring and evaluation

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. (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 United States 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.
The biological control of *Azolla filiculoides* in South Africa: The final curtain

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*Azolla filiculoides* 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*, 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 of 105 sites has been carried out. 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.
Leafy spurge (*Euphorbia esula*) control and soil seedbank composition 19 Years after release of *Aphthona* biological control agents

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*Aphthona* spp. flea beetles were released in two ecological sites of the Little Missouri National Grasslands in southwestern North Dakota in 1999 to control leafy spurge. The change in leafy spurge density and soil seedbank composition was monitored to evaluate the effectiveness of the biological weed control agent and the associated change in plant communities 5, 10, 15 and 19 yr after release in loamy overflow (valleys) and loamy sites (ridges). In 2014, 15 yr after release, leafy spurge stem density had decreased 94% from 110 to 7 stems m⁻² in the loamy overflow sites and 88% from 78 to 9 stems m⁻² in the loamy sites. Leafy spurge represented only 2% and 6% of the loamy overflow and loamy seedbanks in 2004, respectively, compared to nearly 67% and 70%, respectively in 1999. There was a slow shift to re introduction of native species into the seedbank over the last 15 yr. The number of desirable species increased to 21 by 2014 (more than 3 times the number of species in 1999) in the loamy overflow sites, and doubled to 14 species in the loamy sites, while less desirable forb species doubled in both sites. Desirable grass species doubled in the loamy overflow sites by 2014 but remained unchanged in loamy sites. *Aphthona* spp. successfully controlled leafy spurge for over 15 yr without any additional control methods or costs to land managers and resulted in the slow return of a subset of native species.
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. subsp. micranthos (Gugler) Hayek) 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%. We discuss implications of the differing trends in root-mining and seed-feeding biological control agent abundance for the management of C. stoebe.
Attempts to establish *Dichrorampha odorata* on *Chromolaena odorata* in South Africa

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The larvae of *Dichrorampha odorata* (Lepidoptera: Tortricidae) tunnel into the actively growing vegetative shoot tips of *Chromolaena odorata* (Asteraceae). A culture of *D. odorata* was collected in Jamaica and imported into South African quarantine, where it has proved to be adequately host specific for release as a biological control agent against *C. odorata* in South Africa. Following the issue of a release permit in 2013, releases of a total of over 20,000 *D. odorata* have been made at 17 sites across three provinces of South Africa. However, despite the insect being easy to rear in the laboratory, and initial persistence in the field, establishment has apparently not yet been achieved. Given that the origin of the biotype of *C. odorata* invading southern Africa is Jamaica, Cuba or a nearby island, it is unlikely that incompatibility between *D. odorata* and its host plant plays a role in this. It seems likely that climatic incompatibility may be important, and for this reason emphasis was placed on warmer and more humid regions for release. Laboratory trials to examine aspects of the moth’s thermal biology are being undertaken. Rearing temperatures could also be lowered. Lepidoptera can be difficult to establish because of aspects of their biology, and later release techniques have taken this into account (e.g. use of field cages, repeated releases at the same site). If none of these techniques result in establishment, a new culture may be collected in the field in Jamaica and released quickly, to avoid laboratory adaptation.
Management of two Australian invaders, *Acacia longifolia* and *A. pycnantha* in South Africa using biological control - Progress thus far?

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Acacia (Leguminosae: Mimosoideae) ranks amongst the worst invasive tree genera in the world. In South Africa, *Acacia longifolia* (Andrews) Willdenow and *A. pycnantha* Bentham, both of which are native to Australia, were historically regarded as being amongst the worst alien invaders. However, since the 1970s, the utilisation of biological control as a sustainable and long-term management tool for these alien tree weed species has been the primary focus of research. Though seemingly persistent in riparian habitats and on mountain slopes, as well as the substantial regeneration of infestations observed after fire events, we hypothesised that these species are no longer a significant problem due to the long-term presence of biological control in conjunction with clearing efforts and the occurrence of fires. Biological control of these alien invaders has however been restricted to the use of flower- and bud-galling as well as seed-feeding insect agents that only curtail the acacias' capacity to reproduce to avoid negatively affecting others that value and utilize these trees. This compromise ensures that management efforts do not interfere with the beneficial and economically exploitable attributes of these tree species. However, the apparent inadequacy to obtain immediate control and reduction in parent plant densities has created perceptions that biological control of these acacias has been unsuccessful. This study therefore presents the results of post-release evaluation and long term monitoring of biological control efforts against these alien invasive tree weed species in South Africa and assesses the progress made thus far.
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 is compiled. The list of weed targets consist 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.
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 the 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 other many 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.
Session 9-P18 - Post-release monitoring and evaluation

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

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The early stages of biological control agent introduction programs are often characterised 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 sub-optimal. Experimental field releases provide valuable information about the conditions that favour 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 timeframe 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* to Australia. We outline the development 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.
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* King & Robinson 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*, was established at one site in southern KwaZulu-Natal province in the early 2000s and has spread northwards into Mpumalanga province and the neighbouring 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 defences at four 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 introductory country means preservation of defence resources for an appropriate time.
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*, a Chrysomelid leaf-feeding beetle, introduced into New Zealand to control *Tradescantia fluminensis*, 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.
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.
A decade in biocontrol monitoring and release: a South African case study

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The Natural Resource Management programmes (NRM) of the Department of Environmental Affairs (DEA) has funded the research into biological control programmes over the past two decades. The biocontrol implementation programme was initiated in earnest in the year 2000 when the first implementation staff were employed by the Department. In the beginning the implementers reared, released and monitored the biocontrol agents themselves. The programme 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 programmes identified for established agents.
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 programme 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 programme 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.
Session 9-P24 - Post-release monitoring and evaluation

Evaluating establishment and impact of four biological control agents on *Parthenium hysterophorus* in South Africa

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*Parthenium hysterophorus* (Asteraceae: Heliantheae; parthenium) is a serious terrestrial invader globally, negatively impacting on 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 programme. In South Africa, the winter rust fungus *Puccinia abrupta* var. *partheniicola* is present, although not deliberately introduced. The summer rust fungus (*Puccinia xanthii* var. *parthenii-hysterophorae* (Pucciniales: Pucciniaceae) and three insect agents (defoliating *Zygogramma bicolorata* (Coleoptera: Chrysomelidae), stem-boring *Listronotus setosipennis* (Coleoptera: Curculionidae) and seed-feeding *Smicronyx lutulentus* (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. *ithacaee* (Lepidoptera: Sesiidae) and the stem-galler *Epiblema strenuana* (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.
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*, 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* (biotype “stricta”), commonly known as cochineal, has been 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 personal on many aspects such as evaluation, release process, 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 plants that are free of insects. This may continue for a few years. A local grass, *Cenchrus ciliaris*, will be used to protect the soil against possible erosion. The grass will, at the same time, also 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-P1 - Integrated Weed Management and restoration

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, out-competing them for pollinators, and altering native soil fungi and invertebrate communities. In 2006 CABI launched a biological control programme for *I. glandulifera*, and identified a rust fungus *Puccinia komarovii* var. *glanduliferae* 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. Here I will present research that builds on the biocontrol programme 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.
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* and *Smicronyx lutulentus*) 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.
Session 10-P3 - Integrated Weed Management and restoration

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 United States, while the native range of medusahead is 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 the current study is to compare the endophytes of medusahead in the native range to endophytes of medusahead in the introduced range. The results of culture-dependent and culture-independent approaches will be presented.