

SESSION 4: TARGET AND AGENT SELECTION

Biological Control of *Senecio madagascariensis* (fireweed) in Australia – a Long-Shot Target Driven by Community Support and Political Will

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Abstract

Fireweed (*Senecio madagascariensis* Poir.) biological control has a chequered history in Australia with little to show after 20 plus years. Plagued by local impacts, sporadic funding, a poor understanding of its genetics and its origins, and several almost genetically compatible native species, the fireweed biological control program has been faced with numerous hurdles. Hope has risen again, however, in recent years through the staunch support of a very proactive team of local stakeholders and their good fortune of finding themselves in a key electorate. The Australian Department of Agriculture, Fisheries and Forestry has recently funded an extendable two year project for exploration in the undisputed native range of fireweed in South Africa and a detailed search for agents that are deemed to be both effective and unable to attack closely related Australian *Senecio* species. This will be a tall order, but nevertheless is essential to conclude once and for all whether biological control has the potential to reduce the negative effects of the plant in south eastern Australian grazing and dairy country.

Introduction

Fireweed (*Senecio madagascariensis* Poir.) is a toxic, short-lived, perennial, temperate to subtropical climate pasture weed of South African origin that has established and spread in Australia, the USA (Hawaii), Japan, Brazil, Argentina, Venezuela, Columbia and Uruguay (Sindel et al., 1998). It is the focus of a biological control program in Australia (Julien et al., 2012; Radford, 1997) and

Hawaii (Ramadan et al., 2010). The plant contains genotoxic carcinogens in the form of pyrrolizidine alkaloids which cause cumulative chronic liver damage and fatality, especially in monogastric livestock like horses (Sindel et al., 1998; Bega Valley Fireweed Association, 2008). Such toxins can enter the human food chain through nectar incorporated into honey or via dairy products from animals grazing on infested land. The estimated control costs averaged around \$9000 per farm per annum

in Australia, and pasture productivity and profits on infested land were reduced by between 15-50% (Bega Valley Fireweed Association, 2008). Owners of infested land also are at risk of suffering from reduced morale and stress-associated social costs via; a) perception of declining property values, b) increased potential for neighbourhood conflicts, c) regulatory obligations around weed control, d) animal health issues and costs, and e) longer-term business viability and succession issues. There also remains significant debate about whether and when the weed can be controlled using traditional pasture management approaches, such as herbicides and controlled grazing. A high number of land owners, including commercial grazing businesses, have found such interventions to be impractical and the price of chemicals too costly.

In Australia, fireweed is spreading from a core coastal strip in the south-east to the north and to more upland and inland areas, and has certainly not yet spread to all suitable regions of Australia, being still largely absent from the states of Victoria, Tasmania, South Australia and Western Australia (Sindel et al., 2008). As a target for weed biological control, fireweed has many factors in its favour. Its taxonomy and native range are now well understood, being part of a complex of *Senecio* species in the KwaZulu-Natal region of South Africa (Radford et al., 2000; Lafuma et al., 2003). It exhibits much lower abundance in its native range, where competition from native perennial grasses in summer, largely confining the plant to isolated pockets in disturbed fields, dunes and along roadsides (A. Sheppard, pers. obs.). While a community of more than 30 natural enemies can be found on fireweed in Australia (Holtkamp and Hosking, 1993), none of these are very damaging and none of the natural enemies from the native range are already present in Australia, except for a rust fungus that appears to be of Australian origin anyway (Morin et al., 2009). Various other weeds of similar biology and impacts to fireweed have been successfully controlled in Australia using classical biological control, particularly Paterson's curse (*Echium plantagineum* L.) and ragwort (*Jacobaea vulgaris* Gaertn.) (Julien et al., 2012). Finally fireweed, as a target for biological control, has very strong community support in both Australia (Sindel et al., 2011) and Hawaii (M. Ramadan, pers. obs.) which has opened up new opportunities for

international collaboration on this target weed.

Community support in Australia comes from an active landholder group in Bega, in south-east New South Wales (the Bega Valley Fireweed Association; www.fireweed.org.au), which is fortunate to have found itself in a key electorate at both the state and federal level. The federal Member of Parliament for this electorate has since become the Parliamentary Secretary for Agriculture, and has been highly supportive of the fireweed issue, which has led to fireweed biological control gaining funding in the last few years and to the recent successful nomination of fireweed as a Weed of National Significance in Australia.

What makes this plant a difficult target for weed biological control in Australia is the taxonomic close proximity of fireweed to a group of Australian native species in the *Senecio pinnatifolius* (= *S. lautus*) group (Scott et al., 1998; Pelser et al., 2007). Hybridization can occur between these native species and fireweed, even if the progeny are sterile (Prentis et al., 2007). Any natural enemies selected as potential biological control agents for Australia therefore need to be monospecific to *S. madagascariensis*.

Biological control history

Fireweed was declared a target for weed biological control by the Australian Weeds Committee in 1991. At the time, the taxonomy of the target was poorly understood and all the earlier surveys for natural enemies were undertaken in south and eastern Madagascar (Marohasy 1989). These early surveys recorded two moths; a flower-feeding pyralid (*Phycitodes* sp.) and a stem-boring tortricid (*Lobesia* sp.), which were brought into quarantine in Australia. Both, however, failed to be specific enough for release in Australia (McFadyen and Sparks, 1996). One 18-day visit was also made to KwaZulu-Natal province in South Africa as part of these activities in 1991 and identified 11 species of insects feeding on the plant (Marohasy unpublished report - see Table 1 below).

In 2002, Meat and Livestock Australia funded a short project to look at the potential of strains of rust on fireweed in South Africa as potential biological control agents. One such rust, *Puccinia lagenophorae* Cooke, is considered to be native to Australia, but is

Table 1. Natural enemies found on *S. madagascariensis* in South Africa (KwaZulu-Natal) and Swaziland, South Africa.

Nature of attack	Natural enemy type (family)	Scientific name (current knowledge)
Stem-borers	Weevil (Curculionidae)	<i>Gasteroclisus tricostalis</i> (Thunberg)
	Moth (Tortricidae)	<i>Lobesia</i> sp.
	Fly (Tephritidae)	<i>Coelopacidia strigata</i> Bezzi
	Fly (Agromyzidae)	<i>Melanagromyza</i> spp.
Flower feeder	Moth (Pyralidae)	<i>Homeosoma stenotea</i> Hampson
	Moth (Pyralidae)	<i>Phycitodes</i> sp.
	Moth (Pterophoridae)	undetermined
	Fly (Tephritidae)	<i>Cryptophorellia peringueyi</i> (Bezzi)
	Fly (Tephritidae)	<i>Trupanea inscia</i> Munro
	Fly (Tephritidae)	<i>Sphenella austrina</i> Munro
	Fly (Tephritidae)	<i>Telaletes ochraceus</i> (Loew)
	Fly (Tephritidae) small	undetermined
	Fly (Cecidomyiidae)	undetermined
Root feeders	Weevil (Curculionidae)	<i>Proictes longehirtus</i> Fairemaire
	Leaf beetle (Chrysomelidae)	undetermined
Sap suckers	Leaf hopper (Tettigometridae)	<i>Hilda elegantula</i> Gerstaecker
	Plant bug (Miridae)	<i>Ellenia</i> sp.?
	Lace bug (Tingidae)	undetermined
Plant pathogens	Yellow rust (Pucciniaceae)	<i>Puccinia lagenophorae</i> Cooke & hybrids
	White rust (Albuginaceae)	<i>Albugo</i> nr <i>tragopogonis</i>
	Flower smut (Ustilaginaceae)	<i>Ustilago</i> sp.
	Stem blotch fungus	undetermined

cosmopolitan in distribution on a range of *Senecio* species. It is also found in both Australia and South Africa on *S. madagascariensis*. Surveys in KwaZulu-Natal, South Africa were used to collect rust samples at 14 of 25 sites and accessions were tested from samples from eight of these sites (Morin et al., 2009). Taxonomic and genetic sequencing evidence confirmed that these accessions were a mixture of both *P. lagenophorae* and interspecific hybrids with *P. lagenophorae* as one of the parents. These rust accessions were found to attack Australian fireweed plants and some failed to develop on the native species tested, *S. lautus* subsp. *lanceolatus*, in laboratory trials. However, all the South African accessions were either comparable to Australian *P.*

lagenophorae rust isolates, or less virulent, in their response to Australian fireweed plants (Morin et al., 2009).

Biological control – future activities

The scientific case for a continuing biological control program against fireweed in Australia has been quite challenging, driven by the need for both monospecific and effective agents. Although more than 50–85% of national weed biological control programs have led to significant or permanent weed control (Myers and Bazely, 2003), these constraints and the high associated chance of non-target impacts,

suggests a very much lower chance of success. To be conservative, we have presented to the stakeholders that the chances of success might be in the order of 20%. The arguments in favour are as follows:

- a. There have been at least six weed biological control programs in Australia where there are native species in the same genus as the target weed. At least two of these have been successful to some degree; i.e. the programs against *Rumex pulcher* L. and *Jacobaea vulgaris* Gaertn..
- b. Monospecific agents are not hard to find in weed biological control programs. Most targets that are widespread in their native range have at least some natural enemies that are monospecific. Also most plant pathogens used as biological control agents are monospecific and indeed many are weed-genotype or biotype-specific pathotypes. Researchers have also found biotype-specific arthropod agents (e.g. two strains of *Dactylopius opuntiae* (Cockerell) on *Opuntia ficus-indica* (L.) Mill. or *O. stricta* (Haw.) Haw. in South Africa).
- c. No in-depth studies have been completed in the native range of fireweed in South Africa.

Based on these arguments and on a workshop at the first National Fireweed Conference in Bega in 2008, the continuation of the fireweed biological control program was supported by the stakeholders and the federal Department of Agriculture, Fisheries and Forestry. Funding was made available for this in 2009.

The re-started biological control program is now based in South Africa at the University of KwaZulu-Natal and will focus on studies of the following issues:

- a. The community and population ecology of fireweed in its native range pastures, particularly the roles of inter- and intra-specific

plant competition, climate and soil type.

- b. Taxonomic and genetic variation in the target, through surveying the morphological variation of fireweed in KwaZulu-Natal and measuring the associated local genetic variation.
- c. The natural enemy community across sympatric *Senecio* species in South Africa, looking specifically at host use across the different *Senecio* species and natural enemy distribution and abundance.
- d. Experimental manipulations of both fireweed and the natural enemy densities to identify agents that are resource limited and capable of causing high levels of impact. Through this, fireweed growth and reproductive effort, with and without natural enemies, will be measured.

Based on surveys carried out to date and historical surveys made by Marohasy in 1991 and by Mohsen Ramadan on more recent trips to South Africa for Hawaii, Table 1 shows the current list of known natural enemies.

Conclusion

Fireweed biological control in Australia was started nearly 20 years ago, but the amount of investment and effort has been quite limited to date. Work has consisted of a few surveys to a poorly defined native range and the importation and basic testing of two moths and one pathogen, of which only the latter was from the true native range. Two years ago, a strong local support group – the Bega Valley Fireweed Association – organised a National Conference on fireweed and were lucky enough to be heard and find themselves with some political advantage at the 2007 federal election. This has led to some renewed federal funding for fireweed biological control, despite the fact that the prospects for success are seriously constrained by the very close taxonomic affinity of fireweed to a group of native

Senecio species. Scientists argued that the chances of success may be as little as 20%, but a lack of any in-depth studies in the native range of the weed suggested that continuation of the program may still be worthwhile. The program has, therefore, recently moved to the plant's native range in South Africa to seek effective monospecific biological control agents for fireweed.

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Prospects for the Biological Control of Tutsan (*Hypericum androsaemum* L.) in New Zealand

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Abstract

The feasibility for biological control of tutsan, *Hypericum androsaemum* L., in New Zealand (NZ) was assessed. Conventional control methods are impractical and tutsan is not valued by any groups of society. It therefore makes a potentially good candidate for biological control. However, the lack of information about potential agents and the existence of four indigenous *Hypericum* spp. in NZ, including two endemics, are likely to prove challenging.

Introduction

Tutsan, *Hypericum androsaemum* L., is an evergreen or semi-evergreen shrub (up to 1.5 m) of the family Clusiaceae (alternatively Guttiferae). In New Zealand (NZ) tutsan has become a common weed in higher rainfall areas, growing in open forest, forest margins, scrub, waste places and garden surroundings. Tutsan is shade tolerant, unpalatable to stock, and tends to infest areas in which mechanical and/or chemical control options are impractical.

Tutsan's extensive native range includes Europe, Caucasia, Turkmenistan, Iran, Syria, Turkey, north-west Africa and temperate Asia (Davis, 1967; USDA ARS, 2009). The naturalised range includes Australia, NZ, Southern Africa, continental Chile and possibly part of the US (Thomas, 2007).

A climate similar to that of southern France, with average annual temperature of 13°C and annual rainfall of 910 mm, appears optimal for tutsan; however, tutsan can tolerate a wide temperature range (Van Der Veken et al., 2004). It is also tolerant of various soil types and acidity levels (e.g., Hutchinson, 1967). Tutsan is a shade-tolerant species and, in its native range is a component of mature forests (Olano et al., 2002). These findings suggest that large parts of NZ could prove to be

suitable habitat for this species.

Tutsan is a garden escapee in NZ (Healy, 1972) and was first recorded as naturalised here in 1870 (Owen, 1997). The plant is well established throughout NZ (North and South Islands, Stewart Is, Chatham Islands, and Campbell Islands) (Sykes, 1982). It is currently of greatest concern in the Taumarunui District in the North Island of NZ.

In NZ tutsan is considered a major pest in a range of bioclimatic zones from warm- to cool-temperate (ranging from latitude 31° to 50° S, maritime climate, below 600 m with average annual temperatures ranging between 12.5 and 22.5°C). Plant community types identified as prone to invasion by tutsan include shrublands, tussock grasslands and bare land. Tutsan can impact on the structure (i.e., on the dominant growth form of forest, shrubland etc.), or have a "major effect on many native species or on the composition or density of dominant species" (Owen, 1997).

A 1995 survey of weeds of conservation land determined its national distribution status as: "established, widely distributed throughout NZ and extending its range into new habitats and areas". Tutsan is a problem in regenerating forest (Sullivan et al., 2007). Its biological success is mainly attributed to the high seeding ability per plant, seedbank persistence of >5 years, and its tolerance of semi-

shade conditions, hot or cold temperatures, high to moderate rainfall, damage and grazing. In addition, its fleshy fruits are effectively dispersed by birds, and possibly also by goats, possums, and soil and water movement (Whatman, 1967; Owen, 1997). Classical biological control is therefore a desirable option.

In NZ there are four indigenous *Hypericum* spp. (Webb et al., 1988; Heenan, 2008, 2011):

- *Hypericum involutum* (Labill.) Choisy, native to NZ, Australia, Tasmania and New Caledonia
- *Hypericum pusillum* Choisy, native to NZ, Australia and Tasmania.
- *Hypericum rubicundulum* Heenan, endemic to the South Island of NZ (and known from one locality in the North Island) and considered naturally uncommon
- *Hypericum minutiflorum* Heenan, endemic to NZ, restricted to the central North Island Volcanic Plateau and considered nationally critically endangered

A high degree of host specificity would be required of any agent introduced against tutsan, if we were to avoid significant non-target risks to the indigenous *Hypericum* species. There are no other indigenous representatives in the Clusiaceae family in NZ.

History of biological control of tutsan in NZ

Biological control of tutsan in NZ was attempted opportunistically in the late 1940s, using a St John's wort biological control agent *Chrysolina hyperici* (Forst.) (Julien and Griffiths, 1998). Adult beetles were observed feeding on tutsan, and subsequently, an attempt was made to release *C. hyperici* in areas where tutsan was considered a problem. Beetles released on tutsan between 1947 and 1950 all failed to establish on the weed (Miller, 1970).

Early instar larvae of both the lesser and greater St. John's wort beetles, *C. hyperici* and *Chrysolina quadrigemina* (Suffrian) suffered high mortality when offered tutsan in recent no-choice laboratory feeding experiments, and the survivors' development was severely impeded (Groenteman et al., 2011),

confirming that tutsan is a sub-optimal host for the beetles, and explaining why beetles released on tutsan in the late 1940s quickly died out.

History of biological control of tutsan worldwide

The state of Victoria, Australia, initiated a biological control programme against tutsan in the early 1990s. This programme was discontinued at an early stage, prior to any surveys in the native range of the weed being carried out, after the rust fungus *Melampsora hypericorum* (De Candolle) Winter was discovered to have self-introduced there. While the use of *M. hypericorum* as a biological control agent has generated mixed results, the fungus has largely successfully controlled tutsan in Victoria (Bruzese and Pascoe, 1992; McLaren et al., 1997; Casonato et al., 1999; David McLaren pers. comm.).

Objectives

Given the difficulties to control tutsan using conventional methods, and given it is rapidly expanding its range, classical biological control emerges as an attractive option. The objectives of the current study were, therefore, a) to review the literature to identify potential biocontrol agents for tutsan and assess the feasibility of their release in NZ and, b) to assess the prospects of achieving successful biological control of tutsan in NZ.

Methods

Identifying fungal pathogens of tutsan

The information was obtained by searching online databases and Internet sites. Online databases searched were:

USDA Fungus-host database or FDSM (which includes most NZ plant disease records): http://nt.ars-grin.gov/fungal_databases/fungushost/FungusHost.cfm

Fungal Records Database of Britain and Ireland or FRDBI (Cooper, 2006): <http://www.fieldmycology.net/FRDBI/assoc.asp>

IMI fungal herbarium (CABI Bioscience, 2006) <http://194.203.77.76/herbIMI/index.htm>

NZ fungi and bacteria database or NZFUNGI

(Landcare Research, 2009): <http://nzfungi.landcareresearch.co.nz/html/mycology.asp>; this database was also used to determine which species were already present in NZ

In addition, CAB abstracts, Current Contents, ISI Proceedings, Web of Science, Agricola, Science Direct, Google and Google Scholar were searched, using the terms “*Hypericum androsaemum* or tutsan” and sub-searched using the terms “pathogen* or fung*”. Once a list had been created, further information about each fungus was sought in the published literature as well as in the following online databases:

Index Fungorum database (Index Fungorum, 2004): <http://www.indexfungorum.org/Names/Names.asp>

Global Biodiversity Information Facility or GBIF (Global Biodiversity Information Facility, 2009): <http://data.gbif.org/species/>

Identifying arthropod biological control agents for tutsan

Unlike for fungal pathogens, comprehensive online databases for all arthropod herbivores do not exist. However, the following databases were searched:

For Lepidoptera, the Natural History Museum’s world listing (Natural History Museum London, 2007): <http://www.nhm.ac.uk/jdsml/research-curation/research/projects/hostplants/>

Database of Insects and Their Food Plants Biological Records Centre (UK) (Biological Records Centre (BRC), 2009) http://www.brc.ac.uk/dbif/Interpreting_foodplant_records.aspx

Plant-SyNZ™ <http://www.crop.cri.nz/home/plant-synz/database/hostplant.php>

In addition, CAB abstracts, Current Contents, ISI Proceedings, Web of Science, Agricola, Science Direct, Google and Google Scholar were searched using the terms “*Hypericum androsaemum* or tutsan” and sub-searched using the terms “invertebrate* or herbivor*”. Checklists of NZ fauna were referred to, to determine whether any of the species recorded feeding on/infecting tutsan already occurs in NZ.

Results

Extensive searches of the literature and online databases yielded very few records of organisms attacking tutsan. This could reflect scarcity of herbivores and pathogens attacking tutsan; but it could also reflect lack of interest in tutsan on behalf of entomologists and plant pathologists, and consequently a potential array of agents to discover. All but one of the organisms recorded from tutsan were not specific to this species (see also Groenteman, 2009).

Fungi

Only 10 species of fungi have been reported in association with tutsan (Table 1). One was an endophyte, which does not cause disease symptoms. Five others could not be considered either because their host range is too broad or they are unlikely to be sufficiently damaging.

Four other pathogens may hold some potential as biological control agents. The powdery mildew *Erysiphe hyperici* (Waller.) Fr. attacks various *Hypericum* species, and is troublesome for *H. perforatum* L. where the latter is cultivated for its medicinal values (e.g., Radaitienė et al., 2002). It may be worthwhile investigating whether a virulent tutsan-specific strain exists.

Another powdery mildew, *Leveillula guttiferarum* Golovin, has only been recorded from three *Hypericum* spp. That it has not been recorded from the highly studied *H. perforatum* suggests, perhaps, a relatively narrow host range. There is no information regarding the virulence of this pathogen and, its native range is not well matched to NZ climate.

The brown leaf spot *Diploceras hypericinum* (Ces.) Died. was recorded from tutsan in NZ and Japan, and in the Netherlands in the form of *Pestalotia hypericina* Ccs. It attacks other *Hypericum* species and can cause severe dieback in *H. perforatum*. The virulence of this pathogen to tutsan in NZ is not known, but could relatively easily be tested. In the Netherlands, conditions of nearly 100% relative humidity were necessary to create

infection on tutsan in the laboratory (Van Kesteren, 1963) so conditions for natural infection in the field might rarely be met. Developing this pathogen into a bioherbicide is an avenue that could potentially be explored to overcome this limitation; however, this is an expensive pathway, unlikely to be economically viable for tutsan.

Finally, the rust *M. hypericorum* was the most common species recorded from tutsan, including in NZ. *M. hypericorum* was first recorded in NZ in 1952 (Baker, 1955). It is unclear how the fungus has arrived here, and its effectiveness in controlling tutsan is variable (Baker, 1955; Whatman, 1967).

M. hypericorum is also found in Australia, first recorded in Victoria in 1991. By 1992 it had already shown phenomenal potential as a biocontrol agent of tutsan (Bruzzese and Pascoe, 1992). Once a very common and invasive weed in south-western Victoria, by 1997 tutsan was difficult to find in that region, resulting in “possibly the most spectacularly successful example of weed biocontrol ever witnessed in Victoria” (McLaren et al., 1997).

Further attempts to use the rust as a biocontrol agent had mixed results: genetic variation between tutsan populations suggested intrinsic resistance, and various rust isolates varied in virulence (Casonato et al., 1999).

The findings from Australia highlight the importance of compatibility between genotypes and strains of fungal pathogens and their weedy hosts, and suggest that as part of a biological control programme against tutsan in NZ it should be determined what strains of tutsan and *M. hypericorum* are present here and how they compare to those known from Australia. The hypothesis that observed variation in the impact of the rust against tutsan is attributed to genetic variability of the weed, the rust, or both should be examined. In addition, if rust strains from Australia are absent from NZ, their virulence against NZ tutsan should be tested.

Arthropods

Only nine species of insects have been recorded from tutsan, four of which can be immediately precluded as potential agents due the breadth of their host range (Table 2).

The remaining five insect species are oligophagous, but restricted to the genus *Hypericum*.

Four of these species are chrysomelid beetles, two of which, *Chrysolina quadrigemina* Suffrian and *Chrysolina hyperici* Forster, are well established in NZ and their performance on tutsan is poor. *Chrysolina varians* Schaller failed to establish in Australia and North America as a biological control agent against *H. perforatum* (Currie and Garthside, 1932; Currie and Fyfe, 1938; Coombs et al., 2004). San Vicente (2005) mentions tutsan and as host of *C. varians* in Spain, yet does not explicitly treat *H. perforatum* as a host. Whether the Spanish *C. varians* is a biotype adapted to tutsan is perhaps an avenue to pursue. Lastly, *Cryptocephalus moraei* L. thrives on *H. perforatum* but not on tutsan (Tillyard, 1927).

Concluding remarks

Available information about prospective biological control agents for tutsan is slim, and makes it difficult to assess the prospects of successful biological control at this time. However, it is clear that tutsan has never been the target of any extensive surveys, and it is possible that a suite of potentially useful agents would be discovered should such a survey take place.

The genus *Hypericum* has four indigenous representatives in NZ, therefore highly specific agents are likely to be required.

Opposition to biological control of tutsan is unlikely. It is not grown here for medicinal purposes, nor is it highly valued as a garden plant. It is highly unpalatable to stock and therefore not valued for fodder, nor is it valued for beekeeping.

In a significant part of its range in NZ, tutsan is a problem on terrain where mechanical and chemical control methods are impractical. Therefore, bioherbicides are not likely to be a practical (or economic) solution and are not recommended as an avenue of future research for this weed.

Acknowledgements

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Table 1. Fungi recorded on tutsan, *Hypericum androsaemum*, and their potential usefulness for biological control.

Phylum Order Family	Species (and other names) ¹	Range on <i>H. androsaemum</i> ²	Likely to be damaging?	Likely to be host specific? (and comments)	Found in New Zealand/ biocontrol potential?
Ascomycota Botryosphaerales Botryosphaeriaceae	<i>Guignardia endophyl- licola</i> Okane, Nakagiri & Tad. Ito	Japan	Endophytic. Does not cause disease symp- toms in <i>H. androsae- mum</i>	No. Recorded from a wide range of hosts from various plant families	Not yet re- corded here / No
Ascomycota Erysiphales Erysiphaceae	<i>Leveillula taurica</i> (Lév.) G. Arnaud (= <i>Erysiphe taurica</i>)	Iran	Insufficient informa- tion	No. Attacks multiple genera in multiple fami- lies	Yes, exotic / No
Ascomycota Erysiphales Erysiphaceae	<i>Leveillula guttiferarum</i> Golovin	Iran	Insufficient informa- tion	Possibly genus specific. Two records in FDSM ³ : one from <i>H. androsaemum</i> , one from <i>H. helian- themoides</i> ; one record in IF ⁴ from <i>H. scabrum</i>	No / ?
Basidiomycota Pucciniales Melampsoraceae	<i>Melampsora hypericorum</i> (DC.) J. Schröt.	UK, Ireland, Scotland, Canada (BC), Australia, Bulgaria, Japan, New Zealand, USSR	Yes. Has been highly successful in control- ling <i>H. androsaemum</i> in Victoria, Australia. Highly damaging in parts of New Zealand.	Yes, highly specific (to <i>H. androsaemum</i> strains). Note that the species had been recorded from various other <i>Hypericum</i> spp., including <i>H. perforatum</i> ; however, the <i>H. an- drosaemum</i> strain failed to infect <i>H. perforatum</i> in Australia	Yes, since early 1950s / Offers partial con- trol in some areas
Chromista Oomycota Pythiales Pythiaceae	<i>Phytophthora cinnamomi</i> Rands	Japan	Yes, a highly aggres- sive species	No. Attacks many unrelated woody plant spe- cies. Not classified strictly as a fungus anymore, due to a mobile life stage (akin to brown algae). Highly invasive (classified as a 'Key process threatening biodiversity in Australia')	Yes / No!

Phylum Order Family	Species (and other names) ¹	Range on <i>H. androsaemum</i> ²	Likely to be damaging?	Likely to be host specific? (and comments)	Found in New Zealand/ biocontrol potential?
Ascomycota Erysiphales Erysiphaceae	<i>Erysiphe hyperici</i> (Wallr.) S. Blumer	England, Scotland	Yes, considered a troublesome disease of <i>H. perforatum</i> and gets sprayed with fungicides where the latter is cultivated for its medicinal values. A powdery mildew	Yes, at the genus level. FRDBI ⁵ has 8 records from <i>H. androsaemum</i> but 39 from <i>H. perforatum</i> and additional 91 from various other <i>Hypericum</i> spp. FDSM has 149 records from various <i>Hypericum</i> spp., none from <i>H. androsaemum</i>	No / Possibly, if a specific strain is found
Ascomycota Xylariales ⁶	<i>Melomastia mastoidea</i> (Fr.) J. Schröt.	Ireland	No, saprobe ⁷	No, associated with plants from various families	No / No
Ascomycota Xylariales Amphisphaeriaceae	<i>Diploceras hypericinum</i> (Ces.) Died. <i>Pestalotia hypericina</i> Ces. <i>Hyaloceras hypericinum</i> (Ces.) Sacc. <i>Seimatosporium hypericinum</i> (Ces.) B. Sutton	Netherlands (as <i>P. hypericina</i>), New Zealand, Japan	Causes leaf blight and severe stem dieback in <i>H. perforatum</i> . Not virulent to <i>H. androsaemum</i> - requires 100% RH post-inoculation to produce symptoms (in the form of <i>Pestalotia hypericina</i>). Brown leaf spot	Attacks other <i>Hypericum</i> spp. Had been collected from <i>Fragaria</i> (strawberry) plants in Canada (as <i>P. hypericina</i>)	Found in New Zealand as <i>D. hypericinum</i>
Basidiomycota Hymenochaetales Hymenochaetaceae	<i>Hymenochaete cinnamomea</i> (Pers.) Bres.	New Zealand	Probably not. Wood rot (attacks dead and decaying wood, but also live wood). Not likely to be very damaging.	No. Attacks hosts from multiple families.	Yes/No

Phylum Order Family	Species (and other names) ¹	Range on <i>H. androsaemum</i> ²	Likely to be damaging?	Likely to be host specific? (and comments)	Found in New Zealand/ biocontrol potential?
Ascomycota Hypocreales Monoliniaaceae	<i>Verticillium</i> sp. Needs [stat. anam.]	New Zealand	Insufficient information. Plant-pathogenic <i>Verticillium</i> spp. exists in various strains with variation in virulence and host range. They are known to cause severe wilting in susceptible hosts, but no symptoms in tolerant hosts.	Possibly not. <i>Verticillium</i> spp. attack woody hosts of various plant families. A number of <i>Verticillium</i> spp. are listed on the Unwanted Organism register. http://www1.maf.govt.nz/uor/searchframe.htm	Yes/No

¹Many fungi have more than one Latin name because they can produce more than one type of spore. The name given when they are producing 'sexual' spores is called the teleomorph, whereas the stage producing 'asexual' spores is called the anamorph. The two stages often look completely different. Fungi are classified according to their 'teleomorph' name, unless the 'anamorph' is the only form known. So, Table 1 gives the taxonomy of the teleomorph, but column 2 uses whichever name/names were recorded when the fungus was found on *H. androsaemum*. If a fungus was listed under an out-of-date name (synonym) this is also stated in column 2.

²Only the places where the organism was found associated with *H. androsaemum* are listed here. It may also be found elsewhere on other hosts.

³FDMS = USDA Fungus-host database at <http://nt.ars-grin.gov/fungal/databases/fungushost/FungusHost.cfm>

⁴IF = Index Fungorum, World database of fungal names at <http://www.indexfungorum.org/Names/Names.asp>

⁵FRDBI = the Fungal Records Database of Britain and Ireland (FRDBI) at <http://194.203.77.76/fieldmycology/FRDBI/FRDBI.asp>

⁶Insertae sedis = of uncertain taxonomic position within a higher taxonomic order (e.g. Phylum known, but order within that phylum uncertain).

⁷Saprobe: An organism using dead organic material as food and commonly causing its decay (Kirk et al. 2001). Unlikely to cause disease and therefore probably insufficiently damaging to be useful for biocontrol.

Table 2. Records of invertebrates feeding on tutsan *Hypericum androsaemum*.

Order and Family	Species	Type of organism	Range	Likely to be sufficiently host specific?
HEMIPTERA				
Aleyrodidae	<i>Aleyrodes fragariae</i> Walker (=loniceriae)	Whitefly	Europe, Northern Asia, Mediterranean Basin	No. Polyphagous. A pest of strawberry.
HETEROPTERA				
Lygaeidae	<i>Kleidocerys truncatulus</i> ericae (Walker)	Ground bug	Isle of Wight (UK), Dutch West Frisian Isles (as <i>K. ericae</i>)	No. Feeds on <i>Erica</i> spp. and <i>Calluna</i> spp.
LEPIDOPTERA				
Nepticulidae	<i>Ectoedemia</i> (= <i>Fomoria</i>) <i>septembrella</i> (Stainton)	Leaf mining moth	Palaeartic	Possibly not. Feeds on various <i>Hypericum</i> spp. Possibly more common on <i>H. perforatum</i> .
Tortricidae	<i>Ctenopseustis herana</i> Felder & Rogenhofer and <i>C. obliquana</i> Walker	Leafrollers	New Zealand (endemic), Australia (introduced)	No. Highly polyphagous. Pests of many crops
	<i>Planotortrix excessana</i> Walker and P. Octo Dugdale			
COLEOPTERA				
Chrysomelidae	<i>Chrysolina varians</i> (Schaller)	Leaf beetle	Europe (from Spain to West Siberia)	Possibly not. Feeds on various <i>Hypericum</i> spp., and more common on <i>H. perforatum</i> . Possibility of an <i>H. androsaemum</i> biotype in Spain?
	<i>Cryptocephalus moraei</i> (L.)	Leaf beetle	Europe	Possibly not. Feeds on various <i>Hypericum</i> spp. Does not thrive on <i>H. androsaemum</i>
	<i>Chrysolina quadrigemina</i> (Suffrian)	Leaf beetle	From North Africa to Denmark. Introduced to Australia, New Zealand and North America	No. Prefers <i>H. perforatum</i> . Performs poorly on <i>H. androsaemum</i> . Reported feeding on <i>H. involutum</i> (indigenous to Australia and New Zealand) in Australia
	<i>Chrysolina hyperici</i> (Forster)	Leaf beetle	Native to northern and central Europe and western Asia. Introduced to Australia, New Zealand and North America.	No. Prefers <i>H. perforatum</i> . Performs poorly on <i>H. androsaemum</i>

The Use of *Ascochyta caulina* Phytotoxins for the Control of Common Ragweed

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Abstract

Common ragweed, *Ambrosia artemisiifolia* Bess. is an alien weed of North American origin that became invasive in Europe. Although the main concern regarding this plant is its impact on human health, due to abundant production and easy spread of its highly allergenic pollen, *A. artemisiifolia* is also increasingly becoming a major weed in agriculture, being very competitive with crops and difficult to manage. Previous research had led to the identification of a mixture of three toxins from the culture filtrates of *Ascochyta caulina* (P. Karst.) Aa & Kestern, a fungal pathogen of *Chenopodium album* L., having high toxicity against both host and non-host plant leaves, with lack of antibiotic and zootoxic activities. Recently, a two-year research project named ECOVIA has been approved and financially supported by the Regional Governorate of Lombardy (Italy). The main aim of the project is to develop the technologies to obtain a natural herbicide based on the bioactive toxins produced by *A. caulina*, and to study the possibility of its practical use. Among the selected target weeds, *A. artemisiifolia* showed an evident response to the toxin application, probably due to the presence of trichomes on the leaves that allow a quick absorption of the metabolites, with fast appearance of leaf necrosis and plant desiccation. Greenhouse bioassays as well as open field experiments clearly indicate the lethal effects on the young ragweed plants.

Introduction

Common ragweed, *Ambrosia artemisiifolia* Bess. is an alien weed of North American origin that became invasive in Europe. Although the main concern regarding this plant is its impact on human health, due to abundant production and easy spread of its highly allergenic pollen, *A. artemisiifolia* is also increasingly becoming a major weed in agriculture, being very competitive with crops and difficult to manage.

Previous research had led to identify a mixture of three toxins from the culture filtrates of *Ascochyta caulina* (P. Karst.) Aa & Kestern, a fungal pathogen of *Chenopodium album* L., showing a wide range of phytotoxicity against different weed species.

Recently, a two-year research project named the ECOVIA has been approved and financially supported by the Regional Governorate of Lombardy (Italy). The main aim of the project is to develop technologies for obtaining a natural herbicide based on the bioactive toxins produced by *A. caulina*, and to study the potential of its practical use.

During 2010 and 2011, in the framework of the ECOVIA research project, among the 16 weed species we tested, *A. artemisiifolia* was evaluated both in laboratory and semi-field conditions.

Materials and Methods

Toxin production

The fungus, *A. caulina*, a biological control agent of the weed *C. album*, was grown on a liquid defined mineral medium (named M1-D) for 4 weeks in static conditions as previously described (Evidente et al., 1998). Toxins were purified as described (Evidente et al., 1998; 2000) and obtained as a pure mixture containing three main phytotoxic compounds (i.e., ascaulitoxin, its aglycone, and trans-4-aminoproline; Fig. 1).

Laboratory and screen house bioassays

Young potted ragweed plantlets were sprayed uniformly with an 8 mg/ml water solution of the purified fungal toxins by uniform nebulisation of leaf surface. A relative humidity of 100% was maintained for 24 hours after each application. The experiment was successively repeated with a lower concentration of toxins solution (2 mg/ml) on cut leaves in Petri dishes and on potted plantlets.

In order to enhance the efficacy of the toxins, further bioassays were performed by adding a wetting agent to the toxin solution. The following four commercial products, which appear to best fit our needs, were used at the labelled concentration: Biopower®, Adigor®, Etravon® and Codacide®.

Results

A. artemisiifolia showed an evident response to the toxin application. In particular, the 8 mg/ml solution showed a high herbicide effect on young plantlets with 100% leaf damage within 3-5 days after the application (Fig. 2). The sensitivity of the target

weed is likely due to the presence of high numbers of trichomes on the leaves (Grangeot et al. 2006) that allow a quick absorption of the metabolites, with the fast appearance of leaf necrosis and plant desiccation. We obtained a significant damage with cut leaves treated with the 2 mg/ml solution in Petri dishes (Fig. 3). The same solution (2 mg/ml) caused a leaf damage of less than 50% when sprayed on the plantlets, but its phytotoxicity has been significantly improved using the toxin in combination with the Codacide®.

Results presented here show promise that these toxins can be effective in controlling common ragweed. Bioassays are in progress to further develop methods of application of the toxin mixture in order to achieve higher efficacy as a natural herbicide for the control of common ragweed.

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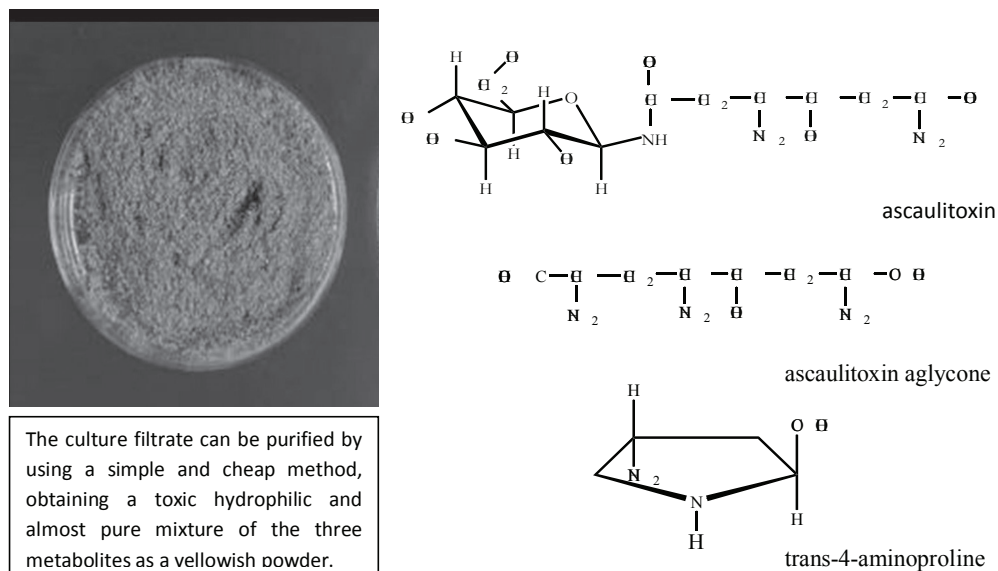


Figure 1. The toxin mixture is a yellowish powder containing three compounds: a caulitoxin, ascaulitoxin aglycone, and trans-4-aminoproline.

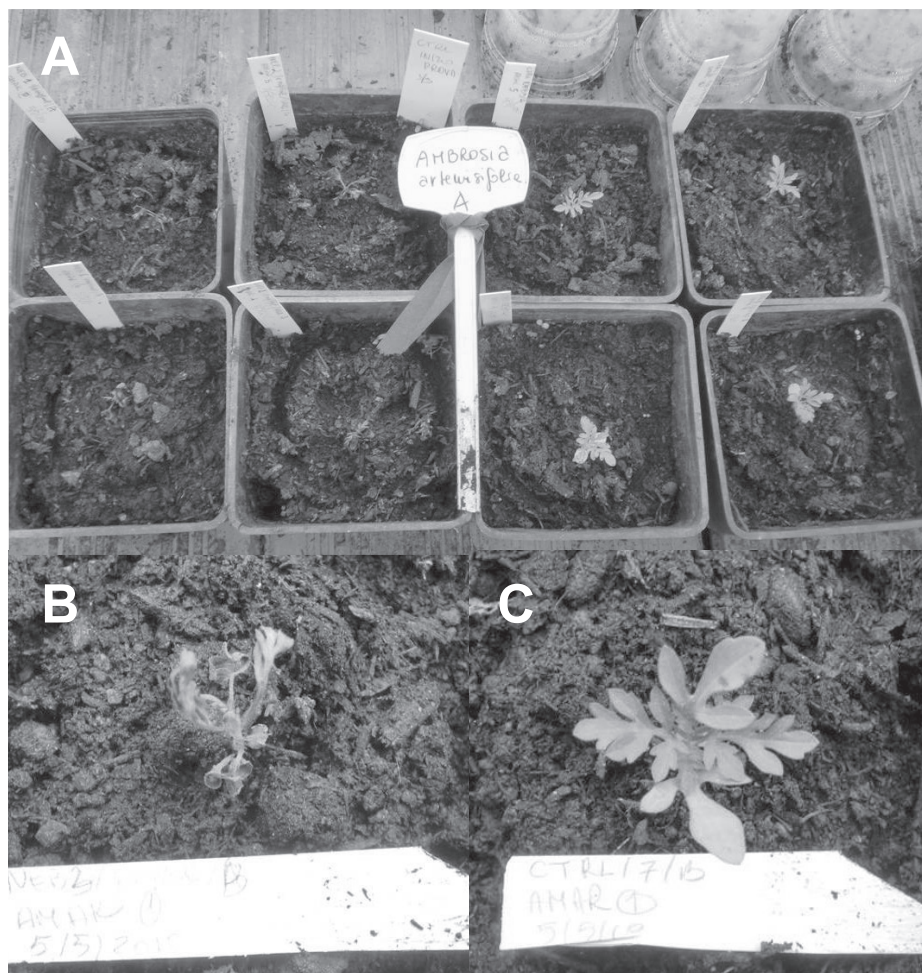


Figure 2. Phytotoxicity of an 8 mg/ml toxin solution on ragweed plantlets: A) treated plants (left) and control plants (right); B) treated plant, C) control plant.



Figure 3. Effects of a 2 mg/ml toxin solution on ragweed cut leaves (control - left, treated - right).

Biological Control of *Hygrophila*: Foreign Exploration for Candidate Natural Enemies

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Abstract

Hygrophila, *Hygrophila polysperma* (Roxb.) T. Anders (Acanthaceae) is an invasive aquatic weed of lotic habitats in Florida, USA. This rooted submerged or emergent plant is typically found in flowing fresh water channels and structured shorelines. *Hygrophila* forms dense vegetative stands that occupy the entire water column, interfering with navigation, irrigation and flood control activities. It is listed as a Federal Noxious Weed and a Florida Exotic Pest Plant Council Category I invasive species. A visible increase in the number of water bodies invaded by *hygrophila* since 1990 suggested that current methods employed to control this weed are inadequate. A previous study confirmed that *hygrophila* is a good candidate for classical biological control. However, little information was available on natural enemies affecting *hygrophila* in its native range. Exploratory field surveys were conducted in a range of habitats in India and Bangladesh during 2008 and 2009. In total, 41 sites were surveyed, including 28 sites in the states of West Bengal and Assam, India and 13 sites in Mymensingh, Bangladesh. The geolocation and altitude of each survey site were recorded. Several collection techniques, e.g. hand picking, Berlese funnel extraction, as well as sweep and clip vegetation sampling, were used to collect natural enemies. A number of insects, including two caterpillars (*Precis alamana* L., Nymphalidae and an unidentified noctuid moth, Lepidoptera) that defoliate emerged plants, an aquatic caterpillar (*Parapoynx bilinealis* Snellen, Crambidae, Lepidoptera) feeding on submerged *hygrophila*, and a leaf mining beetle (*Trachys* sp., Buprestidae, Coleoptera) were collected during these surveys. In addition, a very damaging aecial rust fungus was collected.

Introduction

Florida historically has been vulnerable to invasion by exotic animals and plants. The aquatic weed *hygrophila*, *Hygrophila polysperma* (Roxb.) T. Anderson (Acanthaceae), is one such plant. It is listed as a federal noxious weed (USDA, 2006),

and a Florida Exotic Pest Plant Council Category I invasive species (FLEPPC, 2009). This aquatic plant escaped cultivation by the aquarium trade and is now causing serious problems by displacing native aquatic vegetation (Spencer and Bowes, 1985), primarily in lotic habitats (Spencer and Bowes, 1985; Sutton, 1995). *Hygrophila* is believed

to be native broadly to the southeastern Asiatic mainland (Les and Wunderlin, 1981; Spencer and Bowes, 1985; Schmitz, 1990; Angerstein and Lemke, 1994). This herbaceous perennial weed is capable of forming dense stands and can occupy the entire water column, causing disruption in irrigation and flood control systems (Schmitz and Nall, 1984; Sutton, 1995). Conventional control techniques do not provide effective control of this invasive weed. Mechanical methods may be useful for removing the floating mats, but harvesting increases the number of viable stem fragments that can be transported to other areas where they can infest new water bodies (Sutton, 1995). Established hygrophila infestations are also difficult to control with registered aquatic herbicides (Sutton et al., 1994a; Sutton et al., 1994b). Due to presence of cystoliths (calcium carbonate pustules) in its leaves and stems, hygrophila is not a preferred food plant for triploid grass carp *Ctenopharyngodon idella* Val (Cuvier & Valenciennes) (Pisces: Cyprinidae) (Sutton and Vandiver, 1986). Considering several biological and economic attributes of this weed, biological control may be a viable option for managing hygrophila (Pemberton, 1996, Cuda and Sutton, 2000).

A noticeable increase in the number of public lakes and rivers with hygrophila has occurred in Florida since 1990 (Langeland and Burks, 1999). One of explanations for the recent hygrophila problem is simply a lack of host specific natural enemies attacking the plant, which would give it a competitive advantage over native flora. Surveys of the natural enemies of hygrophila are needed because there is no information available on potential biological control agents of this aquatic plant (Cuda and Sutton, 2000). The objectives of this study were to (i) catalog and georeference historical populations of hygrophila from herbaria records in India; and (ii) identify candidate natural enemies associated with hygrophila in its native range.

Materials and Methods

Cataloging and Geopositioning Herbaria Records

In order to identify areas for field surveys, locality information of hygrophila specimens was collected

from herbaria in the plant's native range. In India, herbaria label information was obtained from The Central National Herbarium, Howrah, India under the Botanical Survey of India. In addition, label information was collected from the Royal Botanic Garden Herbarium, Kew, United Kingdom.

Exploratory Field Surveys in Hygrophila's Native Range

Exploratory field surveys were conducted in September 2008 and September 2010 in a range of habitats in India and Bangladesh to collect natural enemies of hygrophila. In total, 41 sites were surveyed, including 28 sites in the states of West Bengal and Assam, India and 13 sites in Mymensingh, Bangladesh (Fig. 1). The geoposition and altitude of each survey site were recorded. Several collection techniques, e.g., hand-picking, Berlese funnel extraction, sweep and clip vegetation sampling, as well as dissection of plant parts, were used to collect natural enemies. Arthropods collected during surveys were preserved according to standard methods. Specimens were submitted for identification to cooperating systematists with expertise on specific taxa.

Results and Discussion

Cataloging and Geopositioning Indian Herbaria Records

In total, 64 herbaria records of hygrophila were examined and the locality information/ ecological notes recorded from the Central National Herbarium at Kolkata, India. The herbarium's records indicated that hygrophila was collected from 12 Indian states and the majority of samples (26 of 64, or 41%) were from the state of West Bengal in the eastern part of India. The earliest record was dated 1910 and at least one sample was collected at an altitude of 1200 m.

Label information from 41 specimens was collected from the Kew Herbarium, Richmond, Surrey, UK. Records were collected from specimens dating back to the 1800s and early 1900s from Pakistan, Burma, Vietnam, Taiwan, Sri Lanka and Malaysia. This information was helpful in delimiting the native range of hygrophila.

Exploratory Field Surveys in the Native Range

Insects collected from *hygrophila*'s native range belonged to the Orders: Coleoptera (Anthicidae, Buprestidae, Carabidae, Chrysomelidae, Coccinellidae, Curculionidae, Dytiscidae, Hydrophilidae, Noteridae, Scarabaeidae, and Staphylinidae), Lepidoptera (Crambidae, Noctuidae and Nymphalidae) and Hemiptera (Cicadellidae, Delphacidae, Meenoplidae and Tingidae). In comparison to its invasive range, substantially higher numbers of herbivores were associated with *hygrophila* in its native range (Mukherjee, 2011). Table 1 lists the insects collected along with their approximate abundances, trophic status or feeding guild and methods of collection.

Coleoptera

In total, 19 genera of beetles in 11 families were collected during surveys in India and Bangladesh (Table 1). The most important species observed to cause direct damage to *hygrophila* was a leaf mining buprestid beetle *Trachys* sp. (Family: Buprestidae, Fig. 2). This insect was collected in both Assam and West Bengal in India, as well as the Mymensingh area of Bangladesh from terrestrial population of *hygrophila*. Based on field observations, the buprestid completes its life cycle within a single leaf of *hygrophila*. The dorsoventrally flattened, wedge-shaped larva has a characteristic buprestid form with large flattened head and thoracic regions. The larva mines entirely inside *hygrophila* leaves, feeding on leaf tissue from side to side without damaging the upper and lower leaf epidermises, forming a transparent leaf cavity. Larval duration is ~3 weeks. Pupation occurs within the leaf pocket and the pupal duration is ~7 days. The pupa is brownish in color and ~6-7 mm long and 2-3 mm broad. The adult beetle is metallic black in color, 3-4 mm in length and 1-1.5 mm in width. It is important to note that the occurrence of this insect was rare. In total, only 19 larval specimens were collected during the all the surveys. As this insect completes its life cycle within a single leaf, the size of the *hygrophila* leaf may be an important factor limiting its abundance, and damage was usually observed in broader and longer lower leaves of the plant. The average length and width of the leaves attacked by this insect ranged between 3.0 ± 0.5 cm and 1.2 ± 0.2 cm (mean \pm SD)

, respectively. Further studies on *Trachys* sp. will be required in order to understand its life cycle and host range. This information will be essential for determining its usefulness as a potential biological control agent of *hygrophila*.

A weevil, *Bagous luteitarsis* Hustache (Curculionidae) also was collected during these surveys (Table 1) (O'Brien and Askevold, 1995). This is a semi-terrestrial species in the usually aquatic genus and to date there is no available host plant information (Charles O'Brien, personal communication). The genus *Bagous* has been important for classical biological control of aquatic weeds (O'Brien and Askevold, 1995). *B. luteitarsis* only was collected once during the surveys, suggesting that this is not an abundant species. However, considering that this is a semi-terrestrial species, enabling it to attack both terrestrial and submerged forms of *hygrophila*, further studies are needed to evaluate its biological control potential.

Several other phytophagous leaf beetles (family: Chrysomelidae) were collected during this surveys (Table 1). Congeners of some of these insects have been considered as potential biological control agents for other weeds (for example, Kok, 2001). However, as indicated in Table 1, these beetles were collected during sweep sampling or Berlese funnel extraction. Therefore, there is no direct evidence that these insects feed directly and/or exclusively on *hygrophila*.

Hemiptera

In total, ten species in the order Hemiptera, including suborders, Auchenorrhyncha (families Cicadellidae, Delphacidae and Meenoplidae) and Heteroptera (family Tingidae) were collected during these surveys (Table 1). All the Auchenorrhyncha genera are known to be phloem feeders and are major pests of agricultural crops including rice (*Oryza sativa* L). These insects were collected during sweep sampling and probably used *hygrophila* as an alternate host. No information on host range is available on the two tingid species, *Belenus bengalensis* Distant and *Cantacader quinquecostatus* Fieber, collected during this survey (Distant, 1902;1909). It is noteworthy that several tingids have been used as biocontrol agents or reported as feeding on invasive weeds in several classical

weed biological control programs (summarized in Julien and Griffiths, 1998). The above information suggests that tingids could be successful biological control agents. Further studies will be necessary to confirm the feeding damage of *B. bengalensis* and *C. quinquecostatus* on hygrophila.

Lepidoptera

Three lepidopteran species, an aquatic leaf cutter *Parapoynx bilinealis* Snellen (Crambidae: Acentropinae) (Fig. 3), and two foliage feeders, *Nodaria* sp. (Noctuidae: Herminiinae) (Fig. 4) and *Precis almana* L. (Nymphalidae) were collected during the surveys. All three species were observed to cause direct feeding damage to hygrophila.

Larvae of *P. bilinealis* make cases with hygrophila leaves and feed internally. The mature larva is cream colored, ~15 mm in length and with conspicuous branched tracheal gills on all body segments except the prothoracic region (Fig. 3). Presence of branched gills along the body segments is the diagnostic character for this genus (Habeck, 1974). Duration of the larval stage was ~3 weeks. The larva feeds by scraping leaf tissue from the inner surface of the case, rendering it transparent. In some cases, they were observed to extend outward from the larval case to feed on nearby leaves. However, no feeding damage to the stem was observed. Pupation occurs within the leaf case, and the leaf case containing the pupa floats on the water surface. The duration of the pupal stage was ~7 days. The adult moth (wing span ~10 mm, Fig. 3B) is yellowish brown in color with conspicuous white wavy strips on the wings. The insect caused substantial damage to the submerged hygrophila plants. Available host records suggest that insects of this genus tend not to be host specific (for example, see Habeck, 1974). Based on available information, it seems unlikely that *P. bilinealis* is specific to hygrophila, although our suspicions should be confirmed through host range tests.

A noctuid moth, *Nodaria* sp., which attacks both emergent and terrestrial populations of hygrophila, was also recorded during these surveys (Fig. 4). This moth was found at all locations surveyed and was observed to cause complete defoliation of hygrophila plants (Fig. 4C). The fully grown semilooper larva is ~35-40 mm long and ~2-3 mm wide with a reddish-brown dorsal surface and green ventral surface (Fig.

4B). The dorsal surface of the larva changes color from green to reddish-brown with successive molts. Based on field and laboratory observations, this insect has five instars and total duration of the larval stadium is ~14 days. The larva consumes the entire leaf, leaving only the midrib intact (Fig. 4C). Multiple larvae have been observed feeding on the same plant. Pupation occurs on the plant within a pupal case constructed of 3-4 hygrophila leaves tied together by silk. The pupa is brownish-black in color, ~10 mm in length and 5 mm in width. Duration of the pupal stage is ~7 days. The adult moth is grayish-brown with indistinct blackish antemedial, postmedial and subterminal wavy lines on the wings; the wingspan ~25 mm. Field observations confirmed that this insect can be very damaging, and is probably specific to hygrophila as no feeding was observed on other species of nearby plants. Further studies are needed to confirm its host range and determine its biological control potential.

In addition, a nymphalid butterfly, *Precis almana* L., commonly known as the Peacock Pansy butterfly, was found feeding on hygrophila. This insect, which was collected from both India and Bangladesh, also caused complete defoliation. However, because it is a polyphagous species known to feed on wide range of plants (Kehimkar, 2008), it has no value for importation biological control of hygrophila.

Pathogens

In addition to the aforementioned insects, a very damaging aecial rust fungus (Pucciniales: Pucciniaceae, hereafter hygrophila rust) that completely killed hygrophila plants was found during surveys in India and Bangladesh (Fig. 5). Rust infections were observed at all locations surveyed, suggesting that it could be an important natural enemy of hygrophila.

Historically, rust fungi have been used effectively in classical weed biological control programs (summarized in Charudattan, 2001). Most rust fungi have a complex life cycle, involving both sexual (aecial and pycnial spores) and asexual (uredenia, telial and basidial spores) stages (see Kolmer et al., 2009 for detailed description of a rust life cycle). A prerequisite for a rust fungus to be a potential classical biological control agent is that it must be autoecious, whereby the rust completes its life cycle on a single host species. In case of the

hygrophila rust, only the aecial (Fig 5B) and pycnial (Fig. 5C) stages were observed in the field. Repeated attempts to inoculate healthy hygrophila plants with aeciospores from infected plants failed to initiate infection. However, laboratory observations confirmed that aeciospores germinated on tap-water agar medium, producing a normal germ tube (CAE, personal observation). If this rust were cycling through aecia alone (microcyclic), then it would be expected that the aecia would be behaving as aecioid-telia, germinating to produce basidiospores. However, more work is required to elucidate the germination process since this can be influenced by temperature. If this rust is a full-cycled autoecious rust, then it would be expected that the aeciospores would infect the hygrophila, and produce uredinia. Thus, either the conditions following inoculation were not conducive to infection, or the rust is indeed heteroecious, requiring a primary host to complete its life cycle.

In an earlier study, Thirumalachar and Narasimhan (1954) reported that the aecial stage of the heteroecious leaf rust, *Puccinia cacao* McAlp. occurs on *Hygrophila spinosa* (Acanthaceae), a congener of *H. polysperma*. The common grass *Hemarthria compressa* (L.f.) R.Br. (Poaceae) is the primary host of *P. cacao*. They reported that on *H. spinosa*, the rust develops a systemic infection and the infected shoots are paler in color. Interestingly, similar observations were made on hygrophila. Laundon (1963) provided descriptions of all rust fungi infecting *Hygrophila* spp., at that time. When compared, the measurements of aecial and pycnial spores, collected from *H. phlomoides* Nees, *H. salicifolia* (Vahl) Nees and *H. spinosa* T. Anders as reported in Laundon (1963), match closely with those of the hygrophila rust that we collected (Table 2). These results also suggest the rust from *H. polysperma* could be the sexual stage of a different pathotype of *P. cacao*. Cross-inoculation studies to test the ability of aeciospores from *H. polysperma* to initiate infection (uredinia) in a currently unknown primary host (possibly a grass such as *Hemarthria compressa*) is necessary to confirm the identification of this rust fungus. However, field observations in Assam, over two growing seasons and at multiple natural sites, have not resulted in the discovery of a grass infected with uredinia, growing in the vicinity of aecia-infected *H. polysperma* (pers.

comm., K.C. Puzari, 2011). Considering the high level of aeciospore inoculum on *H. polysperma*, and assuming that the rust is the full cycled *P. cacao*, then it would be expected that uredinia would have been found on a native grass, after such long term and intensive observations. Clearly, a more in depth study of the life cycle is required before this potential biological control agent is rejected.

Overall, a number of natural enemies, including two caterpillars (*P. almana* and *Nodaria* sp.) that defoliate emerged plants, an aquatic caterpillar (*P. bilinealis*) feeding on submerged hygrophila, and a leaf mining beetle (*Trachys* sp.) were collected during surveys in India and Bangladesh, part of the native range of hygrophila. Some of these insects, in particular, *P. bilinealis*, *Nodaria* sp. and *Trachys* sp. hold promise as potential biological control agents of hygrophila. Further studies are necessary to determine their host ranges and specificity to hygrophila. In addition, a very damaging aecial rust fungus (*Puccinia* sp.) was collected. Although initial studies suggested that this rust could be the aecial stage of the heteroecious *P. cacao*, detailed cross-inoculation studies, involving its primary host *H. compressa*, are necessary to confirm its identity.

Acknowledgements

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Table 1. Insects collected from *Hygrophila* during surveys in India and Bangladesh, native habitats of *Hygrophila polysperma*.

Taxonomy	Approx. abundance ¹	Trophic level or feeding guild ²	Methods of collection ³
Anthicidae			
<i>Anthelephila mutillaria</i> Saunders	R	Scavenger?	Sweep
Buprestidae			
<i>Trachys</i> sp.	U	Leaf miner	Hand coll.
Carabidae			
<i>Bembidion</i> sp.	R	Predator	Sweep
<i>Clivina</i> sp. Latreille	U	Predator	Sweep
<i>Tachys</i> sp.	R	Predator	Sweep
Chrysomelidae			
<i>Altica</i> sp.	U	Leaf feeder	Sweep
<i>Aspidomorpha</i> sp.	R	Leaf feeder	Sweep
<i>Cassida</i> sp. A	U	Leaf feeder	Sweep
<i>Cassida</i> sp. B	U	Leaf / root feeder	Sweep
<i>Chaetocnema</i> sp.	R	Leaf feeder	Sweep
<i>Lema</i> sp. A	R	Leaf feeder	Sweep
<i>Lema</i> sp. B	R	Leaf feeder	Berlese
<i>Pachnephorus</i> sp.	U	Leaf feeder	Sweep
<i>Philipona</i> sp.	U	Leaf feeder	Sweep
Coccinellidae			
<i>Harmonia</i> sp.	C	Predator	Sweep
Curculionidae			
<i>Bagous luteitarsis</i> Hustache	R	?	Berlese
Hydrophilidae			
<i>Helochares</i> sp.	U	Predator	Berlese
<i>Paracymus</i> sp.	U	Predator	Berlese
<i>Regimbartia</i> sp.	U	Predator	Berlese
Scarabaeidae			
<i>Rhyssemus</i> sp. Mulsant	R	Scavenger?	Sweep
Staphylinidae			
<i>Philonthus</i> sp. Stephens	R	Predator	Sweep
Hemiptera			
Cicadellidae			
<i>Nephotettix</i> sp.	C	Sap feeder	Sweep
<i>Cofana spectra</i> Distant	C	Sap feeder	Sweep
<i>Cofana unimaculata</i> Signoret	C	Sap feeder	Sweep

Table 1. Insects collected from hygrophila during surveys in India and Bangladesh, native habitats of *Hygrophila polysperma*.

Taxonomy	Approx. abundance ¹	Trophic level or feeding guild ²	Methods of collection ³
<i>Hecalus</i> sp.	U	Sap feeder	Sweep
<i>Scaphomonus indicus</i> Distant	U	Sap feeder	Sweep
Delphacidae			
<i>Nilaparvata</i> sp.	C	Sap feeder	Sweep
<i>Perkinsiella</i> sp. Kirkaldy	C	Sap feeder	Sweep
Tingidae			
<i>Belenus bengalensis</i> Distant	R	Sap feeder	Sweep
<i>Cantacader quinquecostatus</i> Fieber	R	Sap feeder	Sweep
Lepidoptera			
Crambidae			
<i>Parapoynx bilinealis</i> Snellen	R	Leaf cutter	Hand coll.
Noctuidae			
<i>Nodaria</i> sp.	C	Leaf feeder	Hand coll.
Nymphalidae			
<i>Precis almana</i> L.	C	Leaf feeder	Hand coll.

¹Approximate abundance: R = Rarely collected, 3 times or less; U = Uncommonly collected, 4-10 times; and C = Commonly collected, >10 times

²Trophic level and feeding guild information for herbivorous species does not imply that species included on this list were actually observed using hygrophila as a food source

³Methods of collection: Sweep = Sweep net sampling; Berlese = Berlese funnel extraction and Hand coll. = larva collected from field and reared to adult

Table 2. Comparison of aecial rust collected from *Hygrophila polysperma* with that collected from *H. phlomoides*, *H. salicifolia* and *H. spinosa* by Laundon, (1963)

	Aecia (diam.)	Aeciospores (diam.)	Pycnia (diam)	Pycniospores
<i>Puccinia</i> sp. collected from <i>hygrophila</i>	228.1 - 398.1 μm	18.43 - 28.4 μm	103.4 - 120.1 μm	Paraphysate
Laundon (1963)	200 - 400 μm	15 - 30 μm	80 - 100 μm	Paraphysate

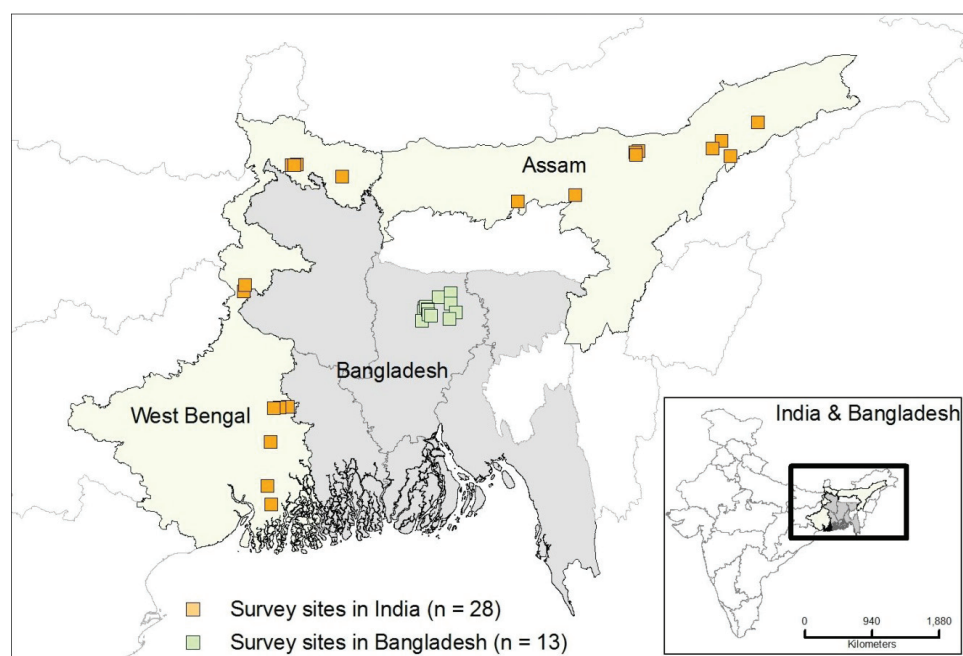


Figure 1. Survey sites in India (n = 28) and Bangladesh (n = 13). In India, surveys were conducted in the states of West Bengal (n = 15) and Assam (n = 13).

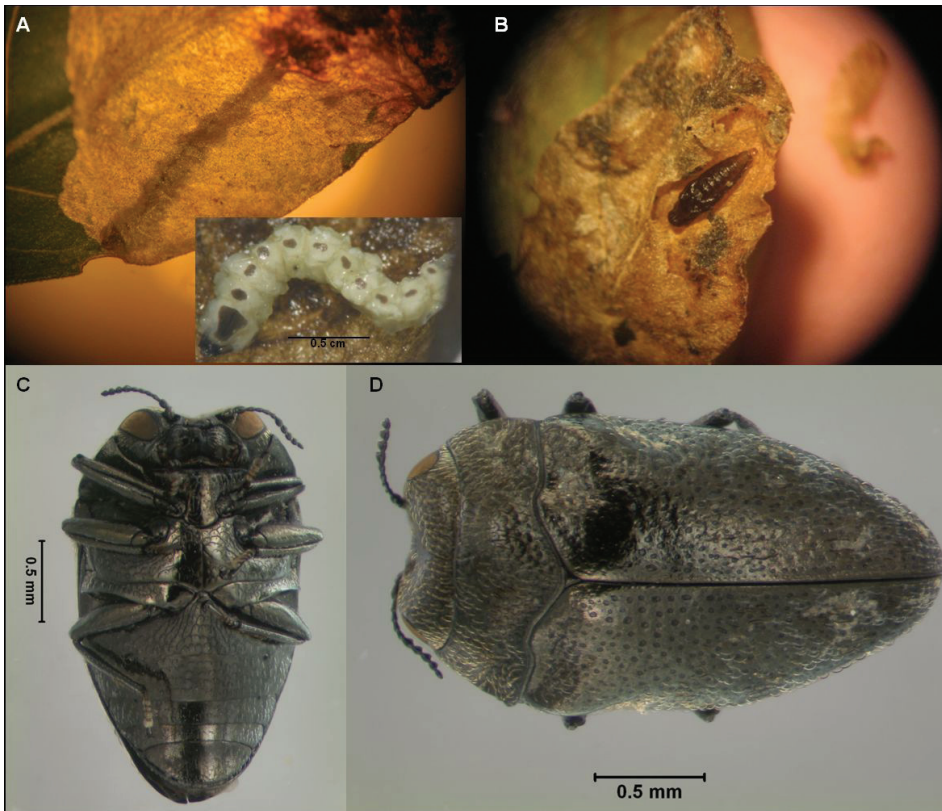


Figure 2. *Trachys* sp. (Coleoptera: Buprestidae) collected from *H. polysperma*; Larva mining inside the leaf cavity, inset: close-up of larva (A); Pupa inside the leaf cavity (B); Ventral and dorsal views of the adult beetle (C-D). Photo credit A. Mukherjee

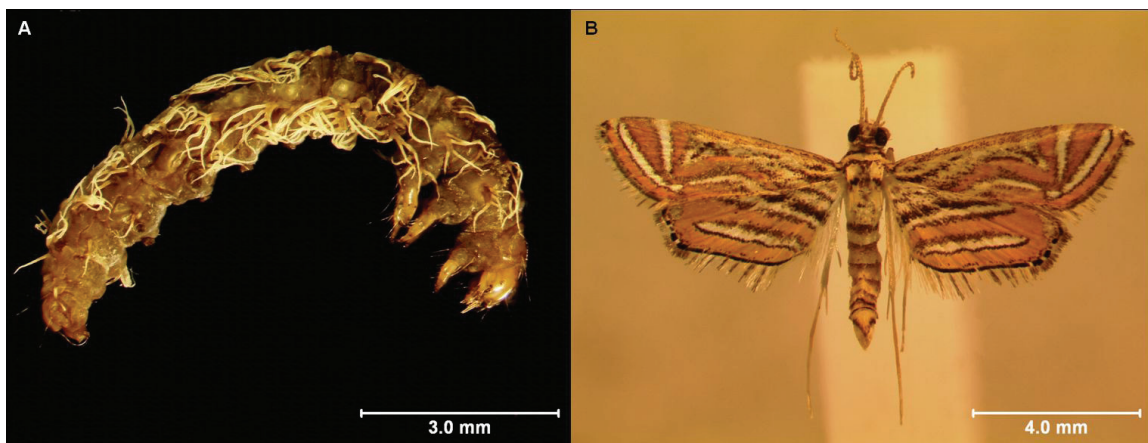


Figure 3. Larva (A) and adult (B) of *Parapopynx bilinealis* (Lepidoptera: Crambidae) collected from *H. polysperma*. Note the presence of branched tracheal gill on larval body – a characteristic of the genus. Photo credit A. Mukherjee

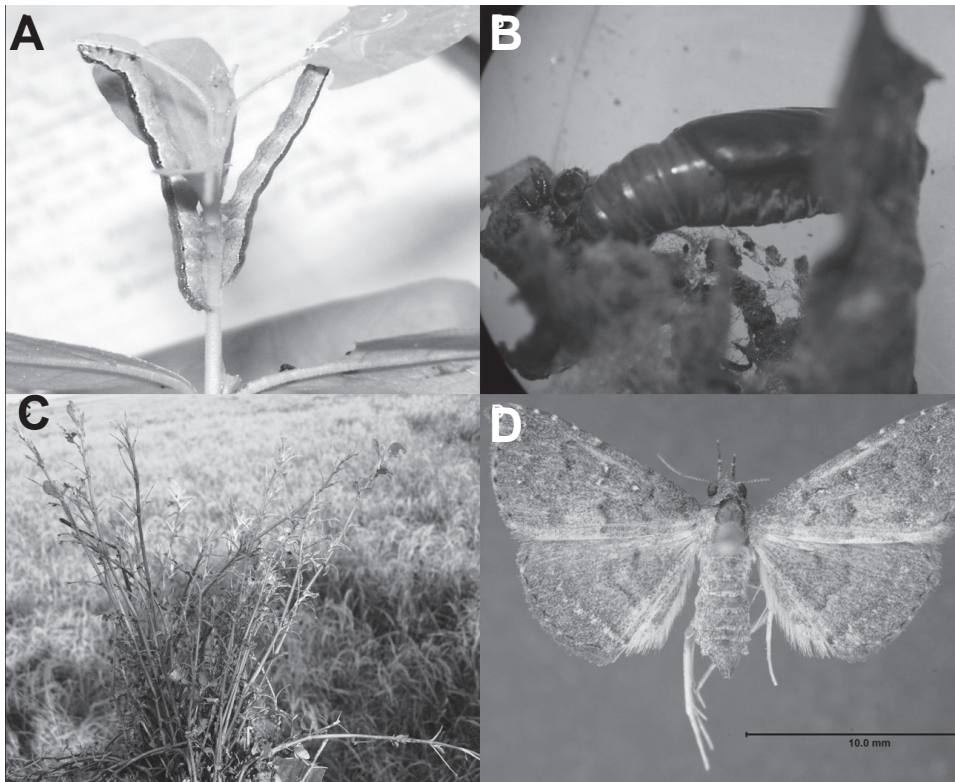


Figure 4. *Nodaria* sp. (Lepidoptera: Noctuidae) collected from *H. polysperma*; Larva feeding on hygrophila leaves (A); Pupa (B); Feeding damage (C); Adult moth (D). Photo credit A. Mukherjee

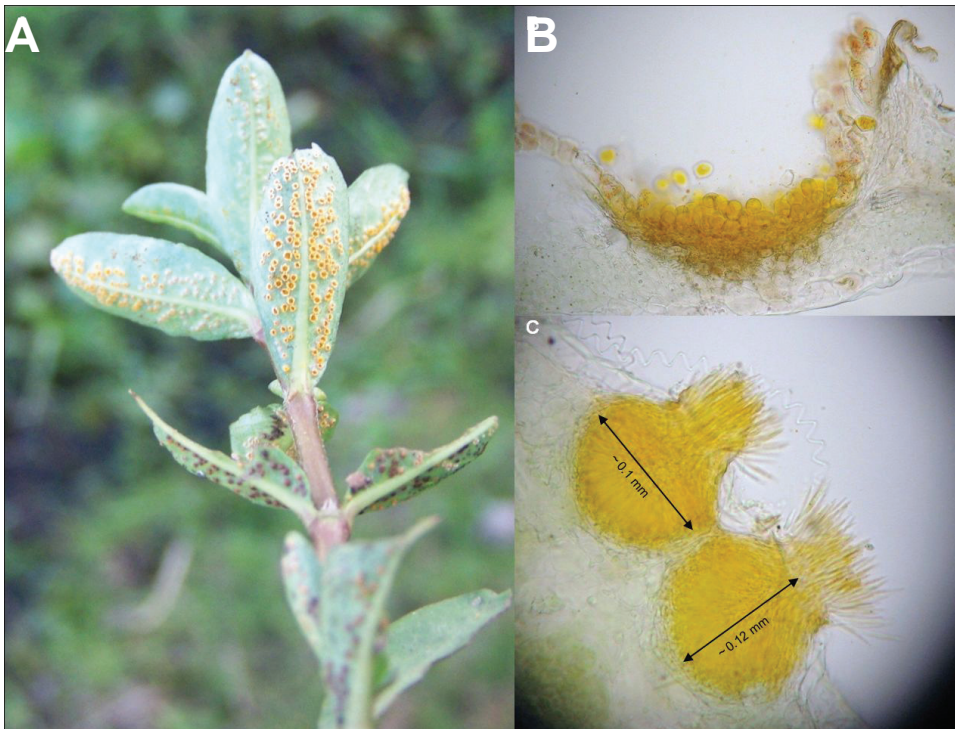


Figure 5. Rust fungus (*Puccinia* sp.) collected from *H. polysperma*. Rust infected hygrophila plant (A); Cross section of aecia (B); Cross section of pycnia (C). Photo credit A. Mukherjee

Biological Control of *Rubus alceifolius* (Rosaceae) in La Réunion Island (Indian Ocean): From Investigations on the Plant to the Release of the Biological Control Agent *Cibdela janthina* (Argidae)

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Abstract

The giant bramble (*Rubus alceifolius* Poir.: Rosaceae), native to Southeast Asia, is one of the most invasive plants in La Réunion. A ten year research program was launched in 1997 with three components: i) genetic diversity, ii) development strategy, and iii) selection of biological control agents. Introduced populations in La Réunion, Mauritius, Mayotte and Australia were clonal and far from the highly variable native populations in Asia, while Madagascar populations appeared intermediate. Seed production is by apomixis in La Réunion Island and by allogamy in the native habitat. Fruit production occurs up to 1,100 m elevation while vegetative multiplication is possible up to 1,700 m. The plant grows in well lighted places, invading forest edges, and all open areas. From surveys of *Rubus* natural enemies in its native range, the sawfly *Cibdela janthina* (Klug) (Argidae) was selected as the most promising biological control agent and studied. The first population was thus released in La Réunion in early 2008 with the agreement of the local authorities for the biological control of *R. alceifolius*. It is now naturalized, spreading and under evaluation.

Introduction

Giant bramble (*Rubus alceifolius* Poir.: Rosaceae) is an invasive Southeast Asian bramble introduced to La Réunion Island (Indian Ocean) in the mid 19th century. In 1892, it was already cited as fatal for the island (Lavergne, 1978), and Rivals (1960) said “it was a real pain for natural environment. Density was so high that regeneration of indigenous forest plants was impossible under *Rubus* thickets.” At that time, he also mentioned that “only a biological solution could destroy this plant and solve the problem”.

Since the 16th century, the native ecosystems of La Réunion Island have undergone rapid

transformation with the introduction of more than 2000 plant species, of which 628 have become naturalised (Lavergne et al., 1999). During their evaluation of the threat posed by invasive plants to the island, Macdonald et al. (1991) underlined that 62 exotic species were major threats, among them was *R. alceifolius*, which topped the list.

For many years, the *Office National des Forêts* attempted to bring the weed under control. But neither mechanical weeding nor chemical control was successful. Control was possible only on small surface areas, and needed to be repeated regularly. Also, the cost was extremely high (at present, €2 million is spent annually on the control of invasive plants) and the use of herbicides in native forests

has raised ecotoxicological concerns. In 1992, the *Conseil Régional* of La Réunion made *R. alceifolius* a priority and decided to fund a research program to develop integrated control methods, with the emphasis placed on biological control. This program started in 1997 and ended in 2006 with the selection of a biological control agent. It was then followed by complementary action funded by the DIREN (Direction Régionale de l'Environnement) of La Réunion until 2009 for the introduction, acclimation and release of the selected biological control agent *Cibdela janthina* (Klug) (Hymenoptera: Argidae).

While much research has been done on the biological control of other species of *Rubus* (Bruzese and Lane, 1996; Evans et al., 1999; Gardner et al., 1997; Groves et al., 1997; Julien and Griffiths, 1998; Nagata and Markin, 1986), little work has been done on tropical *Rubus* species or *R. alceifolius* itself. It concerned its distribution, seed production and spread in La Réunion (Sigala and Lavergne, 1996; Strasberg, 1995; Thebaud, 1989). The chrysomelid *Phaedon fulvescens* Weise, originating in Northern Vietnam, was mentioned as a potential candidate for biological control (Jolivet, 1984). Other natural enemies were an unidentified stick insect and two rust fungi, *Hamasporea acutissima* P.Syd. & Syd. and *Gerwasia rubi* Racib. recorded in Thailand by Taysum et al. in 1991 (unpublished document). All previous experiments carried out worldwide in the biological control of *Rubus* spp., used pathogens.

The project was built around three components. The first concerned the plant's genetic diversity: i) to compare the diversity between the area of introduction and the native range in order to match genetic and geographic origins of the invader, and ii) to determine the degree of specificity needed by biological control candidates and the opportunity for their use throughout the area of introduction. The second component was the biological study of the weed under La Réunion environmental conditions in order to highlight factors for growth, multiplication and spread. The third component aimed to select and study the biological control candidates suitable for introduction and release in La Réunion.

This paper presents a compilation of the project's main findings leading to the proposal for a biological control of the giant bramble in La Réunion using the sawfly *Cibdela janthina*.

Materials and Methods

La Réunion Island (2,512 km² in area) is a volcanic island in the Indian Ocean rising to 3,061 m a.s.l., with considerable climate variations. Rainfall ranges from 8,000 mm per year on the east coast to 500 mm per year on the lowland west coast (Robert, 1986). The island's vegetation comprises four main types that are determined by a combination of rainfall and temperature: semi xerophytic tropical forest, lowland humid tropical forest, mountain humid tropical forest and ericoid vegetation at high elevations (Cadet, 1977).

The giant bramble, *Rubus alceifolius* Poir. (Rosaceae, subgenus *Malachobatus*) was first described as a subspecies of *R. moluccanus* L., but is now considered a separate species (Kalkmann, 1993). It is a shrub with arching or climbing branches up to 5 m long in native areas or up to 15 m in length in areas of introduction. Branches and leaves bear curved prickles and yellow hairs. Stipules are large, orbicular, and deeply digitately divided. Leaves are simple, orbicular to broadly ovate, 10-30 cm in diameter, with 5-7 lobes, and cordate at the base. Inflorescence is terminal, consisting of up to 4 racemes with up to 8 flowers. Flower bud is globular, petals are orbicular, white. Collective fruit is globular, succulent, 1 cm in diameter and containing many red drupelets (Kalkmann, 1993). The giant bramble is exotic in Australia (Queensland), Mauritius, Mayotte, Madagascar and La Réunion Island but is native to Southeast Asia (southeast China, Hainan, Taiwan, Myanmar, Thailand, Laos, Cambodia, Vietnam, Indonesia/Sumatra, Java, Malaysia, Lesser Sunda Islands, Borneo and Sulawesi) (Friedmann, 1997; Kalkmann, 1993; Parsons and Cuthbertson, 1992). At La Réunion it frequently occurs as large stands along forest edges, road and river sides from sea level up to 1,700 m of elevation (Baret et al., 2004).

Genetic diversity studies of *R. alceifolius* were based on 224 specimens: 116 from introduced populations (La Réunion (75), Mayotte (8), Mauritius (7), Madagascar (19) and Australia/Queensland (7), and 108 from native populations (Thailand (59), Vietnam (30), Laos (1), Java (4), and Sumatra (14). Thirty specimens of other *Rubus* species were also evaluated (La Réunion (3), Thailand (12), Vietnam (10), Laos (2), and Sumatra (3). Plant DNA was

extracted and processed following the protocol of Bousquet et al. (1990). Genetic differentiation was assessed by amplified fragment length polymorphism (AFLP), using 4 primer pairs and the restriction enzymes *EcoRI* and *MseI*, as detailed by Amsellem et al. (2000). The genetic distance between individuals was calculated and expressed as the Simple Matching distance (Rohlf, 1993). A tree was constructed according to the Neighbour-Joining Method (Felsenstein, 1993). The study focused on two levels of comparison: individuals and areas (Amsellem et al., 2000).

The reproductive biology of *R. alceifolius* in the native and introduced habitats was assessed and compared using microsatellite markers specific for *Rubus* species (Amsellem et al., 2001a). We compared the reproductive system (fruit set) of *R. alceifolius* in its native range (nine half-sib seedlings derived from different fruits on a single plant sourced from Vietnam), in its area of introduction in Madagascar (three individuals and their half-sib progeny from two localities), and in La Réunion where 44 flowers from several parents were manually fertilized with pollen from other plants (Amsellem et al., 2001b).

Developmental patterns were then studied at La Réunion by conducting architectural and morphometric analyses that described individuals at the five specific growth stages from seedlings to mature plants (Baret et al., 2003a; Baret et al., 2003b). Altitudinal variations in fertility and vegetative growth were assessed by counting flowers, fruits, seeds and leaves in eight randomly located quadrats at six sites from 50 m to 1,500 m a.s.l., and by estimating the soil seed bank (Baret et al., 2004).

Biological control agents were selected from surveys carried out in the native range (Vietnam, Laos, Thailand, China, Indonesia (Sumatra) and Singapore) from 1997 to 2004, and in the area of introduction at La Réunion Island (1998). Altogether, stands of *Rubus* were examined at 309 different locations subject to different climatic conditions, ranging from the lowlands to the highest elevations (2,500 m a.s.l. in Doi Inthanon Mountains, Northern Thailand) and from equatorial (Toba Lake, Northern Sumatra) to tropical climates with fairly cold winters (Guangdong and Hainan mountains in China). *Rubus* natural enemies (arthropods or pathogens) were collected and identified. Symptoms and environmental conditions were recorded. The

most promising ones were collected alive and reared in laboratories in Sumatra, Montpellier and La Réunion for further biological and specificity studies in order to select those most suitable for biological control of *R. alceifolius* at La Réunion.

Results

Rubus genetic diversity and putative origins of *R. alceifolius*

The genetic study of *R. alceifolius* revealed two well-separated groups, both distinct from the out-group corresponding to other *Rubus* species. The first group consisted of all the samples taken within the native range and the second group, all the samples from the area of introduction. A study of the within-area diversity showed relatively marked genetic diversity between individuals from countries of the native range. On contrary, each population sampled in the Indian Ocean islands (La Réunion, Mayotte, Mauritius, and Australia), with the exception of Madagascar, was characterized by a single different *R. alceifolius* genotype. All these genotypes were closely related to individuals from Madagascar where polymorphism was intermediate between the situation in areas of introduction and the native range.

Many authors have discussed the different hypothetical origins of this exotic plant. E. Jacob de Cordemoy (1895) mentioned that it may have been introduced at La Réunion in 1846. Nevertheless a previous text “*Ralliement du 17 août 1892*” cited by Lavergne (1978) specified that this plant came from the Botanical Garden in Calcutta. Other authors thought it was sent from Vietnam (Jolivet, 1984), or that it may either have been introduced first in Mauritius by Commerson on his return from Java in 1768 (Rivals, 1960), or introduced into Mauritius from Madagascar (Vaughan, 1937) and then spread to La Réunion in the 1850s (Rivals, 1960). Our results suggest that *R. alceifolius* was first introduced into Madagascar, perhaps on multiple occasions. The Madagascan individuals were thus the immediate source of the plants that colonized other areas of introduction. Successive nested founder events appear to have resulted in the populations in the area of introduction showing a cumulative reduction

in genetic diversity. The marked genetic differences between the populations in the native range and in the area of introduction prevented us from determining the geographical origin of the alien plant (Amsellem et al., 2000).

Genetic diversity and the weed's biological strategy as pointers for control

The low diversity of the populations found in most areas of introduction suggested that a biological control agent efficient against one individual should be able to attack the entire population on the island.

Both the genetic diversity patterns and differences between half-sib progeny and their maternal parents (revealed by microsatellite markers) showed that in the native range, seeds are produced sexually (Amsellem et al., 2001a; Amsellem et al., 2001b). By contrast, in Madagascar, over 85% of the half-sib progeny resulting from open pollination gave multilocus genotypes identical to those of their respective maternal parents. Seeds thus appear to be produced mostly or exclusively by apomixis in Madagascar. We therefore suggest that Madagascan populations resulted from the hybridization of an introduced *R. alceifolius* and native populations of presumably *R. roridus* Lindley that Kalkman (1993) considered similar and synonymous to *R. alceifolius*. Apomixis was therefore a consequence of this hybridization. In Reunionese populations of *R. alceifolius*, seeds obtained in controlled pollination experiments were all genetically identical to the maternal parents. While genetic variation (microsatellite markers) in Reunionese populations was low, it was sufficient to demonstrate that seeds could not have resulted from fertilization by the pollen donors chosen for controlled pollinations, or from autogamy, but were produced exclusively by apomixis (Amsellem et al., 2001b). This phenomenon was responsible for the clonal population in the area of introduction.

The architectural and morphological studies showed five developmental stages for *R. alceifolius*, differing by several markers such as internode length and diameter, pith diameter, and plant shape. A heteroblastic developmental pattern was thus revealed for the plant, midway between that of a bush and a vine. The results also showed that this species taps environmental resources early in its

development, whereas it “explores” the environment during the adult stage (Baret et al., 2003b).

To determine the invasive capacity of *R. alceifolius*, fertility and vegetative growth were studied at different altitudes on La Réunion Island (Baret et al., 2005). Flowering period duration, seed production, and the seed bank were found to be negatively correlated with elevation (50 – 1,500 m a.s.l.). At a lowland site, fruit production averaged between 30 and 80 fruits m⁻², while no fruits were observed above 1,100 m. The seed bank was greater under *R. alceifolius* patches (>10,000 seeds.m⁻²) than in understories not colonized by the bramble (approximately 3,000 seeds.m⁻²). Seed dispersion in forest was mainly by running water. Although the number of leaves per unit area was similar along the entire gradient studied, the reduced fruiting in upland areas might be offset by an increase in vegetative growth. Monospecific bramble patches in lowland areas may serve as the sources of seed for the colonization of new areas by bird dissemination. Once established at high elevations, the weed grows vegetatively without flowering and multiply by layering, cutting or sucker (Baret et al., 2004).

Selection of potential biological control agents

Fifty one arthropods and four pathogens were recorded and collected during the surveys conducted in the native range and in La Réunion Island. Particular care was taken to select agents on the basis of a combination of criteria (type of damage to and impact on *R. alceifolius*, host specificity, life traits etc.) for further biological and specificity studies. Of the leaf feeders, sawflies from Sumatra and China (*Cibdela janthina*, *C. chinensis* Rohwer, and *Arge siluncula* Konow) appeared to be the most promising. They caused complete defoliation of the weed and seemed to be highly specific. The beetles *Phaedon fulvescens* and *Cleorina modiglianii* Jacoby, found respectively in Vietnam and Sumatra, were also promising. The rust fungus *Hamaspora acutissima* was observed and collected in many places throughout Asia. Only a few common insects were reported in La Réunion Island, but never damaging *R. alceifolius*.

Phaedon fulvescens Weise (Coleoptera: Chrysomelidae) was collected in Northern Vietnam

while feeding on *Rubus* spp. leaves on the edge of mountain forests at 900 m a.s.l. Field observations coupled with biological and host specificity studies in Montpellier generated a wealth of new information about this species. Although not encountered frequently, individuals were very numerous in the populations observed and both adults and larvae damaged the plant by leaf skeletonizing. Eggs were laid separately on the underside of leaves and coated with feces. We also noted that the insect undergoes a two-month summer diapause. Therefore, this insect may only complete a single generation in a year, not three or four as initially thought. Field observations indicated that the beetle was highly specific to *Rubus* species of the *Malachobatus* subgenus. But host specificity tests carried out on *R. apetalus* (indigenous of La Réunion) of the *Ideobatus* subgenus showed that the insect can also feed on this plant and survive throughout its lifecycle. We therefore rejected *P. fulvescens* for the biological control of *R. alceifolius* in La Réunion (Le Bourgeois et al., 2004).

Cleorina modiglianii Jacoby (Coleoptera: Chrysomelidae) was found in Sumatra on several *Rubus* spp. in the shade provided by *Pinus merkusii* Jungh. & de Vriese forests from 700 to 1,200 m. This beetle caused leaf skeletonizing damage to *R. alceifolius* and *R. moluccanus* L. We were unable to find eggs or larvae despite numerous field surveys. Host specificity tests were carried on adults in the laboratory, comparing *R. alceifolius* (Réunion), *R. alceifolius* (Sumatra), *R. apetalus* (L Réunion) and *R. fraxinifolius* (La Réunion). The adults only fed and survived on *R. alceifolius* (Sumatra) and *R. apetalus* (La Réunion). This insect was therefore rejected as a potential candidate for biological control.

Cibdela janthina (Klug) (Hymenoptera: Argidae) was recorded in Sumatra. The insect's behavior was observed in the field while its biological traits were assessed in the laboratory in Sumatra. Mating happened in full light at 30°C and 80% humidity two days after female emergence. Then eggs were inserted into the main nerves of the plant's upper young leaves not yet fully opened. Average fertility was 58 eggs per female, with 84% viability. The eggs hatched after 10 days of incubation. The larvae completed seven instars within 25 to 30 days, and then pupated in a silk cocoon under the leaf litter. Larvae were gregarious during the major part of their development and presented a typical S-shape.

The full life cycle ranged from 48 to 62 days. The insect may complete six generations per year without any diapause. Adult life span was only 7-14 days and they were found not to be feeding under Sumatran environmental conditions; only drinking dewdrops on leaves, while the larvae were feeding on *R. alceifolius* leaves consumed systematically along the branches in a top-down process. Host range tests were conducted on 41 plant species from 13 botanical families chosen on the basis of phylogeny and economic or conservation issues for La Réunion. *Cibdela janthina* appeared to have a very narrow host range, feeding only on *Rubus* species. Starvation tests showed some feeding on certain subspecies of *R. moluccanus* not present in La Réunion, on *R. fraxinifolius* (exotic to La Réunion) and on *R. apetalus* (indigenous in La Réunion). Choice tests showed that the insect mainly prefer to feed on *R. alceifolius*. Temperature conditions that impact on the insect's development should keep *C. janthina* under 1,000 m of elevation while *R. apetalus* is present from 700 m to 1,700 m. Considering these results and the insect's biological features, *C. janthina* was considered as a good potential biological control agent against *R. alceifolius* in La Réunion. Accordingly, a petition was made for permission to introduce and release it.

Hamaspora acutissima P.Syd. & Syd. (Uredinales: Phragmidiaceae) was observed at many locations in Vietnam, Thailand and Indonesia (Sumatra). It was visible on the upper face of leaves as small brown to yellowish spots and on the lower face as bunches of orange paraphyses containing teliospores. Spots were found on isolated leaves and plants, or as intense infestations covering all parts of *Rubus* plants. In cases of marked contamination, leaves were drying and withering. All the *Rubus* mentioned as infected by this fungus belonged to the *Malachobatus* subgenus indicating the narrow host range of the rust. Biological and host specificity studies showed that Asian *Rubus* species belonging to the *Malachobatus* subgenus could sometimes be inoculated. *R. alceifolius* from La Réunion was inoculated but the pathogen stopped growing at the mycelium stage without producing new teliospores. Rust fungi are known to be highly specific (Evans and Gomez, 2004). Our results confirmed that the Reunionese *R. alceifolius* is genetically too different from those in the native range to allow *H. acutissima* attack.

Conclusion

The alien invasive giant bramble found in La Réunion and other areas of introduction is genetically different from those in the native range and probably resulted from hybridization in Madagascar. It has a high growth potential and produces apomictic fruits in the area of introduction. With its marked phenotypic plasticity, the plant is able to fruit below 1,100 m a.s.l. and can grow and multiply vegetatively up to 1,700 m.

Of the biological agents collected in the bramble's native range, the sawfly *C. janthina* showed the best biological and ecological traits and host specificity that could justify its introduction into La Réunion to regulate *R. alceifolius* populations under 1000 m a.s.l. It also appeared to have the most severe impact on weed growth.

Therefore, a petition form to introduction and release of *C. janthina* was submitted in 2006 to the *ad hoc* scientific committee and was accepted by local authorities. Cocoons from Indonesia/Sumatra were introduced in mid 2007 for the rearing of the sawfly and acclimatization at La Réunion. The first population was released on the east coast of La Réunion in early 2008. It is now spreading well, and controlling populations of the giant bramble. Studies of the spread dynamics and impact of *C. janthina* on *R. alceifolius* thickets at the island level are ongoing, as is a study of the impact of the decline of populations of the giant bramble on natural vegetation dynamics.

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Beyond the Lottery Model: Challenges in the Selection of Target and Control Organisms for Biological Weed Control

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Abstract

Biological control scientists have long tried to pick winning combinations of target and control organisms, while minimizing adverse effects on nontarget organisms. What have we learned from recent work and what should be focus on moving forward? Establishment rates for new control organisms are nearing 100% with little room for improvement; however, improving rates of control given establishment and minimizing off-target effects are two areas for improvement. Two ways to keep control organisms effectively on target are to (1) diagnose and exploit weed vulnerabilities using targeted life-cycle disruption (thereby achieving effective biological using fewer control organism individuals and species), and (2) investigate the mix of evolutionary and ecological forces enabling control organisms to exploit new habitats and hosts (thereby better forecasting outcomes of biological control). Continuity of program support, learning from experience, rational regulations and policies, plus developing and exchanging new technology will help achieve these goals.

Bottom-Up Effects on Top-Down Regulation of a Floating Aquatic Plant by Two Weevil Species: The Context-Specific Nature of Biological Control

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Abstract

Predicting the efficacy of prospective biological control agents, the holy grail of weed biological control, while often advocated, is rarely implemented. We examined, *a posteriori*, whether it would have been possible to predict which of two introduced weevil species, *Neochetina eichhorniae* Warner or *N. bruchi* Hustciche, would have been the superior choice for controlling *Eichhornia crassipes* (Mart.) Solms-Laubach. Plant nutrition and competition can alter a plant's ability to sustain or compensate for herbivory and affect a phytophagous insect's ability to reproduce. These factors could also influence efficacy predictions. We therefore conducted three outdoor mesocosm experiments to compare the performance of these two weevils, independently and together, among five fertilizer treatments. A low initial plant density experiment examined their ability to reduce growth and flowering but allowed for density to increase. A high plant density experiment evaluated their ability to lessen biomass and reduce surface coverage. The third experiment began with low plant density but plants were maintained at low density by harvesting a portion whenever coverage exceeded 50% of the water surface. This was intended to minimize intraspecific competition. The effects varied between weevil species, among fertilizer treatments, and among experiments. Interactions between herbivory and fertilizer treatments were apparent and the nature of these interactions varied among experiments. Efficacy therefore seemed nuanced and context specific, requiring extensive assessments of multiple evaluation criteria across a wide range of environmental and ecological conditions. Overly simplistic evaluations risk rejection of effective agents capable of mediating adverse impacts from invasive plant populations. These results also argue against the concept that a single best agent can be identified to control a weed that inhabits a broad range of habitats and conditions.

Predicting Parasitism of Weed Biological Control Agents

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Abstract

We conducted a nationwide survey of parasitism of weed biological control agents in New Zealand (NZ) and found that 19, mostly native, parasitoid species attack 10 weed biological control agent species. Fifteen of these parasitoid species were confined to five agents that possessed “ecological analogues”, defined as a native NZ insect that belongs to the same superfamily as the agent and occupies a similar niche on the target weed. Parasitoid species richness in NZ was positively correlated to richness in the area of origin. However, only agents with ecological analogues contributed significantly to this pattern. Our results support Lawton’s (1985) hypothesis that, to find enemy-free space, selected agents should “feed in a way that is different” and “be taxonomically distinct” from native herbivores in the introduced range.

Learning from Experience: Two Weed Biological Control Programs with Rust Fungi Compared

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Abstract

Rust fungi are the type of pathogens most widely used in classical biological control of weeds. This is primarily because of their typically high level of specificity, the severe damage they can inflict on plants, and efficient wind dispersal capability. They are not, however necessarily the easiest and most effective solutions to pursue for all weed problems. To illustrate the potential pitfalls that can be encountered with rust fungi as biological control agents, I will compare various aspects of the recent programs against bridal creeper (*Asparagus asparagoides* (L.) Druce) (Kleinjan et al., 2004; Morin and Edwards, 2006; Morin et al. 2002, 2006; Turner et al., 2010) and European blackberry (*Rubus fruticosus* L. aggregate) (Evans and Bruzese, 2003; Evans et al., 2011; Gomez et al., 2008; Morin et al., 2011) in Australia. For example, specificity in rust fungi can be too high from a biological control point of view, necessitating the release of a range of rust pathotypes to affect the different genotypes of the weed that exist in the introduced range. Leaf-age resistance of the weed to the rust fungus can drastically limit its impact on individual plants, leading to only minor changes in the weed population dynamics. The growing season and preferred habitat of the target weed, as well as prevailing climatic conditions, can also influence the development of severe epidemics of the rust fungus and consequently its efficiency as a biological control agent.

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Potential Benefits of Sourcing Biological Control Agents from a Weed's Exotic Range

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Abstract

Specialist herbivores that establish in the exotic range of a weed provide a potential source of biocontrol agents. Such agents are more likely to adapt successfully to another novel environment and to be devoid of parasitoids and disease. In addition, in a simplified system lacking many key phytophages, assessment of the impact of potential agents may be easier than in the native range. In a South African program for control of weedy *Acacia* species *Bruchophagus acaciae* (Cameron) has been collected from New Zealand where, having established without parasitoids, it is much more abundant than in its native Australia. New Zealand has accumulated many specialist herbivores of *Acacia* from Australia and one of these, the weevil, *Storeus albosignatus* Blackburn, might have provided a useful biocontrol agent for South Africa's weedy *Acacia* species. This weevil was first recorded in New Zealand in the 1930s where it is now widespread and relatively common, feeding on seeds of several *Acacia* species. However, it was not associated with *Acacia* in Australia until 2009. Although seed-feeding agents of target *Acacia* species were sought in Australia intermittently over a period of more than 30 years and five species of *Melanterius* weevils were introduced to South Africa for control of different weedy *Acacia* species, the program did not collect *S. albosignatus* from *Acacia* species. *Storeus albosignatus* could possibly have provided a more parsimonious solution by limiting seed production of several of the target *Acacia* species. Searching for biocontrol agents in the exotic range will be most useful in areas where potential agents naturally accumulate in the exotic range through relative proximity to the native range, but it is also applicable in areas where establishment occurs through accidental transfer by human agency.

Plant-Mediated Interactions among Herbivores: Considerations for Implementing Weed Biological Control Programs

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Abstract

Complex trophic interactions are common, both in natural and managed ecosystems. One such interaction that has important implications for biological control of weeds involves plant responses to feeding by an herbivore which then impacts one or more other herbivores. Effects may be positive or negative, and mechanisms can be chemical or structural. Knowing if, and to what extent, these indirect plant-mediated interactions occur prior to importing new biological agents can assist with decisions about candidate selection, thus reducing economic and environmental costs, and increasing the overall success rate of weed biological control programs. We examined whether feeding by the musk thistle weevil *Trichosiromus horridus* (Panzer), which attacks the vegetative crown early in the plant's development, alters musk thistle as a resource for the later-arriving weevil *Rhinocyllus conicus* Frölich, which infests flower heads. Minor infestations of musk thistle by *T. horridus* had no effect on *R. conicus* oviposition and subsequent production of new adults. In contrast, heavy infestations of *T. horridus* reduced 1) *R. conicus*-musk thistle synchrony, 2) acceptability of musk thistle to ovipositing *R. conicus*, 3) the quantity and 4) the quality of resource available to *R. conicus* larvae. As a result, the production of new *R. conicus* adults was reduced 63%. Thus, even spatially- and temporally-isolated herbivores can affect one another negatively and in multiple ways. Nevertheless, musk thistle seed reduction was still greater when both weevils were present. Hence, the outcome for biological control programs may not necessarily be adverse because of compensatory trade-offs concerning the relative impacts of the two herbivores on the weed. Recommendations for incorporating protocols to assess potential indirect plant-mediated impacts on weed biological control programs will be given.

The Use of Chemical Ecology to Improve Pre-Release and Post-Release Host Range Assessments for Potential and Released Biological Control Agents of *Cynoglossum officinale*

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Abstract

Unlike their extensive use in pest insect management systems, chemical ecological techniques are not commonly used in biological weed control programs, despite their potential benefits to improve host range predictions. Semiochemicals are the principal communication in insect-plant interactions and are especially important mediators of host selection behavior in response to different chemical cues. While much of pre-release host range testing focuses on differing test conditions to either assess the fundamental host range (no-choice) or the realized host range (choice and field tests), little research is directed at underlying host plant cues triggering or preventing a potential agent female's host choice. This is true for two insects currently used or proposed as biological control agents for *Cynoglossum officinale* L., the root-mining weevil *Mogulones cruciger* Hbst. and the seed-feeding weevil, *Mogulones borraginis* (Fabricius). The former has been released for the control of *C. officinale* in Canada in 1997 but a pest alert has been issued for the insect by regulatory authorities in the USA in 2010 because of risks of non target plant feeding. *M. borraginis*, has a narrower host range and is being proposed for introduction into the USA. Pre-release host range evaluations for *C. officinale* agents are complicated by the fact that several native confamilials are rare and endangered or cannot be grown under greenhouse conditions. To assess the risk of non-target feeding by *M. cruciger* in the USA and predict the environmental safety of *M. borraginis*, we developed a portable system to collect headspace volatiles of native confamilials of *C. officinale*. We use combined gas chromatography and electroantennogram detection (GC-EAD) to test whether the volatile headspace or particular components of it, identified using gas chromatography with mass spectrometry (GC-MS), triggers responses of the antennas of either weevil species. We then verify preliminary GC-EAD results in behavioral trials using a four-area olfactometer. We assert that a major advantage of this approach is the non-destructive assessment of the attractiveness of rare non-target plant species to either weevil species.

Shooting Straight: What Weeds Should We Target Next?

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Summary

Biocontrol is the only realistic option for managing some of our most serious weeds, but projects are typically long-term and costly to develop. Therefore it is critical that targets are objectively selected by assessing the likely return on investment. This can be difficult given that both stakeholders and biocontrol practitioners can have very different views on which weeds should be targeted. Further, the relative importance of a particular weed problem, and how feasible and realistic biocontrol is for the particular species, is frequently not considered. Despite these challenges, little attention has been given to the process of target selection within the biocontrol community.

Target prioritisation can be considered within a matrix that includes weed impact, the feasibility of biocontrol (the process constraints for the establishment and execution of a biocontrol program) and the likelihood of successful outcomes (for example the chance that biological control can mitigate the identified impact). Weed impact can be extremely difficult to evaluate and predict, and any assessment needs to consider the context in which the weed occurs, whether the weed is a passenger or driver of environmental change, what it is impacting (in terms of production, biodiversity and ecosystem services), and the temporal and spatial dimension of invasions. Assessment of feasibility includes an in-depth analysis of constraints and opportunities, ranging from the practical (e.g. access to the native-range), social or political (e.g. acceptance of biocontrol as a management solution), and ecological (e.g. the genetic integrity of the target within its native range and the availability of host-specific natural enemies). Likelihood of success includes determining whether biocontrol can address clearly defined criteria for the successful management of the weed, and whether weed impacts are likely to be mitigated by the available natural enemies.

Australian examples were used to illustrate how such a prioritisation matrix can help identify the next generation of biocontrol targets by (a) helping achieve the right balance between targeting weeds of moderate to high impact where potential agents are readily available and high impact weeds where they may not be, and (b) focusing research around key knowledge gaps preventing the adequate prioritisation of new and existing targets. The biocontrol “industry” needs to demonstrate continued success against important targets if it is to survive, and that this will require science-driven target selection, a balanced portfolio of targets, quantification of potential weed impact, and continued improvement in science and technology.

Does Rise and Fall of Garlic Mustard Eliminate the Need for Biological Control?

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Abstract

Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara and Grande), a European biennial herb introduced to North America 150 years ago is now widespread in temperate forests from East to West and as far north as Alaska and south to Georgia. Garlic mustard's rapid spread, high local abundances, and lack of any beneficial effects spurred development of a biological control program in the late 1990's, which included a long-term monitoring program to develop baseline data on life history and population dynamics of *A. petiolata*. We anticipated that before and after comparisons would allow us to assess changes in *A. petiolata* performance associated with biological control agent releases. After a decade of studies in the East and Midwest, we have seen declines of *A. petiolata* to very low abundance at our permanent monitoring locations (but only in the absence of management efforts). Detailed evaluations of potential mechanisms point to negative soil feedback, i.e. the build-up of soil pathogens that appear to suppress *A. petiolata* while having no apparent negative effect on associated forest understory vegetation. While these effects appear widespread in the oldest invaded areas, similar population declines are not as widespread or apparent in the more recently invaded areas further west. We are now ready to petition the introduction of the first host specific control agent – but is this the right choice of action? At the present time the lack of clear and extensive negative ecosystem impacts of *A. petiolata* and the apparent decline without management intervention suggests that adding biological control agents to the North American fauna will not aid in restoration of herbaceous forest communities.

Unravelling the Identity of *Tamarix* in South Africa and its Potential as a Target for Biological Control

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Abstract

The Old World genus *Tamarix* has become naturalized and invasive in much of the rest of the world. *Tamarix usneoides* E. Mey. ex Bunge is native to south western Africa and indigenous to South Africa, where it is being used for phytoremediation of acid mine drainage from tailings storage facilities on gold mines. However, *T. chinensis* Lour., *T. parviflora* DC. and *T. ramosissima* Ledeb. are all exotic to South Africa, and are hypothesized to be hybridizing among themselves and with *T. usneoides*. Correct specific identification is therefore essential; to clone the indigenous species for phytoremediation use, and to investigate the possibility of biological control of the alien species. *Tamarix* remains one of the more taxonomically difficult genera to identify and when in the vegetative state many taxa are almost indistinguishable. The high incidence of hybridization in *Tamarix* also plays a role in the taxonomic confusion. The Internal Transcribed Spacer (ITS) regions of ribosomal DNA (rDNA) were successfully used to identify the local *Tamarix* species and their hybrids. Insect abundance and diversity were found to be higher on the indigenous *T. usneoides* than on the exotic *T. ramosissima* and its hybrids, suggesting that a potential biological control agent might distinguish between the alien and indigenous species. The potential for successful biological control of *T. ramosissima* in South Africa is discussed.

Origins and Diversity of Rush Skeletonweed (*Chondrilla juncea*) from Three Continents

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Abstract

Rush skeletonweed (*Chondrilla juncea* L.) is an invasive apomictic perennial plant in Australia, South- and North America, accidentally introduced from Eurasia, which shows differential resistance/tolerance to some herbicides and classical biological control agents. Rush skeletonweed biotypes have been locally described using morphology, phenology, isozyme patterns, and resistance to control agents, but studies comparing invasions on different continents and determining exact origins of invasive genotypes do not exist or are lacking in detail. Commonly available molecular tools and bulk analysis capacity now make it possible to determine genetic diversity within invasions and their origins. We investigated over 1000 plants from three invaded continents using highly variable AFLP (Amplified Fragment Length Polymorphism) markers, and found 13 distinct genotypes (three from Australia, three from Argentina, and seven from North America). No genotypes were shared between continents. Certain regions in North America, such as California, contain only one genotype of the weed. We then investigated over 1000 plants from the native Eurasian range to determine, as accurately as possible, origins of the invasive genotypes, including those that are currently resistant to strains of rust (*Puccinia chondrillina* Bubak & Syd.) used in biological control programs. This information can be used to screen for pathogens and other agents that will not be resisted or tolerated by certain rush skeletonweed genotypes. Understanding global intraspecific diversity and exact origins can improve management of differentially-resistant/tolerant weed biotypes, enhance efficacy of future agent selection, and increase cooperation between invaded regions.

Comparing the Population Biology of *Isatis tinctoria* in its Native Eurasian and Introduced North American Range under Different Experimental Treatments

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Abstract

Isatis tinctoria L. is an annual, biennial, or short-lived perennial mustard native to Eurasia. The weed was culturally introduced to North America where it has naturalized and become a noxious weed in several western US states. The goals of this study are to identify the sensitive phenostages of *I. tinctoria* to improve management strategies for the invasive plant and to identify most effective biological control agents. Permanent study plots were established in 2010 at an experimental and a close by natural infestation of *I. tinctoria* in southeastern Idaho. In Europe, the experimental site was established in 2008 in southern Germany. The study site in Germany includes the following treatments: Presence and absence of 1) interspecific competition and 2) biological control insect herbivory. The experimental site in Idaho includes presence and absence of 1) interspecific competition and 2) the native rust pathogen (*Puccinia thlaspeos* Schub.), as treatments. Established with each experimental site were seed bank plots with presence and absence of interspecific competition and seed longevity experiments. In both ranges, interspecific competition had so far the strongest and most consistent effect on plant numbers. In Europe, specific insect herbivores negatively impacted individual plant parameters. We hope that the continuation of the study will allow us to demonstrate effects of treatments on life stages of *I. tinctoria*. Results will ideally help prioritizing candidate insect biological control agents currently studied in the native range and assessing the net effect of the native rust present in the introduced range.

Invasive Exotic Plant Species in Tennessee, USA: Potential Targets for Biological Control

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Abstract

Numerous invasive exotic plant species are well established in Tennessee, located in the southeastern United States, where many of these plant species pose serious economical and environmental threats to agriculture, forestry, natural areas, and urban areas. The threat of some invasive plant species, such as musk thistle (*Carduus nutans* L.), multiflora rose (*Rosa multiflora* L.), and kudzu (*Pueraria montana* var. *lobata* (Willd.) Maesen and S. Almeida), is well documented, and these species are well recognized by growers and landowners. Management programs, primarily focused on chemical, mechanical and cultural controls, have been developed to limit the spread of these exotic plant species, and biological control is a major component of integrated pest management of musk thistle. However, the threat of other species, such as purple loosestrife (*Lythrum salicaria* L.), spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos*), and Canada thistle (*Cirsium arvense* (L.) Scop.), in Tennessee is not as well known, and growers and landowners are not as familiar with these exotic species. In most cases, the state-wide distribution of many of these invasive plant species has not been clearly defined. In addition, the diversity of native and exotic insect herbivores that utilize many of these introduced plant species has not been investigated. Biological control will be explored as a management tactic against selected exotic weeds based upon the 'best fit' for the climate and geography of Tennessee. This poster details invasive exotic plant species established in Tennessee and examines the potential for the integration of biological control into pest management programs directed against selected weed species.

Genetic Variation in a Biological Control Target Weed: The Strawberry Guava Species Complex

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Abstract

Our objective is to characterize the genetic variation in strawberry guava populations in Hawaii, with the goal to inform biological control efforts currently being developed to counter this invasive species complex in native forests. Specimens collected on the islands of Hawaii, Maui, and Oahu were evaluated for fruit and vegetative morphology, ploidy as determined by flow cytometry, and microsatellite variation at three chloroplast SSR loci and three nuclear SSR loci. Results supported three previously recognized taxa and one new category. *Psidium littorale* Raddi was uniform with regard to fruit morphology (yellow, spindle-shaped), ploidy (8x), and SSR polymorphisms, suggesting that it may be a fertile allo-octoploid or a sterile apomict. Similarly, *P. lucidum* Hort. was uniform in fruit morphology (yellow, spherical fruits) and SSR genotype, but showed minor ploidy variation about 6x, suggesting a mostly fertile allo-hexaploid or an apomict with some residual sexual function. *P. cattleianum* Afzel. ex Sabine displayed a single uniform chloroplast and nuclear SSR genotype, but ploidy variation between 6.5x and 7.1x, and red fruit color of variable hue and intensity, suggesting that sexual reproduction is operative in this nominally heptaploid form and that it produces mainly aneuploid progeny. A fourth form (*Psidium* "X") with fruit color (orange) and ploidy range (6.4x to 6.8x) intermediate between those of *P. lucidum* and *P. cattleianum* originally suggested derivation through interspecific sexual crossing or possibly elimination of genetic material in the aneuploid sexual progeny of hybrids or of self- or sib-mated *P. cattleianum*. However, the presence of a unique chloroplast SSR allele found in the orange-fruited forms and not in either of the putative parent species indicates that it is not recently of hybrid origin or directly derived from *P. cattleianum*. The orange-fruited form represents a new taxon not previously described in Hawaii. The SSR uniformity within the four strawberry guava taxa may reflect predominantly apomictic seed production, or simply that our survey employed an inadequate number of marker loci to detect polymorphisms. This apparently modest level of genetic variation may suit the strawberry guava complex in Hawaii to target status for a host-specific biological control agent, such as *Tectococcus ovatus* Hempel.

Demographic Matrix Model for Swallow-Wort (*Vincetoxicum* spp.)

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Abstract

Demographic matrix modeling of plant populations can be a powerful tool to identify key life stage transitions that contribute the most to population growth of an invasive plant and hence should be targeted for disruption (weak links) by biological control and/or other control tactics. Therefore, this approach has the potential to guide the selection of effective biological control agents. We are in the process of parameterizing a five life-stage matrix model in order to generate pre-release agent recommendations for the swallow-wort biological control program. Pale swallow-wort (*Vincetoxicum rossicum* (Kleo.) Barb.) and black swallow-wort (*V. nigrum* L.) are herbaceous, perennial, viny milkweeds introduced from Europe (Apocynaceae-subfamily Asclepiadoideae). Both species are becoming increasingly invasive in a variety of natural and managed habitats in the northeastern United States and southeastern Canada. Black swallow-wort appears restricted to higher light environments, whereas pale swallow-wort infestations occur from the high light environments of open fields to low light forest understories. We are quantifying demographic transitions over 3-4 years of both swallow-wort species in field and, for pale swallow-wort, forest habitats in New York State (N = six populations). Vital rates estimated include seed survival, germination, plant survival to reproductive maturity, and fecundity (viable seeds produced per plant). Data will be presented on model parameters derived to date.

How Many Species of *Salsola* tumbleweeds (Russian Thistle) Occur in the Western USA?

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Abstract

Russian thistle or common tumbleweed, *Salsola tragus* L. (sensu lato), is an alien weedy annual plant that infests over 41 million hectares in the western United States. The taxonomy of this plant has had a long confusing history, with frequent misapplication of the species names kali and australis. Recent studies based on morphology, allozymes and molecular genetics indicate that “Russian thistle” comprises seven distinct species in North America. *Salsola tragus* is probably the most widespread species. *Salsola collina* Pall. occurs primarily east of the Rocky Mountains, *S. paulsenii* Litv. primarily in deserts, and *S. kali* is restricted to ocean shores and is not a rangeland weed. *Salsola australis* R., sometimes reported as “type B”, occurs primarily in California, South Africa and Australia, but has never been documented to occur in Eurasia. Almost all uses of this name before 2008 are probably misapplications. Polyploid hybrids include *S. x gobicola* (includes *S. tragus* and *S. paulsenii* ancestry), which is known from western USA and central Asia, and *S. x ryanii* (includes *S. tragus* and *S. australis* ancestry), which is known only from California. A gall forming midge, *Desertovelum stackelbergi* Mamaev, from Uzbekistan (Sobhian *et al.* 2003. Biol. Control 28: 222-228) and a fungal pathogen, *Colletotrichum gloeosporoides* (Penz), from Hungary (Bruckart *et al.* 2004. Biol. Control 30: 306-311) had much higher rates of attack and damage to *S. tragus* than *S. australis*. Although it was previously believed that all species in the kali section of *Salsola* originated in Eurasia, the presence of 4 indigenous species in Australia suggests a separate clade (Borger *et al.* 2008. Aust. J. Bot. 56: 600–608). It is likely that *S. australis* is native to Australia.

An Initial Focus on Biological Control Agents for the Forest Invasive Species *Prosopis juliflora* in the Dry Zone of Myanmar

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Abstract

Prosopis juliflora (Sw.) DC (*Prosopis* hereafter) was introduced into Myanmar for dry zone greening about 1950 from Israel by Agriculture Research and Development Cooperation (ARDC). *Prosopis* is an aggressive weed of forests in the dry zone of Myanmar. Though regarded useful for fuel wood for rural people, this deep rooted species forms thickets, outcompetes native vegetation, has hard and sharp thorns. It is also capable of vegetative reproduction and produces many coppices when cut, and is hence difficult to eradicate. Its distribution is expanding and control is needed in some areas. Investigation for biological control agents were conducted in Pyawbwe in January, 2010. *Prosopis* appeared to struggle to compete with the climber *Combretum roxburghii* D. Don, and the shrubs *Azima sarmentosa* Benth. and *Lantana camara* L. The use of these plants as competitors to suppress *Prosopis* growth is not desirable as *Prosopis* has benefits for rural people, while these competitors do not. Damage to *Prosopis* was detected in the form of yellowing foliage and damage from pathogens around the cuts made to the woody tissue during harvest of fuel wood. *Fusarium* sp., *Tubercularia* sp. and *Nectria* sp. were identified from these damaged trees. Small-scale trials have been initiated to examine the potential for these fungal pathogens to aid in the biological control of *Prosopis*.

Potential for the Biological Control of *Crassula helmsii* in the U.K.

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Abstract

Crassula helmsii (Kirk) Cockayne, also known as Australian swamp stonecrop or New Zealand pigmyweed, is native to Australia and New Zealand as the common names suggest. Since being introduced to the UK in 1911 as an 'oxygenating' plant for garden ponds, it has been gradually increasing its range both in the U.K. and parts of Europe by escaping gardens and through incorrect disposal by aquarium and pond owners. It has now spread to at least 2000 sites in the UK, particularly threatening conservation sites that are home to rare and endangered organisms. With no dormant period and a high tolerance to a range of temperatures, it can dominate static and slow moving water bodies, as well as bank sides, growing in dense mats as an emergent, submerged or terrestrial form. Once established its impacts can be serious; affecting native biodiversity and impeding water flow. With limited possibilities for chemical control in the EU and the plant's ability to re-grow from fragments as small as 1cm, this weed is particularly difficult to manage. An estimation of the cost of treating 2000 infested sites was estimated to be between €5.8 - 12 million. Little is known of the natural enemy complex on *C. helmsii* populations in the native range, so CABI and collaborators initiated scoping surveys in New Zealand and Australia in 2009. Despite the limited nature of the initial surveys, considerable pathogen and herbivore damage were observed, revealing an assortment of natural enemies associated with this weed. The discovery of two highly damaging stem-mining weevils that were previously unrecorded, suggests more species may be identified in the native range in following future surveys. The discovery of additional natural enemies, to those specified in the literature, bodes well for the future biological control of this species.

The Road Less Taken: A Classical Biological Control Project Operated Through an NGO

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Abstract

Historically, most classical weed biological control projects have been initiated and managed by government departments or agencies. We have successfully developed a project along an alternative route, where the main sponsor is a non-governmental organization. The Alberta Invasive Plants Council (AIPC) was established in 2006 to raise the awareness of invasive plants as a problem in Alberta and to promote cooperative efforts to manage these plants in the province. Later that year, AIPC obtained funding to start a biological control project against common tansy (*Tanacetum vulgare* L.), a toxic, perennial weed native to Europe and Asia which is spreading rapidly in pastures and riparian areas in western Canada and the northern USA. This is a joint US-Canadian project, with funding from a number of state, provincial, federal, and industry sponsors in both countries. The US funding is coordinated by the Minnesota Department of Agriculture, and most Canadian funding is handled through AIPC. The CABI laboratory in Delémont, Switzerland, conducts exploration and agent testing under contract with AIPC, and a private consultant (the first author) coordinates the project, also under contract with AIPC. The project coordinator prepares proposals for ongoing funding, reports on progress to project sponsors, promotes public awareness of the project, and works with CABI to select agents for study and oversee the project. AIPC provides administrative services to the project including contract management, billing, and accounting. An annual consortium meeting is held to review progress and plan future work. This approach has allowed us to enhance the existing Canadian capacity for managing overseas biological control research.

A Reassessment of the Use of Plant Pathogens for Classical Biological Control of *Tradescantia fluminensis* in New Zealand

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Abstract

Tradescantia fluminensis Vell. is an herbaceous plant of the family Commelinaceae, native to South America, and commonly known as small-leaf spiderwort. In 1910, it was introduced into New Zealand and became an aggressive invasive weed in natural ecosystems, causing serious environmental imbalances: in particular, preventing the regeneration of native species, and thus undermining the local biodiversity. The problem caused by *T. fluminensis* quickly led to the use of conventional control measures, but without success. As a result, biological control is considered to be the most promising alternative to mitigate the problems generated by *T. fluminensis*. Since 2003, surveys have been conducted in search of plant pathogens in south and southeastern Brazil. In the first phase of surveys, five new fungal species were added to the known pathogenic mycobiota of *T. fluminensis*: *Ceratobasidium* sp., *Cercospora apii* Fresen., *Kordyana* sp., *Uredo* sp. and *Mycosphaerella* sp. In 2009, a second phase of surveys was performed, and resulted in the addition of four more species of pathogenic fungi to the list: *Colletotrichum falcatum* Went, *Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc. and *Septoria* sp. During this period, *Kordyana* (Brachybasidiaceae = Exobasidiales) - commonly associated with species of Commelinaceae, causing symptoms equivalent to those of white smuts such as *Entyloma ageratinae* Barreto and eVans, the successful biological control agent of *Ageratina riparia* in Hawaii and New Zealand, was assessed to have the most potential, based on field data. Subsequently, pathogenicity tests were conducted to evaluate its potential use as a biological control agent. Besides confirmation of its infectivity to *T. fluminensis*, its specificity to this target species was also demonstrated, among the 70 plant species that were tested. Based on these results, further studies are in progress to clarify the identity of *Kordyana* sp. and to develop a protocol for handling the introduction of the pathogen into New Zealand.

European Insects as Potential Biological Control Agents for Common Tansy (*Tanacetum vulgare*) in Canada and the United States

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Abstract

Common tansy (*Tanacetum vulgare* L., Asteraceae), an herbaceous perennial native to Europe, was introduced into North America as a culinary and medicinal herb. Now widely naturalized in pastures, roadsides, waste places, and riparian areas across Canada and the northern USA, tansy is also spreading in forested areas. It contains several compounds toxic to humans and livestock if consumed, particularly α -thujone, and is listed as a noxious weed in several states and provinces. A biological control program for common tansy is being coordinated by a Canadian-US consortium led by the Alberta Invasive Plant Council and the Minnesota Department of Agriculture, with CABI Switzerland Centre identifying and testing potential agents for efficacy and host specificity. Collection efforts are focused on Eastern Europe (Russia and Ukraine) to maximize the climatic match with the infested areas in North America. Several potential agents are under study, the most promising agent at present being a stem-mining weevil, *Microplontus millefolii* (Schltz.). A root-feeding flea beetle, *Longitarsus noricus* Leonardi, also shows promise, and DNA barcoding is being used to separate this species from morphologically similar species that may emerge as contaminants in host-specificity tests. The leaf-feeding tortoise beetle *Cassida stigmatica* Suffr. is specific to *Tanacetum* but is able to complete development on the North American native *T. bipinnatum* ssp. *huronense* (Nutt.) Breitung; further evaluation of the risk to this species is needed. Life history studies on a stem-mining moth, *Isophrictis striatella* (Denis & Schiffermüller), suggest that it develops mainly in the previous year's dead stems. This may reduce its potential impact as a biological control agent. The effects of chemical and genetic variation in tansy on the feeding and oviposition responses of insects are being studied, and molecular methods are also being used to evaluate the relationships between *T. vulgare* and other species.

The Potential for the Biological Control of Himalayan Balsam Using the Rust Pathogen *Puccinia cf. komarovii*: Opportunities for Europe and North America

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Abstract

Himalayan balsam (*Impatiens glandulifera* Royle) is a highly invasive annual herb, native to the western Himalayas, which has spread rapidly throughout Europe, Canada and the United States since its introduction as a garden ornamental. The plant can rapidly colonize riparian systems, damp woodlands and waste ground where it reduces native plant diversity, retards woodland regeneration, outcompetes native plants for space, light and pollinators and increase the risk of flooding. Current control methods are fraught with problems and often unsuccessful due to the need to control the plant on a catchment scale. Since 2006, CABI and our collaborators have surveyed populations of Himalayan balsam throughout the plants native range (the foothills of the Himalayas, Pakistan and India) where numerous natural enemies have been collected and identified. Agent prioritization, through field observations and host range testing has narrowed the potential candidates down to the rust pathogen, *Puccinia cf. komarovii* Tranzschel. This autoecious, monocyclic pathogen shows great promise, not only due to its impact on the host but also due to its high specificity as observed in the field and preliminary host range testing. The aecial stage infects the hypocotyl of young seedlings as they germinate through leaf litter containing teliospores. This initial infection severely warps the structure of the developing plant. The aeciospores then infect developing leaves to produce the cycling phase (uredia). This severely affects the photosynthetic capacity of the maturing plant, with the potential of reducing seed-set. Late in the season, teliospores are produced which overwinter in the leaf litter. This paper will review the research conducted to-date, including a molecular comparison of *P. cf. komarovii* with other closely related species, the life cycle and infection parameters of the rust and an up-date on the current host specificity testing under quarantine conditions in the UK.

The Scotch Broom Gall Mite: Accidental Introduction to Classical Biological Control Agent?

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Abstract

The gall mite, *Aceria genistae* (Nal.) Castagnoli s.l., an accidentally introduced natural enemy of Scotch broom (*Cytisus scoparius* (L.) Link), was first discovered in the Portland OR and Tacoma WA region in 2005. It has since been reported from southern British Columbia to southern Oregon. Observationally, the mite appears to reduce Scotch broom seed production and at high densities can cause extensive stem die-back and plant mortality. In order to utilize the mite as a classical biological control agent, a study of its host range and potential nontarget attack was started in 2006 and continued in 2008-2010. Over 20 ecologically- and economically-valued species were tested in greenhouse and open-field studies. Surveys of confamilial nontarget plant species naturally co-occurring with mite-infested Scotch broom were also assessed. Mites and gall formations were noted on hybrids and ornamental species of Scotch broom. Under greenhouse tests, gall-like growth and eriophyid mites were found on *Lupinus densiflorus* Benth. (*L. microcarpus*), a species listed as endangered in Canada. One unidentified eriophyid mite and no deformed growth was detected on *L. densiflorus* at naturally occurring populations growing sympatrically with mite-infested Scotch broom on Vancouver Island. The ambiguous taxonomy of the mites found on Scotch broom, gorse (*Ulex europaeus* L.) and *L. densiflorus* has added further complications to the study. The overall project and plans for developing a petition for its approval as a biological control agent will be discussed.

The Impact of the Milfoil Weevil *Eubrychius velutus* on the Growth of *Myriophyllum spicatum* and Other Watermilfoils Native to Europe

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Abstract

Eurasian milfoil *Myriophyllum spicatum* L. (Haloragaceae) is an aquatic submerged macrophyte that has become invasive in several countries outside its native range. In the United States a native weevil *Euhrychiopsis lecontei* (Dietz) (Coleoptera: Curculionidae) is being used as a natural enemy of *M. spicatum*. In Europe, a similar aquatic weevil *Eubrychius velutus* Beck develops on several native milfoil species. Impact studies were conducted on three milfoil species including *M. spicatum* to assess the growth impact of the largely leaf-feeding habit of *E. velutus*. The results indicate that the adult and larval damage significantly reduces the growth rate of plants, even at low beetle densities. Although it may not be specific enough to release in regions that have closely related native milfoils, *E. velutus* may be utilized as a candidate agent elsewhere in the world. The implications of the levels of damage are discussed and compared with the damage characteristics of *E. lecontei*, informing the potential use of the weevil as a classical biological agent.

Field Explorations in Anatolia for the Selection of Specific Biological Control Agents for *Onopordum acanthium* (Asteraceae)

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Abstract

Scotch thistle, *Onopordum acanthium* L. (Asteraceae), is a biannual thistle of Eurasian origin, accidentally introduced in North America in the late 1800s. It occurs in most of the western states especially in rangelands, pastures and disturbed soils. When abundant, it reduces forage availability for livestock and wildlife. Western Europe has previously been explored for prospective biological control agents, and seven species of insects have been released in Australia. However, host specificity requirements in N. America are more stringent for these agents because of the presence of many native *Cirsium* species. Some of the agents released in Australia do not appear to be sufficiently specific to use in N. America. Therefore we decided to conduct foreign exploration further east in areas not previously explored. Since 2007 we conducted several explorations and survey trips to discover new potential biological control agents in Turkey. Among the most promising candidates are three weevils *Larinus latus* (Herbst.), *L. gigas* Petri and *L. grisescens* Gyllenhal that were collected in Central Anatolia. The larvae of these species develop in the flowerheads and destroy most seeds. Other candidates are *Psylliodes cf. chalcomera* Illiger and *Lixus cardui* Olivier, whose larvae develop inside stems and leaves. Preliminary laboratory host specificity tests show a narrow host range of some biotypes of both potential biological control agents. Specimens from these experiments are currently undergoing genetic and morphological study to understand if there are distinct genetic entities not distinguishable by morphological traits. A new species of eriophyoid mite, *Aceria* sp., was recorded for the first time in the vicinity of Isparta, Western Turkey associated with the target weed. It causes stem atrophy and flower bud abortion with a consequent decrease of seed production. An unidentified nematode species causing visible blisters on Scotch thistle leaves was found in Central Turkey.

Potential Biological Control of Invasive Tree-of-Heaven (*Ailanthus altissima*)

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Abstract

The highly invasive tree-of-heaven, *Ailanthus altissima* (Mill.) Swingle, was introduced into Pennsylvania (PA), USA, in 1784 and has since spread across PA and most of the USA. Wherever it is found, *Ailanthus* often dominates a site at the expense of native plant species. However, in 2002-2003, we discovered several stands of dead and dying *Ailanthus* trees within oak-dominated, mixed-hardwood forests in south-central PA. Isolations from symptomatic *Ailanthus* seedlings, root sprouts, saplings, and canopy trees in the field consistently yielded the naturally occurring, soil-borne, wilt fungus *Verticillium albo-atrum* Reinke & Berthold. Identification was based on morphological characteristics, and confirmed using molecular techniques. Potted *Ailanthus* seedlings in the greenhouse and canopy *Ailanthus* trees in the field were inoculated with a randomly selected isolate of *V. albo-atrum*. Classic wilt symptoms quickly developed on inoculated plants, from which *V. albo-atrum* was recovered, fulfilling Koch's Postulates and illustrating that *V. albo-atrum* was highly virulent on *Ailanthus*. As of 2011, *V. albo-atrum* has killed thousands of canopy *Ailanthus* trees in south-central PA. Intermingled non-*Ailanthus* tree species and understory shrub have been generally unharmed. We hypothesize that a naturally occurring strain of *V. albo-atrum* has become host-adapted to *Ailanthus*. As part of risk assessment, we inoculated more than 80 non-*Ailanthus* species (potted seedlings in the greenhouse, as well as trees in the field) with a randomly selected strain of *V. albo-atrum*. Inoculated non-*Ailanthus* species were generally unharmed, again indicating that a pathogenic strain of *V. albo-atrum* may have become host-adapted to *Ailanthus*. In addition, past forest management practices in the area (e.g., clear-cutting large blocks of oaks killed by insect infestations) favored development of dense, nearly monoculture stands of clonal *Ailanthus*, which in turn. Short-range dissemination of *V. albo-atrum* within infected stands of *Ailanthus* likely occurs *via* intraspecific root grafts between diseased and healthy trees within these dense stands. Long-range dissemination of *V. albo-atrum* may be facilitated by *Euwallacea validus* (Eichhoff), an introduced ambrosia beetle that is epidemic on *Ailanthus* trees under stress from *V. albo-atrum* infections.

***Abrostola clarissa* (Lepidoptera: Noctuidae), a New Potential
Biological Control Agent for Invasive Swallow-Worts,
Vincetoxicum rossicum and *V. nigrum***

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Abstract

Pale and black swallow-worts (*Vincetoxicum rossicum* (Kleopow) Barbar. and *V. nigrum* (L.) Moench; Apocynaceae, subfamily Asclepiadoideae), perennial vines native to Eurasia, are now invading natural and anthropogenic habitats in the northeastern U.S.A. and southeastern Canada, threatening natural biodiversity and increasing control costs for land managers. Chemical and mechanical methods have not been adequate to control swallow-worts. In addition, no local American herbivores or pathogens cause significant damage to these weeds. Several potential biological control agents associated with *Vincetoxicum* spp. in Europe have been found and investigated, but none of them have yet been introduced. During explorations for herbivorous insects feeding on *Vincetoxicum* species in the Russian North Caucasus, we discovered a new potential biological control agent, *Abrostola clarissa* Staudinger (Lepidoptera, Noctuidae). *A. clarissa* inhabits low mountains and dry hills, having 1 – 2 generations per season. The biology of this species is similar to that of the closely related *A. asclepiadis*: eggs are laid on the undersurface of the host plant leaves, and larvae feed on the foliage and pupate in the soil. In natural conditions, larvae of this noctuid moth were collected only on *Vincetoxicum* spp. No-choice tests conducted under laboratory conditions showed that larvae of *A. clarissa* voluntarily fed and successfully pupated on *Vincetoxicum nigrum*, *V. rossicum*, *V. hirundinaria* (L.) Pers., and *V. laxum* (Bartl.) C.Koch. Neither feeding nor survival was recorded on other Apocynaceae (11 species of *Amsonia*, *Apocynum*, *Asclepias*, and *Cynanchum*) or on plants from other, more distantly related, families (Rubiaceae, Scrophulariaceae, and Convolvulaceae). We conclude that *A. clarissa* can be considered a highly specific potential biological control agent that undoubtedly deserves further study.

Suitability of Using Introduced *Hydrellia* spp. for Management of Monoecious *Hydrilla verticillata*

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Abstract

Two species of introduced leaf-mining flies, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock, suppress dioecious hydrilla by reducing photosynthesis, thereby impacting biomass production, tuber production, and fragment viability. To determine the flies' suitability for monoecious hydrilla management several studies were conducted. When reared on monoecious hydrilla, *H. pakistanae* survival was reduced by 40 and 26 percent during bioassays and greenhouse rearing, respectively. *Hydrellia* spp. also exhibited a 9 day increase in developmental time when reared on monoecious hydrilla. *Hydrellia* spp. colonization and percent leaf damage between biotypes also differed significantly using small-scale tank experimentation. Initial fly stocking rates were equal, but four weeks after release fly levels and percent leaf damage in dioecious tanks were 5.3 and 2.4 fold higher than on monoecious. Small pond experimentation revealed similar results with fly levels 50-fold higher on dioecious in comparison to monoecious. Most importantly, release of close to 2,000,000 *Hydrellia* spp. at sites on Lake Gaston, NC since 2004 have failed to provide convincing evidence of establishment let alone population increase and impact. Recent anecdotal information from Guntersville Reservoir, AL suggests that shifts from a dioecious hydrilla dominated system to one composed mainly of the monoecious biotype may have been, in part, caused by differential feeding by *Hydrellia* spp. Experiments and field studies conducted since 2004 indicate that the monoecious biotype is not as suitable a host for introduced *Hydrellia* spp. as is dioecious hydrilla. This conclusion is based in part on reduced survival and longer developmental time in bioassay experiments and greenhouse rearing, lower colonization success and population growth in larger outdoor systems, and lack of establishment on Lake Gaston. Underlying mechanisms for such differences are unknown but may be due to overwintering strategies, density of the canopy at the water surface, and lowered nutritional value between the biotypes.

Natural Enemies of Floating Marshpennywort (*Hydrocotyle ranunculoides*) in the Southern USA

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Abstract

The aquatic plant, floating marshpennywort, is invasive to many areas where it has been introduced, including Australia and Europe. Because traditional methods of control are often costly, ineffective, or unsustainable, biological control is being considered as a viable alternative. The first step in initiating a successful biological control program is a survey of natural enemies of the target plant in its home range. Since it is considered native to the United States, floating marshpennywort was surveyed in the Gulf Coast States for insect herbivores and pathogens from 2007-2011. A total of ten insect and five potential pathogenic species were recovered during the surveys. Insects recovered included three weevil (Coleoptera: Curculionidae), two caterpillar (Lepidoptera), and at least one grass fly (Diptera: Chloropidae). Although many of the insect species collected are known to be generalist herbivores, several had unknown diets. The chloropid, *Eugaurax floridensis* Malloch, and nymphalid, *Enigmogramma basigera* (Walker) show the greatest promise as biological control agents because both species are able to cause damage and complete their life cycles on floating marshpennywort. Host specificity testing is needed to determine diet for both species. Potential pathogen genera collected included *Alternaria*, *Pestalotiopsis*, *Colletotrichum*, and *Phoma*. None of the pathogen species collected are likely to be host-specific, but several appear to cause considerable leaf damage to floating marshpennywort and may limit its growth and reproduction, at least to some degree. The potential for these species to be used as mycoherbicides will require further research.

Can We Optimize Native-Range Survey Effort through Space and Time?

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Abstract

Native range surveys are an expensive and time consuming step in biological control projects but are crucial for determination of the arthropod and fungal fauna which provides the basis upon which agent selection can take place. A key challenge when designing and conducting native-range surveys is to decide how best to allocate survey effort through space (the species-native range distribution) and through time (at any one particular site or region). For example, should more effort be spent searching the same sites more extensively or on searching elsewhere, and when should surveying come to an end? *Parkinsonia aculeata* L. is a weed of Australia's rangelands that occurs naturally over a large part of the American tropics and subtropics (USA to Argentina). Native range surveys have been conducted over many years in many parts of this range but in an uneven way with some parts intensively surveyed, some parts minimally surveyed and other parts not surveyed at all. A study commenced in 2008 which used the existing results of surveys to predict where further surveys would be most fruitful. Information used in this study included 1) biogeographic zoning of the Americas according to the insect fauna, 2) a *P. aculeata* climate model, 3) *P. aculeata* species distributions, and 4) the insect fauna collected. We used generalized dissimilarity modelling of *P. aculeata* fauna to provide the data for a technique called survey gap analysis. The survey gap analysis predicted areas which should yield the greatest number of new species. In addition, we used species accumulation curves to predict which existing regions further surveying would yield most new species. We discuss the potential for these and other approaches to improve the allocation of native-range survey effort.

Potential Agent *Psectrosema noxium* (Diptera: Cecidomyiidae) from Kazakhstan for Saltcedar Biological Control in USA

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Abstract

Research was conducted on eight native populations in southeastern Kazakhstan during 1995-2009. This narrow-oligophagous, univoltine, stem-galling, midge fly attacks saltcedar (*Tamarix* spp.) where both are native in Kazakhstan, Turkmenistan, Uzbekistan and western China. It is a candidate for biological control of saltcedars in the western United States and northern Mexico. In nature, the adults of *Psectrosema noxium* Marikovskij emerged from overwintered galls in late April, mated, and laid 1-30 eggs on each of several new foliage buds. The larvae emerged in 3-4 days and immediately entered the same buds. They developed slowly as the buds developed into stems, and formed colonial galls of 1-30 cells visible in early summer that became ligneous by autumn. The larvae pupated in spring in synchrony with *Tamarix* bud-break. Where numerous, they killed branches or even the entire *Tamarix* plant. Outbreak populations were seen only each 5-7 years, reduced between peaks by severe attack by four species of egg and larval parasitoids. In Kazakhstan, we observed galls of *P. noxium* mostly on *T. ramosissima* Ledeb. and sometimes on other saltcedars but never on other genera of Tamaricaceae. In outdoor, uncaged tests at Almaty, females readily accepted accessions of *T. ramosissima* from five U.S. states, for oviposition and the larvae completed their development on it. However, close synchronization between *Tamarix* bud-break and adult midge emergence is critical so that oviposition can take place on just erupted buds. Eggs laid on young shoots produce only undersized galls that fall from the plant by autumn and the larvae die. Females did not oviposit in on athel, *T. aphylla* (L.) H.Karst., a beneficial exotic shade tree in northern Mexico and the U.S., that is not a target for biological control. Methods of culturing *P. noxium* in the laboratory and in field cages were developed if it is introduced into the USA.

**Fungi Pathogenic on *Paederia* spp. from Northern Thailand as
Potential Biological Control Agents for Skunkvine
Paederia foetida (Rubiaceae)**

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Abstract

The skunkvine *Paederia foetida* L. (known locally as Maile Pilau) is one of the major invasive environmental weeds on the Hawaiian Islands. Due to its rapid spread to remote and inaccessible areas, its ability to cause substantial damage to natural ecosystems and the increasing cost of conventional control methods, its management by biological control seems to be the most appropriate means with high success potential. A survey was conducted in its native range in northern Thailand in the fall of 2010, with the aim of locating and identifying potential agents for classical biological control. Diseased tissues of *Paederia* species exhibiting symptoms of necrotic spots/lesions, galls and rusts were imported into the Hawaii Department of Agriculture's Plant Pathogen Containment Facility (HDOA-PPCF) for evaluation. Most of the infected tissues were derived from *Paederia pilifera* Hook.f., from which several fungi were subsequently isolated. Among these fungi were the gall rust (Basidiomycota) *Endophyllum paederiae* (Dietel) F. Stevens & Mendiola, the Hyphomycetes *Pseudocercospora paederiae* Sawada ex Goh & W.H. Hsieh, and two isolates of the Coelomycetes *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Repeated attempts to establish *E. paederiae* on the local skunkvine *P. foetida* failed, indicating that this rust fungus may be too host specific to infect the local species. However, the remaining fungi *P. paederiae* and isolates of *C. gloeosporioides* were amenable to laboratory culture on standard potato dextrose agar. Separate inoculation tests on the local *P. foetida* plants with conidia from each fungal culture showed leaf lesions or necrotic spots, where the respective fungus could subsequently be reisolated. One of the isolates of *C. gloeosporioides* was relatively aggressive, causing leaf chlorosis, defoliation and even shoot tip dieback on the infected *P. foetida* plants. Further studies on the potentials of these fungi as biological control agents on *P. foetida*, such as their effects on other economic plants (host range), culture and pathogenic enhancements by environmental factors etc., are underway inside the HDOA-PPCF.

Preliminary Surveys for Natural Enemies of the North American Native Delta Arrowhead (*Sagittaria platyphylla*, Alismataceae), an Invasive Species in Australia

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Abstract

The perennial, North American native delta arrowhead (*Sagittaria platyphylla* (Engelm.) J.G.Sm., Alismataceae) was introduced to Australia as an ornamental pond plant in the 1950s. Today, it is a serious invader of irrigation channels and natural waterways in south-eastern Australia with up to \$2 million being spent annually on control in the worst affected regions. Delta arrowhead is free from attack by herbivores and pathogens in Australia, where it seeds prolifically for nearly seven months of the year. Recent preliminary surveys in the southern USA identified a number of curculionids in the genus *Listronotus* associated with delta arrowhead, none previously reported on this host-plant. These weevils and a chloropid fly, *Eugaurax* sp. were all found to attack flowers and fruits. Further surveys are planned to complete the catalogue of natural enemy flora and fauna across the native range. Upcoming genetic studies to identify the genetic origin(s) of the Australian populations will underpin future research on the selection of biological control agents for delta arrowhead.

Prospects for Biological Control of *Berberis darwinii* (Berberidaceae) in New Zealand: What are its Seed Predators in its Native Chilean Range?

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Abstract

A native of South America, Darwin's barberry, *Berberis darwinii* Hook, (Berberidaceae) has become an invasive species in New Zealand. It has invaded many habitat types from grazed pastures to intact forests, due in part to its large reproductive capacity. Consequently, as an early step in a biocontrol solution surveys were conducted in its native range for damaging invertebrates utilizing flower buds and seeds. Sampling for potential agents was conducted at 35 sites in southern Chile between Concepción (36°57' S.) and Chiloé (42° 52' S.). At suitable sites flowering or fruiting plants of *B. darwinii* were beaten 5 times onto a beating tray and all weevil species observed were collected. The insect surveys yielded four weevil species on Darwin's barberry. *Berberidicola exaratus* (Blanchard) was the most common and widely distributed seed predator. It was detected at 29 of the 35 sites. *Anthonomus kuscheli* Clark was the most common flower bud feeder and was detected at 13 of the 35 sites. Damage to the seeds and flower buds by these weevils is obvious. Host-testing studies of these two weevil species is continuing in Chile.

Surveys for Potential Biological Control Agents for *Pereskia aculeata*: Selection of the Most Promising Potential Agents

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Abstract

New biological control agents are required for the control of *Pereskia aculeata* Mill. in order to reduce the weed's density to acceptable levels. Data from eight surveys for natural enemies of *P. aculeata* in the native distribution were used to compile a list of insect species associated with the plant. Sixty-two sites were surveyed resulting in a list of 40 insect species associated with the plant. Six prioritization categories were used to identify the most promising potential biological control agents from the suite of insects associated with the plant. Prioritization categories were i) the presence of feeding damage, ii) insect incidence measured as the number of sites at which the insect species was present divided by the total number of sites, iii) the host range of the insect observed in the field, iv) the similarity of the climate where the insect species was found to the climate at the weed's introduced distribution, v) the similarity of the weed genotype to the genotype on which the insect species developed in the native distribution and vi) the mode and levels of damage in the native distribution. The most promising potential biological control agents for *P. aculeata* identified using the various criteria of the prioritization categories are the released biological control agent, *Phenrica guérini* Bechyné (Chrysomelidae), two species of Curculionidae and *Maracayia chlorisalis* Walker (Crambidae). The method used to prioritize the most promising potential biological control agents for future research may be useful when surveying for natural enemies for use as biological control agents for other weed species.

Predicting the Feasibility and Cost of Weed Biological Control

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Abstract

We reviewed previous work related to selection and prioritisation of weed biological control targets and developed and tested hypotheses regarding which attributes make weeds more or less amenable to biological control. Our analyses revealed that biocontrol impacts have, on average, been greater against, biennial and perennial versus annual weeds, plants capable of vegetative reproduction versus plant that reproduce solely by seed or spores, aquatic and wetland weeds versus terrestrial weeds, and plants that are not reported to be weedy in the native range versus those which are known to be weedy in the native range. We incorporated these criteria affecting biological control success into a feasibility scoring framework that should enable practitioners to better prioritise targets for biological control in the future.

USDA-ARS Australian Biological Control Laboratory

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Abstract

The staff of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Australian Biological Control Laboratory (ABCL) conduct exploration in the natural areas of Australia and Asia for insects and other organisms that feed on pest insects and plant species that are invasive in the USA. Based at the Ecosciences Precinct in Brisbane, Queensland, Australia, the ABCL is operated by the USDA-ARS Office of International Research Programs (OIRP) while personnel and facilities in Australia are provided through a co-operative agreement with the Commonwealth Scientific and Industrial Research Organization (CSIRO). Many invasive weeds in the USA such as the broad-leaved paperbark tree, *Melaleuca quinquenervia* (Cav.) S.T. Blake; Old World climbing fern, *Lygodium microphyllum* (Cav.) R.Br.; hydrilla, *Hydrilla verticillata* (L.f.) Royle; and Australian pine, *Casuarina* spp. are native to Australia. However, the native distribution of many of these weed species extends into tropical and subtropical Southeast Asia, including Indonesia, Malaysia, Thailand, Vietnam, Papua New Guinea, India and southern China, as well into the Pacific Islands. With excellent collaborators in these regions, ABCL has the capability to explore these countries to find the most promising biological control agents for these and other targets, and evaluate them under quarantine conditions in Brisbane. Research conducted at ABCL includes determination of the native range of a target, exploration for natural enemies, molecular typing of herbivores, ecology of the agents and their hosts, field host-range surveys and ultimately preliminary host-range screening and impact assessments of candidate agents. The data we gather on potential agents is combined with information about the ecology of the target where it is invasive. Our research seeks to determine what regulates the target in its native environment and evaluates all potential biological control agents particularly those that can mitigate the weed's impact. Organisms with a narrow host range and good regulatory potential are prioritized and intensively investigated. In collaboration with US-based ARS scientists, agents are selected and shipped to the United States for further quarantine studies and possible release.

Potential Biological Control Agents of Skunkvine, *Paederia foetida* (Rubiaceae), Recently Discovered in Thailand and Laos

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Abstract

The skunkvine, *Paederia foetida* L., also known as Maile pilau in Hawaii, is an invasive weed that smothers shrubs, trees, and native flora in dry to wet forests. It disrupts perennial crops and takes over landscaping in moist to wet areas on four Hawaiian Islands. Skunk-vine is considered a noxious weed in southern United States (Alabama and Florida) and also an aggressive weed in Brazil, New Guinea, Christmas and Mauritius Islands. Chemical control is difficult without non-target damage as the vine mixes up with desirable plants. Biological control is thought to be the most suitable option for long term management of the weed in Hawaii and Florida. Skunk-vine and most species of genus *Paederia* are native to tropical and subtropical Asia, from as far as India to Japan and Southeast Asia. There are no native plants in the tribe Paederieae in Hawaii and Florida and the potential for biological control looks promising. A recent survey in October-December 2010, after the rainy season in Thailand and Laos, confirmed the presence of several insect herbivores associated with *P. foetida* and three other *Paederia* species. A leaf-tying moth (Lepidoptera: Crambidae), two hawk moths (Lepidoptera: Sphingidae), a herbivorous rove beetle (Coleoptera: Staphylinidae), a chrysomelid leaf beetle (Coleoptera: Chrysomelidae), a sharpshooter leafhopper (Hemiptera: Cicadellidae), and a leaf-sucking lace bug (Hemiptera: Tingidae) were the most damaging to the vine during the survey period. The beetles are being investigated at the HDOA Insect Containment Facility as potential candidates for biological control of Maile pilau in Hawaii. Initial findings on host specificity, biology, and their potential for suppressing this weed are discussed.

Towards Biological Control of Swallow-Worts: The Ugly, the Bad and the Good

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Abstract

Native to Eurasia, swallow-worts (“the ugly:” *Vincetoxicum rossicum* (Kleopov) Barbarich and *V. nigrum* L. - Apocynaceae) have invaded forested landscapes and prevented native plant regeneration in eastern North America. We first aimed to understand where the invasive populations of both species come from, and then evaluated the impact of potential biological control agents (BCAs). The following phytophagous BCAs have been studied since 2009: *Chrysochus asclepiadeus* (Pallas) (Col., Chrysomelidae), *Abrostola asclepiadis* (Denis & Schiffermuller) and *Abrostola clarissa* (Staudinger) (Lep., Noctuidae). Adults of the beetle feed on leaves while larvae are root feeders, and *Abrostola* spp. larvae are foliage feeders. Genetically, none of the native *V. nigrum* populations analyzed to date possesses exactly the major multilocus genotype detected in the invasive North American populations, in contrast to *V. rossicum*, for which source populations of the invasion are found to be in Ukraine. We performed choice and no-choice specificity tests with French and Russian populations of *C. asclepiadeus*. We evaluated adult herbivory in no-choice tests on three *Vincetoxicum* spp., as controls, and seven *Asclepias* spp.: *C. asclepiadeus* fed on controls but also on *Asclepias tuberosa* L., a monarch butterfly host plant. Choice tests revealed no herbivory outside the genus *Vincetoxicum*. Larval herbivory in choice tests was noticed on all controls, plus *A. tuberosa* and *Asclepias syriaca* L. Similar results were obtained for both populations of *C. asclepiadeus*. Although *C. asclepiadeus* has a severe impact on swallow-worts, herbivory on several *Asclepias* spp. lead us to consider it a “bad” BCA. No-choice tests with larvae of *Abrostola asclepiadis* from France revealed that they died in 5d on all the *Asclepias* spp., but developed to pupa in 23d on *Vincetoxicum hirundinaria* Medik., in 20.6d on *V. rossicum*, and only reached the 3rd instar in 17.8d on *V. nigrum*. Similar results were obtained with *Abrostola clarissa* of Russian origin. Thus, data with *Abrostola* spp. appear promising, and we consider the two *Abrostola* species to have good potential as BCAs against all genotypes of swallow-worts.

Genetic and Behavioral Differences among Purported Species of *Trichosirocalus* (Coleoptera: Curculionidae) for Biological Control of Thistles (Asteraceae: Cardueae)

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Abstract

Trichosirocalus horridus (Panzer) was introduced to North America, New Zealand and Australia for biological control of *Carduus nutans* L. Since then two more species of *Trichosirocalus* have been described (Alonso-Zarazaga and Sánchez-Ruiz. 2002. Aust. J. Entomol. 41: 199-208), and the three species are thought to have different host plant associations: *T. horridus* on *Cirsium vulgare* (Savi) Ten. and possibly on other Carduineae, *T. mortadelo* Alonso-Zarazaga & Sanchez-Ruiz on *C. nutans*, and *T. briesei* Alonso-Zarazaga & Sanchez-Ruiz on *Onopordum* spp. This raises the question of which species were previously released for biological control of *C. nutans*. Subsequent studies by Groenteman et al. (2009, XII International Symposium on Biological Control of Weeds, pp. 145-149.) raises uncertainty about which species are in New Zealand and whether *T. mortadelo* was a valid species. *Trichosirocalus briesei* was introduced to Australia to control *Onopordum* spp. and is being evaluated for introduction to North America. We analyzed part of the mtDNA cytochrome oxidase I (COI) gene sequence of adult specimens representing the three species collected in Spain, Italy, USA, New Zealand and Australia. All specimens morphologically identified as *T. briesei* formed one clade that was clearly distinct from all the other specimens. The COI sequences for specimens of *T. horridus* and *T. mortadelo* were intermixed within the same clade, suggesting that they represent one heterogeneous species. Furthermore, the morphological characters attributed to *T. mortadelo* are of little significance to really isolate two different species, so that we combine them under the name of *T. horridus*. In laboratory choice experiments, specimens from Spain identified as *T. briesei* preferred *Onopordum acanthium* L. to *Carduus* or *Cirsium* spp., whereas those from North America, identified as *T. horridus* preferred *Carduus* spp. but also attacked *Cirsium* spp.

Survey of Dispersal and Genetic Variability of *Tectococcus ovatus* (Heteroptera: Eriococcidae) in the Regions of Natural Occurrence of *Psidium cattleianum* (Myrtaceae)

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Abstract

The species *Psidium cattleianum* L. is considered one of the greatest threats to the ecosystem and biodiversity of the islands of Hawaii. Seeking to control its dissemination, techniques of biological control were used. Among the various species studied, as a biological agent control, *Tectococcus ovatus* Hempel showed a higher level of specificity. This work had as aim to verify the existence of genetic variability among and inside the different populations of *T. ovatus*, using the technique of PCR-RAPD. The analyses were made from females collected in the states of Rio de Janeiro, Paraná, Santa Catarina and Rio Grande do Sul in Brazil. From the eight initiators of PCR-RAPD tested, four were used in the analyses, revealing monomorphic and polymorphic markers with a variable frequency, to the individuals of one place as well as to the individuals of different places. Through the analysis of the grouping of molecular characterization it was possible to verify a formation of two distinctive groups A and B presenting a genetic variability/variable of 44%. The results obtained through the analysis of markers RAPD were useful in the verifying of variation and provided safe information about the levels of variability and similarity amongst and inside the different populations of *T. ovatus*.

Arundo donax – Giant Reed

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Abstract

Arundo donax L., giant reed, carrizo cane, is an exotic and invasive weed of riparian habitats in the southwestern U.S. and northern Mexico. Giant reed dominates these habitats, which leads to: loss of biodiversity; stream bank erosion; damage to bridges; increased costs for chemical or mechanical control along irrigation canals and transportation corridors; and impedes access for law enforcement personnel. Most importantly this invasive weed competes for water resources in an arid region where these resources are critical to the environment, agriculture and urban users. Biological control using insects from the native range of giant reed may be the best option for long-term management. *A. donax* is a good target for biological control because it has no close relatives in North or South America, and several of the plant feeding insects from Mediterranean Europe and known to only feed on *A. donax*.

Foreign Exploration for Biological Control Agents of Giant Reed, *Arundo donax*

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Abstract

Collections of insect-infested *Arundo donax* L. have been made 2000 - 2011 by scientists at the USDA/ARS EBCL facility in Montpellier, France. Pieces of *A. donax* stem, leaves and rhizomes were placed in double wrapped paper bags and kept in tight mesh covered boxes in the EBCL insect quarantine. Emergence of insects was noted and specimens sent to relevant authorities for identification. Samples were taken at the same time for genetic characterization of *A. donax*. Surveys have been made in appropriate areas of Croatia, Bulgaria, Slovenia, France, Spain, Portugal, Canary Isles, Italy, Greece, Crete, Turkey, Morocco, Tunisia, Egypt, South Africa, Namibia, Kenya and Australia. Surveys were in fall and spring to cover the most important growth periods. Site details, locality, altitude, GPS position were recorded. Giant reed rhizomes were unearthed and dissected at some sites for natural enemies. Lengths of rhizome and cut stems and leaves were placed in moisture absorbent bags, cooled, and returned to the EBCL quarantine for emergence. Quadrats (50x50cm) of *A. donax* have been sampled from 6 stands each week for 15 weeks (starting May 5 2003 in 2004 and 2005) in the Montpellier and Perpignan areas of southern France. All *A. donax* within the quadrats was cut, taken back to the laboratory, examined, dissected and documented. Organisms found were where possible reared and adults passed on to appropriate taxonomists. The arthropod herbivores collected from *A. donax* were (in order of most to least common) *Tetramesa romana* Walker (Hymenoptera: Eurytomidae); *Rhizaspidotus donacis* (Leonardi) (Hemiptera: Diaspididae); *Cryptonevra* spp. (Diptera: Chloropidae); *Lasioptera donacis* Coutin (Diptera: Ceccidomyiidae); *Cerodontha phragmitidis* Nowakowski (Diptera: Agromyzidae); *Melanaphis donacis* (Passerini) (Hemiptera: Aphidae); *Aclerda berlesii* Buffa (Hemiptera: Aclerdidae); *Siteroptes* sp. (Acarina: Pyemotidae); and *Hypogaea* sp. (Hemiptera: Aphididae). Only the first four species were found to be sufficiently host specific to warrant further host range testing.