SESSION 1: PRE-RELEASE TESTING OF WEED BIOLOGICAL CONTROL AGENTS
Pre-release Studies and Release of the Grasshopper
*Cornops aquaticum* in South Africa – a New Biological Control Agent for Water Hyacinth, *Eichhornia crassipes*

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Abstract

The grasshopper, *Cornops aquaticum* Brüner (Orthoptera: Acrididae) has recently been released in South Africa as a biocontrol agent for water hyacinth, *Eichhornia crassipes* (Martius Solms-Laubach (Pontederiaceae), the country's worst invasive aquatic weed. The release follows 15 years of pre-release studies to assess *C. aquaticum*’s safety and potential value as a new agent. *Cornops aquaticum* was first introduced into quarantine in South Africa from Manaus, Brazil in 1995. Host specificity testing was completed by 2001 but release of the grasshopper was delayed, initially, due to difficulties in obtaining release permits for weed biocontrol agents. A permit was finally granted in 2007 by which time pre-release efficacy studies had been initiated and new concerns over compatibility of *C. aquaticum* with the *Neochetina* (Coleoptera: Curculionidae) weevils, the most damaging agents in the field in both South Africa and other parts of Africa, had arisen. The efficacy and agent interaction studies were first concluded to guide the decision on whether *C. aquaticum*’s introduction into the country was justifiable. Pre-release impact studies indicated that *C. aquaticum* damage is directly associated with density and that herbivory at relatively low grasshopper densities can disrupt water hyacinth growth and productivity when growing under optimal nutrient conditions. Interaction studies with *C. aquaticum* and *Neochetina eichhorniae* Warner suggested a synergism whereby pairing of these agents, under laboratory conditions, had the greatest negative impact on biomass accumulation compared to the agents alone or other combinations of agents tested. In August 2010, the South African biocontrol community supported a decision to release *C. aquaticum* and field releases began early in 2011. Four initial release sites have been selected to encompass different nutrient and climatic conditions and are being monitored to assess establishment, impact and population dynamics of *C. aquaticum*.

Introduction

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) is a free-floating perennial herb, native to South America that was introduced into South Africa in the early 1900’s via the ornamental plant trade. By the 1970’s it had reached pest proportions in many systems around the country and to date remains South Africa’s worst invasive aquatic weed (Coetzee et al., 2011). A biological control programme for water hyacinth was initiated in 1974 with the release of the petiole-mining water hyacinth weevil, *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae). Following this, an additional four arthropod biocontrol agents were released between 1989 and 1996: a leaf-mining mite, *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae) in 1989; another petiole-mining weevil, *Neochetina bruchi* Hustache and, a petiole-mining moth, *Niphograpta albignutallis*
Warren (Lepidoptera: Pyralidae) in 1990; and a sap-sucking mirid, Eccritotarsus catarinensis Carvalho (Hemiptera: Miridae) in 1996 (Hill and Cilliers, 1999).

While South Africa’s biocontrol programme had a few successes, most water hyacinth sites around the country have been difficult to control biologically. By the end of the 1990’s, after almost 30 years of an active biological control programme, it was clear that success was variable and levels of control were deemed unsatisfactory. Hill and Olckers (2001) outlined several factors that were speculated to disrupt or reduce the efficacy of the biocontrol agents already released, the most important of which were eutrophication, where plant growth rates outpace the damage caused by the biocontrol agents, and incompatibility of the agents with the temperate climate of many regions of the country. A potential solution was to consider new agents in the hope that a better agent could be found or that the correct, complimentary suite of agents had not yet been released.

The neotropical grasshopper, Cornops aquaticum Brüner (Orthoptera: Acrididae) was considered the most promising candidate based on reports on its damage potential from the native range (Perkins, 1974), and its wide distribution in South America, extending to climatically similar regions to South Africa (Adis et al. 2007). The first collections of the grasshopper took place in Manaus, Brazil in 1995 and subsequent collections were made in Trinidad and Venezuela in 1997 and Mexico in 1997. Oberholzer and Hill (2001) studied the host range of C. aquaticum by testing 64 plant species in 32 families and concluded that it is oligophagous within the family Pontederiaceae, with a strong preference for water hyacinth.

Although C. aquaticum was considered safe for release in South Africa based on its host specificity, its release was initially delayed due to difficulties in obtaining release permits. Regulatory authorities delayed granting permits as they lacked in-house expertise needed to critically evaluate release applications. A permit was finally granted in 2007, by which time agent efficacy studies had already been initiated. It was decided to complete this research in order to determine whether the grasshopper’s introduction into South Africa was justified based on its potential to be an effective biocontrol agent. The efficacy results showed strong support for the grasshopper’s release but new concerns over compatibility of C. aquaticum with the Neochetina weevils had arisen. The weevils are the most important water hyacinth biocontrol agents in South Africa as well as other parts of Africa so any disruption to their efficacy or populations would have diminished prospects for the grasshopper’s release.

This paper presents a subset of results from the efficacy and agent interaction studies that primarily motivated the decision to proceed with the release of C. aquaticum into the South African biocontrol programme for water hyacinth. It also summarizes details of the first releases of the grasshopper in South Africa.

**Methods and Materials**

1. **Agent efficacy studies**
   1.1 Effect of water nutrient levels on the impact of Cornops aquaticum herbivory

   Water hyacinth plants were grown in plastic tubs (43 x 31 x 19 cm) in a quarantine glasshouse for a period of four weeks prior to the introduction of the grasshoppers. Each tub contained 15 L of water and two water hyacinth plants and was enclosed with a net canopy. Nutrient levels in the water were manipulated to represent levels of nitrates and phosphates present in South African water bodies. Nitrogen and phosphorus were added as potassium nitrate (KNO₃) and potassium dihydrogen orthophosphate (KH₂PO₄) respectively. Commercial chelated iron was also added at a rate of 1.3g/15L of water. The nutrient levels were classified as eutrophic (high), mesotrophic/eutrophic (medium) and oligotrophic (low) (Table 1) according to the South African Water Quality Guidelines (Holmes, 1996). Water in the tubs was changed once a week to maintain the required nutrient supply to the plants. After the four-week growth period, all daughter plants, dead leaves and stems were removed, and the plants weighed to determine wet weight. Adult C. aquaticum grasshoppers were introduced into the experimental tubs at a density of one per plant and one male/female pair per tub. The treatments were replicated six times and the trial was run for a period of ten weeks. Plants were weighed at termination.
of the trial to determine end wet weight. The effect of nutrient treatment, herbivory by *C. aquaticum* and their combined effect on the difference in wet weight from the start to the end of the trial were analyzed using a two-way ANOVA. Tukey’s HSD test was used as a post-hoc comparison of the means.

### 1.2 Effect of plant nutrient levels on *Cornops aquaticum* (a) survival and (b) fecundity

(a) Twenty-eight newly emerged *C. aquaticum* nymphs were reared on water hyacinth plants grown at the high, medium and low nutrient levels (Table 1) for a period of three months. Water and plant nutrient levels in water hyacinth are highly correlated (Gossett and Norris, 1971) so there was a corresponding increase in plant tissue nutrient levels with an increase in nutrient supply to the plants (Bownes, 2009). The total number of nymphs to survive to adulthood and the proportions of males and females were recorded.

(b) Eight pairs of adult grasshoppers reared in trial (a) were confined in tubs with water hyacinth plants grown at the same nutrient levels on which they were reared. The number of egg packets oviposited by females and the number of nymphs to emerge from each egg packet were recorded. The number of egg packets per female and the number of nymphs per egg packet were compared by one-way ANOVA to test for the effect of nutrient treatment on fecundity of *C. aquaticum*. Tukey’s HSD test was used as a post-hoc comparison of the means.

### 1.3 Density-damage relationships between *Cornops aquaticum* and water hyacinth

The experimental design followed the same protocol as trial 1.1 with the exception that all plants were grown at the high nutrient level, when plant growth and productivity would be optimal. Male and female *C. aquaticum* grasshoppers were introduced into the tubs at a density of 2, 3 and 4 per plant (= four, six and eight grasshoppers per tub). The sexes were separated so that each tub had either only males or only females. Each treatment was replicated six times and two tubs per replicate were used as controls. Water hyacinth plants were weighed at the start and the end of the trial to determine wet weight and the trial was run for a period of eight weeks. The plant biomass data was subjected to a regression analysis to determine the relationship between insect biomass (as the independent variable) and plant biomass (as the dependent variable). Insect biomass was used as a surrogate for insect density since densities of male and female grasshoppers were the same. For this, a random sample of male and female grasshoppers were weighed (males n =47; females n = 50) to obtain a mean wet weight (g) for each sex (Bownes et al. 2010a). The biomass and insect data were fitted to a damage curve, similar to that suggested by McClay and Balciunas (2005), and which is used to identify agents that are not sufficiently damaging to their host plant to justify release. The damage curve relates a critical aspect of weed performance such as growth rate or final biomass to increasing densities of the biocontrol agent.

<table>
<thead>
<tr>
<th></th>
<th>High (eutrophic)</th>
<th>Medium (eutrophic/mesotrophic)</th>
<th>Low (oligotrophic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrates (mgL⁻¹)</td>
<td>7.6</td>
<td>2.52</td>
<td>0.034</td>
</tr>
<tr>
<td>Phosphates (mgL⁻¹)</td>
<td>1.37</td>
<td>0.316</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Table 1. Nutrient concentrations used to represent the range of levels found in South African river systems and impoundments.
Table 2. Treatments testing interactions between *Cornops aquaticum* and two biocontrol agents already released in south Africa, *Neochetina eichhorniae* and *Eccritotarsus catarinensis*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species combination (density/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No insects</td>
</tr>
<tr>
<td>1</td>
<td>CA (1 female) + NE (2 pairs)</td>
</tr>
<tr>
<td>2</td>
<td>CA (1 female) + EC (10 adults)</td>
</tr>
<tr>
<td>3</td>
<td>NE (2 pairs) + EC (10 adults)</td>
</tr>
<tr>
<td>4</td>
<td>NE (2 pairs)</td>
</tr>
<tr>
<td>5</td>
<td>EC (10 adults)</td>
</tr>
</tbody>
</table>

Table 3. Effect of nutrient levels on survival of *Cornops aquaticum* from first instar to adult, and sex ratio of survivors.

<table>
<thead>
<tr>
<th>Nutrient level</th>
<th>Survival to adult</th>
<th>Sex ratio Female:Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>82%</td>
<td>65:35</td>
</tr>
<tr>
<td>Medium</td>
<td>71%</td>
<td>55:45</td>
</tr>
<tr>
<td>Low</td>
<td>64%</td>
<td>39:61</td>
</tr>
</tbody>
</table>

Figure 1. Mean change in wet weight of water hyacinth plants in response to herbivory by *Cornops aquaticum*. Plants grown at high, medium and low nutrient levels for ten weeks. Means with the same letter are not significantly different (Tukey's HSD, $P<0.05$). Error bars represent the standard error of the mean.
Figure 2. Mean fecundity of *Cornops aquaticum* females measured as (A) the no. of egg packets per female and (B) the number of nymphs to emerge from each egg packet. Means with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$). Error bars represent the standard error of the mean.
2. Agent interaction studies

*Effect of Cornops aquaticum on populations and feeding damage of the Neochetina weevils and the impact of combinations of these agents on water hyacinth growth and productivity*

**(a) Manipulative, small-scale experiment**

One water hyacinth plant per treatment was grown in an 8 L bucket in a temperature controlled quarantine glasshouse. Nitrates and phosphates were added to the water at a rate of 2 mg L⁻¹ and 0.29 mg L⁻¹ respectively. Flowers and ramets were removed from the plants two weeks prior to the start of the trial to allow for the plants to recover and stabilise. Nutrients and water were replaced after the two-week recovery period and 25 days subsequent to this. Table 2 shows the combinations and densities of the insect species that were tested. Plants were weighed at the start and end of the trial to determine wet weight and were sampled weekly to record insect activity such as the number of *N. eichhorniae* feeding scars. Each treatment was replicated 10 times and the trial was run for a period of 50 days. To analyze the effect of the different insect treatments on the change in wet weight of water hyacinth plants, the biomass data were compared by one-way ANOVA. The mean number of weevil feeding scars when in combination with *C. aquaticum* and *E. catarinensis* and alone were also compared by one-way ANOVA. Tukey’s HSD test was used as a post-hoc comparison of the means.

**(b) Pond experiment with an already established weevil population**

The trial was conducted in a 1300 L portable pool (215 x 45 cm) housed in a semi-quarantine glasshouse and enclosed with a net canopy to confine the insects to the plants. The pool was 100% covered with water hyacinth and had a combined density of 2.6 (± 0.87) adult *N. eichhorniae* and *N. bruchi* per plant that were resident for a period of 3 months prior to the introduction of the grasshoppers. At the start of the trial, 97 adult *C. aquaticum* (46 females: 51 males) were released onto the plants which equated to 0.3 grasshoppers per plant. A random sample of five water hyacinth plants were destructively sampled fortnightly and the following plant and insect parameters were measured: number of leaves per plant, number of ramets per plant, proportion of petioles mined by *Neochetina* larvae and the total number of larvae recovered. The means of each parameter were compared over time by one-way ANOVA and Tukey’s HSD test used as a post-hoc comparison.

**Results**

1. Agent efficacy studies

1.1 *Effect of water nutrient levels on the impact of Cornops aquaticum herbivory*

Nutrient treatment (F2;29 = 48.53; P < 0.0001) and herbivory (F1;29 = 81.80; P < 0.0001) had a significant effect on the change in wet weight of water hyacinth plants (Fig. 1) from the start to the end of the ten week trial. The interaction between nutrient supply and herbivory was also significant (F2;29 = 5.56; P = 0.009). Plant tolerance to herbivory by *C. aquaticum* increased with an increase in nutrient supply to the plants however feeding by the grasshoppers significantly reduced biomass accumulation at all three nutrient levels.

1.2 *Effect of plant nutrient levels on Cornops aquaticum (a) survival and (b) fecundity*

(a) Survival of *C. aquaticum* nymphs to adulthood was influenced by plant nutrient levels which also had an effect on the proportions of males and females to be reared through to adulthood (Table 3). Higher levels of nitrogen in the plant tissue (Bownes, 2009) elicited higher rates of survival and greater numbers of females survived to adulthood in the high nutrient treatment (Table 3).

(b) Nutrient treatment had a significant effect (F2;20 = 26.06; P < 0.0001) on fecundity of female grasshoppers that were reared and maintained, after pairing at adulthood, on plants grown at the high, medium and low nutrient levels, with fewer egg packets being produced at the low nutrient level. Nutrient treatment had a significant effect (F2;18 = 7.58; P = 0.0041) on the number of nymphs to hatch from egg packets of females (Fig. 2). The mean number of nymphs per egg packet increased with an increase in nutrient supply to the plants although
only the high and low treatments were statistically significantly different from one another.

1.3 Density-damage relationships between *Cornops aquaticum* and water hyacinth

The relationship between plant biomass at the end of the trial as a function of increasing *C. aquaticum* biomass was curvilinear (Fig. 3). Biomass of water hyacinth plants decreased with an increase in feeding intensity by the grasshoppers. Exponential regression best described the relationship between plant yield and insect biomass and was highly significant ($F_{6;43} = 73.20; P < 0.0001$) accounting for 75% of the variance (Bownes et al. 2010a).

2. Agent interaction studies

*Effect of Cornops aquaticum on populations and feeding damage of the Neochetina weevils and the impact of combinations of these agents on water hyacinth growth and productivity*

(a) Manipulative, small-scale experiment

A combination of *C. aquaticum* and *N. eichhorniae* was the only treatment to significantly reduce biomass accumulation of water hyacinth plants compared to control plants ($F_{5;54} = 3.62; P = 0.0068$). Although the other combinations of insects or treatments with *N. eichhorniae* and *E. catarinensis* alone hampered biomass accumulation relative to insect-free plants, none of these differences were statistically significant (Fig. 4). The presence of *C. aquaticum* had no effect on *N. eichhorniae* feeding intensity compared to when the weevils were alone or in combination with the mirid *E. catarinensis*.

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![Figure 3](image-url)

Figure 3. Regression of *Cornops aquaticum* biomass (g) and final weight (kg) of water hyacinth plants after eight weeks. Insect biomass is represented by a mean weight of male or female grasshoppers multiplied by the respective density.
Figure 4. Mean change in wet weight of water hyacinth plants exposed to different combinations of insect biocontrol agents (*Cornops aquaticum*, *Neochetina eichhorniae* and *Eccritotarsus catarinensis*). Means with the same letter are not significantly different (Tukey’s HSD, $P < 0.05$). Error bars represent the standard error of the mean.

Figure 5. Mean numbers of *Neochetina eichhorniae* feeding scars when tested alone and in combination with *Cornops aquaticum* and *Eccritotarsus catarinensis*. Means with the same letter are not significantly different (Tukey’s HSD, $P < 0.05$). Error bars represent the standard error of the mean.
Figure 6. Mean numbers of ramets (daughter plants) and leaves produced by water hyacinth plants over time in response to feeding by the Neochetina weevils and Cornops aquaticum. Means with the same letter are not significantly different (Tukey’s HSD, $P < 0.05$).

Figure 7. Proportions of petioles mined by Neochetina larvae and numbers of Neochetina larvae per water hyacinth plant when in combination with Cornops aquaticum. Means with the same letter are not significantly different (Tukey’s HSD, $P < 0.05$). Error bars represent the standard error of the mean.
Interestingly, the presence of *E. catarinensis* caused a reduction in feeding of *N. eichhorniae*; however the differences in the number feeding scars were not statistically significant (Fig. 5).

(b) Pond experiment with an already established weevil population

Plant productivity, as measured by the number of leaves and ramets per water hyacinth plant decreased over time, although this was only statistically significant for the number of leaves per plant ($F_5;24 = 12.39; P < 0.0001$). Although there were no significant differences, ramet production of the water hyacinth plants ceased after 5-6 weeks (Fig. 6), and by the 10th week, most of the plants in the pool had died back. *Neochetina* larval activity increased over time after the introduction of *C. aquaticum*. There was a significant increase in the proportion of petioles mined ($F_5;24 = 6.80; P = 0.0004$) and in the total number of larvae ($F_5;24 = 24.38; P < 0.0001$) recovered from the plants from the start of the trial to the last sampling event (Fig. 7).

Discussion

The results from these and other studies (Bownes, 2009; Bownes et al. 2010b) strongly suggested that *C. aquaticum* has the potential to be a valuable biocontrol agent for water hyacinth in South Africa and the following conclusions were made: (1) *C. aquaticum* has the potential to reduce populations of water hyacinth under eutrophic nutrient conditions and that these nutrient conditions should have a positive effect on their population dynamics; (2) the damage caused by *C. aquaticum* is density-dependent in that increasing densities will lead to a corresponding reduction in water hyacinth growth and productivity, supporting the conclusion that it would be sufficiently effective to warrant release (McClay and Balciunas, 2005); (3) *C. aquaticum* does not appear to have a negative effect on feeding and populations of the *Neochetina* weevils; and (4) an apparent synergism between *C. aquaticum* and the *Neochetina* weevils could potentially lead to better levels of control of water hyacinth in South Africa. On the basis of these conclusions and on the fact that a substantial amount of time and resources had been invested in developing this agent, a decision was made in August 2010 to proceed with the release of the grasshopper.

For the initial releases and monitoring of *C. aquaticum*, four sites were selected to encompass a range of both nutrient and climatic conditions such as high or low water nutrient conditions and temperate to sub-tropical climates. All four sites were monitored for at least nine months prior to release in order to evaluate site-specific conditions such as microclimate, water nutrient conditions and the status of the plants and insect biocontrol agents already present. The first release took place in January 2011 followed by a second release in March 2011. Three hundred adult and late instar nymphs were released at each site. Sites were monitored three months post-release, but to date evidence of establishment of the grasshoppers has not been found.

With assistance with mass rearing from the South African Sugar Research Institute (SASRI) which has a specialized rearing facility for insect biocontrol agents, repeated releases will take place during the summer of 2011/2012. All sites will be monitored on a quarterly basis to determine establishment and efficacy of *C. aquaticum* as well population dynamics of the insect biocontrol agents already present on water hyacinth in South Africa.

Acknowledgements

The Working for Water (WFW) Programme of the Department of Environmental Affairs is gratefully acknowledged for funding research on this agent. Prof. Martin Hill and Prof. Marcus Byrne are thanked for their guidance on certain aspects of the pre-release research which contributed to a PhD degree. The authors also thank WFW implementation officers, Daleen Strydom and Ryan Brodvig for their assistance with locating suitable field release sites and with field work.

References

Australia’s Newest Quarantine for Weed Biological Control

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Abstract

Two of Australia’s leading weed biological control groups, from the Queensland Government’s Alan Fletcher Research Station and CSIRO’s Long Pocket Laboratories, recently relocated to the new world-class Ecosciences Precinct at Dutton Park, Brisbane. There they share a purpose built quarantine facility of QC3 standard. Built on the level 5 rooftop of the Precinct, the 400 m² quarantine facility has six research suites, each consisting of an 11 m² laboratory and a 30 m² air-conditioned glasshouse. These research areas are supported by controlled environment rooms, storage areas, a room for controlled environment cabinets and an unpacking room. One research area is isolated for working with plant pathogens or extremely small arthropods requiring “shower out” procedures. Special features of this state-of-the-art facility include double glazing of laminated glass of the glasshouses, HEPA filtration, “pass through” autoclaves and fumigation chamber, and a heat transfer, continuous treatment system for liquid waste. The QC3 facility includes dedicated mechanical services rooms on the loft above and the floor below for easy access for maintenance. Approximately 3000 m² of non-quarantine on-site plant growth facilities support the QC3. The facility was approved as a QC3 facility at its first inspection. The immediate approval of the facility was attributed to several factors, including benchmarking existing quarantine facilities worldwide, high standards of materials and building expertise, and detailed communication between project staff, certifying agencies, construction and specialist consultants and scientists throughout the whole process. Within the next few years, this facility will become one of only two quarantines in Australia designed for weed biological control research.

Introduction

Secure quarantine facilities are a cornerstone requirement of any classical biological control program, allowing researchers to undertake host testing of foreign organisms and ensuring security control of any unintended organisms in imported packages. Quarantine requirements have changed over time and will continue to change into the future, probably with tighter controls. When Koebele sent his first insects (without any host testing) to Hawaii in 1902 (Perkins and Swezey, 1924) the packages were first opened in the corridor before someone suggested that it might be better to open them in a room with the door closed! By the time insects for prickly pear were brought to Australia in the 1920s,
a special quarantine area (though crude by today's standards) had been constructed at what became the Alan Fletcher Research Station in Queensland (Dodd, 1940). Today a very high standard of quarantine containment is required in all countries with significant biological control programs (Radcliffe et al., 2003; Agostino et al., 2004; Ferrar et al., 2004; Anon, 2005; Adair and Irwin, 2008).

Quarantine facilities have also assumed greater importance with time because budgetary constraints have resulted in a greater proportion of total research being undertaken in the home country and because there is a much higher requirement to test against native plant species (Fisher and Andres, 1999).

Strong investment in new quarantine facilities over the last decade or so has resulted in new facilities for biological control being constructed in Canada (De Clerck-Floate et al., 2000), England, South Africa, New Zealand, Brazil and the United States.

This paper describes a new quarantine facility constructed within the $270 million Ecosciences Precinct, which replaced quarantine facilities at the Queensland Government's Alan Fletcher Research Station and CSIRO Ecosystem Science's Long Pocket Laboratory. The Ecosciences Precinct itself is a newly constructed, state-of-the-art science facility housing approximately 1000 scientists and staff from CSIRO, two Queensland Government agencies, and the University of Queensland. It was developed to replace several aging research facilities in the Brisbane area and forms part of a science corridor within South East Queensland.

Quarantine Philosophy

Quarantine Level

Very early in the planning and after advice from the regulating agencies it was decided to build the entire quarantine at the Australian Quarantine and Inspection Service (AQIS) defined Quarantine Containment level 3 (QC3). A major consideration was that future quarantine requirements may be more stringent and retrofitting an existing facility to a higher standard is very undesirable. Essential features of QC3 are that there is an interlock space at the entrance, exhaust air is HEPA-filtered (meaning that glasshouses can't use evaporative air conditioning systems), liquid waste must be sterilized by heat and areas within the quarantine envelope are built to air-tight standards and are held at negative air pressure. While QC3 gives a high level of certainty of containment, it has the disadvantages that it is sophisticated, expensive-to-build and incurs high running and maintenance costs.

Pathogen (Micro) area

Regulatory authorities advised that QC4 level was necessary for certainty of approval to import exotic pathogens but that pathogens might be approved on a case by case basis for a QC3 quarantine, providing certain extra features were present. Those features included a shower-out for exiting staff and heat treatment of liquid waste. It was therefore decided to separate one suite of the facility from the others and to provide this suite with its own entrance (Figure 1). This suite was to be reserved firstly for pathogens, tiny arthropoda such as mites or thrips, or any other agent requiring extra caution or separation from the other projects.

Insect (Macro) area

This area, with one common entrance and five independent research suites, is designated for standard insect work (Figure 1). Each suite includes a small laboratory attached to a 30 m² glasshouse with refrigerated air conditioning. In addition to these suites, the Macro area has shared spaces including three controlled environment rooms (CERs), a room housing four controlled temperature cabinets, an unpacking room, and a storage room.

Waste Disposal

We were advised by the regulators that heat sterilization was the preferred option for treatment of liquid waste. This was problematic because of energy costs and particularly because a facility with several glass houses could generate considerable quantities of liquid waste. There were then issues such as continuous versus batch systems to consider. The best option appeared to be the selection of an Actini® with its continuous flow, heat transfer system.

Most solid waste would be treated by sterilization in pass-through autoclaves. Each area has such an autoclave.

Some solid wastes, laboratory equipment such as insect cages, books or other paper products can be removed from the quarantine though a 3-door,
pass through fumigation chamber. This chamber is of generic design and is capable of using any one of several fumigants. A hazard and operability study (HAZOP) is presently being undertaken before the fumigant of choice, methyl bromide, is used.

Management
The Ecosciences Precinct quarantine facility replaces two facilities; the Queensland Government’s Alan Fletcher Research Station and the CSIRO’s Long Pocket Laboratory and now houses teams from both agencies. The new facility is managed as an integrated shared facility to maximize resources and functionality of the facility.

The Quarantine Manager, jointly funded by CSIRO and the Queensland Government, is responsible for the functioning of the infrastructure and adherence to protocols of all personnel using the facility, regardless of their affiliation.

Standard Operating Procedures (a 100 page document) detail quarantine protocols and procedures for quarantine users and maintenance staff.

Collaboration
This QC3 facility is one of the most innovative, leading-edge structures built within the Ecosciences Precinct. Successful commissioning of the facility means CSIRO and Queensland Government scientists now work together to develop safe and sustainable methods to manage the spread and impact of the worst weeds and insect pests which threaten the environment and Australia’s rural industries.

Because of the structural complexity in QC3 laboratories and glasshouses, the need to meet very high containment standards and requirement for certification through the AQIS, these types of facilities have a high risk of building failure.

Collaboration and cohesive communication were critical success factors for the design, construction, certification and commissioning processes for the QC3 facility at the Ecoscience Precinct. This was achieved by continual engagement and regular feedback loops, scheduled workshops and programmed visual inspections by CSIRO and Queensland Government scientists (users), consultants (architects, mechanical, electrical, hydraulics engineers and other specialists) and construction teams throughout the six years from project brief to construction completion. In addition, scientists were actively engaged in benchmarking world’s best practices in QC3 construction and working with consultants and the construction team to test and source suitable materials and construction methodologies, including problem solving with regard to infrastructure technologies such as heat treatment and material containment. A List-server, which is still operating, was set up to facilitate email discussion about quarantine problems with quarantine managers around the world.

Maintenance
Discussions with other quarantine facility managers taught us the importance of building a structure with easy access to all associated mechanical, electrical and hydraulic equipment. We built a floor above the facility in which some of this equipment is housed. A floor below the facility houses much of the remaining equipment.

Supporting infrastructure
The quarantine is supported on the rooftop by both airconditioned and evaporatively cooled non-quarantine glasshouses and shadehouses. These are used to grow test plants and also to mass rear insects approved for release. All of the rooftop infrastructure is supported by potting areas in the basement.

Some Design Details
The Actini® liquid waste system
Liquid waste from the quarantine facility is decontaminated by passing through an Actini® system that treats the liquid by holding it at 145°C for 260 seconds. Energy consumption is minimized by a heat transfer system. The system is capable of treating 4000 L per week, which generously allows for contingencies. In addition a storage tank can hold 4000 L of untreated waste.

Autoclaving
Pass-through Getinge® autoclaves with 415 L sterilizing chambers service both Micro and Macro areas. They are programmed so that the outer door can only be opened after a sterilizing event has taken place. The requirement for sterilization is 121°C for
15 minutes if the core temperature is measured and 121°C for 30 minutes where the core temperature is not measured.

**Fumigation**

The fumigation chamber has been built to use a number of approved fumigants. It is a custom built, pass-through chamber that services both Macro and Micro areas. This is achieved by the three door design. These doors are sequenced electronically to prevent cross contamination between Macro and Micro areas and to ensure that contaminated material is fumigated before the outer door is opened. In essence, once either of the inner doors is opened a fumigation event must occur before any of the other doors can be opened. Once a fumigation event has occurred and the chamber has been unpacked from the outside, it can be used as a pass back facility to take materials and equipment back into the quarantine areas.

**Air handling system**

There are 18 air handler units in the facility, with all glasshouses and all CERs having independent units. This design, and also the manipulation of supply and exhaust dampers, allow each suite to be fumigated separately by either injecting gas (through dedicated fumigation ports) or heating liquid formaldehyde (using dedicated power points) while allowing the remainder of the facility to operate normally.

Negative pressures are achieved with a Variable Speed Device system, maintained at -15 Pa in the external corridor, -25 Pa in the airlocks, -50 Pa in the internal corridor, -65 Pa in laboratories and Macro CERs and -75 Pa in the glasshouses and Micro area CER.

All exhaust air is passed through HEPA filters situated in plant rooms on Level 4, Level 5 and the loft above and outside the quarantine envelope. There are 39 HEPA filters mounted in 21 stainless steel HEPA boxes that can be opened on the clean side for annual integrity testing.

**Glasshouses**

The glasshouses are each 30 m² in size and are provided with compressed air, carbon dioxide and reverse osmosis water. Liquid waste drains from the centre of each room to the Actini® treatment system. The roof of the glasshouse has a 27° slope from 4.5 m high on the south side to 2.7 m high at the north side.

The roof is shaded by a retractable internal blind controlled by a pneumatic device. Its operation is controlled from the Building Management System (BMS) using an algorithm based on ambient temperature, humidity and solar radiation measured by an independent weather station in the building. The BMS can be over-ridden by the user. External roll up blinds have been retrospectively fitted on the vertical glass walls to intercept radiant heat, which was a problem particularly in the winter months.

The glasshouses are fully enclosed in a double glazing system. This allows a panel of the inner or outer glass to be replaced, in the event of damage, without disruption to the building function. An entire prototype glasshouse was built to test the system before construction. The internal laminated pane of glass consists of one 4 mm thick layer of glass on each side of a 1mm PVB layer. The external laminated pane of glass consists of one 5 mm thick layer of glass on each side of a 4mm PVB layer. A replaceable desiccant prevents condensation in the 300 mm space between the glass panes.

**Laboratories**

Each laboratory is fitted with a sink, benches, under bench cabinets, above bench shelves, and a space for an item of equipment such as a refrigerator. Each laboratory is 11 m² except the Micro laboratory, which is 18 m² to fit the various additional pieces of equipment needed for working with plant pathogens. Each laboratory is provided with compressed air and carbon dioxide.

**Controlled Environment Rooms**

There are four CERs, varying in size from 9 to 12 m². Each has its own air handling and dehumidifying unit and is illuminated by 24 metal halide lamps housed in a space above the room, separated by a glass barrier ceiling which allows the light to penetrate but isolates the heat generated by the lamps. Maintenance of the lamps is from outside the quarantine envelope. We have experienced excellent growth of tropical plants in the rooms. Each CER is provided with compressed air and reverse osmosis water.
Entry and Exit

Each quarantine area is accessed by passing through the airlock and change room, which are accessed from a foyer outside the quarantine envelope. On entry, the first room (airlock) in each quarantine area is blackened and is kept dark except when in use. This airlock is fitted with a black light insect trap. The outer door of this airlock is considered the boundary of the quarantine envelope. The next room is a changing room. Electronic locks ensure that only one door in a set of airlocks can be opened at any one time. An air curtain operates above the door from each change room to the corridor within their respective quarantine areas.

Toilets

A toilet is provided only in the Micro area because staffs are more likely to remain in quarantine for long periods when shower out protocols apply. The toilet is for urination only because the Actini® system cannot handle paper.

Safety

Emergency door release buttons for all interlocking doors, push buttons to isolate reticulated services such as gas, water and electricity, automatic exhaust of the fumigation if a leak is detected in the fumigation chamber, and visible and audible alarms for pressure deviations have been installed to enhance personal safety. All these devices and other sensors throughout the areas of the quarantine are monitored though the BMS. High priority BMS alarms are sent to the SMS alarm messaging service, resulting in callouts any time during the day or night. In addition, an intercom system provides immediate communication to the security desk.

Acknowledgements

The successful construction of this quarantine facility is due to the enthusiastic support of many people and organizations. We particularly thank the project teams, architects (Hassell & Co), engineering consultants, building contractor (Watpac), the quarantine consultant (Neil Walls) and regulatory agencies (AQIS and Biosecurity Australia) for all their unstinting efforts. We would also like to thank our colleagues around the world for showing us over their facilities and ensuring that we benefited from past experiences.

References


Figure 1. Schematic diagram of the quarantine facility
Host Specificity of an Italian Population of *Cosmobaris scolopacea* (Coleoptera: Curculionidae), Candidate for the Biological Control of *Salsola tragus* (Chenopodiaceae)

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Summary

Russian thistle, *Salsola tragus* L. (Chenopodiaceae) is a troublesome weed infesting the drier regions of western North America. It is native to Central Asia and infests rangelands and semi-arid pasture lands, croplands, residential, disturbed and industrial areas. *Cosmobaris scolopacea* (Germar) is a weevil distributed in Eurasia and North America, and generally associated with plant species of the family Chenopodiaceae. The larvae feed and pupate within the stems of the host plant, and the adults emerge in the following late spring. From preliminary host range testing carried out at the ENEA-BBCA facilities in Rome, Italy, it appeared that *C. scolopacea* might harbour different host races, one being potentially more specific to the target and only present in Sicily, Italy. To determine species boundaries and reveal population structure at the intraspecific level, a phylogeographic study using the mitochondrial COI gene was conducted on specimens collected in the native range (Italy, Spain, Iran, Bulgaria, Turkey) and the U.S.A. The study confirmed the presence within the species of a highly divergent Sicilian lineage that has only been reared from *Salsola kali* L. The degree of specificity of this particular lineage and hence host race status is being tested through host specificity testing. Preliminary results seem to indicate that this Sicilian lineage can be at least a true *Salsola* host race, opening doors for further testing as a biological control agent for Russian thistle.

Introduction

*Salsola tragus* L. (*sensu lato*), together with other closely related Russian thistle species, is a troublesome weed in the drier regions of western North America (Young, 1991). It infests range and semi-arid pasture lands as well as cropland, agricultural, residential and industrial areas. As a crop weed it can cause yield losses of greater than 50% in spring wheat (Young, 1988). It is also a host for several crop pests, and the tumbling plant skeletons can fill irrigation canals and pile against fences (Goeden and Ricker, 1968). A biological control program started during 1970s and there is still a need for effective agents (Goeden and Pemberton, 1995; Smith et al., 2006).

Larvae of *Cosmobaris scolopacea* (Germar,
(Coleoptera: Curculionidae) were recorded and reared from plants of *Salsola kali* L. (Chenopodiaceae) near Catania (Sicily, Southern Italy) by Gaetano Campobasso (pers. comm., 2000). Despite the fact that the stem boring weevil is known as a cosmopolitan pest species of several Chenopodiaceae crops, a screening for the evaluation of the host range of this population was started in the early 2000s by G. Campobasso, performing mainly open field host range observations by dissecting native and crop Chenopodiaceae occurring in sympatric conditions with the target weed.

Starting in 2008, we decided to continue the screening of the weevil, by the combination of three different approaches: morphological taxonomy, genetic analysis and ecological bioassays. The purpose of the present study was to compare for these two last aspects different populations of *C. scolopacea* to reveal the existence of cryptic species and /or host races within the species, and to determine if any are potentially suitable as a biological control agent for the target weed.

**Material and methods**

**Field sampling**

Weevil larvae were collected from *Salsola* spp. in Eurasia, i.e. Sicily, Central Italy, Central Spain, Central Turkey, Bulgaria, Iran, and in the US (California) from 2008 to 2010. In most of the sites, larvae were collected also in the stems of other Chenopodiaceae (often *Chenopodium album* L. and rarely on *Halimione* spp.). Stem dissection was conducted in situ, with some of the larvae preserved in absolute ethanol for genetic studies and others transferred to artificial diet (Tomic-Carruthers, 2009) for adult emergence to use for morphological study. Adults have been collected in two locations in Sicily (Simeto and Eraclea Minoa, respectively) to carry out host range tests in laboratory conditions at the BBCA facilities.

**Molecular and phylogenetic analysis**

Weevils were collected as adults and larvae from a total of i) 29 populations throughout the Eurasian native distribution range from Italy to Northern Iran and ii) 2 populations in North America, and iii) across three major host plants in the Chenopodiaceae family, i.e. the Russian thistle (*Salsola* spp.), *C. album* and *Halimione* spp. Also included in this study as an outgroup was a dried specimen of *Cosmobaris discolor* (Boheman, 1836) collected by E. Colonnelli in South Africa in 2007 on *Chenopodium* sp. Weevils were preserved in absolute ethanol and stored at -20°C before DNA extraction. Genomic DNA was extracted from single specimens using either the CTAB protocol (Doyle and Doyle, 1987) or the DNeasy Blood and Tissue DNA extraction kit (Qiagen S.A, Courtaboeuf, France) following the manufacturer's protocol. A ~830 bp section of the mitochondrial cytochrome oxidase c subunit I (COI) gene was amplified through Polymerase Chain Reaction (PCR) in a 9700 Perkin Elmer thermal cycler (Applied Biosystems) using primers C1-J-2183 and TL2-N-3014 (Simon et al., 1994) and PCR profile: 5 min at 94°C, 5 cycles of 30s at 92°C, 30s at 48°C, 1 min at 72°C, followed by 25 cycles of 30s at 92°C, 30s at 52°C, 1 min at 72°C, and 7 min at 72°C. PCR products were sequenced on both strands at Genoscreen (Lille, France) on ABI 3130XL automatic sequencers (Applied Biosystems, Foster City, CA, USA). Alignments of consensus sequences were manually edited with Bioedit 7.09 (Hall, 1999). A dataset of 79 sequences of 638bp of length was obtained for *C. scolopacea* sensu lato. To determine species boundaries and reveal discontinuities among lineages at the intraspecies level, an analysis method based on haplotype relationships (i.e. statistical parsimony) was chosen (TCS; Clement et al., 2000). Under the 95% parsimony criterion haplotype network resulted in three unconnected networks (data not shown). To provide a framework for understanding the evolutionary relationships between all populations and between these haplotype networks, a phylogenetic analysis was performed on the same dataset. Modeltest version 3.7 (Posada and Crandall, 1998) was used to determine the model of nucleotide substitution that fitted the data best. The hierarchical likelihood ratio (hLRT) test as implemented in Modeltest selected the HKY+G model as the best fit for our dataset. The Maximum Likelihood analysis was conducted under PhyML 3.0 (Guindon et al., 2010), and bootstraping was calculated from 100 replicates. Genetic divergence levels within and between networks and species were
determined by calculating un-corrected $p$ distances in PAUP*4.0 (Swofford, 2002).

**Host range experiments**

Laboratory host range choice-tests were carried out during 2010, testing one population from Eraclea Minoa, Western Sicily. Bioassays were carried out in Petri dishes in a climatic cabinet at 21-26°C and with a 14:10 h L/D cycle, confining one female with one stem of *S. kali* (SAKA) plus one stem of one of the following plant species: *Kochia scoparia* (L.) Schrader (KOSC), *Chenopodium album* (CHAL), *Suaeda taxifolia* (P. C. Standley) P. A. Munz (SUTA) and *Bassia hyssopifolia* (Pallas) Volk (BAHY).

**Results and Discussion**

The ML phylogenetic tree obtained on the basis on the 79 COI sequences of *C. scolopacea* sensu lato that was rooted with *C. discolor* as an outgroup is presented in Figure 1. Three major highly diverged mitochondrial clades supported with high bootstrap values above 93% can be observed, and are equivalent to the three unconnected haplotype networks obtained under TCS. One clade (A) contained the nine North American specimens from one site in California and one site in Prince George’s County, Beltsville, Maryland, corresponding to two haplotypes. Specimens from *S. tragus* and *C. album* at the Brentwood, CA site shared the same haplotype. Genetic divergence within this clade was very low (0.06%). A second clade (B), also named the Sicilian clade, contained the 18 samples collected in Sicily and that were associated with *S. kali* and *Halimione* sp. hosts. It contained three haplotypes, and its intraclade genetic divergence averaged 0.25%. The third clade (C) contained the remaining 52 samples distributed across Eurasia and that were associated with *Salsola* and *Chenopodium* host plants. A total of 24 haplotypes belong to this clade which has the highest intraclade genetic divergence among the three, averaging 0.56%. The genetic divergence between the three clades ranged from 8.5% between the “American” clade (A) and the “C” clade to 9.2% between the Sicilian clade (B) and the “C” one. These values are nearly of the same order as those observed between the outgroup species *C. discolor* and any of the three clades. First described by Casey in 1920, the American lineage has been assumed to be *C. americana* Casey (Casey, 1920; O’Brien and Wibmer, 1982). In the present day, from gathered morphological data, it is admitted that *C. americana* Casey is a synonym of *C. scolopacea* and should not retain its separate name (Colonelli, pers com.). However, to determine whether the extent of the divergence is sufficient for the three clades to be considered cryptic species, sub-species, host races or biotypes, further research is likely required in the future.

The presence of a highly divergent lineage of *C. scolopacea* in Sicily that was collected mainly on *S. kali* supported Campobasso’s hypothesis that Sicilian populations may be more host specific than other populations of *C. scolopacea* in Eurasia. We therefore conducted host range testing of specimens from the Sicilian clade, collecting individuals as adults on *S. kali* (the Sicilian subclade highlighted in grey in the ML tree). Two-way choice oviposition experiments carried out during 2010 in Petri dishes showed a clear preference of the weevil for *S. kali*, with occasional oviposition on *B. hyssopifolia*, *C. album* and *S. taxifolia* (Table 1).

Table 1. Oviposition preference by *Cosmobaris scolopacea* in two-choice tests.

<table>
<thead>
<tr>
<th>Plant spp.</th>
<th>No. of reps</th>
<th>eggs on A (mean)</th>
<th>Std. Error</th>
<th>eggs on B (mean)</th>
<th>Std. Error</th>
<th>Wilcoxon Signed Ranks Test (Z)</th>
<th>Asymp. Sig. (2 tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAKA+BAHY</td>
<td>7</td>
<td>2.00</td>
<td>0.76</td>
<td>0.57</td>
<td>0.43</td>
<td>-1.279</td>
<td>0.201</td>
</tr>
<tr>
<td>SAKA+KOSC</td>
<td>6</td>
<td>3.00</td>
<td>0.82</td>
<td>0.00</td>
<td>0.00</td>
<td>-2.226</td>
<td>0.026</td>
</tr>
<tr>
<td>SAKA+CHAL</td>
<td>6</td>
<td>2.33</td>
<td>0.61</td>
<td>0.17</td>
<td>0.17</td>
<td>-2.214</td>
<td>0.027</td>
</tr>
<tr>
<td>SAKA+SUTA</td>
<td>9</td>
<td>1.78</td>
<td>0.43</td>
<td>0.11</td>
<td>0.11</td>
<td>-2.354</td>
<td>0.019</td>
</tr>
</tbody>
</table>

*BAHY = Bassia hyssopifolia, CHAL = Chenopodium album, KOSC = Kochia scoparia, SAKA = Salsola kali, SUTA = Suaeda taxifolia.*
Figure 1. Phylogenetic tree inferred by the maximum likelihood method based on mitochondrial COI sequences of various populations of *Cosmobaris* spp. Bootstrap scores (100 replicates) are indicated along the branches. The letters following the sample names refer to the host plants (S: *Salsola* spp.; H: *Halimione* spp.; C: *Chenopodium album*). The weevil populations used for the host specificity testing were from the “Sicilian” sub-lineage (gray-shaded block). The scale bar below the ML tree indicates the number of substitutions per site.
As recently and extensively reviewed by Gaskin et al. (2011), for weed biological control practitioners, there is a reasonable consensus that specialized lineages, including morphocryptic ones, whatever the stage of speciation they represent, are now considered one of the best routes for efficacy as regards their host specificity and perhaps safety. Hence, depending upon future results, the *Cosmobaris* weevil could be yet another example of phytophagous weevils that have several host races or cryptic species, some being strictly specific to a targeted weed, and hence opening the door for potential use in biological control (Fumanal et al., 2004; Antonini et al., 2008; Gaskin et al., 2011).

**Acknowledgements**

We want to remember and thank Gaetano Campobasso, USDA ARS Research Entomologist, passed away 3 years ago, who recorded for the first time the weevil damage on Russian thistle in Sicily. We gratefully thank Alessio De Biase (University of Rome “La Sapienza”) and René Sforza (USDA-ARS EBCL) for reviewing the manuscript; and Fatiha Guermache (USDA-ARS EBCL) for her assistance with the molecular analysis.

**References**


Biological Control of Chilean Needle Grass (*Nassella neesiana*, Poaceae) in Australasia: Completion of Host Range Testing

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Abstract

*Nassella neesiana* (Trin. and Rupr.) Barkworth (Chilean needle grass, CNG, Poaceae) is a Weed of National Significance in Australia and a declared pest plant in parts of New Zealand. Studies have been conducted in Argentina to identify potential biological control agents (pathogens) for this species. The rust *Uromyces pencanus* Arthur & Holw has been selected for having the greatest potential: it is highly host specific and can cause significant damage to the target weed. Most of the host range testing for the selected isolate (UP27) is now complete. No pustules have developed on any of the test species other than the target. However, there has been some development of the rust, with formation of haustoria, in *Piptatherum miliaceum* (L.) Cosson and several *Austrostipa* spp. In addition, some of the inoculated plants of *P. miliaceum* showed peculiar macrosymptoms (“blisters”) somewhat resembling pustules. Resistance mechanisms were observed to occur in all these species, probably explaining why the rust could not develop further to produce spores. As *Austrostipa* and *Piptatherum* are very closely related to *Nassella* (all in the tribe Stipeae) these results are not unexpected. It is surprising though that penetration, with little further development, was observed in other congeneric *Nassella* spp. tested. Testing of various isolates of *U. pencanus* revealed they varied greatly in their ability to attack different populations of CNG. *U. pencanus* isolate UP27 is able to infect eight out of the nine tested Australian populations and one out of three from New Zealand. An isolate has yet to be found that is able to infect those CNG populations resistant to UP27: those tested so far have failed. Authorities in New Zealand have recently approved the importation of *U. pencanus* for release in that country. An application to release the rust in Australia will be prepared soon.

Introduction

*Nassella neesiana* (Trin. and Rupr.) Barkworth (Chilean needle grass, Poaceae) is a perennial tussock-forming grass that is indigenous to Argentina, Bolivia, Chile, Ecuador, southern Brazil and Uruguay (Rosengurt et al. 1970). In Australia, it is both a serious environmental weed (Carr et al. 1992; McLaren et al. 1998) and a problem weed of agriculture (Grech 2007) and is a Weed of National Significance (WONS) (Thorpe and Lynch 2000). It is widespread in Victoria, NSW and the ACT with...
recent outbreaks occurring in Queensland, SA and Tasmania (Snell et al. 2007). *N. neesiana* is also a serious weed in New Zealand (Bourdôt and Hurrell 1992). Small populations occur in the North Island but the worst infestations occur in the Marlborough region, near the top of the south island.

Difficulties in controlling Chilean needle grass by chemical and cultural methods have led to investigations into the possible use of pathogens for biological control of this species in Australia and New Zealand. The rust *U. pencanus* has the greatest potential as: it is relatively easy to manipulate; it persists as urediniospores; it is highly host specific; and, can cause significant damage to the target weed (Giordano et al. 2009, Anderson et al. 2010a). We discuss the results of the host range testing carried out to study the specificity of the rust. We also report the outcome of the application to introduce the rust to New Zealand.

### Materials and Methods

A host specificity test list of 58 grass species was developed that included significant Australian and New Zealand native and commercially important grass species selected according to their taxonomic relatedness to *N. neesiana*. Testing was conducted in part at CERZOS in Bahía Blanca, Argentina, where the project is based, and part in a quarantine facility located at IMYZA-INTA, Castelar, Buenos Aires, Argentina, where all species exotic to Argentina had to be tested (Anderson et al., 2010b).

An isolate (UP27) of the rust fungus that originated from a field site in Bahía Blanca was selected on the basis of its virulence against Australian accessions of *N. neesiana* (Anderson et al. 2006). Batches of 4-5 test plant species were screened at one time, with a total of 8 plants being tested for each test species whenever availability permitted. Dry urediniospores mixed in talcum powder (ratio 1:30) were brushed onto the adaxial side of two leaves per plant, which were later sprayed with water. Accessions of *N. neesiana* from the Australian Capital Territory (ACT) were included in each test as positive controls. Inoculated plants were maintained at 18-20°C under a 12h (D:L) photoperiod and 100% relative humidity (RH) for 48h, after which they were kept under the same conditions, but at 70% RH for four weeks. Four weeks was selected because there is generally a two-week gap between inoculation and spore production on positive control plants. All inoculated plants were then inspected for external symptoms of infection. Also, samples were taken for internal microscopic examination at one week after inoculation and at the end of each experiment (four weeks). These samples were cleared and stained so as to make it easier to distinguish between fungal and plant tissues under the microscope using a modification of the Bruzzese and Hasan (1983) method (Flemmer et al., 2010). Each species was screened at least twice.

### Results

Most Australian accessions of *N. neesiana* proved to be susceptible to isolate UP27, with development of normal uredinia on infected leaves. However, a collection of *N. neesiana* from Ballarat (Victoria) did not become infected. Plants from only one out of the three tested accessions from New Zealand (Marlborough) proved to be susceptible, while those from the other two (Hawke’s Bay and Auckland) did not become infected. There were no pustules formed on any of the other species tested. Peculiar symptoms that looked like blisters, and somewhat resembled pustules, were formed on some of the inoculated leaves of *Piptatherum miliaceum*. Microscopic examination revealed these “blisters” were composed of plant rather than fungal tissues and were formed in response to rust penetration at some infection sites. Here both hyperplasia and hypertrophy seem to occur. On a few other species leaf spots were formed on inoculated leaves i.e., small yellow specks on congeneric *Nassella* species and small black spots on several *Austrostipa* species. Table 1 shows a summary of the results obtained on 50 tested species. These are listed in decreasing order of taxonomic relatedness with *N. neesiana*, the target weed. Details of the interactions of the rust and host cells at a microscopic level and the resistance mechanisms recorded will be presented elsewhere.

### Discussion

As a result of the inoculation experiments using *U. pencanus* isolate UP27, no pustules developed on any test species other than the target *N. neesiana*. 
Table 1. Host specificity *Uromyces pencanus* on Poaceae species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Macroscopic Symptoms</th>
<th>Microscopic Symptoms*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nassella neesiana</em> (Trin. &amp; Rupr.) Barkworth [ACT]</td>
<td>Pustules</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Bacchus Marsh, Vic]</td>
<td>Pustules</td>
<td>NE</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Ballarat, Vic]</td>
<td>None</td>
<td>NE</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Clifton Springs, Qld]</td>
<td>Pustules</td>
<td>X X X X</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Fitzroy flats, NSW]</td>
<td>Pustules</td>
<td>X X X X</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Goulburn, NSW]</td>
<td>Pustules</td>
<td>NE</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Laverton, Vic]</td>
<td>Pustules</td>
<td>NE</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Thomastown, Vic]</td>
<td>Pustules</td>
<td>NE</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Truganina, Vic]</td>
<td>Pustules</td>
<td>NE</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Auckland, NZ]</td>
<td>None</td>
<td>X X (X) (X) X</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Hawke's Bay, NZ]</td>
<td>None</td>
<td>X X</td>
</tr>
<tr>
<td><em>N. neesiana</em> [Marlborough, NZ]</td>
<td>Pustules</td>
<td>X X</td>
</tr>
<tr>
<td><em>N. charruana</em> (Arechav.) Barkworth</td>
<td>None</td>
<td>X (X) X (X)</td>
</tr>
<tr>
<td><em>N. hyalina</em> (Nees) Barkworth</td>
<td>Yellow leaf spots</td>
<td>X X (X) X</td>
</tr>
<tr>
<td><em>N. leucotricha</em> (Trin. &amp; Rupr.) R.W. Pohl in Barkworth</td>
<td>Yellow leaf spots</td>
<td>X (X) X (X) (X) X</td>
</tr>
<tr>
<td><em>N. tenuissima</em> (Trin.) Barkworth</td>
<td>None</td>
<td>X X (X) X</td>
</tr>
<tr>
<td><em>N. trichotoma</em> (Nees) Hack. ex Arechav. [Dalgety, NSW]</td>
<td>Yellow leaf spots</td>
<td>X X X</td>
</tr>
<tr>
<td><em>N. trichotoma</em> [N. Canterbury, NSW]</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Achnatherum caudatum</em> (Trin.) S.W.L. Jacobs &amp; J. Everett</td>
<td>None</td>
<td>X X (X) X (X)</td>
</tr>
<tr>
<td><em>Austrostipa aristiglumis</em> (F. Muell.) S.W.L. Jacobs &amp; J. Everett</td>
<td>None</td>
<td>X X X X</td>
</tr>
<tr>
<td><em>A. bigeniculata</em> (Hughes) S.W.L. Jacobs &amp; J. Everett</td>
<td>None</td>
<td>X X X (X) X</td>
</tr>
<tr>
<td><em>A. breviglumis</em> (Hughes) S.W.L. Jacobs &amp; J. Everett</td>
<td>Black leaf spots</td>
<td>X X X X X (X)</td>
</tr>
<tr>
<td><em>A. elegantissima</em> (Labill.) S.W.L. Jacobs &amp; J. Everett</td>
<td>Black leaf spots</td>
<td>X X (X) X X</td>
</tr>
<tr>
<td><em>A. eremophila</em> (Reader) S.W.L. Jacobs &amp; J. Everett</td>
<td>Black leaf spots</td>
<td>X X X X X (X)</td>
</tr>
<tr>
<td><em>A. flavescens</em> (Labill.) S.W.L. Jacobs &amp; J. Everett</td>
<td>Black leaf spots</td>
<td>(X) (X) (X) (X) (X)</td>
</tr>
<tr>
<td><em>A. mollis</em> (R.Br.) S.W.L. Jacobs &amp; J. Everett</td>
<td>None</td>
<td>X (X) X (X) X</td>
</tr>
<tr>
<td>Species</td>
<td>Macroscopic Symptoms</td>
<td>Microscopic Symptoms*</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>----------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td><em>A. nitida</em> (<em>Summerh. &amp; C.E. Hubb.</em>) <em>S.W.L. Jacobs &amp; J. Everett</em></td>
<td>Brown leaf spots</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td><em>A. nullanulla</em> (<em>J. Everett &amp; S.W.L. Jacobs</em>) <em>S.W.L. Jacobs &amp; J. Everett</em></td>
<td>Black leaf spots</td>
<td>X (X) X (X) X X (X)</td>
</tr>
<tr>
<td><em>A. rudis</em> (<em>Spreng.</em>) <em>S.W.L. Jacobs &amp; J. Everett</em></td>
<td>None</td>
<td>X X (X) X (X)</td>
</tr>
<tr>
<td><em>A. setacea</em> (<em>R.Br.</em>) <em>S.W.L. Jacobs &amp; J. Everett</em></td>
<td>None</td>
<td>X (X) X (X) X (X)</td>
</tr>
<tr>
<td><em>A. scabra</em> (<em>Lindl.</em>) <em>S.W.L. Jacobs &amp; J. Everett</em></td>
<td>None</td>
<td>X X (X) X (X)</td>
</tr>
<tr>
<td><em>A. verticillata</em> (<em>Nees ex Spreng.</em>) <em>S.W.L. Jacobs &amp; J. Everett</em></td>
<td>None</td>
<td>(X) (X) (X)</td>
</tr>
<tr>
<td>Piptochaetium napostaense (<em>Speg.</em>) Hack.</td>
<td>Yellow leaf spots</td>
<td>X X X (X)</td>
</tr>
<tr>
<td>Piptatherum miliaceum (<em>L.</em>) <em>Coss</em></td>
<td>Yellow leaf spots, &quot;blisters&quot;</td>
<td>(X) X X (X)</td>
</tr>
<tr>
<td>Avena sativa <em>L.</em></td>
<td>None</td>
<td>(X) (X) X</td>
</tr>
<tr>
<td>Brachypodium distachyon (<em>L.</em>) <em>P. Beauv.</em></td>
<td>None</td>
<td>X X (X) (X)</td>
</tr>
<tr>
<td>Bromus catharticus <em>Vahl.</em></td>
<td>Yellow leaf spots</td>
<td>X X X X</td>
</tr>
<tr>
<td>Dichanthium aristatum (<em>Spreng.</em>) <em>C.E. Hubbard</em></td>
<td>None</td>
<td>X (X) X X X X</td>
</tr>
<tr>
<td>Elymus scabrifolius (<em>Döll</em>) <em>J.H. Hunz.</em></td>
<td>Yellow leaf spots</td>
<td>X (X) X (X)</td>
</tr>
<tr>
<td>Eragrostis curvula (<em>Schrad.</em>) <em>Nees</em></td>
<td>None</td>
<td>X (X) (X) X</td>
</tr>
<tr>
<td>Festuca arundinacea <em>Schreb.</em></td>
<td>None</td>
<td>X X X X (X)</td>
</tr>
<tr>
<td>Hordeum vulgare <em>L.</em></td>
<td>Yellow leaf spots</td>
<td>X X X</td>
</tr>
<tr>
<td>Lolium perenne <em>L.</em></td>
<td>None</td>
<td>X X X X</td>
</tr>
<tr>
<td>Phalaris aquatica <em>L.</em></td>
<td>Yellow leaf spots</td>
<td>X X X (X) (X)</td>
</tr>
<tr>
<td>Poa ligularis <em>Nees ex Steud.</em></td>
<td>None</td>
<td>X X X (X)</td>
</tr>
<tr>
<td>Secale cereale <em>L.</em></td>
<td>None</td>
<td>X X X X</td>
</tr>
<tr>
<td>Triticum aestivum <em>L.</em> cv. ACA 303</td>
<td>None</td>
<td>X X X X X X</td>
</tr>
<tr>
<td>T. aestivum cv. Arriero</td>
<td>None</td>
<td>X X X X X X</td>
</tr>
<tr>
<td>T. aestivum cv. Guapo</td>
<td>Yellow leaf spots</td>
<td>X X X X</td>
</tr>
<tr>
<td>T. aestivum cv. Liquén</td>
<td>Yellow leaf spots</td>
<td>X X X X</td>
</tr>
<tr>
<td>T. aestivum cv. Malevo</td>
<td>Yellow leaf spots</td>
<td>X X (X) X X</td>
</tr>
<tr>
<td>T. aestivum cv. Sureño</td>
<td>None</td>
<td>X X X X</td>
</tr>
<tr>
<td>Species</td>
<td>Macroscopic Symptoms</td>
<td>Microscopic Symptoms*</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>T. aestivum unknown cv.</em></td>
<td>Yellow leaf spots</td>
<td>X</td>
</tr>
<tr>
<td><em>Microlaena stipoides</em> (Labill.) R.Br.</td>
<td>None</td>
<td>(X)</td>
</tr>
<tr>
<td><em>Oryza sativa</em> L.</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Phyllostachys aurea</em> Riviere &amp; C. Riviere</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Phragmites australis</em> (Cav.) Trin. ex Steud.</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Austrodanthonia geniculata</em> (J.M. Black) H.P. Linder</td>
<td>None</td>
<td>(X)</td>
</tr>
<tr>
<td><em>Chloris gayana</em> Kunth.</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Cynodon dactylon</em> (L.) Pers.</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Sporobolus rigens</em> (Tr.) Desv.</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Aristida pallens</em> Cav.</td>
<td>Yellow leaf spots</td>
<td>X</td>
</tr>
<tr>
<td><em>Bothriochloa springfieldii</em> (Gould) Parodi</td>
<td>None</td>
<td>(X)</td>
</tr>
<tr>
<td><em>Cymbopogon citratus</em> (DC.) Stapf.</td>
<td>None</td>
<td>(X)</td>
</tr>
<tr>
<td><em>Paspalum dilatatum</em> Poir.</td>
<td>Yellow leaf spots</td>
<td>X</td>
</tr>
<tr>
<td><em>Pennisetum clandestinum</em> Hochst. ex Chiov.</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Sorghum halepense</em> (L.) Pers.</td>
<td>None</td>
<td>X</td>
</tr>
<tr>
<td><em>Zea mays</em> L.</td>
<td>None</td>
<td>X</td>
</tr>
</tbody>
</table>

* 1= normal spore germination; 2= abnormal spore germination; 3= normal appressoria; 4= abnormal appressoria or non-stomatal appressoria; 5= penetration not observed; 6= penetration, two to four infection hyphae formed from substomatal vesicle, growth cessation; 7= penetration + contact with plant cells, growth cessation; 8= penetration + contact with plant cells + thickening of cell walls, growth cessation; 9= haustoria; 10= abundant intercellular mycelia. ( )= observation was infrequent; ?= observation was doubtful; NE= not examined.
Moreover, one of the tested Australian accessions of *N. neesiana* and two from New Zealand (Auckland and Hawke’s Bay) did not become infected either. It should be noted that results regarding these two accessions from New Zealand are not conclusive as only four plants from Auckland were available for testing and those from Hawke’s Bay belonged to only one site. Fortunately, the Marlborough population of *N. neesiana*, which is susceptible to UP27, is the infestation in New Zealand that most requires a biological control agent. An isolate has yet to be found that is able to infect plants from populations not susceptible to UP27.

Different types of leaf spots were formed on inoculated leaves of several test species but there appears to be no direct relation between the presence of these symptoms and the level of development reached by the rust within inoculated leaves. Leaf spots were formed on leaves where no penetration was recorded, and conversely, there were instances of no apparent symptoms on leaves where penetration by the rust was later confirmed under the microscope (Anderson, unpublished). There was some development of the rust within the leaves of *P. miliaceum* and several Austrostipa spp, in which a few haustoria and some development of intercellular mycelium was observed, but no such development was recorded in congeneric *Nassella* species. This was unexpected, as typically, the taxa most susceptible to a pathogen are the plants most closely related to its preferred host (Wapshere 1974). Still, development of the rust within leaf tissues only occurred in grasses belonging to the same tribe as the target weed and the pathogen could only complete development on some populations of *N. neesiana*. Conducting host range studies in artificial conditions can predispose plants to infection (Parker et al., 1994), and lead to an over estimation of the field host range. Overall, our results suggest that the rust is very unlikely to cause any damage to non-target plants in the field. Several different resistance mechanisms were observed during these studies which will be discussed in detail elsewhere. Such mechanisms include: abnormal germination; incorrect appresorium positioning; inhibition of growth shortly after penetration; thickening of host cell wall in response to the presence of, or contact with, fungal hyphae; necrosis of cells in the proximity of the penetration area; and, encasement of haustoria by deposition of cell material (Heath, 1981, Heath 1982 & Heath, 1997). In most cases, more than one mechanism was recorded on samples of a single inoculated leaf. These plant defenses probably account for the failure of the rust to produce pustules on all of the tested species. On the basis of these findings, authorities in New Zealand have recently approved the importation of *U. pencanus* isolate 27 for the control of *N. neesiana* in that country. This is a historic achievement as this is the first time a pathogen has been approved for release on a grass.

**Acknowledgments**

This research was made possible by the financial support provided by the Australian Commonwealth Government through the Rural Industries Research and Development Corporation “The National Weeds and Productivity Program”. The New Zealand contribution to the project was funded by a national collective of regional councils and the Department of Conservation. CERZOS-CONICET and IMYZA-INTA are thanked for providing laboratory and glasshouse facilities in Bahía Blanca and Buenos Aires, Argentina.

**References**


Finding the Weapons of Biomass Destruction—Identifying Potential Biological Control Agents by Applying Principles of Chemical Co-Evolution

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Abstract

The history of the biocontrol of weeds has many parallels with that of biocontrol of insects in that the practice began without an extensive conceptual or theoretical framework. Among the first scientific attempt at using natural enemies to control weeds was undertaken in Hawaii. Soon after Lantana camara L. was introduced into Hawaii in 1858 for ornamental purposes, it became a noxious weed. Shortly thereafter, lantana was suffering from the effects of infestation by the exotic scale Orthezia insignis Browne and by the turn of the century Hawaiian Sugar Planters’ Association members were transporting the scale all over the islands. Albert Koebele was sent by the Association in 1902 to Mexico, from which he forwarded 23 insect species to Honolulu, 8 of which, representing six families in three orders, became established. For decades thereafter, suites of prospective agents were imported to areas of non-indigeneity in the hope that, either individually or collectively, they would have the desired effect of restricting weed growth and expansion. In the intervening century, a deeper understanding of phytochemical constraints on hostplant utilization has developed and principles derived from studies of plant-herbivore chemical coevolution have considerable potential for informing the design and implementation of weed biocontrol programs, in particular in anticipating nontarget risks. Among the predictive indicators, reflective of coevolutionary adaptations, are: 1. phylogenetic patterns of host usage, as evidenced by literature records; 2. behavioral adaptations that express dependence (e.g., taxonomic restriction of kairomones); 3. physiological limitations of plant response (e.g., galls); 4. ecological dependence on unique phytochemistry for defense against predators (e.g., sequestration); and 5. dependence on abiotic activators of plant defenses. Studies of coevolutionary interactions between herbivorous insects and their hostplants, independent of the economic status of the plants, thus can contribute meaningfully to the construction of a theoretical framework to aid the weed biocontrol community.
Molecular Analysis of Host-Specificity in Plant-Feeding Insects: Phylogenetics and Phylogeography of Fergusonina Flies on Australian Paperbarks

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Abstract

Molecular phylogenetics has been widely used by evolutionary biologists to explore patterns of host-plant specificity in phytophagous insects. This approach has also been used in biological control research where it can provide critical information during pre-release exploration of the potential utility of an insect against a weed target. Most importantly, molecular phylogenetics can resolve species limits, reveal cryptic species, and assess host-specificity within and among closely related species. Intra-specific phylogeographic analysis can provide additional information on the suitability or lack thereof of particular species for use in biological control. Melaleuca quinquenervia (Cav.) S.T.Blake., the “broad-leaved paperbark,” is an Australian wetland tree that has become an important invasive weed within Florida, including the Everglades. The search for potential biological control agents within Australia, discovered undescribed Fergusonina gall flies (Diptera: Fergusoninidae) feeding on M. quinquenervia and its relatives. Using molecular phylogenetics, we explored species limits and host-specificity in this group of flies from nine species of Australian paperbarks with the aim of assessing host specificity. In most cases, species delimited by molecular data were monophagous, feeding on a single host species. Further analysis of Australian populations of Fergusonina turneri Taylor the species on the invasive paperbark, suggest that pre-release agent selection may also need to consider phylogeographic structure of natural populations of potential agents.
Selection of Test Plant Lists for Weed Biological Control with Molecular and Biochemical Data

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Abstract

The initial steps of weed biological control programs involve the determination of the host range of a prospective agent prior to consideration for release. Accurately predicting the host range of a potential agent is fundamental to this process. This may be conducted first in the country of origin in open field testing (Briese et al., 2002) and later under controlled environmental conditions in quarantine (Zwolfer and Harris, 1971; McFadyen, 1998). Initially a plant test list is established composed of species that are taxonomically related to the weed and species of economic and ecologic importance from the area where the weed is a problem (Wapshere, 1974). This centrifugal / phylogenetic testing procedure involves “testing plants of increasingly distant relationship to the host until the host is circumscribed” (Wapshere, 1974) and is based upon the assumption that host shifts occur to plants of similar taxa (Ehrlich and Raven, 1964; Mitter and Farrell, 1991). Typically rare species are also included in the plants tested. As useful as this process is it potentially overlooks unrelated plant taxa that share similar secondary plant metabolites. Recent evidence indicates that chemical similarity may be a better predictor of host use than are phylogenetic relationships (Becerra, 1997; Wahlberg, 2001). Although little evidence may exist from weed biological control projects (Schaffner, 2001), species with secondary metabolites similar to the target weed should be included in the test list as they may contain the behavioral cues used by these specialized herbivore species to locate hosts and initiate feeding (Wheeler, 2005). As useful as the centrifugal / phylogenetic testing procedure may be, it potentially overlooks distantly unrelated plant taxa that share similar secondary plant metabolites.

References

Successfully Eliminating Parasitic Gregarines from *Neolema ogloblini* (Coleoptera: Chrysomelidae) - a Biological Control Agent for *Tradescantia fluminensis* (Commelinaceae)

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Abstract

*Tradescantia fluminensis* Vell. (Commelinaceae) was introduced into New Zealand (NZ) as a house plant but is now a serious under storey weed of indigenous forest. Surveys for potential biological control agents in SE Brazil, starting in 2005, identified a rich natural enemy biota including herbivorous insects and plant pathogens. Routine screening of the first insect agent to be host range tested, the leaf beetle *Neolema ogloblini* (Monros) (Chrysomelidae), revealed high levels of a gregarine (sporozoan protozoan) gut parasite. This appeared to reduce beetle fecundity, longevity and general vigor, potentially compromising its biological control efficacy. Depending on the host specificity of the gregarine, it could also threaten NZ fauna. In NZ biological control agents can only be released from containment if they are shown to be free from unwanted associated organisms. We report on two years of increasingly intensive attempts to obtain a gregarine-free population of *N. ogloblini* including use of highly hygienic field collection methods in Brazil to get clean material at source, surface sterilization of eggs, use of cages with HEPA-filtered air in containment, and attempts to improve our gregarine detection methods by gut dissection and DNA probes (both of which proved less easy, and more expensive than anticipated). In December 2010 we finally released *N. ogloblini* from containment after showing we had three consecutive generations of beetles tested negative for gregarines. Success was achieved by repeated sub-culturing. Firstly, eggs were collected as hygienically as possible from single female beetles (each having been paired with a single male). Then each larva was reared in solitary containment but with poor hygiene to ensure that any low level of gregarine infection would be expressed sufficient to minimize the risk of getting false negatives in subsequent testing. All lines testing positive were eliminated. Final crossing of lines before release from containment was carried out in an attempt to restore lost heterozygosity and overcome any inbreeding depression or adaptation to laboratory conditions.
Metabolic Profiling: A New Tool in the Prediction of Host-Specificity in Classical Biological Control of Weeds?

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Abstract

Current host-specificity testing for the selection of environmentally safe weed biological control agents is based on the molecular phylogeny of the weed. According to the centrifugal phylogenetic theory, non-target species closely related to a target weed should be at greatest risk of attack by a biological control agent, as they are biochemically and morphologically more similar to the target, and therefore more likely to share the cues used by specialists to select their host. However, a molecular phylogeny is not always a suitable surrogate for phenotypic traits at the species level. For example, the potential weed biological control agent Ceutorhynchus cardariae Korotyaev (Coleoptera: Curculionidae) investigated for the invasive Brassicaceae plant Lepidium draba L., attacks plant species distantly related to L. draba under no-choice conditions, revealing a disjunct fundamental host range. The aim of this study is to compare the reliability of a phenotypic phylogram with a genetically based one for predicting host use by C. cardariae. We used data of feeding and oviposition trials for 23 test plant species/populations, differing in susceptibility to C. cardariae attack. Host preference of C. cardariae was assessed using different phylograms based either on genetic distance between test plant species or various combinations of phenotypic traits, such as chemical profile and physical attributes. Patterns of susceptibility to C. cardariae among the different trees were compared using different measures of phylogenetic correlation. Principles discovered could be used to explain and potentially predict the host range of other biological control agents.
Individual Variation in Insect Response Causes Misleading Interpretation of Host Specificity Tests

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Abstract

Host specificity tests, conducted prior to the introduction of broom seed beetles (\textit{Bruchidius villosus} (F.)) to New Zealand for biocontrol of \textit{Cytisus scoparius} (L.) Link, failed to detect their ability to oviposit and develop into adults on the closely related non-target host plant \textit{C. proliferus} L.f. Tests conducted with individual beetles indicated that the failure of the original host specificity tests resulted from high levels of variation in beetle oviposition preference and relatively low levels of replication. The occurrence of a host range expansion was discounted, but New Zealand beetles showed a higher preference for \textit{C. proliferus} than newly imported UK beetles, indicating some adaptation to the novel host. Although beetles still strongly preferred the target host \textit{C. scoparius}, beetles reared from \textit{C. proliferus} scored more highly on several key performance criteria than beetles reared from \textit{C. scoparius}, indicating the potential for increasing use of the non-target host. Implications for host-testing species with high levels of individual variation are that individuals, rather than groups, should be tested and that higher levels of replication are needed to ensure detection of low-level effects. Also, a strong preference for the normal host shown in laboratory testing may not be a sufficient indication of host specificity in the field. It is suggested that changes to host specificity testing protocols are needed, including a more conservative approach to interpreting low levels of non-target use.
Simulated Herbivory May Underestimate the Effects of Natural Herbivory: A Case Study with Dyer’s Woad

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Abstract

Weed biological control critics and advocates alike have expressed a strong desire for improved predictive ability in the selection of effective agents. Consequently, studies on plant response to herbivory have become increasingly important in risk assessments. The first objective of our study was to assess the response of Dyer’s woad (Isatis tinctoria L.) to damage by a root-crown mining weevil (Ceutorhynchus rusticus Gyllenhal.) currently investigated as a biological control agent for North America. Manipulating phytophagous insects can be logistically challenging and simulated herbivory is frequently advocated as a technique for replacing natural herbivory. However, mechanical damage does not always produce the same response as herbivore feeding, in particular when trying to mimic internal feeding organisms. A second objective was therefore to evaluate whether artificial herbivory can reproduce C. rusticus attack on dyer’s woad. In addition, both natural and simulated herbivory were combined with two levels of plant competition from the North American grass Festuca idahoensis Elmer. Dyer’s woad reacted to both types of herbivory by an increased production of secondary shoots. These shoots were however thinner and shorter and both biomass and seed production were reduced compared to control plants. Simulated herbivory caused similar effects as natural herbivory, but the magnitude of impact was lower compared to natural herbivory. F. idahoensis only had a weak effect on Dyer’s woad, while Dyer’s woad reduced biomass of F. idahoensis. Weevil attack on Dyer’s woad increased the biomass of F. idahoensis, while simulated herbivory had no effect on grass biomass. In conclusion, the results of our study confirm 1) the potential of C. rusticus as an effective biological control agent for Dyer’s woad and 2) revealed that simulated herbivory was able to mimic effects of natural herbivory, but that it underestimated the magnitude of effect in our study system.
Does Nitrogen Influence Host Choice by a Biological Control Insect?

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Abstract

Previous studies have demonstrated the importance of nitrogen as a component of host plant quality for phytophagous insects, with some insects preferring and performing better on hosts of higher nitrogen content. Although some researchers have investigated the role of nitrogen in host species choice by insect pests, there has been little exploration of how nitrogen may influence host choice patterns, and thus risk assessment, for insects used in weed biological control. The root weevil, *Mogulones crucifer* Pallas (Coleoptera: Curculionidae), was first released in Canada in 1997 against the rangeland weed, houndstongue (*Cynoglossum officinale* L.) (Boraginaceae). Both pre and post-release investigations have documented non-target attack on closely-related Boraginaceae species, albeit at a lower level than on houndstongue. Field and laboratory studies also have shown how fertilization of houndstongue with nitrogen can increase *M. crucifer* population size and weevil feeding and oviposition. As a next investigative step, laboratory studies were conducted using houndstongue and the native North American borage species, *Hackelia floribunda* (Lehm.) I.M.Johnst., to determine how the addition of nitrogen may alter non-target choice by *M. crucifer*. Two single-choice experiments (adult feeding and oviposition) were conducted using greenhouse grown houndstongue and non-target plants of either low or high nitrogen content. Leaves from individual plants were paired in small containers so that all possible combinations of plant species and nitrogen level were replicated for each experiment. Laboratory-reared female weevils at their ovipositional peak were added to each container and left for 24 hours to feed (1 female) or 48 hours to oviposit (2 females), before data collection. The results showed no effects of either species or nitrogen level on the amount of feeding by weevils. Although there was some oviposition preference shown for high nitrogen houndstongue, overall, the preference for houndstongue was greater than for the non-target species regardless of nitrogen level.
Neoclassical Biological Control: Will the Introduction of a New Association Contribute to the Control of *Myriophyllum spicatum* in South Africa?

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Abstract

While South Africa has a long and successful history of classical biological control of floating aquatic weeds, investigations into biological control against submerged plant invasions have only recently been initiated. *Myriophyllum spicatum* L. was first recorded in South Africa in the 1880s, yet the need to control it only became obvious in the 2000s, following its explosive growth on the Vaal River, one of South Africa’s largest and most important rivers. *M. spicatum*, which is indigenous to Europe, Asia and North Africa, is one of the most important waterweeds in continental USA, causing millions of dollars to be spent on its control. Despite surveys for natural enemies in its regions of origin, no suitable agents have been found. However, a successful biological control program based on a new association has been implemented in the USA with a native North American weevil, *Euhrychiopsis lecontei* Dietz. which prefers *M. spicatum* over its natural host plant. Based on the experience in the USA, *E. lecontei* has been imported to South Africa as a candidate agent for *M. spicatum*. The use of new associations is inherently risky and is appropriate only where the target weed has few or no native relatives in the area of introduction. There is only one indigenous plant in the milfoil family in South Africa, *Laurembergia repens* P.J. Bergius (Haloragaceae), and host specificity tests show that *E. lecontei* cannot complete development on it. Further, this is a unique program for weed biological control, because preliminary amplified fragment length polymorphism (AFLP) analysis of the ITS region showed the South African *M. spicatum* populations to be genetically distinct from US samples, which is mirrored by differences in performance of *E. lecontei* on these populations. Implications for neoclassical biological control are discussed.
A Review of Interactions between Insect and Fungal Biological Control Agents of Water Hyacinth and Our Recent Studies

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Abstract

A large number of biological control agents have been released for the management of water hyacinth, *Eichhornia crassipes* (Mart.) (Solms-Lauchab) (Pontederiaceae), an aquatic weed of socio-economic importance. Integrated biological control is a holistic approach aimed at minimizing weed population while simultaneously maintaining the integrity of the ecosystem and reducing reliance on any single agent. In South Africa six arthropod and one fungal biological control agents have been released and several indigenous fungal agents have been reported. With the presence of these biological control agents interaction between host plants, their herbivores and pathogens could play an important role in control of the weed. Such studies have been largely ignored and impacts of biological control have been mostly analyzed separately, thereby neglecting mutualistic or antagonistic interactions between these bioagents and possible joint effects on the host. We studied the possible mutualistic or antagonistic effect between mirid, *Eccritotarsus catarinensis* (Carvalho) and phytopathogen, *A. zonatum* (Sawada) W. Gams. at different insect inoculation load and culture age of pathogen respectively. Our 21 day old culture of *A. zonatum* was found to be the most virulent for all inoculation loads of *E. catarinensis*. At lower inoculation loads of 5 and 10 *E. catarinensis* per plant, the disease initiation and disease index was significantly higher than that at higher insect inoculation loads of 15 and 20 mirids per plant at same culture age of the fungi. The study brings to light the fact that the co-existence of the arthropod and fungal biological control agents can be both beneficial and detrimental to each other depending on their interactions with the host plant. Successful combination of the biological control agents can be applied under field conditions with higher chances of success.
Host-Specificity Testing of *Liothrips tractabilis* (Thysanoptera: Thripidae), a Candidate Biological Control Agent for *Campuloclinium macrocephalum* (Asteraceae) in South Africa

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Abstract

Pompom weed, *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), originates from Central and South America and was first detected in South Africa in 1962. In the 1980s *C. macrocephalum* started slowly extending its range and in the 1990s and 2000s it entered a dramatic expansion phase. An invasive of grasslands, savannas and wetlands, *C. macrocephalum* reproduces and spreads via numerous wind-dispersed seeds. Studies have highlighted the significant negative impact the weed has on biodiversity. A biological control program was initiated against the weed in 2003. Two rust fungi and nine insect species have been found to be associated with the plant in its native range. Of these, two insect species, *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Thripidae) and *Cochylis campuloclinium* Brown (Lepidoptera: Tortricidae), and one pathogen, *Puccinia eupatorii* Dietel (Uredinales: Pucciniaceae) were rated (based on damage, range and abundance) as having the most potential. The stem-deforming thrips, *L. tractabilis*, was selected as the first agent to be tested. Field host range surveys (15 species - one Lamiaceae and 14 Asteraceae) and laboratory host-specificity testing (43 species in 11 tribes in the Asteraceae) were conducted in Argentina and quarantine in South Africa, respectively. In the native range, no signs of thrips activity were recorded on any of the species surveyed. In laboratory no-choice trials, feeding damage and/or oviposition was recorded, albeit at lower levels than on the *C. macrocephalum* controls, on 14 test species in four tribes. Paired-choice trials were conducted on the 14 species that were positive in the no-choice trials. No feeding or oviposition was recorded on any of the test species, whereas the control plants were heavily attacked. *Liothrips tractabilis* is therefore considered to be suitably host-specific to *C. macrocephalum* and permission for its release is currently being sought.
Developing Biological Control for Common and Glossy Buckthorn

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Abstract

*Rhamnus cathartica* L. (common buckthorn) and *Frangula alnus* L. (glossy buckthorn) (Rhamnaceae) are both shrubs and small trees of Eurasian origin which have become invasive in North America. Over the past nine years, eight potential common buckthorn biocontrol agents have been studied and discarded due to lack of host-specificity, including five leaf-feeding moths. Among the other 15 leaf-feeding moths known from buckthorn in Europe, only one or two species might be specific enough to deserve further attention. There will be no further research on glossy buckthorn biocontrol agents as initial research found no promising agents. The most specific species studied so far is the leaf-margin curl galler psyllid *Trichochermes walkeri* Foerster which is known only from *R. cathartica* in Europe. Other potentially specific candidate species are the psyllids *Cacopsis thamnicolla* Foerster. and *Trioza rhamni* Schrank. and the seed-feeding cecidomyiid fly *Wachtiella krumholzi* Stelter. Thus there are only a few candidate agents left. In addition, the detection of ‘*Candidatus Phytoplasma rhamni*’ Marcone et al. in *T. walkeri* adults raises several questions that will need to be addressed before further considering sap-suckers for biological control of *R. cathartica*. Populations of the psyllid species as well as *Rhamnus* spp. in Europe and North America have been collected to detect the presence of phytoplasma. First results indicate the presence of the phytoplasma in most psyllid populations and its host plant in Europe. We are planning in a second step to determine whether *T. walkeri* transmits ‘*Candidatus Phytoplasma rhamni*’. Studies of buckthorn seedling mortality in Europe may identify additional potential biocontrol agents, such as pathogens.
Evaluating the Potential for Biological Control of Swallow-Worts 
(*Vincetoxicum nigrum* and *V. rossicum*) in Eastern North America

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Abstract

Two European species of swallow-worts, *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopov) Barbarich are now naturalized in eastern North America, and considered invaders of natural areas and abandoned pastures. Herbivore surveys conducted since 2006 in Switzerland, France, Germany and Ukraine located four potential biological control agents on *V. hirundinaria* (L.) Pers.: the leaf-feeding noctuid *Abrostola asclepiadis* Denis & Schiffermüller, the leaf-feeding chrysomelid *Chrysolina aurichalcea asclepiadis* (Villa), the root-feeding chrysomelid *Eumolpus asclepiadeus* Pall., and the seed-feeding tephritid *Euphranta connexa* Fabricius. Surveys also documented the first occurrence of the leaf feeding moth noctuid *Hypena opulenta* Christoph. on *V. rossicum* in Ukraine. No herbivores have been found in Europe on *V. nigrum*. A petition is currently being prepared for release of *H. opulenta* against *Vincetoxicum* in North America (Weed et al., in these proceedings) while *C. a. asclepiadis* has been found to be too polyphagous to be considered as a potential agent. *E. asclepiadeus* overwinters as larvae in the soil; generation time ranged from one to three years in common garden experiments in Switzerland. A few native North American non-target species in the genus *Asclepias* support complete larval development of this species. In no-choice adult feeding and reproduction tests with potted plants, naïve *E. asclepiadeus* females were able to produce fertile eggs on two *Asclepias* species. *E. asclepiadeus* will occasionally oviposit in the vicinity of non target plants in the presence of *Vincetoxicum* although very little or no adult feeding was recorded from potted non-target plants under choice conditions. In an impact study with *V. rossicum* and *V. nigrum*, root herbivory at larval densities of 20 and 60 larvae/plant stunted shoot height by 4 and 6 cm and reduced plant biomass by 30% and 70% respectively. We are planning to screen additional populations of *E. asclepiadeus* and to continue working with *E. connexa* and *A. asclepiadis*. Additional surveys on *V. rossicum* and *V. nigrum* are also being considered.
Laboratory and Open-Field Tests on *Abia sericea* (Hymenoptera: Cimbicidae) – a Candidate for Biological Control of Teasels (*Dipsacus* spp.)

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Abstract

Invasive teasels (*Dipsacus* spp.) are widespread in the USA (43 states) and listed as noxious in five states. The cimbicid sawfly *Abia sericea* L. (Linné, 1758) is under evaluation as a potential agent for biological control of teasels. *A. sericea* lays its eggs under the epidermis of the leaves of *Dipsacus* plants and the larvae feed on the leaves. Laboratory and open-field experiments to evaluate the host specificity of the sawfly were conducted from 2007-2010 at Agricultural University of Plovdiv, Bulgaria. In the laboratory, potted plants from twelve plant species belonging to the families Dipsacaceae, Caprifoliaceae, Valerianaceae, Apiaceae, Asteraceae, and Brassicaceae were tested in multi-choice oviposition and feeding tests. They were arranged in plastic cages measuring 40x40x20 cm, with each cage containing one *Dipsacus laciniatus* L. plant and seven plants of different species. Individual females were released in each cage to oviposit. Number of eggs laid, number of larvae hatching and larval feeding were recorded. Eggs were laid only on *D. laciniatus* plants with one exception – on *Valeriana officinalis* L., although no larvae hatched from the latter. Larval feeding was observed only on *D. laciniatus*, *Knautia arvensis* (L.) Coult. and *Scabiosa* sp. (all Dipsacaceae). An open field test was conducted in 2010 with seven plant species from the families Dipsacaceae, Caprifoliaceae, Valerianaceae, Apiaceae, and Brassicaceae arranged in a pseudo Latin square design with a distance of 70 cm between the plants within rows. Third- and fourth-instar larvae were released in June at a rate of 1 or 2 per test plant. Adults were released on the plants several times in June-July to lay eggs. In the open-field test eggs were laid and larvae fed only on *D. lacianius*. 

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Biology and Fundamental Host Range of the Stem Boring Weevil
*Apocnemidophorus pipitzi* (Coleoptera: Curculionidae),
a Candidate Biological Control Agent for Brazilian Peppertree

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Abstract

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), was introduced into Florida, USA, from South America as an ornamental in the 1840s. It eventually escaped cultivation and has become a serious threat to the state’s biodiversity, especially over large areas of the Everglades. In the 1980s, this invasive weed was targeted for classical biological control because of the extent of the infestation and the absence of native congeners in the continental USA. In March 2006, a survey for new natural enemies of Brazilian peppertree was conducted in southeastern Paraguay. A stem boring weevil identified as *Apocnemidophorus pipitzi* (Faust) was collected from the plant at several locations. The insect also has been reported from Argentina, Brazil and Uruguay. Adults are defoliators and feed mainly on the upper surface of subterminal leaflets, where they produce a characteristic notching pattern. Weevils were transported under permit to the Florida Biological Control Laboratory in Gainesville, FL. A laboratory colony of *A. pipitzi* was established in April 2007 by caging the adults on cut branches of Brazilian peppertree supplemented with leaf bouquets. This insect is the first stem borer of Brazilian peppertree successfully reared under laboratory conditions. To date, over nine generations of the weevil have been produced in the laboratory, with over 10,000 adults emerging in the fifth generation. Females deposit eggs singly inside the stems and larvae feed under the bark where they damage the vascular cambium. There are five instars, pupation also occurs inside the stem, and a new generation of adults emerges in 3-4 months. Host specificity tests were conducted with 77 plant species in 39 families and 7 orders. The results showed that *A. pipitzi* can reproduce only on Brazilian peppertree and the congeneric Hardee peppertree, *Schinus polygamus* (Cav.) Cabrera, which is invasive in California. The results of laboratory host range tests indicate that *A. pipitzi* is a *Schinus* specialist. A petition to release this insect in Florida for classical biological control of Brazilian peppertree was submitted to the federal interagency Technical Advisory Group for Biological Control Agents of Weeds.
Biology, Host Specificity, and Larval Impact of *Hypena opulenta* (Lepidoptera: Noctuidae): A Promising Biological Control Agent of Swallow-Worts (*Vincetoxicum*) in North America

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Abstract

A classical biological control program has been initiated against the invasive European swallow-worts *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopov) Barbarich in North America. After its discovery in southeastern Ukraine attacking leaves of *V. rossicum*, the noctuid moth *Hypena opulenta* (Christoph) was transported to quarantine to initiate studies on its life history, host specificity, and larval impact. In the laboratory, adults of *H. opulenta* begin oviposition two days after emergence and produce approximately 600 eggs. Larvae develop through five instars and overwinter as pupae. Pupal diapause is facultative, resulting in at least two generations per year. Longevity and fecundity of females raised on *V. nigrum* and *V. rossicum* were similar and they showed no oviposition preference among *Vincetoxicum* species. Of the 74 plant species tested (distributed among 43 genera within 9 families), *H. opulenta* larvae completed development only on *Vincetoxicum*. *H. opulenta* averaged over 75% survival on all *Vincetoxicum* species, indicating that both target weeds are suitable hosts. Partial development occurred on two plants in the Urticaceae. In the impact study, feeding by two larvae per plant caused reductions in aboveground biomass to *V. rossicum* resulting in decreased reproductive output (flower, seedpod, and seed production). Only flower production of *V. nigrum* was negatively affected by larval feeding. The results of this study indicate that *H. opulenta* apparently poses little risk to native North American plants and is a promising agent against forested populations of *V. rossicum*. A petition is currently being prepared for release against *Vincetoxicum* in North America.
Phenotypes of Common Crupina (Crupina vulgaris),
Synchronization of Bolting, and Yield Effects of Leaf Removal
and Inoculation by Ramularia crupinae

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Abstract

Common crupina (Crupina vulgaris Cass.) is an annual plant of major importance in
the Western United States. There are two varieties of crupina, i.e., var. vulgaris and var.
brachypappa, that occur in North America. Only by artificial plant vernalization, is it
possible to synchronize bolting between varieties for comparative studies. Successful
vernalization was achieved in this study by germinating seeds and growing transplants at
10°C with an 8 hr photoperiod for a minimum of one month. Typical plant phenological
development, i.e., seedling, rosette, bolt, bud, flowering, and seed stages, results for both
varieties. Use of this protocol has enabled comparative studies on susceptibility of both
varieties at the same time. Because crupina reproduces only by seed, an attempt was made
to determine which plant part (or parts) provides photosynthate for seed fill. If such can
be identified, then climatic conditions that occur at that stage of growth can be estimated
and used to determine if conditions would be favorable for disease when the plant is most
vulnerable. Either selected leaf removal or inoculation of various plant parts (or growth
stages) by Ramularia crupinae Dianese, Hasan & Sobhian was used in these tests. Clear
evidence of the importance of cauline leaves was found in two leaf removal experiments.
Although reductions in seed yield and other parameters resulted from inoculations with R.
crupinae, the importance of plant part was less clear than in the detached-leaf experiments.
One reason for this difference is that symptom development under greenhouse conditions
requires from 10 days to 2 weeks, so effects from infection of crupina on yield parameters
may be manifested at a slower rate than when leaves are detached. Although R. crupinae was
damaging and caused seed yield loss in these studies, more profound effects may result from
inoculations, either at earlier stages of plant development or after multiple inoculations.
An Update on Biological Control of Invasive Hawkweeds in North America

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Abstract

European hawkweeds (Pilosella spp.) have been introduced into New Zealand and North America where several species have become problematic. In the early 1990s, CABI Europe-Switzerland initiated a biological control project on behalf of the Hieracium Control Trust in New Zealand for mouse-ear hawkweed, Pilosella officinarum (L.) F.W.Schultz & Sch. Bip. (= Hieracium pilosella). Five insect species were eventually released in New Zealand. Since 2000, CABI has also been investigating natural enemies for use against invasive alien hawkweeds in North America, namely meadow hawkweed, P. caespitosa Chiov. (= Hieracium caespitosum) and orange hawkweed, P. aurantiaca (L.) F.W.Schultz & Schultz-Bip. (= Hieracium aurantiacum). In contrast to New Zealand, where all existing hawkweeds are naturalized, native Hieracium spp. occur in North America, thus limiting the number of species that can be considered for introduction. A gall wasp, Aulacidea subterminalis Niblett, which attacks the stolon tips of several Pilosella species has proven to be very specific. Regulatory authorities in the USA and Canada have recently approved release of the agent in time for releases in spring 2011. A TAG petition for a root-feeding hoverfly, Cheilosia urbana Meigen. is currently being drafted. One additional candidate agent investigated is Aulacidea pilosellae Kieffer, which galls the midrib of leaves, stolons and flower stalks of several Pilosella spp. Two forms of the wasp that differ in life history are known. Wasps located in the northern distribution range are univoltine, whereas wasps in the southern range are bivoltine. The two forms also appear to differ in their host range. Molecular analyses are currently underway to determine the level of genetic differentiation between the two forms. Apart from the candidate agents listed above, we believe that the prospects for finding further effective and safe European insects for hawkweed biological control in North America are unlikely. Should further agents be required, we suggest that pathogens, especially the rust Puccinia hieracii (Röhl.) H. Mart. should be re-evaluated.
Searching for New Potential Agents for an Old Problem: Field Bindweed (*Convolvulus arvensis*)

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Abstract

In the 1980s, two biological control agents were released for field bindweed (*Convolvulus arvensis* L.) management in North America: the bindweed moth *Tyta luctuosa* Denis & Schiffermüller (Lepidoptera: Noctuidae), and the gall mite *Aceria malherbae* Nuzzaci. (Acari: Eriophyidae). While establishment for the moth has not been confirmed, the mite is established in several U.S. states and in Canada, but impact is variable. In 2009, the search for additional potential agents for the US was revived. We currently focus on the stem-boring fly *Melanagromyza albocilia* Hendel. (Diptera: Agromyzidae) and the root-mining flea beetle *Longitarsus pellucidus* Foudras. (Coleoptera: Chrysomelidae). The agromyzid has two generations per year and field observations revealed that attacked shoots often dry up and die. Unfortunately, we were not able to obtain oviposition of the fly under lab conditions in 2010, and so no host-specificity tests could be conducted. Adults of the flea beetle readily laid eggs, and we started to conduct no-choice larval transfer tests with ten test plant species, eight native to North America. Adults emerged from at least three test species; these will be exposed under multiple-choice conditions in 2011. Apart from the agromyzid and the flea beetle, there are at least five additional insects with biocontrol potential, i.e. a defoliating leaf beetle, two leaf and flower feeding moths, and two root-mining sesiid moths. We are also revising the original test plant list. The mite had been tested on 48 plants species in a wide range of families, including many economically important, but unrelated, crop species. Taking into account changes in the emphasis of host range testing since the 1980s and new information on the phylogeny of the family Convolvulaceae, the revised list will mainly contain native North American species and ornamentals and crop plants in the family Convolvulaceae.
Field Garden Experiments to Assess the Host Specificity of *Aceria solstitialis* (Acari: Eriophyoidea), Potential Biological Control Agent for *Centaurea solstitialis* (Asteraceae)

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Abstract

*Centaurea solstitialis* L. (yellow starthistle) is an annual noxious weed that currently infests millions of acres of rangelands, non-cultivated and natural areas in the Western USA. It displaces native plant communities reducing plant diversity and forage production for livestock and wildlife. *Aceria solstitialis* L. is an eriophyoid mite found exclusively in association with *C. solstitialis* in Turkey and Bulgaria. This mite damages bolting plants causing stunting, leaf curling and incomplete flower development. During 2008 and 2009, two open field tests were conducted in Bulgaria, to study the mite’s dispersal behavior and host range. The experiments were conducted on plots of 100 m² at the experimental field of Agricultural University of Plovdiv. Five plant species were included in the experiment: *C. solstitialis* (infested and not-infested), *C. diffusa* Burm.f., *C. cyanus* L., *Carthamus tinctorius* Mohler, Roth, Schmidt & Boudreaux, and *Cynara scolymus* L. The plants were infested with mites before transplanting them in the field. An infested leaf cutting, with at least 30 mites, was placed on each test plant except on the negative control (*C. solstitialis* not-infested). Results of these field experiments showed that *A. solstitialis* mites were present in high population densities only on intentionally infested *C. solstitialis* and *C. cyanus.*
Open Field Experiment to Assess the Host Specificity of *Lixus cardui* (Coleoptera: Curculionidae), a Potential Candidate for Biological Control of *Onopordum acanthium* (Asteraceae)

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**Abstract**

Scotch thistle *Onopordum acanthium* L. (Asteraceae) is native to Europe and Asia and has been introduced to temperate climates elsewhere, including North America and Australia. In the US the weed is most problematic in the semi-arid parts of the Northwest, California and Nevada. *Lixus cardui* Olivier is a weevil that lays its eggs in the flowering stem of Scotch thistle in cavities chewed by ovipositing females. The larvae burrow, feed and pupate within the stem. An open field experiment, to evaluate the host specificity of the weevil, was conducted on a small experimental plot at the Agricultural university of Plovdiv, Bulgaria, in 2010. Nine plant species, belonging to the family Asteraceae, were arranged in a pseudo Latin square design with a distance of 1 m among the plants in the rows. Most plants were provided as rosettes, which were transplanted from the field in Southern Bulgaria during April and early May (*O. acanthium*, *Cirsium arvense* (L.) Scop., *Arctium lappa* L., *Carduus acanthoides* L., *Carthamus tinctorius* L.and *Centaurea cyanus* L.). Some were sown in the lab and then transplanted to the experimental plot (*Cynara scolymus* L., *Silybum marianum* (L.) Gaertn. and *Helianthus annuus* L.). Adult *L. cardui* were collected in May and June in the area around Plovdiv and were released in the experimental field, one or two on each plant. At the end of August the plants were dug out, except those which did not bolt (*C. scolymus* and *A. lappa*). The stems were dissected and examined for larvae, pupae or adults of *L. cardui*. The results of dissections showed that all the Scotch thistle plants were damaged by the weevil, while its presence was never registered in any other test species. Specimens from these experiments are currently undergoing genetic and morphological studies to understand if we are in the presence of different genetic entities not distinguishable by morphological traits.
Targeting Ecotypes of *Hydrellia lagarosiphon* in Pre-Release Studies Using Adult Longevity, Reproductive Performance and Temperature Tolerance

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Abstract

A leaf-mining fly, *Hydrellia lagarosiphon* Deeming (Ephydridae) was identified as a potential biological control candidate for the submerged aquatic weed *Lagarosiphon major* Ridl. Moss ex Wager (Hydrocharitaceae). Larvae feed on the leaves and cause significant damage to shoot tips reducing the photosynthetic potential of the plant. Three populations of *H. lagarosiphon* were collected from different sites across the native geographic range in South Africa varying in altitude. As part of the pre-release testing the variation in adult longevity, reproductive performance and extreme temperature tolerance of the three fly populations were assessed under laboratory conditions maintained at 22:16°C in a day:night photoperiod of 15:9h. The mismatch between performance in native and introduced ranges in classical biological control is considered an important factor reducing the efficacy of biocontrol agents. To assess temperature tolerance for different fly populations critical minima trials were run using a range of pre-treatments and plunge protocol into discriminating temperatures before scoring survival. The implications for targeting specific populations in the native range as a better ecological match to conditions in Ireland and parts of Europe are discussed.
Developing Biological Control for Perennial Pepperweed in the U.S.: Progress So Far

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Abstract

Perennial pepperweed, *Lepidium latifolium* L. (PPW), is a mustard of Eurasian and Central Asian origin that is invading natural and cultivated habitats in North America and is difficult to control with conventional means. A project investigating the potential for biological control of PPW was started in 2004. Based on field collections in various countries within the native range of PPW, 113 phytophagous organisms were sampled or reared, five of which were prioritized as potential biological control agents: the root-mining weevil *Melanobaris* sp. n. pr. *semistriata* Boheman (Coleoptera, Curculionidae), the gall-forming weevil *Ceutorhynchus marginellus* Schultze (Coleoptera, Curculionidae), the stem-mining flea beetle *Phyllotreta reitteri* Heikertinger (Coleoptera, Chrysomelidae), the gall-forming eriophyid mite *Metaculus lepidifolii* Monfredo & de Lillo (Acari, Eriophyidae) and the stem-mining fly *Lasiosina deviata* Nartshuk (Diptera, Chloropidae). Host-specificity testing in quarantine at CABI Europe-Switzerland started in 2006 with the first three of the potential agents. In addition, several field tests were conducted in the native range of the organisms in Russia and Turkey. A summary of results so far will be presented and the potential of the organisms as biological control agents discussed.
What’s Been Happening in Our Containment Facility?  
The Old and the New

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Abstract

Landcare Research, formerly Department of Scientific and Industrial Research (DSIR), has worked on the biological control of weeds for the past 90 years. During this time 63 species of insects have been imported into our containment facility for re-phasing, host testing, pathogen screening and rearing before being released into the New Zealand environment. Some species were imported and not released for various reasons. These included, inherent diseases, population collapse in containment, used for experiments only, regulations allowing host testing only. Table 1 (See the URL below) shows which species have been assessed for establishment, which species were tested for diseases and those which tested positive. Additionally, the table shows which importations were approved for release, when and by which authority, and which importations were never released. Families of insects introduced into our quarantine facility:

- Acari = 2
- Chrysomelidae = 16
- Curculionidae = 9
- Tephritidae = 5
- Pyralidae = 3
- Oecophoridae = 3
- Tortricidae = 3
- Nymphalidae = 2
- Scythrididae = 2
- Tingidae = 2
- Cerambycidae = 1
- Psyllidae = 1
- Agromyzidae = 1
- Syrphidae = 2
- Cecidomyiidae = 2
- Bruchidae = 1
- Eupterotidae = 1
- Crambidae = 1
- Cynipidae = 1
- Pteriophoridae = 2
- Arctiidae = 1
- Cosmopterigidae = 1

Parts of the plant targeted for attack by these potential biological control agents were: stems, 15; leaves, 30; roots, eight; and flowers, one. Nine insect species were released to attack seeds. Six seed-feeding agents, three root feeders, one stem feeder and four foliage feeders, were shown to have an impact on the target plant either in the laboratory or in the field. During 1920 to 2011 most introductions have occurred during the 1980s (16), 1990s (24), and 2000s (23).

There have been 18 weed species (listed below) that these insects have been imported to attack as biological control agents. Of the weed species selected as targets for biological control 11 were environmental (i.e. not productive sector weeds), three solely pastoral while four were both pastoral and environmental weeds. The 18 weed species include: Cytisus scoparius (L.) Link, Jacobaea vulgaris Gaertn., Ulex europaeus L., Cirsium arvense (L.) Scop., Carduus nutans L., Cirsium vulgare (Savi) Ten., Passiflora tripartite (Juss.) mollissima (Kunth) Holm-Niesen & P.M. Jørg., Hieracium pilosellae (L.) F.W. Schultz & Sch. Bip., Chrysanthemoides monilifera (L.) Norlindh, Loniceria japonica Thunb., Tradescantia fluminensis Vell., Araujia hortorum E. Fourn, Calluna vulgaris (L.) Hull, Clematis vitalba L., Alternanthera philoxeroides Griseb, Solanum mauritianum Scop., Ageratina riparia (Regel) R. M. King & H. Rob., and Hypericum perforatum L. Twenty-one potential agents were imported but not released and 10 of these were not released because they failed host range testing experiments. Only three species were not released because of disease risk. Up until 1991, 21 species were internally approved for release from quarantine by the director of DSIR. Only one species was refused approval for release in
1991 by an external agency, the Ministry of Agriculture and Fisheries (MAF). From 1991 to 1998 MAF approved the release of 8 species from quarantine before the existence of the Environmental Risk Management Authority New Zealand (ERMA NZ). ERMA NZ (now the Environmental Protection Authority, EPA) was formed and began presiding over releases of exotic organisms in 1998 and has since approved the release of 16 species of biological control agent for seven weed species.

Table 1 can be located and viewed at the following URL:

References

Biological Control of Garlic Mustard, *Alliaria petiolata*, with the Root and Crown-Boring Weevil *Ceutorhynchus scrobicollis*

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Abstract

Biological control of garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande, testing indicates potential for long-term management of this invasive biennial weed. Extensive host specificity trials with the potential biological control agent *Ceutorhynchus scrobicollis* Nerensheimer and Wagner, a root and crown-boring weevil, have been conducted at CABI Europe-Switzerland and at the University of Minnesota. To date, we have tested native Brassicaceae species representing the majority of Brassicaceae tribes present in North America, as well as tribes containing cultivated or ornamental species, a total of 72 species in all. Representative species from twenty-two additional plant families have been tested, primarily plants growing in the same habitat as garlic mustard. Results of these tests indicate that *C. scrobicollis* is a highly specific herbivore. Female *C. scrobicollis* do not exhibit normal oviposition behavior on plant species outside of the Brassicaceae. Within the Brassicaceae, *C. scrobicollis* has been found to have a narrow host range. After applying to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) for approval to field release *C. scrobicollis*, we are currently testing additional native mustards at the reviewers’ request. We hope to complete additional tests by May 2011 and submit supplemental data to TAG this summer.
Pre-release Efficacy Assessments of the Leaf-Mining Fly
*Hydrellia lagarosiphon*, a Candidate Biological Control Agent
of the Submerged Weed *Lagarosiphon major*

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Abstract

Releasing biological control agents that suppress plant populations is essential in achieving effective control. Pre-release efficacy assessments were conducted on the leaf-mining candidate agent *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) to ensure that the agent will incur sufficient damage to control *Lagarosiphon major* Ridl. Moss ex Wager (Hydrocharitaceae) in Ireland. Larval densities were manipulated with limited resources available to determine the carrying capacity of shoot-tip fragments and the resultant impact on the growth and viability of *L. major*. Both potted plants and shoot-tip fragments of varying lengths were used representing rooted field infestations and fragments that are essential for short and long-distance dispersal. The results indicate that leaf damage increased with larval densities, but that competition for limited resources resulted in a maximum of between 3-4 larvae being supported by each shoot tip. Of the parameters measured for the effects on subsequent growth of *L. major*, shoot tip length and shoot biomass were negatively affected by larval damage at all densities and at higher damage levels in particular, failed to recover over time. Plants compensated for larval damage through the initial production of side-shoots and roots. However over time, at larval medium (3) and high (5) densities, these parameters were negatively affected and failed to recover. Establishment of *L. major* was compromised at medium (3) and high densities (5) of larval damage and of those that did establish at these densities were significantly impacted in terms of root and shoot biomass. Sustained damage induced by consecutive generations of *Hydrellia* showed increasing negative effects on rooted plants at both low and high larval densities in terms of root and shoot biomass. The pre-release efficacy studies suggest that *H. lagarosiphon* will contribute to the suppression of plant growth at relatively low larval densities per shoot-tip.
Biology and Preliminary Host Range of *Hydrellia lagarosiphon*, a Potential Biological Control Agent against *Lagarosiphon major*

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Abstract

A recently discovered fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydridae) was investigated in South Africa as a possible biological control agent for *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) in Ireland and other parts of the world where it is invasive. Impact studies under laboratory conditions show that *H. lagarosiphon* larvae destroy approximately 20 leaves of *L. major* before pupation, and restrict the formation of side branches, an important aspect of vegetative spread of the weed. Investigations in the field showed that *H. lagarosiphon* was the ubiquitous and most abundant species associated with *L. major* in South Africa as it was found at every field site (n= 29) where *L. major* was recorded. High larval abundance was also recorded, with a majority of shoots investigated containing larvae (58%) at densities of up to ten larvae per shoot. Furthermore, examination of its field host specificity showed *H. lagarosiphon* to be specific to *L. major* as it was not recorded from any other co-occurring aquatic or semi-aquatic species. Due to the difficulty in managing *L. major* in its invaded range, *H. lagarosiphon* could be considered a valuable candidate biological control agent.
Host Range of Two Chrysomelid Beetles, *Zygogramma signatipennis* and *Z. piceicollis*, Biological Control Candidates for *Tithonia rotundifolia*

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Abstract

The weedy red sunflower *Tithonia rotundifolia* (Mills) S.F. Blake (Asteraceae: Heliantheae), originally from Mexico, has become invasive throughout the humid and sub-humid tropics of South America, South East Asia and tropical and subtropical Africa, including South Africa. In South Africa, *T. rotundifolia* is declared a category 1 weed, and was targeted for biological control in 2007. Host-specificity tests showed that the two leaf-feeding beetles, *Zygogramma signatipennis* Stål and *Z. piceicollis* Stål (Chrysomelidae: Chrysomelinae), were the most damaging and promising biological control agents for *T. rotundifolia*. During no-choice tests on 29 plant species in eight plant families, *Z. signatipennis* laid overwhelmingly on *T. rotundifolia*, with 79.67 eggs deposited on the target weed versus 33.5 and 2.5 deposited on its congener *Tithonia diversifolia* (Hemsl.) A.Gray and *Helianthus annuus* L. (Asteraceae), respectively. *Tithonia rotundifolia* was also found to be the most suitable host for the other chrysomelid, *Z. piceicollis*, during no-choice tests, depositing 56 eggs on this plant versus 29 and 7.5 eggs on *T. diversifolia* and *H. annus*, respectively. Further larval survival tests showed that both *Zygogramma* species were able to complete development only on *T. rotundifolia* but not on *T. diversifolia* and *H. annus*. During multi-choice tests including nine plants species in three plant families, both *Zygogramma* spp. strongly preferred *T. rotundifolia* to other plant species. Based on host-specificity tests and surveys conducted in the native range, the two *Zygogramma* spp. appear to be sufficiently host-specific to be released against *T. rotundifolia* in South Africa.
Biological Control of Silvery Threadmoss (*Bryum argenteum*) in Turfgrass, Nursery Crops, and Hardscapes

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Abstract

Silvery threadmoss (*Bryum argenteum* Hedw.) has become an increasingly problematic weed of golf courses, particularly since the loss of mercury and other heavy metal based pesticides. Though not labeled for moss control, they were used extensively on golf course putting greens as fungicides and at the same time controlled moss. To meet golfer demand for firmer, faster playing surfaces superintendents have decreased mowing heights, requiring increased passes of equipment over the green. This, along with decreased nutrient inputs and an open turf canopy contributes to moss encroachment on putting greens. Currently, few labeled products exist for moss control driving turf managers to use off-label substances including peroxides, baking soda, and detergents. These desiccate moss and may severely injure turfgrass even with careful applications. Hand removal of moss is also a common practice. The only commercial herbicide labeled for control is carfentrazone applied at 6.7fl oz/A, which does not completely eradicate moss, so sequential applications are required once moss recovers. Aside from turf, silvery threadmoss can also be a weed problem of containerized nursery crops as well as nursery growth pads and stone hardscapes. With no professional products labeled for moss control in these systems there are several potential niche markets for an effective biological control of silvery threadmoss. A naturally occurring microorganism has been discovered that effectively controls moss on putting greens without causing injury to the most commonly managed turf species, creeping bentgrass and annual bluegrass. We are evaluating this organism for all three niche markets. Testing includes fulfillment of Koch’s postulates, pathogen characterization to determine the site of action on silvery threadmoss and evaluation of host specificity in *Bryum* and related genera. Studies conducted this season will evaluate non-target effects on desirable plant species in the turfgrass and nursery industries and naturally occurring mosses in the landscape.
Estimating Density Dependent Impacts of the Arundo Scale,
Biological Control Agent for the Invasive Giant Reed

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Abstract

The sap-feeding armored arundo scale (*Rhizaspidiotus donacis* (Leonardi)) has been permitted for use as a biological control agent for giant reed (*Arundo donax* L.), a non-native, highly invasive woody grass that infests waterways and riparian areas of the southwestern US and Mexico. We used a nested factorial design within a controlled greenhouse setting to (1) test the hypothesis that pressure from natural enemies can interrupt the net primary production of giant reed by disrupting water and nutrient transport and detrimentally affecting the photosynthetic ability of the plant and (2) build a predictive model of the density dependant impacts of the arundo scale on plant growth to inform a biological control program. Different densities of the immature stages of two distinct genotypes of the arundo scale were administered to individually-potted ramets of the same genotype of the target weed. Growth parameters of plant such as shoot height and number of nodes, number of shoots, number and length of side shoots were measured monthly for six months, or after one generation of the scale. Insect-induced plant physiological stress was estimated with monthly measurements of light reflectance using a spectroradiometer, and by analyzing differences in leaf gas exchange among the different treatments at the end of the experiment. At six months, all plants were destructively sampled to count the density of mature scale adults and to extrapolate biomass accumulation and allocation of the test plants. Initial results suggest a scale density-dependent effect on both total plant biomass and water use efficiency. *Arundo* plants with severe infestations of scale insect exhibit reduced photosynthesis and tend to have a slower rate of growth than control plants with no insects or plants with low levels of scale density. If this trend continues, this biological control agent may prove to be an effective tool to curb the negative ecological and social impacts of this weed, especially if present in high densities. These results may help inform an inundative approach to weed biological control.
Morphological and Molecular Identification of White Blister Rust Collected from Perennial Pepperweed in Nevada and California

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Abstract

Perennial pepperweed (PPW, *Lepidium latifolium* L.) is a cruciferous plant native to Eurasia that is a noxious weed in the western USA. In northern Nevada, PPW plants in the field are commonly infected with white rust fungus (*Albugo* sp.), exhibiting white pustules on the leaves and stems of mature plants in summer. Molecular taxonomic identification of the *Albugo* species encountered on PPW in Nevada and preliminary host-specificity tests were performed to assess the potential of this fungus as a bioherbicide for control of PPW. Using genus-specific PCR primers (DC6 and LR-0), a region of rDNA including sections of ITS1, 5.8S ribosomal RNA gene, and ITS2 were amplified, subcloned into the pGEM®-T vector, and sequenced using the T-7 promoter and SP6 upstream primers. A BLAST search matched DNA sequences of the Nevada isolate of *Albugo* sp. with five voucher isolates of *Albugo candida* (Pers.) Kuntze (98% identity, E value=0.0) as well as three isolates of *Albugo lepidii* A.N.S. Rao (99% identify, E value=0.0). Thus, this Nevada isolate has significant variations within its rDNA sequence from that of both *A. candida* and *A. lepidii* and its identity remains somewhat ambiguous. Preliminary host-range tests under both greenhouse and growth chamber conditions have shown that the Nevada isolate infected PPW but there were no symptoms on any of 12 varieties of cruciferous vegetables during tests of up to one month. These preliminary results suggest that the Nevada *Albugo* isolate may represent a previously unknown pathotype with high host-specificity on PPW. Further studies on the host-specificity and pathogenicity of Nevada white rust isolates, particularly for early-season application to new PPW growth, will be necessary to better understand the potential benefits and risks of using this fungus as a bioherbicide against PPW in Nevada.
Preference and Damage by the Stem-Boring Moth, 
*Digitivalva delaireae* – a Potential Biological Control Agent 
of Cape-Ivy, *Delairea odorata*, on its Two Varieties in California, USA

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Abstract

Cape-ivy, *Delairea odorata* Lem. (Asterales: Asteraceae), is a perennial vine native to South Africa, and was introduced in the eastern United States (U.S.) in the 1850s as an ornamental. The plant is now well established in natural areas and has become a serious pest in coastal regions of California and upland Hawaii, as it is an aggressive climbing vine that can form solid covers which can block light and smother native vegetation. The U.S. Dept. of Agriculture, Area Research Service (USDA-ARS) has initiated a biological control project targeting Cape-ivy, and host-range testing of a potential agent - the stem-boring moth *Digitivalva delaireae* Gaedike & Krüger (Lepidoptera: Acrolepiidae), is nearly complete. In both South Africa and California, two morphological varieties (stipulate and astipulate) of Cape-ivy exist. The stipulate variety is most common in both South Africa and California, therefore host-range tests of Cape-ivy were conducted on plants of this variety. We are currently studying preference, as well as the effect of infestation by *D. delaireae* on the development of both Cape-ivy varieties found in California, and whether preference and damage inflicted on Cape-ivy by *D. delaireae* differs between varieties. Results of choice preference tests showed that *D. delaireae* infested 4% more leaves on the astipulate variety, though this difference is minimal, it is significant (P = 0.01). Tests assessing the effect of damage by *D. delaireae* on Cape-ivy development, on both varieties are on-going.
Potential of the Seed-Feeding Weevil *Cissoanthomonos tuberculipennis* for Biological Control of Balloon Vine *Cardiospermum grandiflorum* in South Africa

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Abstract

Balloon vine *Cardiospermum grandiflorum* Swartz (Sapindaceae), originally from South and Central America and now invasive in South Africa, was one of the five emerging weeds targeted for biological control in 2003. In search of potential biocontrol agents, exploratory surveys were conducted in northern Argentina from 2005 to 2009. These surveys, which included other plant species in the genus *Cardiospermum* and other native Sapindaceae, were aimed at determining the distribution and ecological host ranges of the natural enemies associated with balloon vine. The seed-feeding weevil *Cissoanthomonos tuberculipennis* Hustache (Coleoptera: Curculionidae) was one of the two insect species found to be restricted to balloon vine, and was widespread throughout the north eastern part of Argentina, particularly in Misiones province. Open-field tests, conducted under natural conditions in the native range, also showed that *C. tuberculipennis* was restricted and highly damaging to its natural host *C. grandiflorum*, with up to 54% of balloon vine fruits damaged by the beetle between September 2008 and April 2010. Host-specificity tests, including no-choice, paired-choice and multi-choice tests, showed that *C. tuberculipennis* fed and reproduced only on *C. grandiflorum*. Failure of *C. tuberculipennis* to feed and reproduce even on the three congeners of *C. grandiflorum* during host range studies proves beyond reasonable doubt that the weevil is highly host-specific to the target weed, and therefore poses no threat to non-target plant species. As *C. tuberculipennis* is monophagous and has a very short life cycle with a highly damaging larval stage, it is the best candidate for biological control of this weedy creeper in South Africa and elsewhere. It is strongly recommended that permission be granted for the release of *C. tuberculipennis* from quarantine for the biological control of *C. grandiflorum* in South Africa.
Artificial Diet for Completing Development of Internal Feeding Insects of Plant Stems and Roots as an Aid for Foreign Exploration

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Abstract

Internal-feeding insects can be effective biological control agents of invasive alien weeds, but it is usually difficult to rear field-collected immature stages to the adult stage to facilitate identification and establishment of laboratory colonies. The development of effective diets and rearing systems could greatly aid the discovery and evaluation of root- and stem-feeding insects for biological control. We developed and tested a system for rearing adult insects from field-collected larvae that is useful for foreign exploration. We adapted a previously developed artificial diet for *Hylobius transversovittatus* Goeze, the purple loosestrife root weevil, and tested the system on a root-feeding weevil, *Ceratapion basicorne* (Illiger), dissected from *Centaurea solstitialis* L. (yellow starthistle) plants in Turkey. The diet ingredients were modified to reduce microbial contamination, and the container size and style of top were chosen for ease of use and to reduce diet desiccation. Gouging the diet at the container sides facilitated insect survival and permitted easier monitoring of developmental progress. The method also worked with varying success for a variety of other beetles (Buprestidae, Cerambycidae, Chrysomelidae, Curculionidae), moths (Noctuidae, Pyralidae) and flies (Chloropidae) dissected from a variety of plant species (Apiaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Elaeagnaceae). However, the diet was not successful for rearing adults of the yellow starthistle stem-boring flea beetle, *chalcomera* Illiger, which normally pupates in soil. The diet probably can be further modified to better suit insects associated with a particular plant species and/or plant parts that differ in critical physical and/or chemical properties.
First Insect Agents Evaluated for the Biological Control of 
Parthenium hysterophorus (Asteraceae) in South Africa

L. Strathie and A. McConnachie

Abstract
The annual herbaceous plant, Parthenium hysterophorus L. (Asteraceae: Heliantheae) (parthenium), has wide-ranging negative impacts on crop and animal production, biodiversity conservation, and human and animal health in Africa, Asia and Australia. Parthenium is an increasing problem in southern and eastern Africa, causing severe economic losses in some countries. In 2003, South Africa became only the third country globally, after Australia and India, to implement a biological control program against this weed. Relying on the experience of the Australian program, three agents were selected and imported into quarantine in South Africa, for evaluation of their suitability for release. They included the leaf rust fungus Puccinia xanthii Schwein. var. parthenii-hysterophorae Seier, H.C. Evans & A. Romero (Pucciniaceae), that, after evaluation, was released in South Africa in 2010, the leaf-feeding beetle Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae) and the stem-boring weevil Listronotus setosipennis Hustache (Coleoptera: Curculionidae). No-choice tests were conducted and, later, multiple choice tests resolved results of non-target feeding and/or oviposition under no-choice conditions for several plant species, for both Z. bicolorata and L. setosipennis. However, up to eight Helianthus annuus L. (Asteraceae: Heliantheae) (sunflower) cultivars were accepted by Z. bicolorata and L. setosipennis for feeding and/or oviposition under choice conditions, necessitating the investigation of larval development. Finally, further analyses were used to quantify the risk, which was demonstrated to be very low, to non-target plant species that were suitable for feeding and/or oviposition under laboratory conditions. Both agents have since been provided to Ethiopia for a biological control program there. The stem-galling moth Epiblema strenuana (Walker) (Lepidoptera: Tortricidae) and the seed-feeding weevil Smicronyx lutulentus Dietz (Coleoptera: Curculionidae) have recently been imported for evaluation in South Africa, as experience elsewhere has demonstrated that a suite of agents is required to achieve effective biological control of parthenium under different environmental conditions and in different regions.
Host Specificity Testing of *Archanara geminipuncta* and *A. neurica* (Lepidoptera: Noctuidae), Candidates for Biological Control of *Phragmites australis* (Poaceae)

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Abstract

Two European stem-boring moth species (*Archanara geminipuncta* Haworth and *A. neurica* Hübner.) are evaluated as possible biological control agents for introduced *Phragmites australis* (Cav.) Trin.ex Steud. in North America. A particular challenge in the *Phragmites* biocontrol program is the existence of a native subspecies *P. australis* (Cav.) Trin.ex Steud. subsp. *americanus*. We have developed a sequence of quarantine-testing procedures, first transferring neonate larvae onto young shoots for five days. If plants are successfully attacked, larvae are given a range of stem sizes and tested for ability to complete larval development. This approach quickly eliminates most plant species as unsuitable hosts with the exception of several native haplotypes and a few test plant species which are exposed for larval development. In addition, open-field tests are underway at CABI in Switzerland concentrating on potential oviposition preference of the moths between introduced *P. australis* and the native subspecies.
Foreign Exploration and Host Testing of Brazilian Pepper 
(*Schinus terebinthifolius*) Biological Control Agents

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Abstract

Brazilian pepper is among the worst environmental weeds in Florida and other areas of the US. This species occupies diverse habitats causing many environmental problems including decreased biodiversity of the infested areas. Although chemical controls are known and used to control this invasive species, biological control presents an attractive alternative when practiced safely. The native range of this species includes eastern Brazil, northeastern Argentina, and eastern Paraguay. The USDA/ARS Invasive Plant Research lab with colleagues at the Universidade Regional de Blumenau in Brazil, and the South American Biological Control Lab in Argentina have been searching for and testing insects that will be safe and effective at controlling this weed in the US. Surveys in South America have discovered many new insects including new moth, wasp, and caterpillar species. Several of these species are undergoing testing to determine suitability and safety for release in the US. These include the Thripidae thrips, *Pseudophilothrips ichini* Hood, the Attelabidae beetle *Omolabus piceus* (Germar), two Gracillariidae moths *Eucosmophora schinusivora* Davis and Wheeler and *Leurocephala schinusae* Davis and Mc Kay, the Notodontidae moth *Tecmessa elegans* Schaus, the Geometridae moth *Oospila pallidaria* Schaus, and the Gelechiidae moth *Crasimorpha infuscata* Hodges. Additionally several new species have yet to be described including the Braconidae wasp *Allorhogas* n. sp. and an unknown Gelechiidae stem galling moth.
Foreign Exploration and Host Testing of Chinese Tallow Biological Control Agents

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Abstract

Chinese tallow, *Triadica (=Sapium) sebifera* (L.) Small (Euphorbiaceae) or popcorn tree is among the worst environmental weeds in the southeastern US. This species occupies diverse habitats causing many environmental problems including decreased biodiversity of the infested areas. Although chemical controls are known and used to control this invasive species, biological control presents an attractive alternative when practiced safely. The native range of this species primarily includes central and southern China. The USDA/ARS Invasive Plant lab, colleagues at the Australian biological control lab, and the Chinese Academy of Science have been conducting foreign surveys searching for insects that will be safe and effective at controlling Chinese tallow in the US. Surveys have revealed many new herbivores throughout the native range of these species. These include many new weevil, thrips, psyllid, eriophyid mites and lepidopteran species. Several of these species are, or have undergone preliminary testing to determine suitability for release. These include the Attelabidae beetle *Heterapoderopsis bicallosicollis* (Voss), the Chrysomelidae beetle *Bikasha collaris* (Baly), and the Noctuidae moth *Gadirtha inexacta* Walker. Host testing conducted in China indicates all three species have narrow host ranges. Quarantine testing is underway in the US and indicates the defoliator/root feeding *B. collaris* herbivore is the most suitable biological control agent. Additionally, a new stem galling wasp (Eulophidae; Tetrastichinae) has been colonized in China and will undergo testing as available.
Performance of *Hydrellia pakistanae* (Diptera: Ephydridae) and *Hydrellia* sp. on the South African Biotype of *Hydrilla verticillata* (Hydrocharitaceae)

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Abstract

*Hydrilla, Hydrilla verticillata* L.f. is a submerged aquatic plant of Asian and Australian origin that is invasive in the U.S.A. and South Africa. Two leaf-mining flies, *Hydrellia pakistanae* Deonier and *Hydrellia* sp., are currently under evaluation as candidate biological control agents for *H. verticillata* in S.A. Because of high genetic diversity within the species, biotype matching for the biological control program was an important consideration. *H. pakistanae* originates on a dioecious biotype of hydrilla from India and *Hydrellia* sp. originates on monoecious hydrilla from Singapore, close to the region of origin of S.A. *H. verticillata*. Based on this, it was assumed that *Hydrellia* sp. is the more suitable candidate however any differences in performance between the two cultures needed quantification before rejecting *H. pakistanae*. Various fitness parameters were measured in a quarantine glasshouse with a min/max of 22/28 °C and a growth chamber with a constant temperature of 27°C and a 16:8 day: night period. *Hydrellia* sp. had a shorter development time and significantly higher egg to adult survival, fecundity and adult longevity compared to *H. pakistanae* under both experimental conditions. *H. pakistanae* fitness parameters were also compared to those measured for this species in the U.S.A. By this comparison, *H. pakistanae* doesn't perform as well on S.A. monoecious *H. verticillata* as it does on the U.S. dioecious form. The results from this study in conjunction with results from host specificity testing suggest that *Hydrellia* sp. is the better candidate for release against *H. verticillata* in S.A. However, the damage potential of each candidate agent will be measured through impact trials before making a final decision on whether *H. pakistanae* should be rejected as a biological control agent for *H. verticillata* in S.A.