Endophytic Fungal Flora from Eastern White Pine Needles and Apple Tree Leaves as a Means of Biological Control for White Pine Blister Rust

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White pine (*Pinus strobus* L.) is one of the most valuable timber species in eastern Canada and it is highly susceptible to white pine blister rust caused by *Cronartium ribicola* J.C. Fisher. A biocontrol agent effective against *C. ribicola* would be a useful tool for the management of young white pine plantations and naturally regenerating stands. Inhibition of teliospore production or basidiospore development in the early infection stages on pine needles would provide effective biocontrol. We report here on the biodiversity of fungal endophytes found in needles of *P. strobus* consisting of some 91 putative species, nursery tests of 63 isolates of needle endophytes to inhibit infection on white pine seedlings, and in vivo test trials of two isolates of *Microsphaeropsis arundinis* against *C. ribicola* on *Ribes glandulosum* leaves. Seven of the endophytes tested were found to inhibit infection by *C. ribicola*. The most interesting species of fungal endophyte, labeled Species A, is represented by two isolates in our collection. Control seedlings presented on average more than ten yellow spots whereas seedlings treated with Species A had fewer than 0.2 spots per seedling. The apple scab biocontrol agent *Microsphaeropsis arundinis*, strains P-176 and P-130, showed great potential for inhibiting *C. ribicola* on red currant leaves. Strain P-130 infected on average 96.4% of the uredinia and strain P-176 was similarly effective with 89.2% of the uredinia being infected.

Investigations for the Biological Control of Cogongrass

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Cogongrass (*Imperata cylindrica*) is a rhizomatous perennial grass of tropical and subtropical regions. It infests over 0.5 billion ha worldwide. Cogongrass was introduced accidentally at Mobile, Alabama, as packing material from Japan in 1912 and intentionally from the Philippines into Mississippi and Florida in 1921 as a potential forage grass and for erosion control. Cogongrass was unacceptable as a forage and it was too weedy for erosion control. Now cogongrass infests about 1 million ha in Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, Texas, and Virginia, where it has become a pernicious weed of agricultural, forestry, urban, and natural areas. Currently, the only effective

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4-14 July 1999, Montana State University, Bozeman, Montana, USA
Neal R. Spencer [ed.]. (2000)

control for cogongrass is repeated applications of non-selective herbicides and/or tillage. Three skipper butterfly (Lepidoptera: Hesperiidae) species, *Ancyloxypha numitor*, *Atalopedes campestris*, and *Hylephila phyleus* were discovered feeding on cogongrass leaves. In caged greenhouse feeding experiments at Stoneville, Mississippi, all three species fed, pupated, and emerged as adults; however, only two of 10 *H. phyleus* larvae reached adulthood. At larval maturity, *A. campestris* larvae were about twice the length and four times the weight of *A. numitor* larvae. Only *A. campestris* was effective in reducing cogongrass foliage by as much as 50% at one larva per pot (10 cm diam). Because all three species generally feed on grasses, including bermudagrass, corn, johnsongrass, sugarcane, St. Augustine grass, and several native grasses, it is unlikely that *A. campestris* could be used in biological control of cogongrass unless the life cycle was terminated prior to the next generation. Several methods including parasitism, radiation, or sterile hybrids may allow augmentative biological control of cogongrass with *A. campestris* or other general grass-feeding lepidopterous larvae. Additional research is being conducted at Stoneville, Mississippi, to investigate the potential for biological control on cogongrass.

Promising Native/Adventive Pathogens and Insect Agents for the Biological Control of Houndstongue in Canada

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Houndstongue (*Cynoglossum officinale*) is an introduced noxious weed that is a serious problem on the rangelands of central British Columbia (BC), Canada. This biennial is a concern because it invades newly-established pastures, grows in place of valuable forage, has barbed seeds which attach to cattle causing irritation, represents potential market losses, and is toxic to livestock. Biocontrol is considered by cattlemen a feasible and economic long-term solution to the control of houndstongue. In addition to releasing European insects for its control, we have conducted surveys in BC in search for native or adventive insects and pathogens found attacking houndstongue. The most promising organisms for integration into a biocontrol program are pathogens and include the pycnidial fungus *Phoma pomorum*. This fungus is highly host specific and causes early loss of older leaves on houndstongue rosettes and reduced plant biomass. The fungal pathogen *Fusarium acuminatum* was shown to seriously damage the root system of houndstongue which led to severe stunting of plants. The fungus *Erysiphe cynoglossi* causes powdery mildew on the leaves of houndstongue and has a significant negative impact on growth and reproduction. Seed production was reduced by about 50% in field tests. A new pathovar of the bacterium *Pseudomonas syringae* was discovered that was specific to houndstongue. A preliminary field study indicated that *P. syringae* reduces winter survival and vigour in houndstongue. Several plant viruses also were shown to infect houndstongue, causing stunting and reproductive losses in some instances. Sphere, rod, and potyvirus-like virus particles have been located within plant material using the transmission electron

microscope. Among the insects found feeding on houndstongue, the defoliating Arctiid moth, *Platyprepia virginalis*, is damaging to young rosettes despite a broad host range. The spittle bug, *Philaenus spumarius*, damages the central buds of rosettes and prevents further growth.

Potential Benefits of Using Native Insects for Biological Control - the Case of *Platyomopsis humeralis* (Cerambycidae) on *Mimosa pigra* in Northern Australia

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Species of native herbivorous insects occasionally extend their range of host plants to include introduced weeds; however, numbers rarely reach levels where the insects are considered to have potential as biological control agents. Reasons for this include the fact that they are usually polyphagous, and hence not closely adapted to the new host, or that they are accompanied by their natural enemies, preventing them from reaching high population levels. In the Northern Territory of Australia, over a hundred species of Australian native or naturalised insects have been recorded on *Mimosa pigra*, a woody weed introduced from tropical America. One of the most abundant of these is the cerambycid beetle (*Platyomopsis humeralis*) which can cause significant damage to *M. pigra*. Reasons for the possible success of this species on *M. pigra*, including a taxonomic survey of its native host range, are examined. Comparisons are made between the levels of parasitism and rates of development in its native and introduced hosts. Data on the phenology and impact of *P. humeralis* on *M. pigra* are provided. Preliminary trials on the redistribution of the insect to sites where it is rare or absent are described, and the potential benefits in using the species as a biological control agent are discussed.

Some Structural Responses of Canada Thistle (*Cirsium arvense*) to Infection by an *Alternaria* sp.

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The infection process of an *Alternaria* sp. on its host, Canada thistle (*Cirsium arvense*), was studied for structural responses to infection using light, fluorescence, and electron microscopy. Irregularly-shaped crystals were frequently observed within epidermal, palisade, and mesophyll tissues in the vicinity of an infection site and fan-like crystalline structures, which appeared to form a collar surrounding penetrating appressoria,

Proceedings of the X International Symposium on Biological Control of Weeds
4-14 July 1999, Montana State University, Bozeman, Montana, USA
Neal R. Spencer [ed.]. (2000)

were occasionally seen. Histochemical staining showed both heavy deposition of lignin on host cell walls at infection sites and callose fluorescence within penetrated epidermal cells. Using scanning electron microscopy and energy dispersive x-ray micro-analysis, very high levels of silicon were detected in epidermal cells directly below appressoria, often forming entirely silicified infected cells which were resistant to collapse upon air-drying. Most non-infected tissues contained only low levels of silicon. This apparent mobilisation of silicon to infection sites appeared to occur more readily within younger, rather than in older, leaf tissues. The implications of these structural reactions for limiting or reducing the rate of infection are being evaluated further.

Are We Using the Best Release Strategies? An Assessment of Weed Biocontrol Introductions Made in Oregon

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The manner in which biocontrol organisms are introduced, including the size and number of releases, as well as the distribution of releases in space and time, can influence the probability of population establishment. We summarize and critically examine current and past introduction strategies used for weed biocontrol agents in the state of Oregon, where records of release number, locations of releases, and current status of control agents has been maintained by the Oregon Department of Agriculture. The data set consists of nearly 4,000 records of individual releases occurring over the last 3 decades for 60 biocontrol agent species against 23 target weeds. By examining patterns of success and failure of individual releases we uncover the relative importance of (1) using large releases, (2) using many releases, (3) spreading releases spatially, and (4) repeating releases through time.

Prospects for the Classical Biological Control of Garlic Mustard (*Alliaria petiolata*): an Environmental Weed in North American Forests

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Garlic mustard (*Alliaria petiolata*) is a biennial herb of European origin which invades forest communities in the eastern United States, the Midwest, and southeastern Canada. A

survey of herbaria and literature revealed that garlic mustard spread exponentially in the last few decades in Illinois. Large, well established populations are difficult if not impossible to control with conventional methods, because of plant recruitment from the seed bank. Therefore, a biological control project was initiated in spring 1998. A literature review revealed 69 phytophagous insect species and 7 fungi to be associated with garlic mustard in Europe. Six insect species were selected as potential biological control agents due to records of their restricted host range: four curculionids, one flea beetle and one agromyzid. Emphasis will be placed on root-feeders and species that attack both phenostages, rosettes and bolting plants, to prevent garlic mustard from escaping attack. Therefore, the following three species are of special interest: *Ceutorhynchus alliariae* and *C. roberti* (Coleoptera, Curculionidae), and the flea beetle *Phyllotreta ochripes*. The two weevils mainly feed in the shoots of garlic mustard, but were also observed to mine in the petioles and root crown of rosettes. Larvae of *P. ochripes* mine in the root of bolting plants and the root crown and petioles of rosettes. During the first field surveys in Europe, the three species were common at all field sites investigated, and heavily attacked plants were observed to die prematurely. Another species of interest is *Ceutorhynchus scrobicollis*, which apparently develops in the plant over winter. A survey to find this species with a more eastern distribution will be carried out this spring. A preliminary test plant list was established and sent to some 150 interested parties in the US and Canada for comments. The revised list will be submitted to TAG in spring 1999, and host-specificity tests will start in 2000.

Leafy Spurge Biological Control Exploration for Natural Enemies from the Leaf Beetle Genus *Aphthona* Chevrolat (Coleoptera: Chrysomelidae: Alticinae)

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Invasive weeds, such as leafy spurge, cause great economic losses each year in the United States. Control of such weeds by plant feeding insects holds great promise as a way to reduce or eliminate these losses. Flea beetles, particularly *Aphthona* species, are one group of insects that feed on these plants and have been useful in biological control. Six Palearctic species of *Aphthona* have already been released in North America, but additional species are needed to control the weeds in a variety of habitats. For the purpose of collecting new, potential biological control agents, field work was conducted in Russia in June and July of 1998. Three major regions were explored: Krasnodar (northwestern Caucasus), Novosibirsk (Western Siberia), and Irkutsk (Eastern Siberia). During this work, a new species of *Aphthona* was discovered and its larvae were reared by the Biological Control Group, Zoological Institute, St. Petersburg, Russia. Also, ten previ-

Proceedings of the X International Symposium on Biological Control of Weeds
4-14 July 1999, Montana State University, Bozeman, Montana, USA
Neal R. Spencer [ed.]. (2000)

ously described *Aphthona* species were collected. Six of them are locally abundant and have the ability to control leafy spurge in natural conditions. New distributional and host plant data for several *Aphthona* species was collected. To the best of our knowledge this was the first attempt to create a multidisciplinary team including (1) biological control specialist, (2) systematist, specialist in the group, and (3) field person. The team proved its effectiveness. Four weeks of explorations in Russia yielded six potential biological control agents from the leaf beetle genus *Aphthona*.

South African Rusts with Potential to Control Two Major Environmental Weeds in Australia

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Exotic weeds such as *Asparagus asparagoides* (bridal creeper) and *Chrysanthemoides monilifera* (bitou bush/boneseed) pose a major threat to biodiversity and conservation in Australia's temperate natural ecosystems. *Asparagus asparagoides* is a climber which establishes itself in relatively undisturbed vegetation, producing dense mats of rhizomes and tubers. *Chrysanthemoides monilifera* is a woody evergreen shrub growing up to 2-6 metres. During extensive field surveys in South Africa in the early 1990's, two rust fungi were identified as potential classical biological control agents for *A. asparagoides* and *C. monilifera*. The macrocyclic and autoecious rust fungus *Puccinia myrsiphylli* infects the cladodes (leaves) and stems of *A. asparagoides*. Severely diseased plants shed infected cladodes prematurely and produce few or no fruits. The rust is commonly found in the winter-rainfall, even-rainfall and summer-rainfall regions of South Africa, wherever *A. asparagoides* occurs. The microcyclic, systemic rust fungus *Endophyllum osteospermi* (formerly referred to as *Aecidium osteospermi*) infects the immature foliage and stems of *C. monilifera*. One to two years after infection, plants develop systemically infected witches' broom symptoms with multiple, swollen stems and short internodes, and small and slightly chlorotic leaves. Infected branches produce few or no fruit and usually die within 1-4 years. The systemic nature of *E. osteospermi* is a desirable characteristic for biological control purposes as, once the fungus is established within the host, the infection is retained until the death of the witch's brooms. The rust occurs widely in the winter-rainfall and even-rainfall regions of South Africa. The host-ranges of *P. myrsiphylli* and *E. osteospermi* are currently being determined experimentally. Should high specificity towards the target weeds be confirmed permission to release the rust fungi in Australia will be sought.

Biological Control of Russian Thistle

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Russian thistle, *Salsola kali* (Chenopodiaceae), was introduced into the USA from Russia in the late 1800s and has become one of the most troublesome weeds in the drier regions of North America.

Explorations during 1996 and 1997, with financial support of CDFA, in Turkey, Uzbekistan, China, and France, resulted in discovery of several promising natural enemies for biological control of the weed. The five top candidates are prioritized here:

1- The mite, *Aceria salsolae* DeLillo and Sobhian (Acari : Eriophyidae). Studies carried out in Turkey showed that the mite attacks the Russian thistle from California and did not attack the other six species or varieties tested, including sugar beet, table beet, and spinach.

2- *Piesma salsolae* (Piesmidae). This is a gregarious species and heavily damages its host plant. According to the available literature and field observations, its host range is restricted to Russian thistle.

3- *Gymnancella canella* Denis and Schiffemueller (Pyralidae). The larvae of this moth have serious negative effects on seed production. According to the available literature and field observations, the host range of the species is restricted to Russian thistle

4- The gall midge *Desertovellum stackelbergi* (Cecidomyiidae). Heavy gall formation reduces plant size and seed production. The species has been reported only on Russian thistle.

5- *Uromyces salsolae*, a rust disease. Infected plants remain stunted and produce fewer seeds. Its host range seems to be restricted to Russian thistle.

Arthropods Associated with Tropical Soda Apple, *Solanum viarum*, in the Southeastern U.S.A.

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Proceedings of the X International Symposium on Biological Control of Weeds
4-14 July 1999, Montana State University, Bozeman, Montana, USA
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Tropical Soda Apple (TSA), *Solanum viarum*, is an invasive shrub native to Argentina, Brazil, and Paraguay. Its abundant fruits and seeds are disseminated by cattle, wildlife, and movement of contaminated seed, hay, and sod, which has facilitated its spread to Asia, Africa, and North and Central America. TSA was introduced into the U.S.A. in Florida in the 1980's. Its rapid dissemination has led to infestation of more than 0.50 million ha (1.25 million acres) of agricultural and natural areas in Florida and eight other states (Alabama, Georgia, Louisiana, Mississippi, North Carolina, Pennsylvania, South Carolina, and Tennessee). Investigations of arthropods associated with TSA have been conducted in the southeastern U.S.A. to identify potential biological control agents and any niches they might occupy. More than 75 spp. of arthropods were collected from TSA. Many species were incidental, but approximately 2/3 were phytophagous. Some of these herbivores included polyphagous pests such as *Heliothis virescens*, *Pseudoplusia includens*, and *Nezara viridula*. Several oligophagous foliovores that fed on TSA are also pest species, such as *Manduca sexta*, *Leptinotarsa decemlineata*, and *Keiferia lycopersicella*. Other native oligophages specializing on Solanaceae also fed on TSA, such as a mirid called the suckfly, *Tupiocoris notatus*, and a pyralid, *Lineodes integra*. *Tupiocoris notatus* caused severe leaf chlorosis on TSA and was found throughout the growing season, with populations reaching maximum densities in Autumn (up to 500/plant). *Lineodes integra* cut and tied leaves and damaged TSA foliage in the field and completely defoliated potted TSA plants in the greenhouse. In the southeastern U.S.A., impact of herbivory of existing arthropods on TSA is apparently limited as this weed continues to grow, reproduce, and spread at an alarming rate. Several niches are available for exploitation by imported South American natural enemies of TSA.

Screening Foreign Plant Pathogens for Biological Control of *Polygonum perfoliatum*, Mile-A-Minute (MAM)

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More than 200 fungal isolates have been collected from MAM in China, and 30 have been screened for biological control of this alien invasive weed, which is of potential importance in the eastern United States. Pathogenicity assays were conducted in the containment greenhouse facility at the FDWSRU. Inoculation methods included: spraying foliage with fungal spore suspensions, drenching soil, or stabbing plant stems with infested toothpicks. Isolate SM1-2, collected from a wilting MAM plant in Liaoning Province, China, caused wilting and death in 50% of toothpick-inoculated plants within one week.

Isolate SM1-2 has not sporulated either on Potato Dextrose Agar or Potato Carrot Agar. Symptoms were not observed on plants inoculated with SM1-2 using a soil drench. Research continues to confirm these results and to identify additional candidate isolates for evaluation as biological control agents.

Selection of Natural Enemies for the Biological Control of *Chromolaena odorata* in South Africa

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Chromolaena odorata (Asteraceae), a neotropical shrub, has invaded many areas of the Old World tropics and subtropics. Its rapid spread and increasingly negative impact on biodiversity in South Africa prompted the initiation of a biological control programme in 1988. The suite of insects and pathogens prioritised for this programme was recently revised. Vegetative parts of the plant are still being targeted in preference to reproductive structures, although capitulum-attacking insects may be considered at a later stage. It is envisaged that a combination of the newly-prioritised leaf-, stem-, and root-attacking candidates, most of which destroy rather than modify plant tissue, will reduce photosynthetic ability and act as a resource sink. Defoliators that are being, or recently have been, investigated are *Pareuchaetes insulata* (Lepidoptera: Arctiidae), *Actinote thalia pyrrha* (Lepidoptera: Nymphalidae), and the leaf-miner *Calycomyza flavinotum* (Diptera: Agromyzidae). *Pareuchaetes pseudoinsulata* has been released in the Northern Province during the past wet summer season, and *P. insulata*, which may be more climatically suited to the highly seasonal regions, is soon to be released. The stem-tip borer *Melanagromyza eupatoriella* (Diptera: Agromyzidae), a stem-galling *Conotrachelus* sp. (Coleoptera: Curculionidae), and the stem-borer *Lixus aemulus* (Coleoptera: Curculionidae) are being investigated because of their effective damage to the photosynthetic stems of *C. odorata*. *Longitarsus horni* (Coleoptera: Chrysomelidae: Alticini) has been targeted because of the larval damage to roots, especially those of young plants. Several fungal pathogens, including *Septoria ekmaniana* (Coelomycetes) and *Mycovellosiella perfoliata* (Hyphomycetes), have been collected from the neotropics but thus far have developed poorly on the southern African form of *C. odorata*. This form is dissimilar to those that have invaded elsewhere and its exact origin is unknown. It is desirable to determine the origin to ensure greater compatibility of insects and especially pathogens with the weed. Thus far, plant specimens from parts of the Caribbean seem most similar in appearance to the local form of *C. odorata*.

Proceedings of the X International Symposium on Biological Control of Weeds
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Biological Control of Houndstongue (*Cynoglossum officinale*) with the Plant Pathogen *Pseudomonas syringae* pv. *tagetis* Synergized with Pelargonic Acid

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Pseudomonas syringae pv. *tagetis* (Pst) is the causal agent of apical chlorosis of Canada thistle (*Circium arvense*) and has been investigated as a biological control agent for that weed. Pst produces mild chlorosis on some non-host plants when applied with the surfactant Silwet L-77, but most plants recover after a few weeks. A formulation of pelargonic acid (Scythe) works as a contact herbicide and kills annual weeds, but perennial weeds generally recover. Houndstongue (*Cynoglossum officinale*) is an exotic, biennial, poisonous weed infesting rangelands in the Western United States. A 4% solution of Scythe applied at 945 l/ha necrotizes houndstongue foliage, but healthy regrowth is initiated within two weeks. When Pst applications are followed by Scythe, a synergistic interaction occurs and plants are killed. The effect is optimized when conditions are warm and sunny. Two years of field tests were performed with four treatments: Pst alone, Scythe alone, Pst + Scythe, and an untreated control. In the spring of 1998, evaluations of the 1997 applications indicated 100% kill of houndstongue in plots treated with Pst + Scythe. Significant reductions in houndstongue were also observed in Scythe alone and Pst alone treated plots. The same treatments were applied in 1998, but under cool, cloudy conditions at the time of application. The Pst + Scythe treatment resulted in a 31% reduction in houndstongue. Evaluations will be conducted in the spring to determine the long-term effects of the treatments. Demonstration plots applied in 1998 during warm and sunny conditions were very effective at controlling houndstongue.