Efficacy and Plant Response to *Ralstonia solanacearum*, a Potential Bioherbicide for Control of Kahili Ginger (*Hedychium gardnerianum*)

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Ralstonia solanacearum was tested for its efficacy as a bioherbicide for the alien kahili ginger (*Hedychium gardnerianum*) in Hawaiian forests. This weed was inoculated with an aqueous suspension (1 x 10^6 cells/ml) of the ginger-infecting strain of the bacterium, combined with an organosilicone surfactant, in 10 x 10 m^2 plots in heavily invaded *Metrosideros* wet forests of Hawai'i Volcanoes National Park. Treatments consisted of spraying mechanically wounded and non-wounded rhizome mounds and shoots in both winter and summer. Controls were wounded only. In addition, half of the wound treatments received a second application of the potential bioherbicide on regrowth after 6-8 weeks. No infections were observed in non-wound treatments. In the wound treatments, both rhizome and stem infections were observed in both the surfactant and non-surfactant based inoculum within 6-8 weeks. Shoot symptoms included interveinal chlorosis, water soaking, and epinasty. Rhizome symptoms included water soaking and decay of infected tissues. Seedlings germinating in treatment plots were also affected by the bacterium, causing death and stunting. Following inoculation, the number of stems produced on rhizome mounds varied within treatments. Some mounds had no regrowth, while others resprouted similarly to the non-treated controls. Many surviving shoots were much reduced in height and failed to mature or flower. Average infection rates of emerging shoots following the second application with and without surfactant were 35% and 2%, respectively. Although treatment responses were inconsistent, preliminary results suggest that effective control of kahili ginger can be accomplished using *R. solanacearum* as a bioherbicide with limited wounding assistance to enhance spread of the bacterium and build up inoculum levels in the soil. The use of *R. solanacearum* as a bioherbicide is a possible alternative to existing control strategies that are not practical on a large scale. However, application in areas where water runoff could occur to commercial edible ginger (*Zingiber officinale*) plantings should be avoided.

Biological Control of the Weed Hemp Sesbania with *Colletotrichum truncatum*

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Hemp sesbania (*Sesbania exaltata*) is a problematic weed in row crops throughout much of the southeastern U.S. An isolate of the fungal pathogen *Colletotrichum truncatum* (NRRL Accession No. 18434) was discovered on the Southern Weed Science Laboratory Experimental Research Farm and has been extensively evaluated over the past several years for use as a bioherbicide against this weed. Various invert and vegetable oil emulsion formulations developed in our laboratory eliminated or greatly reduced free moisture requirements, and have consistently provided 85-95% control of weeds in field trials. Granular formulations ("Pesta") have also provided similar levels of control in replicated field tests. Inoculum consisting of fungal conidia and microsclerotia produced on various crop grains remained viable for at least seven years when stored under refrigeration.

Assays for Predicting Mycoherbicide Formulation Compatibility

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Formulation is one of the major factors that determine mycoherbicide effectiveness and marketability. There are many choices for formulation components, ranging from registered agricultural products to novel substances such as sunscreens, humectants, and starches. Generally the first step in developing a mycoherbicide formulation is compatibility screening with the agent. Compatibility testing of these products, alone and in combinations, can consume a great deal of time and resources. If a rational approach to screening, based on adjuvant chemical structure, were developed, formulation development could be accelerated significantly. The objectives of this research are to examine the relationship between adjuvant chemistry and biocontrol agent species, and to identify a rapid, accurate laboratory screening assay. Thirty-three commercial and experimental adjuvants were used with four *Alternaria* species in this study. Three assays were compared: 1) a standard germination assay was performed by inoculating adjuvant-incorporated water agar plugs with spores briefly submerged in water, and assessing percent germination after 8 hours incubation; 2) a hydrated germination assay was performed by hydrating spores for 8 hours in the formulation before inoculating plain water agar plugs; 3) a radial growth rate assay was conducted by measuring the fungal growth rate on adjuvant-incorporated PDA. The four species reacted similarly to the adjuvants, and discreet responses to different chemical classes were observed. However, the correlation of results between the three assays was inconsistent. To determine which laboratory assay best predicted actual performance, select adjuvants were tested further by rating disease development of a single *Alternaria* agent in the presence of the adjuvants on Canada thistle (*Cirsium arvense*). Preliminary results suggest that a reliable and quick laboratory assay can be developed.

A New Biocontrol Agent, the Stem Feeder Beetle *Thamnurgus euphorbiae* Küster (Coleoptera: Scolytidae) from Italy to Control Leafy Spurge in the U.S.

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Thamnurgus euphorbiae Küster (Coleoptera: Scolytidae), a univoltine stem feeder recorded from *Euphorbia characias* L. in Italy, was selected as a candidate for biological control of leafy spurge (*Euphorbia esula*) in North America. Its potential host range was studied at the USDA-ARS-EBCL Rome laboratory from 1992 to 1998. Of 40 plant species or varieties in 13 families tested, the beetle oviposited on and completed its life cycle only on plants in the subgenus *Esula* of the genus *Euphorbia*. Six North American *Euphorbia* species, including *E. incisa* and *E. robusta*, trees in the families Pinaceae and Ulmaceae were also tested against *T. euphorbiae* and none of them received damage or eggs. The restricted host range suggests use of this beetle as a biological control agent against leafy spurge in North America and therefore, release in the field is proposed. A petition of introduction was prepared and submitted to the Technical Advisory Group to introduce and release this beetle in the U.S.

Biological Control of *Hypericum androsaemum* with *Melampsora hypericorum*

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The rust fungus, *Melampsora hypericorum*, is a biological control agent for the weed tutsan (*Hypericum androsaemum*). Rust infection occurs readily on some tutsan populations but there is no or negligible infection on other tutsan populations. Several possible reasons for this occurring were investigated.

In this study, light, scanning and electron microscopy techniques were used to examine the life cycle of *M. hypericorum*. Urediniospores were the only spore type found and these had distinct, compact ornamentation on the spore surface that was echinulate, where a conical projection was supported by a broader warty base.

To determine the genetic variation between rust isolates obtained, RAPD-PCR was the

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only technique that amplified products in repeatable reactions. The OPM-01, -02, and -06 primers amplified the greatest numbers of polymorphic bands. Four rust isolates from Victoria were analysed, with all being genetically different.

To determine genetic variation between tutsan populations, RAPD-PCR and RAMS were used. RAPD-PCR using primers OPA-02, -07, -08, and 10 produced the greatest numbers of polymorphic bands and showed that all tutsan populations tested (two from New South Wales and four from Victoria) were genetically different.

A cross-inoculation trial was undertaken in controlled glasshouse conditions with all four rust isolates and five tutsan provenances. Myrtleford tutsan plants were most highly susceptible to infection and the Nursery rust isolate the most highly virulent. Infection of plants only occurred when the temperature was at 20°C, not 15 - 25°C.

A viability test and germination trial was undertaken on tutsan seeds. All seeds tested were viable but there were differences in germust fungus.

It was concluded that the lack of infection in some tutsan populations was probably due to variations in rust virulence and tutsan susceptibility, with these probably being genetically determined.

Liquid Fermentation, Delivery System, and Efficacy Testing of the Mycoherbicide *Fusarium oxysporum* M12-4A Against *Striga hermonthica*

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Striga hermonthica (Del.) Benth., a hemiparasite of sorghum and other cereal crops, causes severe losses in grain yield and is a threat to food production in many areas of Africa. *Fusarium oxysporum* Schlet. emend. Synd. & Hans strain M12-4A isolated in Mali from diseased *S. hermonthica* tissue significantly reduced *Striga* emergence under laboratory and field conditions. Optimal conditions for a liquid fermentation process to stimulate *F. oxysporum* M12-4A chlamydospore production were investigated. Fermentors with 1% sorghum straw powder, as substrate, exposed to a temperature of 21°C under black light for 21 days yielded the highest colony forming units (CFU). Fermentor-harvested inoculum can be directly applied into the planting furrow or coated on sorghum and other cereal crop seeds with arabic gum prior to planting. Under laboratory conditions, arabic gum solutions (10, 20 and 40% w/v) used in the sorghum seed pelleting process were found to enhance chlamydospore germination, mycelial growth, and increase inoculum potential by stimulating production of secondary chlamydospores. In a pot trial experiment, under natural conditions, fermentor-harvested *Fusarium* inoculum, applied directly to the soil or on sorghum seeds, was found to control *Striga* emergence. The delivery system proposed is a simple step application procedure: seeding.

Vegetation Problems and Biological Control Efforts at U.S. Army Installations

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In 1996 the Army Environmental Center expressed a concern with problem noxious weeds at Army installations in the United States and abroad. A survey was conducted in 1997-1998 of over fifty Army installations to determine which noxious plants were present and how these problem plants were being managed. All the installations reported some type of vegetation problem and in over 90% of the cases the problem vegetation identified was exotic. No single species appeared as the most prevalent across all the installations. One striking factor noted was that extensive problems occurred with rangeland plant species at almost all installations, particularly those devoted to troop training. The use of biological control as a management method for problem weeds was reported from over 50% of the installations. Follow-up discussions showed that these responses were inflated and did not reflect the true picture of their management operations, but work was being conducted on musk thistle, purple loosestrife, leafy spurge, diffuse knapweed, spotted knapweed, alligatorweed and other exotic plants. The Army Material Command reported the extensive use of *Rhinocyllus conicus* and *Trichosirocalus horridus* on musk thistle. Although releases were made 10 to 20 years ago, no post release evaluations were conducted because of restricted access and high security at these installations. Examination of field populations of the agents at two of the Army Material Command locations in 1998 showed extensive weevils still present on the remnant musk thistle populations.

Water-Dispersible Granules Containing Mycoherbicide Agents

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Water-dispersible granules (WDGs) are applied by spraying after disintegration and dispersion in water. Their use with agrochemicals is increasing because of good shelf life, easy pouring and measuring, and low dust. WDG formulations should also be useful to deliver mycoherbicide agents. We have prepared WDGs containing several agents by extrusion and fluid bed drying. A water-insoluble food source for the fungal agent was included in the formulation to form a film on the target weeds and, possibly, provide some protection from adverse environmental conditions. Because the dew period hurdle must be addressed, other adjuvants were also incorporated. An 80% control of tropical soda apple (*Solanum viarum*) was obtained in the greenhouse using an as yet unidentified Hyphomycetes.

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Fungi Isolated from *Orobanche* Spp. in Ethiopia, and Their Potential Use for Biological Control

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For the isolation, identification, and exploration of microoganisms as potential biocontrol agents of the parasitic weed *Orobanche* (broomrape), a survey was conducted for the first time in Ethiopia (Ziway, Nura Era, Merti Jeju, and Malima farms). All identified fungal isolates from infected plants of *O. ramosa* L. and *O. cernua* (Loefl.), which parasitized tomato (*Lycopersicon esculentum* Mill.) and belonged to the genus *Fusarium*: including *F. gibbosum*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. solani* var. *solani*, and others not determined on the species level. Two of them, *F. nygamai* and *F. proliferatum*, have been identified for the first time as pathogens of *Orobanche* spp. *Fusarium gibbosum*, *F. nygamai*, *F. oxysporum*, and other *Fusarium* spp. significantly reduced the number of emerged *O. ramosa* shoots when applied at a rate of 10 gkg^{-1} of soil in pot under glasshouse conditions. The same isolates significantly decreased the dry weight of *O. ramosa* and resulted in a significant increase of tomato dry weight by 30.3%, 30.3%, 32.1%, and 30.3%, respectively. In root chamber experiments, *F. oxysporum* and *F. nygamai* significantly increased the percentage of dead *O. aegyptiaca* tubercles when applied at a rate of 5×10^5 spores ml^{-1}. The former killed 81% and 82% of the tubercles after 10 and 15 days of inoculation, while the latter killed 41% and 48%, respectively.

U. S. Army Pilot Project for Biological Control of Canada Thistle and Spotted Knapweed in Colorado

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Research to control the spread of and reduce existing populations of Canada thistle (*Cirsium arvense* (L.) Scop.) and spotted knapweed (*Centaurea maculosa* Lam.) at the

Fort Carson Military Reservation located near Colorado Springs, Colorado, was initiated in 1997. Seven heavily infested sites were selected and were sampled for natural enemies. Additional candidate species were obtained from Colorado Dept. of Agriculture, USDA-APHIS in Spokane, WA, USDA-APHIS and Forestry Sciences Laboratory at Montana State Univ., and the Pennsylvania Department of Agriculture, and released for biological control of these selected weeds beginning in June 1997. These insects will be monitored and redistributed as they become established. GPS mapping was conducted to determine the extent of weed infestations at the release sites and will be used to monitor the spread of newly established biocontrol agents and reduction in weed infestations.

Controlling Canada Thistle with *Pseudomonas syringae* pv. *tagetis*: Factors Influencing Efficacy

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Pseudomonas syringae pv. *tagetis* (Pst) causes apical chlorosis in Canada thistle (*Cirsium arvense*) and other composite weeds due to the production of tagetitoxin, a RNA polymerase III inhibitor that blocks chloroplast biogenesis. Previous research demonstrated the potential of Pst as a biological agent for controlling Canada thistle in soybean. Multiple inundative foliar applications of Pst in an aqueous suspension containing an organosilicone surfactant (Silwet L-77) provided good control of Canada thistle without causing injury to soybean. A better understanding of factors regulating the efficacy of Pst on Canada thistle is needed to develop a formulation that will control this weed with a single application. The dynamics of the endophytic Pst population and tagetitoxin production in Canada thistle leaves were measured following inundative application. The optimum concentration of Silwet L-77 for obtaining maximum foliar penetration of Pst was approximately 0.3% (v/v). Initial endophytic populations in mature leaves were directly related to the concentration of Pst applied (10^6 -10^9 cfu/ml). Within 48 h after foliar application of 10^9 cfu/ml Pst, endophytic populations reached a plateau of approximately 10^8 cfu/gram fresh weight. During a two week period following foliar application, endophytic populations declined to levels approximating those measured at the time of application. In some cases, Pst was able to move from mature, sprayed leaves to newly developed leaves via the vascular system. Tagetitoxin production, as measured by chlorosis of newly developed leaves 10 days after Pst application, was variable. Pst concentrations greater than 10^6 cfu/ml were required to get good symptom development. Approximately 60% of plants treated with 10^9 cfu/ml Pst exhibited chlorosis in newly developed leaves.

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Control of St. John's Wort and Round-Leaved Mallow in Non-Cultivated Habitats with *Colletotrichum gloeosporioides*

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The use of *Colletotrichum* spp. as biocontrol agents has largely focused on their potential use as commercial mycoherbicides. In addition, some may have potential in classical biological control where, after release, they establish and persist to provide long-term weed control. Two such successful examples on introduced weeds have been studied in Nova Scotia. The first, *C. gloeosporioides* f. sp. *hypericum* (*Cg-h*) on the perennial St. John's wort (*Hypericum perforatum*) caused 40 to 96% mortality of mature plants in several non-arable habitats. Seedling mortality approached 100% and infected seedlings did not survive winter. The pathogen may have originated from the local *Hypericum* flora and all St. John's wort biotypes collected across North America appeared equally susceptible. *Cg-h* occurs ubiquitously in Nova Scotia and may be selectively transmitted by *Chrysolina hyperici*. *Chrysolina* adults contaminated with the pathogen were an effective means of disseminating the disease. The second example involves round-leaved mallow (*Malva pusilla*), an annual weed increasingly imported into Nova Scotia in western feed grains along with its pathogen, *C. gloeosporioides* f. sp. *malvae*. When applied as a mycoherbicide to micro-plots of mallow in habitats subjected to mowing or cattle trampling, e.g. orchards, pastures, or barnyards, the pathogen was widely disseminated over several hectares within one season and has provided effective control since 1996. These examples demonstrate that these pathogens, and others, can provide long-term control in stable habitats and profoundly affect the weediness of some species.

Successful Biological Control of Water Hyacinth (*Eichhornia crassipes*) in Papua New Guinea by the Weevils *Neochetina bruchi* and *Neochetina eichhorniae* (Coleoptera: Curculionidae)

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Water hyacinth invaded Papua New Guinea (PNG) prior to 1962. Attempts to eradicate the weed failed and it spread throughout the country. In 1986 the biological control agent *Neochetina eichhorniae* was released and became established. A project began in 1993 to locate and control the weed using manual control of small isolated infestations and biological control of all other infestations. Manual removal, recommended at over 90

locations, was successful at 54 locations where no regrowth occurred. Biological control was instigated at 132 locations. The agents released were the weevils, *N. eichhorniae* and *N. bruchi*, and the moths, *Niphograpta albiguttalis* and *Xubida infusella*. *N. albiguttalis* failed to establish while *X. infusella* established at one site. The two *Neochetina* weevils became widely established, and where populations remained undisturbed on water hyacinth growing on large water bodies, successful control resulted. On Sepik River lagoons, weevil damage reduced flowering and decreased the production of offshoot ramets which caused large mats of the weed to break up and then sink or to be flushed away in smaller sections. Damage caused by the weevils increased over three to six years culminating in mats collapsing over a 12 month period in 1994/95. The weed cover on Sepik lagoons decreased dramatically despite continued spread. The improvement to village life was equally dramatic with the return to better levels of nutrition, health and activity. In another region, in a pond where *N. eichhorniae* was released during 1986, control of the weed has been maintained since 1991. Elsewhere, in a lake with elevated nutrient levels, water hyacinth cover was reduced from 70% to less than 30% in two and a half years by both *Neochetina* species. This study presents unambiguous examples of the value of *Neochetina* species as control agents for water hyacinth.

The Development of Mycoherbicides for the Management of Parasitic Weeds of the Genus *Striga* and *Orobanche* - a Review and Recent Results

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Parasitic weeds of the genus *Striga* and *Orobanche* are causing considerable yield losses in cropping systems south of the Sahara and in the WANA-region. Their management is unsatisfactory since present control methods are still not efficient enough to control the underground development stages of the parasites. Hence, most of the control methods do not lead to a yield increase in the first years of application. Fungal antagonists of the genus *Fusarium*, especially *F. oxysporum*, which have been isolated from diseased plants of *Striga hermonthica* and *Orobanche cumana*, have been proven to be highly pathogenic to all developmental stages of the parasites including seeds. In addition, they are highly host specific and non-pathogenic to a wide range of crops tested, thus meeting two important criteria to be used as mycoherbicides. Applied as solid medium in field trials, these fungi were shown to be highly effective, but the inoculum amount has to be reduced to a practicable level. Therefore, different formulations (alginate with nutrient amendments; wheatflour-kaolin granules - 'Pesta') with fungal propagules have been investigated. Compared to the untreated control, 'Pesta' applied in dosages of 0.4 and 1 g per pot (2 kg of soil) reduced the emergence of *Striga* and *Orobanche* shoots by 80% and 71%, respectively. Formulations on the basis of alginates have been advantageous to

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maintain the viability of *F. oxysporum* over a time period of at least 12 months at room temperatures (±20°C). Future studies are aiming to further develop the formulations and to test their efficacy under field conditions.

Stumpout™ - Commercial Production of a Fungal Inoculant to Prevent Regrowth of Cut Wattle Stumps in South Africa

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Many of the introduced Australian *Acacia* species, including black (*A. mearnsii*) and golden (*A.pycnantha*) wattle, are invasive and have infested large areas in South Africa. In the course of experimental trials, naturally infected dead black wattle stumps were found to be colonized by the basidiomycete *Cylindrobasidium laeve*. Tests were conducted by the PPRI Weed Pathology Unit, Stellenbosch, on the effectiveness of this fungus as a biological control agent of cut wattle stumps. Results of field trials showed mortality of treated stumps of both *A. mearnsii* and *A. pycnantha* to be greater than 80% (reaching 90 and 100% in some cases) within 6-12 months of treatment. The product Stumpout™ was registered in 1997 for use as a fungal inoculant to treat and kill wattle stumps. The limited market for the product has dissuaded large business interest in Stumpout™; however, there is a regular demand for the product from conservation organizations and landowners. To meet this demand, Stumpout™ is produced in a small factory on the premises of PPRI Weed Pathology Unit, Stellenbosch, and distributed to clients on request. Clients receive small sachets of the product, consisting of live basidiospores of the fungus *Cylindrobasidium laeve* in an oil formulation. The product is diluted in sunflower oil and 1-2 ml is painted onto the fresh cut surface of the tree stump. The stumps die within a year of treatment. Currently tests are being carried out in order to determine the efficacy of the product against various other alien weed tree species.

Release and Colonization of the Bindweed Gall Mite, *Aceria malherbae*: A Field Bindweed Biological Control Program for the Texas High Plains

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Aceria malherbae Nuzzaci is a gall-forming eriophyid mite imported from Greece for biocontrol of field bindweed. The mite injures plants by producing galls on leaves, petioles, stems and roots. Infested plants are yellowed, have deformed leaves, reduced vigor,

and new growth is killed. The mites can kill whole plants, limit seed production and reduce vegetative spread. *A. malherbae* overwinters on the roots of bindweed, and is present in the spring when bindweed growth begins, where it can quickly build up and suppress new growth. After an initial quarantine period at the USDA-ARS Insect Quarantine Facility at Temple, TX, *A. malherbae* was released at Bushland, TX, in 1989. Approximately 5,800 mites were released in May and 9,000 mites were released in June. By June 1992, 52% of the bindweed crowns at the release site were infested with mites and the mites had moved 9.5 m from the release site. By July 1996, mites had spread over one km from the release site and reduced the bindweed infestation by 50%. Mites have spread at a rate of about 0.125 km/yr. In 1998, a program was initiated at the US Department of Energy's Pantex plant in Amarillo, TX, to redistribute the mite within the confines of this installation. Initial success has been excellent. The release sites in 1998 were inadvertently mowed; however, this "accident" proved to be beneficial. Over a period of 83 days, the mites moved 60-164m (0.73-2m/day) from the point of release at the mowed sites. In contrast, the "natural" movement at the Bushland release site averaged 0.35m/day. In essence, the mites dispersal was increased by 2-6x at the mowed sites. It is thought that since the mites are so small, mite-infested bindweed "clippings" act as good host material for distribution. Releases, redistribution, and monitoring will continue throughout 1999.

Desiccation Tolerance in Microbial Herbicides

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An isolate of *Colletotrichum truncatum* which controls *Sesbania exaltata* and an isolate of *Alternaria alternata* which causes foliar disease on *Amaranthus retroflexus* were selected for a study of the effects of nutrition (C:N ratios of 5:1, 15:1, 40:1) during conidiation on their desiccation tolerance, pathogenicity and conidial ultrastructure. The extracellular matrices (ECMs) produced around germlings were also studied. Conidia of *C. truncatum* grown at C:N of 5:1 showed better desiccation tolerance, higher germination on the host, and more disease expression than those grown at other C:N ratios. Similar effects were found for *A. alternata* conidia grown at C:N of 15:1. ECMs were not visible by either scanning or transmission electron microscopy. However, staining with fluorescent-lectin conjugates and colloidal gold revealed ECMs in both fungi. In the case of *C. truncatum*, concanavalin A (ConA) bound to both ungerminated conidia and germ tubes. Wheat germ agglutinin (WGA) did not label the germ tubes but bound strongly to most appressoria and conidia. Neither soybean agglutinin nor *Ricinus communis* agglutinin reacted with conidia and appressoria but both lectins stained germ tubes. Partial labelling of some appressoria by *Dolichus biflorus* agglutinin was observed. *Ulex europaeus* agglutinin and peanut agglutinin did not label germlings. Colloidal gold staining and silver enhancement revealed the presence of basic proteins around germlings. For *A. alternata*, ConA and WGA strongly labelled the germ tubes, but not conidia. Other lectins showed no reaction with germlings. Colloidal gold staining and silver enhancement showed pro-

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teinaceous materials around the germ tubes of the fungus. Taken together, the C:N ratios of the conidiation media had no effect on the appearance of ECMs around germlings of the isolates. Thus, the observed differences in conidial desiccation tolerance are not explained by differences in ECM production by these fungi.

Fusarium sp. as a Potential Biocontrol Agent for *Egeria densa* and *Egeria najas*

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The submerged mycrophytes *Egeria densa* and *Egeria najas* are natives in Brazil but have become serious weeds in artificial water reservoirs, causing extensive losses in energy production and increasing the cost of maintenance procedures in hydropower plants. Biocontrol studies on these two weeds began in 1995 through field collections for pathogens in natural infestations in Pantanal, a western swampland area, and in Tietê, Paraná, and Paranaíba river basins. Pathogenicity studies showed a good potential for a strain of the fungus *Fusarium* sp. The symptoms consisted of restricted stem necrosis and foliar chlorosis, intensifying progressively until a complete breakdown of the structural integrity of plant tissues occurred. Culture filtrates of fungus produced phytotoxicity, with symptoms similar to those promoted by the fungus. The active substance has not yet been identified. The fungus was cultivated in different natural substrates (rice, wheat and sunflower grains, and minced corn). Rice was selected as the most suitable medium due to the fast growth of the fungus, the ease of drying and grinding, the high efficacy of the inoculum, and the high longevity under storage conditions (more than 8 months at 4°C). Bioassays of different concentrations of *Fusarium* -rice inoculum (0.1, 0.3, 0.5, 0.7, and 1.0 gL^{-1}) showed high weed mortality from 0.5 gL^{-1} for both weed species. The toxicity was lost when the filtrate was diluted 1:8, using an inoculum suspension 5.6 gL^{-1} for 24 hours at 25°C. The duration of the phytotoxic activity was studied through daily exposure of *E. densa* and *E. najas* shoot tips in nutrient solutions enriched with rice-*Fusarium* inoculum (0.7 gL^{-1}). The results showed a reduction in the phytotoxicity symptoms from the seventh day, mainly in *E. najas*. The plant-toxin kinetics were studied in *E. densa*, exposing the shoot tips for different periods in inoculated water (0.7 gL^{-1}) before a severe washing and plant cultivation in sterile nutritive solution. The symptoms suggested that *E. densa* had accumulated a lethal dose after 6 hours of continuous exposure. Studies are being conducted to maximize the toxin production through specific conditions for fungus growth and to increase the list of useful plants tested for specificity. The specificity of the fungus was tested on 14 cultivated species and 11 aquatic plants, but only *Hydrilla* sp. developed symptoms.

South Dakota Correctional Facility Supports Captive-Rearing Efforts for Biological Control Agent of Purple Loosestrife (*Lythrum salicaria* L.)

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Lythrum salicaria, an exotic wetland dependent plant, is listed as a noxious weed under several South Dakota and Nebraska weed laws. Originally from Eurasia, *L. salicaria* invades disturbed wetland sites, displacing native vegetation. *Lythrum salicaria* is currently found along most major river systems in Nebraska and South Dakota. Two species of leaf-eating beetles, *Galerucella calmariensis* (L.) and *Galerucella pusilla* (Duftschmidt) have been approved in the United States as biological control agents for the control of *L. salicaria*. Following established protocol, weed control personnel from Nebraska and South Dakota began a pilot project in 1996 to captive-rear both *Galerucella* spp. in a nursery setting. Project success led federal, state and local government agencies in 1998 to apply for and receive a National Fish and Wildlife Foundation grant to expand and improve the nursery. The Springfield State Prison became a cooperator in 1998, providing nursery space and inmate labor to rear beetles. Prison efforts also supported additional nurseries in Nebraska. Nursery efforts dramatically increased the number of beetles released at control sites and reduced overall noxious weed control expenditures. Project success, support, and professional interest for this project has led to 3 independent research projects, 6 additional nurseries in 1999, and continued cooperation with the Springfield State Prison.

Controlling Leafy Spurge Using *Aphthona* Flea Beetles: One Year After Mass Release

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Leafy spurge (*Euphorbia esula* L.) is one of the most important exotic weeds in the Western United States and it continues to expand its range. Many states, such as New Mexico, are at the edge of the leafy spurge expansion. Scattered populations of leafy spurge now occur in the state. Although it is commonly accepted that biological control agents acting alone cannot eradicate a host population, they can reduce a host population to very low levels, especially in the case of inundative strategies. The objective of this study was to determine the potential of using *Aphthona nigriscutis*, and *A. czwalinae/lacertosa* flea beetles to control isolated patches of leafy spurge. Two study sites (Barker and Tusas) were established in northern New Mexico in May 1997. At each site, twenty-four

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5m x 5m patches of leafy spurge were delineated. Five, 0.25m^2 subplots were permanently marked for estimating leafy spurge stem density and biomass in each patch. One of four treatments was assigned to each plot in a completely randomized block design. Treatments were 1) control (no beetles), 2) low density (1,000 beetles per plot), 3) medium density (2,000 beetles per plot), and 4) high density augmentations (4,000 beetles per plot). Leafy spurge stem density and biomass were estimated three times during each of the 1997 and 1998 growing seasons. Flea beetles established at both sites and resulted in visible, although variable, reductions in leafy spurge. At Tusas all three beetle densities resulted in significant decreases (30 to 51%) in the number of stems per plot and a reduction in biomass (53 to 61%) at the medium and high density treatments. At Barker only the high density treatment resulted in decreases in stem number (37%) and biomass (61%) after one year.

Mycobiota of *Centaurea cyanus* and *Ascochyta doronici* as a Probable Agent of the Biocontrol of this Weed

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In the territory of Russia *Centaurea cyanus* is a harmful weed in the grass, vegetable, and grain crops. The long period of flowering, large number of viable seeds (up to 6700 for one plant), high emergence, absence of period of rest, presence of a pappus for distribution by wind — all these allow the weed to be quickly distributed. The study of the micobiota of *Centaurea cyanus* was undertaken with the purpose of revealing pathogenic fungi, probable biocontrol agents of this weed.

The tax of plant samples, infected by fungi was made in 1993 - 1995 in the Leningrad area (Russia) and in the Ukraine (Sumy and Khmelnitsky regions). The pathogenicity of some micromycetes for *Centaurea cyanus* was confirmed by artificial inoculation of plants in a glasshouse. The impact of leaf spot caused by *Ascochyta doronici* on 1, 2 and 3-week *Centaurea cyanus* seedlings was investigated in small plots. Plants were inoculated by spraying with spore concentrations x1,000,000 spores/mL.

Five species of fungi were identified on *Centaurea cyanus: Ascochyta doronici, Bremia centaureae, Chaetomium globosum, Penicillium purpurogenum,* and *Puccinia cyani. Ascochyta doronici* is the causal agent of the *Centaurea cyanus* leaf spot. In a glasshouse the Ascochyta leaf spot latent period was 5-7 days. After 14 days the disease severity was 42%. In small plots the symptoms were identified after 10 days. The increase of the seedlings age from 1 to 3 weeks resulted in a decrease in the parameters of plant development. The average losses of dry biomass were 39.2%, 27.8%, and 24.4%; of plant height were 25.9%, 28.8%, and 12%; the bud numbers were 1.63, 1.59, and 1.36 times smaller accordingly for 1-week, 2-week, and 3-week plants. No significant differences in the plant development between 1- and 2-weeks seedlings were evident.

Ascochyta doronici has potential as a biocontrol agent for *Centaurea cyanus*. This pathogen is most effective for the control of 1- to 2-week old seedlings.

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Field Observations and Biological Notes of the Entomofauna Associated with *Convolvulus arvensis* L. in Slovakia

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During 1997 and 1998, periodical surveys were carried out to screen the entomofauna associated with field bindweed, *Convolvulus arvensis,* in Slovakia.

From a list of 79 insect species belonging to the 15 families (from Coleoptera, Lepidoptera, Diptera) attacking field bindweed, the fly *Melanagromyza albocilia* Hendel (Diptera: Agromyzidae), two flea beetles *Longitarsus pellucidus* (Foudras), and *L. longipennis* Kutschera (Coleoptera: Chrysomelidae) and four species of tortoise beetles, *Cassida* spp. (Coleoptera: Chrysomelidae) were selected as promising biocontrol agents.

Melanagromyza albocilia larvae were found boring into stems of the field bindweed in many locations. The potential of the fly is related to the narrow host range showed by insect species of the family Agromyzidae. The presence of the larvae can be detected only with plant dissection. The tunnel causes the death of the infested sprouts, with a decrease of the reproductive capacity and photosynthesis. The total number of infested and dried sprouts caused by the fly ranged from 30 to 50% (August - October). In some cases, abundance of pupae was up to 6 pupae per plant.

Five species of parasites, *Aneuropria foersteri* Kieffer (Hymenoptera: Diapriidae), *Sphegigaster truncata* Thomson (Hymenoptera: Pteromalidae), *Macroneura versicularis* (Retzius) (Hymenoptera: Eupelmidae), *Chorebus leptogaster* (Haliday) and *Bracon fulvipes* Nees (Hymenoptera: Braconidae) were reared from pupae and larvae of the fly.

In fact, besides the primary damage, the fly exposes field bindweed to secondary damage by opening the "door" to several pathogens, such as fungi and bacteria, with consequent infections which can increase the damage to plants.

Phytotoxic Metabolites Produced by *Drechslera avenacea*, a Potential Mycoherbicide of Wild Oats

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Wild oats are serious weeds of many crops, both cereals as well as dicotyledons, and are widely distributed across a range of environments and climates. Recently, *Drechslera*

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avenacea (Curtis and Cooke) Shoem, a fungus isolated from diseased wild oat plants in Australia, has been considered a potential agent for biological control of these noxious weeds.

Considering that *D. avenacea* belongs to a well-known toxin-producing genus, and the possible use of fungal toxins and/or their derivatives as an alternative or in addition to the use of pathogens in weed biocontrol, it seemed of interest to ascertain the production of toxic metabolites by *D. avenacea*, carry out their isolation and chemical and biological characterization, and evaluate the potential in Integrated Weed Management strategies.

For the production of toxic metabolites, the fungus has been grown on a defined medium in static conditions for 4 weeks. The culture filtrate, toxic when assayed both on host and non host leaves, has been extracted using organic solvents having different polarity, and purified by the use of HPLC. Preliminary observations showed that the main toxic metabolites have lyposoluble nature and low molecular weight. Toxic filtrate has been sprayed alone or in combination with the fungus and a selective herbicide at reduced dose on wild oat grown in sowing plates in the greenhouse to evaluate the possible use in weed biocontrol.

The preliminary results of the chemical and biological characterization of fungal metabolites and their possible use in weed control programmes will be shown.

A Passive Sorting Device for Separating *Aphthona* Species Biological Control Agents of Leafy Spurge from Larger Insects, Seeds and Plant Debris Associated with Crude Insect Sweep Net Collection

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In 1998, USDA-APHIS-PPQ, in support of Team Leafy Spurge activities on *Euphorbia esula*, developed a passive sorting system for the purpose of mass collecting and distributing large numbers of *Aphthona* species flea beetles. Enhancing some ideas and methodologies that had been previously utilized in various forms, technicians at the Bozeman Biological Control Station produced a light, portable, free standing, and easily operable sorting unit from inexpensive and readily available materials. Dubbed the 'Aphthona Accelerator', this sorter can be set up and operating in minutes. This passive sorting device is driven by the typical behavior of *Aphthona* flea beetles to orient upwards and move toward light. The flea beetles actually separate themselves from the larger insects, seeds, and plant debris that are also collected in field net sweepings, by moving through a fine mesh basket to a slick funnel and eventually into a collection jar. It is possible to sort several hundred thousand flea beetles with this device in just a few hours, leaving nearly all of the extraneous material behind. The 'Aphthona Accelerator' can easily be operated by one technician, freeing up everyone else to sweep up more flea beetles!

Biological and Molecular Characterization of *Ascochyta caulina*, a Biocontrol Agent of *Chenopodium album*

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Chenopodium album, commonly called lambsquarters or fat-hen, is an important and widely distributed weed in the world. It is considered to be the most dangerous weed in 10 major crops because of the abundance and longevity of its seeds. *C. album* has developed resistance to some chemical herbicides, and for this reason is considered a good target for biological control. Recently the use of a fungus, *Ascochyta caulina*, as a biocontrol agent of this weed has been proposed, and the main phytotoxic metabolite produced by the fungus, named ascaulitoxin, has been identified and biologically characterized.

Within a European project involving several research institutions (FAIR5 PL97-3525 - "Optimizing biological control of a dominant weed in major crops"), having the aim of studying and improving the practical application of *A.caulina*, more than 50 strains of this fungus, isolated from fat-hen leaves, stems, and seeds collected in different European locations, were supplied to our institute in order to study their molecular and biological differences.

Strains of the fungus were grown in a defined liquid medium for 4 weeks at 25°C and the phytotoxic activity of their culture filtrates was evaluated using different biological assays. A preliminary screening was conducted to assess some molecular differences and to identify good primers useful for further studies on strains. Several decamers of arbitrary base composition were screened on *Ascochyta* isolates from diverse origin. Isolates displaying polymorphic bands have been further characterized by sequencing variable ribosomal regions (ITS1/ITS2, 28S).

Preliminary results on differences in toxin production and genetic variability between isolates will be shown.