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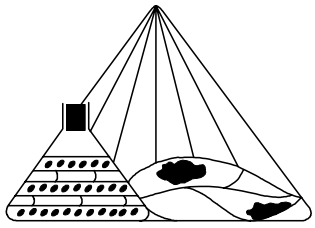

Proceedings

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Battling the fragrant invader: mass production, application, and implementation of biological control for kahili ginger (*Hedychium gardnerianum*)

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Kahili ginger (*Hedychium gardnerianum*) is on the IUCN's list of the "World's 100 worst invasive species", invading tropical and sub-tropical wet forests in areas where it has been introduced as an ornamental plant. The wilt-causing bacterium *Ralstonia* (=*Pseudomonas*) *solanacearum* has been demonstrated as a viable biological control agent for this weed and has recently been established in the field. This bacterium has significant potential in controlling this weed if effective application and mass production methodology can be developed for the biocontrol agent. To address this need, research into the development of mass production methodology and field-testing of new application techniques for the biocontrol of kahili ginger with *R. solanacearum* have been initiated in the wet forests of Hawai'i. Three objectives are being investigated in this study: 1) develop and enhance methodology for mass production of the biocontrol agent; 2) evaluate host resistance among local and international populations of kahili ginger; and 3) evaluate the efficacy of *R. solanacearum*-encapsulated alginate beads and bioherbicide spray. An overview of the kahili ginger biocontrol program and the results of these investigations will be discussed. In addition, information on technology transfer and implementation will also be presented.

Biological control of aquatic weeds of rice in Australia using *Rhynchosporium alismatis*

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Australia produces 1 million tonnes of rice annually, the 85% of which are exported. The majority of Australian rice is aerially sown. This practice has increased the importance of aquatic weeds and their control. Plants species in the Family Alismataceae including *Damasonium minus* (R.Br.) Buch. (starfruit), *Alisma lanceolatum* and *A. plantago-aquatica* are significant weeds of rice in Australia. Of these species, *Damasonium minus* is regarded as the most important weed. The control of starfruit is almost exclusively reliant on the use of only one herbicide (Londax®) which has contributed to the emergence of herbicide-resistant weed biotypes throughout Australian rice growing areas. This resistance and the potential for the contamination of waterways by synthetic herbicides have spurred the search for alternative weed control strategies. As a component of an integrated management system for this weed, the endemic fungus, *Rhynchosporium alismatis*, which causes disease in starfruit plants, is being investigated as a potential mycoherbistat. This paper will report on the basic biology of the fungus in terms of the production of conidia in liquid and solid media, the infection process on starfruit, the production and infectivity of chlamydospores and the environmental conditions that affect the disease. Additionally, an overview of the research will be given including results from studies on genetic variation in the host and the pathogen, details of the infection process on host and non-host species, formulation and field-based yield experiments. The integration of the use of this fungal-based mycoherbistat with cultural and chemical strategies for weed control in rice will contribute significantly to the sustainability of the rice industry in Australia.

WOW emulsion formulation for bioherbicides

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A new liquid formulation for application of bioherbicides is described. It is based on a water in oil in water (WOW) emulsion and contains about 5% oil. It is made by emulsifying an invert emulsion into water. The formulation retains water for extended periods without having disadvantages associated with other formulations such as invert emulsions. The formulation was successful in controlled environment experiments and in the field under dryland conditions with no dew when used with *Collectotrichum orbiculare* on *Xanthium spinosum.*
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Evaluation of *Phoma macrostoma* for control of broadleaf weeds in turgrass

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Ten fungal strains of *Phoma macrostoma* were isolated from chlorotic leaf lesions on Canada thistle (*Cirsium arvense*) in five provinces across Canada. Pathogenicity was assessed on Canada thistle by two methods: i) a foliar spray application (4 ml/pot of a 10^6 conidia/ml suspension) with a 48 hr dew period, and ii) an application of a colonized agar mat to the soil. There was no chlorosis or fresh weight reduction compared to the untreated control when the fungus was applied as a foliar spray, but there was significant chlorosis and fresh weight reduction when the fungus was applied to the soil. The isolates were evaluated for bioherbicidal activity on several weed and crop species under greenhouse conditions using the agar mat test. The isolates demonstrated some bioherbicidal activity on most of the dicotyledenous plants tested, but not to the monocots. The weed species best controlled were Canada thistle, dandelion, scentless chamomile, chickweed and white clover. Field tests were conducted to evaluate dandelion control in turfgrass by applying 0-1000 g/m^2 of granular fungal inoculum to soil at 3 locations in Canada. There was 70-80% control of dandelion at the rate of 63 g/m^2, 90% control at 125 g/m^2, and 100% control at the higher rates. These biocontrol strains of *P. macrostoma* are being further investigated for the control of dandelion and other broadleaf weeds in turfgrass.

Microbes and microbial products for biological control of parasitic weeds

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Orobanche ramosa L. is a parasitic weed species whose distribution interests mainly the Mediterranean basin, North Africa and Asia where, together with *O. aegyptiaca* Pers., infests about 2.6 millions hectares of Solanaceae, above all tobacco, potato, tomato and eggplant. In the last years it has been recorded for the first time in countries as Australia, Central America or United States, attacking Crucifereous species, as rapeseed, and legumes. They are responsible of both qualitative and quantitative damages through interfering with water and mineral intake, that can reach 33% for tobacco. Traditional control methods have been tried by various workers on different crops but none has proved to be effective whereas is considered a good target for biological control. Considering the importance of *O. ramosa* in Italy, mainly on tomato, tobacco and cabbage, a national project on "Biological control of *Orobanche* spp. using phytopathogenic fungi and their toxic metabolites" was funded by the Italian Ministry of Scientific Research. During extensive surveys in fields heavily infested by *O. ramosa* in Southern Italy, aiming to find potential mycoherbicides, a large number of fungi was isolated. Fifty three strains belonging to 15 different species were selected to further assess their pathogenicity and the virulence against broomrape. All the strains were tested using a plastic bag system, in the presence of tomato as host plant. Some of the tested strains were quickly able to cause necrosis and rot of the attached tubercles, and were further tested in pots in greenhouse. Among them, in particular a strain of *Fusarium oxysporum* Schlecht. and of *F. solani* (Mart.) Appel & Wollenw. resulted very promising, being able to strongly reduce either the number and weight of emerging broomrape shoots, or the number of tubercles attached to the host roots. Furthermore, all the strains were grown both in liquid and solid media and the obtained extracts were chemically analysed and biologically assayed with different aims: find new metabolites with the ability to inhibit the germination of *O. ramosa* seeds to be used as natural herbicides; estimate the production of fusaric and dehydrofusaric acids by *Fusarium* strains and evaluate their possible involvement in the disease as virulence factors and their practical use as biomarkers to make easier the selection of potential mycoherbicides; ascertain the production of toxins known as hazardous mammalian toxins, as a preliminary assessment of the risk in releasing potential mycoherbicides into the environment.

Tobacco mild green mosaic virus: a virus-based bioherbicide

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Tropical soda apple (*Solanum viarum*; TSA) is a serious noxious weed in pastures, sod fields, and natural areas in Florida and other states in the southeastern United States. During a search for a biocontrol agent for this weed, we discovered that Tobacco mild green mosaic *tobamovirus* (referred to herein as tobacco mild green mosaic virus or TMGMV; ICTV decimal code 71.0.1.0.011; = tobacco mosaic virus U2 strain) causes a systemic, hypersensitive response and kills seedling and mature TSA plants. Younger plants are killed faster than older plants. Inoculated plants developed necrotic foliar lesions, systemic necrosis of petioles and stem tips, and systemic wilting in rapid succession, beginning 12-14 days after inoculation. TSA is also susceptible to Tomato mosaic *tobamovirus* and Tobacco mosaic *tobamovirus* (strain U1), but these viruses induce only nonlethal mosaic and/or mottle symptoms. Among 31 solanaceous plants screened against TMGMV in a greenhouse, only *Capsicum annuum* (most of the 23 cultivars tested), a previously known host tof this virus, developed hypersensitive reaction comparable to that seen on TSA. Other hosts were symptomless or exhibited systemic mosaic symptoms or local lesions. In repeated field trials, TMGMV caused 83 to 97% mortality of TSA plants of different sizes and ages. Typically, hypersensitive reaction is expressed as necrotic foliar spots; lethal systemic hypersensitive reaction to virus infection is uncommon and usually occurs in seedlings. Thus, TMGMV has the unique capacity to kill TSA plants of all ages and therefore can be used as an highly effective biological control for TSA. Attempts are underway to develop and register TMGMV as the first virus-based bioherbicide.

Microencapsulation: an answer to the formulation quandary?

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Internationally only a handful of bioherbicides are available for use. The integration of bioherbicides into the weed management system has not taken place. This has been due, in part, to difficulties in the formulation of these biological control agents. The biological control agents targeted for use, as bioherbicides, have largely been fungi, which are susceptible to a range of environmental conditions such as UV light, low humidity and temperature. With improved formulations these factors may be overcome resulting in enhanced delivery and action of bioherbicides. The techniques to formulate bioherbicides vary widely, with the final product taking on many forms, from granules to emulsifiable concentrates. To date no one technique has been applicable to the range of organisms that are dealt with. A solution to this has been found in the microencapsulation of organisms via spray drying. Spray drying is particularly applicable to bioherbicide technology, as it may be adapted to a number of different organisms and forms of an organism. Bacteria, bacterial spores, fungal hyphae and fungal spores as well as some yeast have all been successfully spray dried. Once encapsulated the biological control agents are available to be further manipulated into suitable forms for application. The application form required will change with the plant-bioherbicide system, and may range from granules to water-soluble powders. It is the adaptation of this technology to bioherbicides that the study presented here focuses, using the model system of *Phomopsis* sp. to control saffron thistle (*Carthamus lanatus* L.).

Saffron thistle is a significant weed in Australia. It thrives on terrain and pastures that are not valuable enough to warrant extensive chemical control mechanisms, and so it is left unchecked to build up further in the seed bank. The application of a suitable bioherbicide is an attractive, economic, alternative. A suitable fungal bioherbicide *Phomopsis* sp. has been identified, which unfortunately, does not readily produce spores. We are therefore developing a novel formulation technique using spray drying which encapsulates hyphal fragments, resulting in artificial spores. Reported in this study are the results of spray drying the fungus *Phomopsis* sp., it will include the viability and pathogenicity of the hyphal fragments contained within the formulation. Preliminary work has established that at temperatures of 110°C, 120°C and 130°C the mean survival of the hyphal fragments remains above 60%. The median size of the particles produced by the spray dryer in experiments so far is 8.36 microns. This may prove to be too small to be practical but with the addition of other components to the system and further optimisation of the parameters used in the spray dryer the particle size should increase to an acceptable size range of between 50 and 70 microns.

Evaluation of *Ascochyta caulina* for biological control of *Chenopodium album*

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Ascochyta caulina is a plant pathogenic fungus that, under natural conditions, causes necrotic spots on the leaves and stems of *Chenopodium* plants. The objective of the present study was to estimate the effect of weed growth stage, relative humidity, dew period and plant nitrogen supply on the biocontrol activity of *A. caulina* against *C. album*. In greenhouse experiments, replicated groups of *C. album* plants in 10-cm pots sprayed with different isolates of *A. caulina* 2, 3, 4, 5 and 6 weeks after planting. Both disease development and pathogen-induced dry weight reduction decreased with plant age. The efficacy of all isolates tested was reduced by high leaf-to-air vapour pressure deficit. A dew period of grater than 6 hours was required to cause significant disease development in *C. album*. Disease development was positively related to increasing plant tissue nitrogen.

Survey of diseases of alligator weed in eastern Australia for their bioherbicide potential

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Alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.) (Amaranthaceae) is an amphibious herbaceous perennial weed. The fungus *Nimbya alternantherae* has been associated with alligator weed in Brazil and species of the *Fusarium* genus were found on alligator weed in China. Both fungal species showed a very damaging effect on the weed and appeared to be good candidates for further studies as biocontrol agents.
In eastern Australia, where alligatorweed has become a serious invader of wetland areas, surveys have never been carried out on this weed. Although the fungus *N. alternantherae* has been previously reported on *Alternanthera denticulata* R. Br. (lesser joyweed), an Australian native and on *Alternanthera sessilis* (L.) DC. (sessile joyweed or Mukunuwenna), from two locations in Queensland, the fungus has never been found associated with alligator weed in Australia.
To establish what diseases occur on alligatorweed in eastern Australia, surveys were undertaken and fungi were evaluated for their potential as mycoherbicides for this weed.

Evaluating *Fusarium tumidum* and *Chondrostereum purpureum* as mycoherbicides for gorse

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Summary
Field studies in New Zealand revealed that the fungus *Chondrostereum purpureum* applied as mycelium on agar to cut stems of gorse (*Ulex europaeus*) significantly reduced the number and length of living regrowth shoots in November 2002, averaged over twelve treatment occasions (May 2001 to April 2002). The response varied with time of year of fungus application. The addition of an invert oil emulsion, with or without *Fusarium tumidum* conidia, further reduced the number of living regrowth shoots to almost nil.

Introduction
Previous studies in New Zealand have shown two plant pathogenic fungi can infect gorse. *F. tumidum* conidia applied as a suspension to the foliage of young gorse plants caused infection and mortality (Fröhlich et al., 2000, 2001) and *C. purpureum*, a wood rotting fungus, caused infection to cut woody stem sections in the glasshouse (I.H. Harvey pers. comm.).

The efficacy of these pathogens on gorse was examined in field studies in Canterbury in the South Is. and Auckland in the North Is of New Zealand (Fröhlich et al., 2001). Preliminary results of the South Is. work are presented here.

Materials and methods
Individual gorse bushes were treated each month from May 2001 to April 2002. The four treatments were two isolates of *C. purpureum* mycelium on agar, agar without mycelium and an untreated control, each applied to two freshly decapitated woody stems per bush. The *C. purpureum* isolates were applied as 15 mm colonised agar discs placed in contact with the cut stem surface and wrapped with Parafilm. The agar-only treatment was also applied and wrapped as described, while the cut stems on the control bushes were left uncovered. The Parafilm was removed after one month. All of the adventitious shoots within 10 cm of the cut end of the stems were counted, measured and classified as living or dead monthly after treatment until May 2002, then 3-monthly after that.

In a second experiment the combined effects of *C. purpureum* and *F. tumidum* were investigated. The *C. purpureum* treatments were the same as those used in the first experiment as described above, applied once in May 2001 to two cut gorse stems per bush. In November 2001 three separate additional treatments were applied to the adventitious regrowth shoots on the stems treated earlier; these were *F. tumidum* conidia in an invert oil emulsion, the invert oil emulsion without conidia and water alone as a control. Application was by pressurised sprayer. These bushes and stems were assessed at the same frequency as in the first experiment.

The data presented here are the mean number and length of living regrowth shoots per two treated stems, that were present in November 2002.

Results and Discussion
In the first experiment, the number and length of living regrowth shoots was significantly lower on stems treated with isolates of *C. purpureum*, than for the control and agar treatments when averaged over the twelve treatment months (May 2001 to April 2002) (Table 1). The responses did not differ for the two *C. purpureum* isolates, nor was there any difference between the control or agar treatment. However, the effect of *C. purpureum* on shoot survival varied with the time of year of fungus application. Stems treated with *C. purpureum* in the months of May, June, August, September and October 2001 and February and March 2002 had significantly fewer living shoots than the controls

while in other months the number of shoots was not reduced. The fungus evidently was not effective in the coldest winter month (July) or in the hottest summer months (December and January).

Table 1. Mean number and length of living adventitious shoots per two decapitated gorse stems, in November 2002, averaged over twelve monthly treatment occasions from May 2001 to April 2002. Data transformed to square roots for analysis, back-transformed means are in parentheses.

Treatment	Number of living shoots	Shoot length (mm)
Control	1.65 (2.7)	16.1 (259)
Agar	1.68 (2.8)	16.1 (259)
C. purpureum Isolate A	0.94 (0.9)	13.8 (190)
C. purpureum Isolate B	1.11 (1.2)	13.6 (185)
LSD (5%)	0.35	2.3
Control/Agar vs *C. p.* isolates	***	**

In the second experiment *C. purpureum* isolates reduced the number of surviving regrowth shoots to a similar extent as in the first experiment (Table 2). In the absence of *F. tumidum,* both *C. purpureum* isolates had significantly fewer living shoots than the control and agar treatments. Almost no living shoots remained after treatment with *C. purpureum* plus *F. tumidum* conidia in the oil formulation, or the oil formulation alone (Table 2) indicating that the emulsion alone was extremely phytotoxic to the gorse shoots.

The effects of *C. purpureum* were not observed until at least 6 months after application. At the time of writing (January 2003) the infection does not appear to have progressed beyond the treated stems. Although these results indicate that *C. purpureum* can reduce the vigour and survival of regrowth shoots of gorse, further investigation is needed to determine if either of these pathogens has sufficient efficacy to be of use as a mycoherbicide against gorse.

Table 2. Mean number of living adventitious shoots per two decapitated gorse stems in November 2002, after treatment in May 2001 with *C. purpureum* and in November 2001 with *F. tumidum.* Data transformed to square roots for analysis, back-transformed means are in parentheses.

C. purpureum treatment	*F. tumidum* treatment		
	Control	Formulation	*F. tumidum* in formulation
Control	1.57 (2.5)	1.00 (1.0)	0.28 (0.1)
Agar	1.90 (3.6)	0.68 (0.5)	0.53 (0.3)
C. purpureum Isolate A	0.75 (0.6)	0.40 (0.2)	0.00 (0.0)
C. purpureum Isolate B	0.63 (0.4)	0.00 (0.0)	0.28 (0.1)
LSD 5%	1.03		
Control/agar vs *C. p.* isolates	***		
Interaction *C.p.* and *F. t.*	ns		

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Evaluation of the efficiency of *Cercospora caricis* for control of purple nutsedge

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There is no current information on the potential of *Cercospora caricis* as a bioherbicide against purple nutsedge, *Cyperus rotundus* L., in crops. Therefore, the purpose of the present work was to determine the efficiency of this fungus against the mentioned weed in a corn field. A randomized block design was followed, with five repetitions and six treatments which were: one, three and five applications of *C. caricis*; one application of the herbicide 2,4D; two hoeing procedures; and one untreated control. Plots of 1.5 m by 1.0 m were planted with corn, with two planted rows separating the blocks in a field infested with purple nutsedge. A suspension of 75 g/l of fresh *C. caricis* mycelium (2000 l/ha) and 3.0 l/ha of herbicide 2,4D were applied on purple nutsedge plants at the 4 to 9 leaves stage, 22 days after corn planting. Four further applications of the fungus were carried out at 14 days intervals. The second hoeing was done 22 days after the first one. According to an analysis of variance, reduction of dry weight of the aerial parts of purple nutsedge treated with this fungus was less pronounced than that obtained in treatments with herbicide 2,4-D or hoeing. However, in visual terms, the treatment with five applications of the fungus was as efficient as any other. On the other hand, reduction of dry weight and number of purple nutsedge tubers following three applications of the fungus was as efficient as the herbicide 2,4-D or mechanical removal, indicating that *C. caricis* could, in the future, be integrated into systems for management of this weed.

Phomopsis amaranthicola as a post-emergence bioherbicide in peppers (*Capsicum annuum* and *C. frutescens*) and eggplant (*Solanum melongena*)

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Pigweeds (*Amaranthus* spp.) are among the most abundant weeds occurring in vegetable crops throughout the world. Biological suppression of pigweeds is desirable in organic and/or conventional production systems in which selective chemical herbicides are lacking, limited or not efficacious. In several field experiments, the fungus *Phomopsis amaranthicola* was evaluated as a post-emergence bioherbicide to control *Amaranthus lividus* in bell pepper (*C. annuum*), and *A. dubius* in Caribbean-bonnet pepper (*C. frutescens*), and eggplant (*S. melongena*). In all experiments, the fungus was sprayed at run-off volume on the weed/crop canopy at a rate of 1.0-1.5 million conidia per ml. Pigweeds that survived inoculation with *P. amaranthicola* were allowed to interfere with the crops season-long. In eggplant and Caribbean-bonnet pepper, spraying *P. amaranthicola* 10 days after weed emergence (DAE) caused about 30% mortality in different population densities of *A. dubius*, and resulted in yield loss reductions of about 25% in pepper and 16% in eggplant, as compared to the untreated weedy crops. In the bell pepper experiments, the results were similar when using a *Psyllium* mucilloid or an agricultural oil (PCC-588) as a surfactant in the spraying mix. In bell pepper, two applications of *P. amaranthicola* (10 and 20 DAE) were more effective than one application (10, 20, 30, or 40 DAE) in suppressing *A. lividus* growth and interference with the crop. When *P. amaranthicola* was applied more than twice, improvements in pigweed control and pepper yield were negligible. Maximum weed mortality, growth suppression, and yield-loss reduction in these crops were obtained with 1 or 2 early applications of the fungus (10 DAE in eggplant and Caribbean-bonnet pepper and 10 and 20 DAE in bell pepper). Further enhancement in the efficacy of *P. amaranthicola* as a post-emergence bioherbicide may be possible through the use of improved formulations.

Assessment of *Dactylaria higginsii* as a postemergence bioherbicide for purple nutsedge (*Cyperus rotundus*) in bell pepper (*Capsicum annuum*)

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In Florida and other tropical and subtropical regions, purple nutsedge (*Cyperus rotundus* L.) is the most troublesome weed in peppers (*Capsicum* spp.) grown in soils without methyl bromide fumigation. *Dactylaria higginsii* has been previously shown to reduce purple nutsedge growth and competitive ability, but little information is available regarding how those effects translate into crop yields. Therefore, a field study was conducted to determine the effect of repeated applications of *D. higginsii* on the growth of purple nutsedge and the yield and grade of bell pepper. The results showed that weed-free bell pepper produced the highest yield, and weedy bell pepper without *D. higginsii* treatment the lowest. One application of *D. higginsii* 8 days after weed emergence (DAE) reduced purple nutsedge growth and increased overall bell pepper yield and the proportion of large and extra large fruit, as compared to untreated purple nutsedge-infested pepper. Application of *D. higginsii* (8 and 18 DAE) twice resulted in the same yield of large and medium size fruit as in the weed-free crop, although the yield of extra large ("fancy") fruit was lower than in the weed-free crop. The data indicated that to use *D. higginsii* as an effective post-emergence herbicide, its efficacy per application must be enhanced (i.e., increased fungal virulence, conidia survival, and penetration into nutsedge leaves) and/or more than two applications of this potential bioherbicide are necessary to suppress purple nutsedge interference to acceptable levels (<10% yield loss). The environmental conditions during the study were very adverse to *D. higginsii,* with low humidity and high daytime temperatures. More suppression of purple nutsedge and higher yields are likely to occur following application of *D. higginsii* under more favorable weather conditions.

Interactions of *Pyricularia setariae* with herbicides for control of green foxtail

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Under controlled environment, *Pyricularia setariae* Niskada controlled green foxtail [*Setaria viridis* (L.) Beauv.] effectively when applied at high doses. This high-dose requirement might indicate low infection efficiency or virulence. In this study, herbicides were explored as potential synergizers, and products from 6 herbicide groups recommended for the control of green foxtail were tested at 1/10 label rates with the fungus. In greenhouse studies, foxtail plants at 3- and 5-leaf growth stages were sprayed initially with herbicides, and with the fungus at a sublethal rate ($2x10^7$ spores/ml and 200 L/ha) 48 h later using a cabinet sprayer. Treated plants were given 24-h dew immediately after fungal inoculation, then kept in the greenhouse for 6 more days. Plant fresh weight taken at the end of trial was used as an indicator of weed control. In general, synergy was observed more consistently between the fungus and sethoxydim or propanil, while interactions with tralkoxydium, imazethapyr, quinclorac, glyphosate, or glufosinate were slightly more variable. Four herbicides showing more consistent interactions were tested further at 1/10, 1/4, and 1/2 label rates, respectively, with the fungus using a similar procedure. In the greenhouse, quinclorac and propanil were more efficacious and synergistic at 1/2 label rate. Sethoxydim at 1/2 label rate was highly effective and the addition of the fungus showed little benefit for weed control. While the herbicide was synergistic at 1/4 and 1/10 rates with the fungus, the weed-control efficacy was significantly higher with the 1/4 rate. On green foxtail at the 5-leaf growth stage, the fungus-sethoxydim (1/4 rate) combination reduced plant fresh weight by 90.9% when compared to non-treated controls, whereas the fungus or herbicide alone achieved 70.4% or 76.7% reduction. Imazethapyr showed only marginal synergy with the fungus, regardless the rate used. In a field trial, tank-mixing application of the fungus with sethoxydim at 1/4 label rate enhanced the control of green foxtail significantly when compared to the fungus or herbicide treatment alone.

International Mycoherbicide Programme for *Eichhornia crassipes* control in Africa (IMPECCA)

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The IMPECCA Programme was established to develop a mycoherbicide for the control of water hyacinth in Africa, using indigenous fungi. Ultimately, a mycoherbicide could replace the broad-spectrum herbicides which are routinely used for controlling this weed but which have raised environmental concerns. Moreover, a mycoherbicide could potentially be used in conjunction with existing insect biocontrol agents to improve the overall effectiveness of the control measures.

The IMPECCA programme encompasses a number of African national institutes and partners, who undertook surveys for pathogens of water hyacinth and pathogenicity testing. The surveys have shown that the weed is attacked by large numbers of generalist fungi; genera such as *Myrothecium, Alternaria, Acremonium, Fusarium* and *Rhizoctonia* being most frequently encountered. Of these, only *Alternaria eichhorniae* has shown the desirable traits of virulence and host specificity.

Isolates of *A. eichhorniae* have been tested in the laboratory and field using novel formulations to help overcome the rapid growth and considerable regenerative abilities of water hyacinth. The results support the hypothesis that unless a foliar pathogen is capable of rapidly colonising and destroying tissue, either through the production of a potent systemic toxin, or by targeting the growing point of the plant, then it will be unlikely to provide effective control. The situation is further compounded by the limited ability of A. eichhorniae to initiate new infections due to its poor natural dispersal.

Only species of *Rhizoctonia* have shown the ability to rapidly to kill the plant, but these generally lack host specificity. However, this genus warrants further research since there are recent examples where regulatory authorities have allowed the use of such agents with broader host ranges.

Other areas of potential research highlighted by this programme include the classical biological control approach. Preliminary surveys have shown a much greater diversity of fungi in the native range of the plant, including a number of biotrophic and hemibiotrophic species. Significantly, many of the cosmopolitan genera such as *Alternaria* and *Cercospora* are either absent or infrequently encountered.

Are bioherbicides compatible with organic farming systems and will businesses invest in the further development of this technology?

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The organic industry is the fastest growing sector of U.S. agriculture today. According to the most recent estimate by the U.S. Department of Agriculture, Economic Research Service, the land devoted to certified organic crop and pastureland reached 2.3 million acres in 2001. This represents approximately 0.3 percent of total U.S. farmland. In the Third Biennial National Organic Farmers' Survey, published in 1999 by the Organic Farming Research Foundation, weed control was identified as the greatest obstacle to the implementation of organic production systems and weed management was identified as the number one priority area for research. According to the U.S. National Organic Program Standards, management practices for pest control must be incorporated into a crop production plan that includes crop rotation, nutrient management, sanitation, and physical and mechanical control measures. For weed control, approaches may include the use of biodegradable mulches, mowing, hand weeding or grazing. Plastic mulch may be used for weed suppression if the mulch is removed at the end of the growing season. While plastic mulch is permitted and does provide control of many weed species, there is considerable concern about the cost of removal of plastic mulch as well as limitations related to disposal. If the documented production plan has been implemented and weed control still remains a significant limitation to production, a biological control agent, used as an inundative input, could then be applied. Any material that would be used as a carrier for the agent or as an adjuvant at application must either be a natural product or included on the National List of allowed synthetic substances. The language of the National Standard requires that the researcher interested in the development of a biological control agent for weeds in organic production be intimately familiar with the production practices implemented and the weeds that still remain a problem to control. The selection of a system to work on is then highly specialized to the regional organic production system, rather than being considered as an additional market for an existing weed biological control agent. If there is a perceived problem with non-target effects, based on a broad spectrum of activity, the agent will not be acceptable in the organic system. If an agent has the potential to interfere with host plants for beneficial insects, for example, this would not be an acceptable biocontrol agent. Regardless of whether the target market is organic or conventional, high levels of efficacy as well as the ability to produce large quantities of inoculum inexpensively are important requirements. Although the number of tools that are available to organic growers seems limited, the assumption that they will accept a biological control agent that has marginal impact is false. There is a need for this technology and targeted research that involves input from local organic growers and certifying agents can significantly increase the success of a bioherbicide.

Virulence enhancement of bioherbicides

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We report a novel methodology for virulence enhancement of fungal and bacterial pathogens for biological control of target plants. Described is a selection process to obtain phytopathogenic microorganisms that excrete selected amino acids. Pathogenicity studies demonstrate that certain specific amino acid excreting plant pathogens show much greater virulence against target plants than do corresponding wild type strains. Host range evaluations of so obtained mutants did not reveal any increase of virulence towards non-target plants. This novel approach to enhancement of microbial herbicides can be used across a broad spectrum of microbial groups to improve the efficacy of biocontrol. We describe the use of selected and biochemically marked mutants of plant pathogenic microorganisms that overproduce one or more inhibitory amino acids to enhance control of target plants.

Indigenous fungal pathogens – a potential additional tool for the management of *Rhododendron ponticum* L. in the UK

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Rhododendron ponticum L. (Ericaceae), purportedly introduced to the British Isles from the Iberian Peninsula over 200 years ago, has been a highly valued ornamental. However, within the past 40-50 years *R. ponticum* has developed into an invasive category 4 species. Areas particularly severely affected are the western parts of the British Isles, e.g. parts of Wales (Snowdonia National Park) and Scotland, Lundy island as well as Western Ireland.

Current control methods for *R. ponticum* involve mechanical and chemical treatments e.g. cutting and burning of plants, and application of herbicides. These methods are labour intensive and expensive and difficult to sustain over larger areas of infestation. Hence, biological control using indigenous fungal pathogens may be a useful additional tool in the management strategy of *R. ponticum*. Fungal pathogens naturally occurring on *R. ponticum* in the British Isles could be exploited using the inundative approach which involves mass production and formulation of the pathogen and, in the case of *R. ponticum*, application as a site selective stump treatment. This method has already been successfully implemented against woody invasives in the Netherlands, using indigenous strains of the wood-rotting basidiomycete *Chondrostereum prupureum,* and is currently being developed in other countries such as Canada and New Zealand.

A disease outbreak on *R. ponticum* in Windsor Great Park, Berkshire has been investigated and *Cryptosporiopsis* sp. was isolated from affected plant material. The potential of this pathogen as a biocontrol agent will be assessed. Further surveys will be undertaken in the UK in order to assess the distribution of this pathogen as well as to identify other potential fungal agents for the control of *R. ponticum*.

Strategies for optimisation of the biocontrol agent *Ascochyta caulina* *

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The plant pathogen, Ascochyta caulina (P. Karst) v.d. Aa & v. Kest., is a potential biological control agent of Chenopodium album L., one of the major weeds in European spring-sown crops. In spite of 60-70% weed kill during favourable conditions in early field trials, further maximisation of efficacy was desired. The major aim of our work has been to optimise the biocontrol efficacy of A. caulina using different strategies, such as searching for more aggressive isolates, optimisation of humidity conditions to ensure fungal infection and production of desiccation-tolerant fungal propagules by liquid fermentation.

Hundred-and-twenty strains were isolated from seeds, stems and leaves of *C. album* collected in different locations and habitats throughout Scandinavia, and tested for aggressiveness in greenhouse. Possible relations between aggressiveness and origin (location, habitat and plant part) were studied to ease the search for more aggressive isolates later. Several techniques to enhance the efficacy of *A. caulina* under field conditions were investigated in field trials conducted in cruciferous crops in the period 1999-2001. The most aggressive isolates were applied alone or in mixtures with low rates of compatible herbicides (10% of recommended field rate) as part of an integrated weed management strategy. In advance, the compatibility had been examined to ensure that inhibition of spore germination would not occur. In addition, a formulation with psyllium and polyvinyl alcohol, that had significantly enhanced the disease severity of *A. caulina* in earlier greenhouse studies, was examined. Treatments took place late in the evening at the 4-6 true-leaf stage of the *C. album* plants to exploit the night dew, and coverage of the treated plots was also tested as a mean to increase the dew and temperature conditions. Investigation of using rain or irrigation to extend the dew period, required for successful germination of the *A. caulina* spores, was carried out in greenhouse by exposing *C. album* plants, treated with *A. caulina* spores, to artificial rain at different intensities and intervals after inoculation. The disease severity of rain-exposed plants was compared to that of a control that received no rain-treatment.

Opposite to previous work employing pycnidiospores produced on solid medium, the potential of growing *A. caulina* in submerged cultures were studied. Liquid fermentation offers a homogenous environment that easily can be manipulated with regard to nutrients and environmental factors. By optimising the growth medium, fast production of infectious propagules with increased fitness in terms of biocontrol efficacy and stability as formulated agent, can be achieved. Initial studies with submerged cultures of *A. caulina* in various media compositions with regard to carbon-to-nitrogen ratio and carbon content, generated compact hyphal masses or microsclerotia (MS). The fungal propagules were mixed with diatomaceous earth and air-dried at room temperature to 2-5 % moisture content. The effect of the media compositions on the yield, desiccation-tolerance and stability were studied. The biocontrol efficacy of the MS preparations was evaluated in preliminary climate growth chamber studies. Percentage of emerged *C. album* seedlings in soil amended with *A. caulina* MS, was compared to that of a heat-killed control.

* Due to future publications, the results will only be presented orally.

Where did it go wrong? Why is the concept of bioherbicide suffering from limited success?

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We all felt the excitement with the success of Collego and DeVine in the late 70s and earlier 80's. This early success was followed by intensive, relatively well funded research in many counties. We gained significant knowledge in many fields including: pathogenicity, physiology, host specificity, formulation, mass production, but we have failed to deliver the final product. How many new bioherbicides have reached the market? Count them – by my count two or perhaps three: Biochon™ and StumpOut™, Camperico™ is reported to be marketed in 2003. Some herbicides have been registered and not made it to market (BioMal™, Chontrol™) due to production difficulties. Others just have not made it (Coltru™, Casst™, Velgo™). Several other bioherbicides are in government review (Smolder™, Myco-Tech™) and others will be submitted for registration soon. When will we be successful? Can we solve the formulation and production problems? Can we increase the virulence of our bioherbicides? Can we satisfactorily answer all the regulatory questions? Can we raise the capital (1 to 2 million dollars) to complete registration requirements and launch a bioherbicide product? Yes to all of the above – only if we choose the right market niche and the right organism. Presently our bioherbicides cannot compete directly with chemical herbicides. Public pressure and government action to restrict or ban the use of chemical pesticides may be the help we need.

Bio-herbicides, Bio-pesticides and their Market in Japan

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We have the second biggest pesticides market in the world (3.2 billion US$ in 2001). There are 30 kinds of commercial bio-pesticides available, including natural enemies. Only one bio-herbicide, which is for annual bluegrass (*Poa annua*) control in turf field, has been commercialized. The second expected bio-herbicide, which is for barnyard grass (*Echinocloa* sp.) control in paddy field, is under work for registration. The market of bio-pesticides is 11 million US$, 0.3 % of the total pesticides market in Japan. Moreover, sales of each bio-pesticide are small compared to chemical pesticides. Recently consumers tend to prefer organic agricultural products because of their safety so that some farmers (pesticides consumers) are trying to use organic growing methods because the harvested products provide a higher income. The Japanese standard for producing organic agricultural products was published in 1999. By that standard, the use of bio-pesticides has been authorized as pest control agents for organics. Several bio-pesticide candidates are being applied into official field trials now, but there are no bio-herbicide candidates as of yet.

List of abstracts