



Xth International Bioherbicide Group Workshop

WAIKOLOA BEACH MARRIOTT
RESORT & SPA

Waikoloa, Hawai'i, USA
September 10, 2011

IBG supported by:



Preface

Introduction

Welcome to the Xth Workshop of the International Bioherbicide Group (IBG) at the Waikoloa Beach Marriott Resort & Spa in Waikoloa, Hawai'i, September 10, 2011. IBG meets every two years usually as a satellite workshop to a discipline-related international meeting. This year we are meeting in conjunction with the International Symposium on Biological Control of Weeds. IBG provides a forum for communication and collaboration among scientists from governments, universities, and industry who are interested in using microorganisms and their microbial metabolites for the development of bioherbicides and the implementation of inundative weed management strategies. IBG puts out a web-based newsletter 1-2X per year to keep colleagues informed about new weed problems and new research advances. To subscribe to the IBG email list go to <http://ibg.ba.cnr.it/>.

This document is the proceedings of the conference and contains a list of workshop participants, workshop program, the abstracts of the oral presentation, and sponsors of the meeting. It will be placed on the IBG website after the meeting. With the exception of minor grammatical and formatting corrections, the content of the abstracts submitted by the authors has been unaltered.

On behalf of all participants, our gratitude goes to Stuart Falk from The Scotts Company and Yaowei Kang from Novozymes for their generous sponsorship to this meeting. They have made it possible for us to get together and share our ideas and experiences in a relaxed informal setting. We also thank the speakers who stepped forward and volunteered presentations to give us an interesting program for the day. Lastly, we thank Maurizio Vurro, our dedicated colleague and volunteer, who maintains the website and prepares the IBG Newsletter for everyone to enjoy.

Karen Bailey
Chairperson of IBG

Sponsors of the Xth IBG Workshop



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IBG Workshop Agenda

Time	Location	Activity	Speaker	Title
8:30 – 8:45	Naupaka I Ballroom	Registration		
8:45 – 9:00		Welcome	Karen Bailey	Plan for the day
9:00 – 9:30		Session 1	Sue Boyetchko	The Biopesticide Innovation Chain: How can we structure biopesticide development from a research perspective?
9:30 – 10:00			Gavin Ash	Using genomics to advance biopesticide development
10:00 – 10:30	Naupaka Lanai	Refreshments		
10:30 - 11:00	Naupaka I Ballroom	Session 2	Graeme Bourdot	Developing a bioherbicide for <i>Cirsium arvense</i> – an international collaboration between New Zealand and Canada
11:00 – 11:30			Louise Morin	Unsuccessful weed biocontrol initiatives: lessons learned
11:30- 12:00		IBG Business Meeting	Karen Bailey	Revitalization of IBG – is it still useful? Next meeting location and coordinator
12:00 – 13:30	Naupaka Lanai	Lunch		
13:30 – 13:50	Naupaka I Ballroom	Session 3	Russ Hynes	Improvements to granular formulations for the delivery of bioherbicides
13:50 – 14:10			Shen Qiang	<i>Sclerotium rolfsii</i> strain SC64 isolated from Canadian goldenrod (<i>Solidago canadensis</i>), a biological control agent for a potential mycoherbicide
14:10 – 14:30			Angela Post	Biological control of silvery threadmoss (<i>Bryum argenteum</i>) on golf course putting greens
14:30 – 14:50			Robert Barreto	Title coming
14:50 – 15:30	Naupaka Lanai	Refreshments		
15:30 – 15:50	Naupaka I Ballroom	Session 3 cont'd	Marion Seier	Tracking the origins of White Tip disease of <i>Cirsium arvense</i>
15:50 – 16:10			Karen Bailey	Infection of dandelion (<i>Taraxacum officinale</i>) by the bioherbicide <i>Phoma macrostoma</i>
16:10 – 16:30			Stan Bellgard	Innulative approaches to pampas grass control
16:30 – 17:00		Open Discussion and Brainstorming on Innulative Weed Control Issues		
17:00		Adjourn		

The Biopesticide Innovation Chain: How Can We Structure Biopesticide Development from a Research Perspective

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Biopesticide technology has emerged as a viable and environmentally-friendly pest management tool in agriculture. However, the process of developing biopesticides involves a series of critical steps to take it from the theoretical to reality. Although simplistic at the onset when at the early discovery phase where the search and screening of potential candidates has begun, the R&D process can be daunting and convoluted. We offer a model we call the Biopesticide Innovation Chain which describes a stepwise process that takes us through a journey of early discovery of a biopesticide candidate while considering social and economic issues in order to make informed decisions. It also encompasses the utilization of platform technologies such as fermentation, formulation, and application technology and progresses into relatively unfamiliar territory of industry scale-up, regulations, commercialization and finally towards adoption. Working as a cohesive, multidisciplinary team, AAFC scientists take advantage of our collective expertise and utilization of our AAFC federal lab infrastructure, while building partnerships with other AAFC scientists in complementary disciplines, universities, and industry to develop products following the Biopesticide Innovation Chain. The AAFC R&D model is unique and an excellent strategy for delivery of new biopesticide products. The challenges that accompany the process of developing a biopesticide are highlighted with examples that are being utilized by AAFC scientists with this novel R&D model.

Using genomics to advance biopesticide development

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It can be argued that the success of biopesticides could be increased through greater information about the pathogenic agent, the host and their interaction. New genomic sequencing technologies are becoming cheaper and more accessible and can generate massive amounts of data in a relatively short time period. For example, in 2010, Life Technologies released its new Ion Torrent sequencer, which can be used to generate preliminary sequence information of small organisms in hours. Genomic approaches to biopesticide research for the management of molluscs, insects and plants will be given.

Developing a bioherbicide for *Cirsium arvense* – an international collaboration between New Zealand and Canada

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Cirsium arvense (L.) Scop. is an agricultural weed of economic significance in the temperate zones of both the Northern and Southern Hemispheres. The failure to date of classical biological control using various phytophagous insects (deployed in Canada, US and New Zealand) has prompted research into the potential of naturally-occurring pathogens as bio-herbicides. However, none of the pathogens considered to date (in the fungal genera *Alternaria*, *Ascochyta*, *Colletotrichum*, *Erysiphe*, *Fusarium*, *Phoma*, *Stagonospora*, *Verticillium*, *Phyllosticta*, *Puccinia*, *Sclerotinia*, *Septoria*, *Phomopsis*, sterile fungi, the bacterium *Pseudomonas syringae* pv. *tagetis*, mycoplasma-like organisms and the 16SrIII-B subgroup phytoplasma) have emerged from the 9-link “bioherbicide innovation chain” (Bailey and Falk, 2011. Pest Technology 5 (Special Issue 1): 73-79) as a commercially viable product.

Against this background, and with the ecological knowledge that population size in *C. arvense* in a pasture is linearly related to over-wintered root mass (Bourdôt et al., 1998), the New Zealand pastoral industry funded a national field survey looking for fungal pathogens that impact on root growth. Undertaken during 2005-06 and 2006-07, the survey discovered a plant-pathogenic fungus (identity confidential pending patenting), hitherto not been reported from *C. arvense*, to be relatively common in root, stem and leaf tissues of the thistle. Preliminary research by AgResearch in New Zealand revealed that its spores are readily produced in culture and rapidly result in disease when applied in water to the weed's foliage in the glasshouse and in the field. Having completed the “discovery” and “proof-of-concept” stages (Stages 1 and 2 of the bioherbicide innovation chain), “technology development” was begun in August 2009 via a formal collaboration between AgResearch and Agriculture & Agri-Food Canada.

In this paper we present the progress made to date through the “discovery”, “proof-of-concept” and “technology-development” stages of this international collaboration.

Unsuccessful weed biocontrol initiatives; lessons learned!

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This presentation was first presented at the 1st Bio-Protection Symposium on Managing Pests: The future of biocontrol on August 31, 2011, Lincoln University, New Zealand.

Abstract

As around 40% of pesticides used worldwide are herbicides, there is a major impetus to develop alternative, more environmentally-friendly control methods for agricultural weeds. For more than three decades, there has been consistent and primarily academic research into the development of plant pathogens as bioherbicides. However, only a few products have been successfully commercialised.

Key hurdles encountered in bringing bioherbicides to market have included: the inability of the pathogen to kill or severely damage the target weed; market potential too small to justify the large R&D investment required; technical challenges hindering the development of cost-effective production and formulation systems; and unreliable field efficacy.

Lessons learned over the years, and being taken on board by today's researchers, include:

- An early market analysis to gauge potential demand for a bioherbicide for a particular target should go hand in hand with the initial discovery and proof-of-concept stage.
- Pending a virulent candidate pathogen is identified and commercial feasibility is a viable proposition, the proof-of-concept stage should focus on addressing key issues pertaining to the future registration of the bioherbicide to streamline subsequent product development.
- The involvement of an industry partner with the appropriate expertise and interest in developing biological products is highly recommended to advance to the product development stage.
- A team comprising expertise from various scientific and business disciplines can make all the difference in solving technical and commercialisation problems faced along the way.

Finally, the focus on highly host-specific foliar fungal pathogens for bioherbicide development is increasingly being challenged, with the most recent bioherbicides registered or pending registration based on viruses or fungi capable of infecting a range of weeds when applied to the soil.

Improvements to granular formulations for the delivery of bioherbicides

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Granular formulations were developed for *Phoma macrostoma* and *Pseudomonas fluorescens* BRG100, bioherbicides for broad leaf and grass weeds. The effect of i) formulation water activity (a_w) on survival of *P. fluorescens* BRG100 and, ii) starch amendments on disintegration and dispersal of bioherbicides from granular formulations were investigated. The long-term refrigerated storage stability of *P. fluorescens* BRG100 was examined in pesta granules dried to different a_w , 0.3, 0.5 and 0.8. Drying pesta to 0.3 a_w stabilized the population of *P. fluorescens* BRG100 for 16 months at $8.5 \log_{10}$ cfu/g. When pesta was dried to 0.8 a_w , *P. fluorescens* BRG100 population decreased to $7.3 \log_{10}$ cfu/g over six months. The impact of starch addition (corn, pea, rice and potato) and concentration (13% and 26%, wt/wt) to formulated granules containing *P. macrostoma* and *P. fluorescens* BRG100 on the disintegration rate of the granules was determined with laser diffractometry. The order of fast to slow disintegration following starch amendment was pea>potato>corn> rice. Increasing pea, potato and corn starch content from 13 to 26% promoted faster disintegration of the granules, conversely, increasing rice starch content decreased disintegration. For example, half-life disintegration profiles were determined with pea starch amended pesta (26% w/w) being most rapid, 0.8 minute, rice starch (26% w/w) amended pesta was slowest, 4 minutes and non-amended pesta, 2.5 minutes. The ability to produce granular formulations with different disintegration and bioherbicide release characteristics provides the formulator with the potential to custom make the formulation to the specifications of the active ingredient is delivered to the pest when it is most susceptible.

***Sclerotium rolfsii* strain SC64 isolated from Canadian goldenrod (*Solidago canadensis*), a biological control agent for a potential mycoherbicide**

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A strain SC64, identified as *Sclerotium rolfsii* based on morphological characters, was isolated from damaged plants with a destructive stem rot disease symptom sporadically occurred among an invasive alien Canadian goldenrod (*Solidago canadensis* L.) populations in Nanjing city, Jiangsu province of China. ITS sequence (684bp) analysis through initial amplifying from the pathogen with the universal primers ITS1 and ITS4, and comparing to those of related species acquired from GenBank database further confirmed the identification of this strain. The biological characteristics were studied on different media and mass production was developed. It had a broad host-range mainly on most of broad-leaved weeds and a few monocotyledon weeds, but not for turf grass and grassy crops. Biocontrol bioassays showed that isolate SC64 caused 70.5-87.7% mortality rate and 78.7-91.6% fresh weight reduction under turfgrass, wet direct seeding rice and invasive alien composite weeds. *S. rolfsii*, the first recognized causal agent of stem rot on Canadian goldenrod in China, may be a potential to be developed into a mycoherbicide for this weed and other broadleaf weeds.

Biological control of silvery threadmoss (*Bryum argenteum*) on golf course putting greens

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Silvery threadmoss (*Bryum argenteum*) has become an increasingly problematic weed of golf courses, particularly since the loss of mercury and other heavy metal based pesticides. Though not labelled for moss control, they were used extensively on golf course putting greens as fungicides and at the same time controlled moss. To meet golfer demand for firmer, faster playing surfaces, superintendents have decreased mowing heights, requiring increased passes of equipment over the green. This, along with decreased nutrient inputs and an open turf canopy contributes to moss encroachment on putting greens. Currently, few labelled products exist for moss control driving turf managers to use off-label substances including peroxides, baking soda, and detergents. These desiccate moss and may severely injure turfgrass even with careful applications. Hand removal of moss is also a common practice. The only commercial herbicide labelled for control is carfentrazone applied at 6.7fl oz/A, which does not completely eradicate moss, so sequential applications are required once moss recovers. Aside from turf, silvery threadmoss can also be a weed problem of containerized nursery crops as well as nursery growth pads and stone hardscapes. With no professional products labelled for moss control in these systems there are several potential niche markets for an effective biological control of silvery threadmoss.

A naturally occurring microorganism has been discovered that effectively controls moss on putting greens without causing injury to the most commonly managed turf species, creeping bentgrass and annual bluegrass. We are evaluating this organism for all three niche markets. Testing includes fulfillment of Koch's postulates, pathogen characterization to determine the site of action on silvery threadmoss and evaluation of host specificity in *Bryum* and related genera. Studies conducted this season will evaluate non-target effects on desirable plant species in the turfgrass and nursery industries and naturally occurring mosses in the landscape.

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Tracking the Origins of White Tip Disease of *Cirsium arvense*

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Cirsium arvense - commonly known as creeping thistle in its indigenous range in the UK, and Canada thistle in its invasive range in North America - was first reported to be affected by a distinctive disease associated with chlorosis or bleaching of the shoots in Canada in 1994. This white tip disease was found to be caused by *Phoma macrostoma* - characterised by pycnidia with wide ostioles and red pigment in the vegetative hyphae - which, typically, is a weak fungal pathogen of woody plant species. The white tip isolates can readily be distinguished by their genetic profiles and the ability to produce unique phytotoxins (Macrocidins A & B). One of these Canadian isolates (94-44B) forms the basis of a bioherbicide targeted at weeds of turf grass, which will soon be marketed in North America. Recent efforts to trial the product in the UK have been problematic, based on the assumption that it incorporates an alien fungus. However, unpublished data indicated that the disease is already present in the UK and it was posited, therefore, that the causal agent is an indigenous pathogen of *Cirsium arvense* which was introduced accidentally with its exotic host into North America. Here, we report on the evidence obtained from surveys in the UK, and from subsequent pathogenicity and phylogenetic studies in Canada, which offers proof of this hypothesis. *Phoma macrostoma* was consistently isolated from *Cirsium arvense* showing characteristic symptoms of white tip disease in the UK. The disease appears to be restricted to the eastern and southern counties of England. Most isolates of *Phoma macrostoma* from the UK were found to have the same bioherbicidal activity and a similar genetic make-up as the Canadian isolate 94-44B. Isolates of *Phoma macrostoma* from white tip disease of *Cirsium arvense* in Canada and the UK occupy a unique clade: phylogenetically distinct, but morphologically indistinguishable from the type of *Phoma macrostoma*.

Infection of dandelion (*Taraxacum officinale*) by the bioherbicide *Phoma macrostoma*

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The fungal species *Phoma macrostoma* (Montagne) was isolated from *Cirsium arvense* (L.) Scop.) collected from roadsides and ditches in several provinces of Canada. Isolates demonstrated the ability to cause photobleaching of leaves and root inhibition to many broadleaved weeds in the family Asteraceae when applied to the soil as a broadcast granular formulation. Previous research demonstrated that symptoms are caused by phytotoxic metabolites called macrocidins. In nature, *P. macrostoma* is reported to be a weak, wound pathogen or endophyte and does not compete very effectively with other fungi in the environment. To better understand the bioherbicide's role in a soil environment, a histopathology study was conducted to observe how the fungus emerges from the granule and infects a susceptible weed like dandelion (*Taraxacum officinale* Weber ex F.H. Wigg.). Mature dandelion plants were inoculated with granules containing *P. macrostoma*. After 7 and 28 days, roots were cleaned and fixed in FAA prior to polyclonal antibody labeling, root sectioning and staining with Pianese IIIB. Microscopic examinations showed growth of mycelium from granules was directed towards whole dandelion roots. Entering via the root hairs, the fungus moved intercellular, without penetrating plant cells, from the surface to the root interior. Mycelium proliferated in the central core and by the vascular trachea of the root. Mycelium did not penetrate the tracheal core. The exterior root cell structure remained intact but the interior cell structure collapsed in the presence of the dense fungal mycelium. A photographic series will be presented to support the interpretation that *P. macrostoma* passively enters the weed root and releases macrocidins which are taken up by the vascular system causing photobleaching and disrupts internal root cell structure causing root inhibition.

Natural enemies of pampas *Cortaderia selloana* and *C. jubata* –survey results from New Zealand (naturalised-range) and Argentina (home-range)

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The South American pampas grasses *Cortaderia selloana* and *C. jubata* are serious weeds in New Zealand: especially in pine plantations and indigenous plant communities where they successfully invade bare ground (including cliffs). A national survey of invertebrate fauna and microbes associated with pampas grass in New Zealand, was carried out between September 2008 and February 2010. No specialised pampas grass invertebrates were found during the survey. Plant pathogens found included a range of previously recorded, generalist grass pathogens (Table 1 on next page). No specialist pampas pathogens were recovered. A preliminary survey for potential biological control agents for pampas grass has been undertaken in Buenos Aires, Argentina. A very similar suite of plant pathogens was recovered from this brief Argentinean survey. We have ranked the pathogens found to date in terms of their biocontrol potential in the following matrix Table 1. More comprehensive surveys for potential biocontrol agents will be undertaken in South America pending molecular studies currently underway to try to find areas with pampas that is a good match for New Zealand host-plant material.

Natural enemies of pampas *Cortaderia selloana* and *C. jubata* –survey results from New Zealand (naturalised-range) and Argentina (home-range) abstract cont'd.

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Table 1. List of pathogens found on pamapas grass.

Plant pathogen	Classical potential (Score out of 10)
<i>Alternaria alternata</i> (Fr.) Keissl.	0 (not host specific)
<i>Arthrrium sacchari</i> (Speg.)	0 (not host specific)
<i>Ascochyta pinodes</i> L.K. Jones	0 (would damage pea-crops)
<i>Atracidymella muscivora</i> M.L. Davey & R.S. Currah	0 (NZ has some very special mosses; not a good idea to introduce a moss pathogen)
<i>Aureobasidium</i> Viola and Boyer (2 spp.)	0 (unlikely to be host specific)
<i>Claviceps</i> sp. Frederickson, Mantle & de Milliano	5 (could be host specific, may need to move it around to get it started, or to find a strain in South America that is more damaging than the one we have)
<i>Epicoccum purpurascens</i> Ehrenb.	0 (not pathogenic)
<i>Fusarium</i> Link. (3 spp.)	0 (<i>Fusarium</i> spp. have not been used as classical biocontrol agents to-date. Two potential obstacles = slow spread (via water splash) and production of mammalian toxins)
<i>Leptosphaeria coniothyrium</i> (Fuckel) Sacc.	0 (unpopular with commercial berry growers)
<i>Leptosphaerulina chartarum</i> Cec. Roux	0 (host range too broad)
<i>Magnaporthe grisea</i> (T.T. Hebert) M.E. Barr	0 (attacks native toetoe <i>Austroderia</i> spp.)
<i>Microdochium phragmitis</i> Syd.	0 (not host specific)
<i>Neofusicoccum australe</i> Slippers, Crous, & M.J. Wingf.	0 (host range too broad)
<i>Nigrospora</i> Zimm. (2 spp.)	0 (host range too broad)
<i>Paraphaeosphaeria michotii</i> (Westend) O.E. Erikss. (5 spp.)	0 (host range too broad and damage too little)
<i>Phoma</i> Desm. (6 spp.)	0 (host range probably too broad, would be difficult to identify and damage unlikely to be sufficient)
<i>Pyrenophora semeniperda</i> (Brittleb. & D.B. Adam) Shoemaker	0 (host range too broad, already widespread here in NZ)
<i>Stagonospora</i> Sacc. sp.	0 (not damaging enough)

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