INTERNATIONAL BIOHERBICIDE GROUP

IBG NEWS

June 2009

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CHAIRMAN'S COMMENTS

Hello everyone. I would like to introduce myself as the new chairperson for our IBG group. I work as a scientist with Agriculture and Agri-Food Canada in Saskatoon, Saskatchewan, Canada. Since 1994, I have been developing bioherbicides for control of broadleaved weeds like Canada thistle and dandelion. I particularly enjoy exploring new aspects of the organism's biology and fermentation/formulation processes that influence its field performance. At our research institution we have a large group working on a number of aspects related to the development of bioherbicides and other biopesticides, to not only develop specific products but to also develop platform technologies that can be applied to many situations. Many of you may already know my cohorts, Sue Boyetchko, Gary Peng and Russ Hynes.

On behalf of IBG members I would like to express our appreciation to Joe Neal, Bill Bruckart, and John Lydon for organizing the IX International Bioherbicide Group Workshop in Orlando Florida. There was a great turn out for the presentations and everyone enjoyed the getting out into the Florida sunshine to see biocontrol sites and the local scenery. I believe there were about 45 people from 12 countries attending.

For those who were not able to attend, the business meeting had discussion on 3 issues. First, Maurizio has agreed to stay on as the newsletter editor and we greatly appreciate his willingness to continue. BUT, all members to need to help and send him short submission to keep our communications alive and vibrant. The newsletter goes out to about 200 people, so please take 10 minutes twice a year to make a contribution.

Secondly, the website is looking outdated and need to be upgraded. We need to raise about $5,000 to do this. I will initiate a fund raising campaign in September, so if any one has any potential sponsors in mind, send me their contact information and I will send them a letter.

Thirdly, we are planning where and when the next IBG meeting will be held. Some possibilities included 2011 in Hawaii with the International Biocontrol of Weed Symposium, 2012 in China, or 2010 in Brazil. The vote taken was for 2011 in Hawaii. However, there was small attendance at the business meeting and some later comments reflected that the decision may not be representative of the larger membership. So, I will be compiling a list of potential meeting places and then put the decision to the membership via an email response. So, if you know of suitable conferences being held in your area and want to include IBG as complimentary meeting, please let me know about them for the list.

I am looking forward to my term as Chairperson for this group and talking to you about our interests in bioherbicides.

Regards,

Karen Bailey
(e-mail: Karen.Bailey@agr.gc.ca)

THESIS

Assessment of the biocontrol agent *Fusarium oxysporum* for controlling *Orobanche ramosa* in tobacco fields

PhD study of Eva Kohlschmid at the University of Hohenheim, Agroecology in the Tropics and Subtropics, Prof. J. Sauerborn.
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SUMMARY

The obligate root parasite *Orobanche ramosa* L. (branched broomrape) occurs in many parts of the world. Branched broomrape has a broad host range and causes serious damage to economically important crops. It is becoming an increasing problem in oilseed rape (*Brassica napus* L.) in France, while in Germany it mainly infests tobacco (*Nicotiana tabacum* L.) and hemp (*Cannabis sativa* L.). In the last decade broomrape infestation has extremely increased in German tobacco growing areas. Application of conventional control methods against *Orobanche* L. species is limited due to their complex biology, *i.e.* the plant reproduces by mean of tiny and long-living seeds, its very close affiliation with the host plant, and the fact that the plants can be hardly detected before they have irreversibly damaged the crop. Management strategies should focus on reducing the soil seed-bank and interfere with the parasite's early development stages. In this context, the idea of using fungal pathogens - especially of the genus *Fusarium* – as biocontrol agents against broomrapes has a long history. The application of a soil borne phytopathogenic fungus has several advantages: it can be very host-specific and able to destroy also the underground stages of parasitic plants, including the seeds, which can contribute to decrease the soil seed-bank every year.

The objectives of the present study were to investigate the impact of a novel *Fusarium oxysporum* (FOG) isolate for controlling *O. ramosa* in tobacco fields, with regard to its efficacy and host specificity under controlled environmental conditions. Subsequently, biocontrol efficacy was assessed under field conditions in Germany, using different formulation and application techniques. RAPD (Random Amplification of Polymorphic DNA) marker-based assay was conducted in order to identify the biocontrol agent from other *F. oxysporum* strains, to verify the utilised monitoring methods based on morphological criteria and to obtain information about the potential spread of the biocontrol agent into the environment.

The fungus affected all developmental stages of the parasite. Already *Orobanche* seed germination was significantly reduced by 40% in the presence of fungal conidia *in vitro*. The number of underground developmental stages of the weed was reduced by 55% in root chambers compared to the non-treated control. In pot experiments, soil application of a granular formulation of the fungus resulted in a reduction of number and biomass (DM) of *Orobanche* shoots by more than 90%. Spraying of a conidial suspension on aboveground *Orobanche* shoots caused the death of 75% of them within two weeks. In greenhouse experiments, the *Fusarium* treatment combined with the resistance-inducer BTH resulted in the lowest rate, in number and biomass (DM), of emerged *Orobanche* shoots. Data from initial host-range experiments indicate that the isolate is very host-specific, not even attacking shoots of other *Orobanche* species.

Under field conditions, FOG reduced number and biomass (DM) of *Orobanche* shoots between 50% and 70% in three consecutive years (2006–2008) and decreased seed production from emerged shoots in fungus-treated plots. However, in contrast to the results of the greenhouse experiments, no further reduction could be observed when the biocontrol agent was combined with the resistance inducer BTH in the field. Accompanying greenhouse studies revealed some fungistatic effects of the field soil which partly explain the reduced efficacy (-40%) in the field compared to results obtained under controlled conditions. Also climatic conditions seemed to have a great influence in this regard. Laboratory experiments showed a better fungal survival rate under comparatively dry

soil conditions (20% of water saturation) and a temperature optimum between 25-28°C. This could be an explanation for the higher control efficacy of the pesta treatment in the first-year field experiment, when climatic conditions correlated best with optimal growth conditions for FOG.
In-furrow application and broadcasting of the inoculum after tobacco planting as well as pre-planting subsoil application decreased the number of emerged *Orobanche* shoots, but did not differ among each other at the end of the experiment. Broadcasting was more effective at the beginning of the field trial. The application of pesta granules did not show consistent results throughout the seasons, meaning that the 50% reduction of *Orobanche* biomass (DM) in the first year could not be repeated in the following years (20-30%). An alginate formulation introduced in the second year performed better than the pesta formulation. However, the combination of pesta granules with alginate pellets had the highest reliable control efficacy (60-70%) of all treatments in two seasons compared to the untreated control. FOG populations declined strongly (70-90%) over a three months period and fungal population counts in soil samples did not show a close correlation to the actual biocontrol efficacy. Tobacco yield was not increased in the first year, but tobacco biomass (DM) was significantly higher in most of the fungal treatments in 2007.
FOG revealed fragment patterns clearly differentiable from another *Fusarium oxysporum* strain (pathogenic to *Orobanche cumana*) when tested with 20 RAPD primers. The RAPD technique was further applied to compare the original FOG isolate with 45 *Fusarium* isolates collected from necrotic *Orobanche* shoots and soil samples obtained from fungus-treated and untreated plots in the field experiments, as well as from a field in 10 m distance. Thirty isolates showed the same fingerprints as the original FOG isolate. All isolates gained from soil of fungus-treated plots were genetically identical to FOG, whereas in soil samples of the control plots, FOG could be only detected to a very limited extent. All isolates collected from plants or soil of the neighbour field were clearly differentiated from the biocontrol agent. Results verified the population counts obtained with the dilution plate method and indicate that the biocontrol agent has only a limited mobility and persistence in soil. However, since seven out of eight *Fusarium* isolates from *Orobanche* shoots from control plots were 100% identical with FOG, a short-distance aboveground spread seems to occur, possibly by rain-splash or human/animal activity.
As a conclusion, it can be stated that FOG has a potential for controlling *O. ramosa*. Moreover, the fungus has the ability to reduce the parasite's seed production. Since it became obvious that abiotic and biotic conditions as well as its formulation and application have a great impact on the performance of the fungus, future research should try to better understand the interactions in that complex system, in order to be able to optimise and stabilise biocontrol efficacy.

PEOPLE & PLACES

Dr. Russell Hynes is presenting the following paper at the annual Canadian Society of Microbiologists meeting in Montreal, Quebec in June 2009.
(e-mail: Russell.Hynes@AGR.GC.CA)

Formulation development for bioherbicides *Phoma macrostoma* and *Pseudomonas fluorescens*

Russell K. Hynes, Karen L. Bailey, Susan M. Boyetchko • Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, CANADA S7N 0X2

Abstract: The effect of fluidized bed drying duration on *Pseudomonas fluorescens* strains BRG100 and FW02 survival was examined in a granular formulation, pesta. Reducing the water activity (A_w) of pesta from 0.9 to 0.3 stabilized the population of both strains. The population of *P. fluorescens* in

pesta dried to an A_w of 0.9 decreased from about 9 $\log_{10}$ cfu/g to 7.3 $\log_{10}$ cfu/g over six months, whereas, at A_w of 0.3 the population remained at 8.5 $\log_{10}$ cfu/g over six months. Protocols were developed for granule disintegration by particle sizing measurements using a Mastersizer 2000 and dispersion by migration studies with granulated bioherbicidal microorganisms in a laboratory soil/sand system. Disintegration of granular formulations of *P. macrostoma* and *P. fluorescens* were studied by determining the effect of incorporating various amounts of sodium, potassium and magnesium salts, sugars, starches, emulsifiers, and Metasperse (ICI Corp.). Conclusions from these studies indicated that salts, sugars and emulsifiers had very little effect of granule disintegration, however, starch source, such as, corn, pea, rice or potato, and concentration significantly modified the disintegration rate of the granules and concomitant dispersion of the microorganisms from the granules. The population of a green fluorescent protein transformed isolate of *P. fluorescens* BRG100 was used to monitor dispersion of this microorganism in the soil columns.

NEW ARRIVALS IN THE COMMUNITY

Georgina Lucy Barton is born on May 23rd and weighed 3.5 kg (7 lbs 13 oz).
We wish her all the best
(e-mail: jane.barton@ihug.co.nz)

BIOHERBICIDE RESEARCH

Biocontrol of Californian thistle (*Cirsium arvense*) by mowing in the rain in New Zealand

This project, led by Agresearch plant pathologist Dr Bob Skipp and funded by Meat & Wool New Zealand, began with a country-wide survey of 150 farms in 2005-6 to identify pathogens occurring naturally on Californian thistle that may have potential as biocontrol agents. The roots and shoots of diseased plants were sampled in the field and sent to Bob's laboratory where isolations from

these tissues yielded a variety of bacterial and fungal organisms. One of the fungal pathogens of interest that was isolated from the thistle roots from about half of the farms was *Verticillium dahliae*. The spores of *V. dahliae* are dispersed by rain-splash and this led us to speculate that it may be the causal agent responsible for farmer observations that mowing Californian thistle in the rain can lead to its demise. To test this idea we revisited some of the surveyed farms in autumn 2008 to seek the cooperation of pastoral farmers in a field experiment.

We asked these farmers to mow a patch of the thistle in the rain and another patch during dry weather, while a third patch was left un-mown. Estimates of the ground cover of the thistle, and samples of stems and roots, were taken from within the patches before and after the mowing in the autumn and again in the following spring of 2008. The experiment was repeated in new thistle patches on those same farms in summer 2008. The final results from the summer mowing will be known later in 2009 when we will be able to test the hypothesis that mowing in the rain brings about the demise of the thistle and that *V. dahliae* plays a role in this.

The AgResearch team are also investigating the biocontrol potential of some other fungi and bacteria that were found during the survey. One of the fungi is of particular interest because its spores are easily cultured, and they can infect the thistle without added nutrients, which makes it an attractive prospect as a mycoherbicide.

Contacts:
Bob Skipp: bob.skipp@agresearch.co.nz
Graeme Bourdôt: graeme.bourdot@agresearch.co.nz
Geoff Hurrell: geoff.hurrell@agresearch.co.nz

Californian thistle plant infected by *Verticillium dahliae*

Farmer mowing Californian thistle in the rain at Fairlie (May 2008)

Plot of Californian thistle being mown in the rain at Lincoln (Dec 2008)

Development of a pellet-based mycoherbicide *Plectosporium alismatis*

PhD student, Kashif Zeeshan, is currently developing a pellet-based mycoherbicide in liquid culture in the laboratory LUBEM, UBO, France under the supervision of Dr. Sophie Cliquet and Dr. Denis de la Broise. His work is on the fungus, *Plectosporium alismatis* which attacks Alismataceous weeds of rice. *P. alismatis* produces conidia and chlamydospores on artificial media. In certain liquid media, we observed that *P. alismatis* had a tendency to produce aggregates (clumps) which are a complex structure, consisting of conidia, chlamydospores and hyphae. These aggregates were heterogeneous in size and were not suitable to be used directly. We, recently, develop a new medium, named as "Aggregate Production Medium (APM)", which promotes the homogeneous production of pellets (spherical forms). These pellets, containing conidia, chlamydospores and hyphae, seems promising to develop a suitable mycoherbicide in combination with other herbicides and/or fertilizers. Different mycoherbicidal formulations based on these pellets were prepared in our lab and their survival studies are under process. Recently, Mr. Zeeshan got the studentship for going and working in Charles Sturt University (CSU), Australia with Dr. Ash. The study work will be on the use of novel technique of micro-encapsulation in order to develop a mycoherbicide based on these pellet-based cultures.

(e-mail: kashifalvi212@hotmail.com)

RECENT ARTICLES

Cipriani M.G., Stea G., Moretti A., Altomare C., Mulè G., Vurro M., 2009. Development of a PCR-based assay for the detection of *Fusarium oxysporum* strain FT2, a potential mycoherbicide of *Orobanche ramosa*. *Biological Control*, 50: 68-74.

Elzein, A., F. Brändle, G. Cadisch, J. Kroschel, P. Marley, M. Thines, 2008. *Fusarium oxysporum* strains as Potential *Striga* Mycoherbicides": Molecular Characterization and Evidence for a New *forma specialis. The Open Mycology Journal*, 2: 89-93.

Elzein, A., J. Kroschel, G. Cadisch, 2008. Efficacy of Pesta granular formulation of *Striga*-*mycoherbicide Fusarium oxysporum* Foxy 2 after 5-year of storage: step towards practical *Striga* control in Africa. *Journal of Plant Diseases and Protection*, 115 (6): 259-262.

Elzein, A., J. Kroschel, P. Marley, & G. Cadisch, 2009. Does vacuum-packaging atmosphere or co-delivered amendments enhance shelf-life of *Striga*-mycoherbicidal products containing *Fusarium oxysporum* f.sp. s*trigae* during storage? *Biocontrol Science and Technology*, 119 (4): 349-367.

Martínez Jiménez M., Sandoval Villasana A.M., 2009. Evaluation of toxicity of *Cercospora piaropi* in a mycoherbicide formulation by using bacterial bioluminescence and the Ames mutagenicity test. *Mycopathologia*, 167: 203-208

Vurro M., Boari A., Evidente A., Andolfi A., Zermane N., 2009. Natural metabolites for parasitic weed management. *Plant Management Science*, 65 (5): 566-571.

IBG WORKSHOP - ORLANDO

Sue Boyetchko (e-mail: Sue.Boyetchko@AGR.GC.CA)

EDITOR'S CORNER

Dear All,

Thanks for the contribution received for the preparation of this issue of the bulletin.

In particular, please let me thank:

- Karen Bailey
- Jane Barton
- Susan Boyetchko
- Abuelgasim Elzein
- Geoff Hurrell
- Russell Hynes
- Eva Kohlschmid
- Maricela Martínez Jiménez
- Kashif Zeeshan

Despite the quite low number of contributions, also this time I have prepared this issue of the newsletter, always hoping that in this way and with this obstinacy, the future issues will contain much more contributions and information.

Please remind that this bulletin is prepared on a voluntary basis and it contains only the information sent by the newsletter subscribers, under their responsibility. The newsletter is not an official journal and cannot be considered exhaustive. Please also remind that the mailing list can be used as a moderated list for distributing information related to weed biocontrol at any time during the year.

Please feel free to deliver the newsletter to any person that could be interested in it, or invite him/her to subscribe the mailing list.

Thanks

Regards

Maurizio