

WORKSHOP

Biocontrol of Weeds with Pathogens

Saturday 1 February 2003

Venue: Fitzgerald Room
Canterbury Agriculture & Science Centre
Lincoln
New Zealand



Photo: *Ranunculus acris* one week after treatment with *Sclerotinia sclerotiorum*

The purpose of this workshop was to discuss international progress and constraints in the deployment of plant pathogens in weed control.

The workshop was held on Saturday 1 February 2003 at the Canterbury Agriculture and Science Centre, Lincoln, Canterbury, New Zealand, on the weekend before the 8th International Congress of Plant Pathology.

Organising committee:

Graeme Bourdôt – AgResearch, Lincoln

Geoff Hurrell - AgResearch, Lincoln

Von Johnson - AgResearch, Lincoln

Hugh Gourlay – Landcare Research, Lincoln

Jane Barton - Contractor to Landcare Research, Auckland

Editors of proceedings:

Graeme Bourdôt

Shona Lamoureaux - AgResearch, Lincoln

Workshop participants

Ash, Gavin

E-mail: gash@csu.edu.au

Barton, Jane

E-mail: jane.barton@ihug.co.nz

Beed, Fen

E-mail: f.beed@cgiar.org

Bourdôt, Graeme

E-mail: graeme.bourdot@agresearch.co.nz

Britton, Kerry

E-mail: kbritton01@fs.fed.us

Bruckart, William

E-mail: wbruckart@fdwsr.ars.usda.gov

Caesar, Anthony

E-mail: caesara@sidney.ars.usda.gov

Casonato, Seona

E-mail: casonatos@landcareresearch.co.nz

Charudattan, Raghavan

E-mail: rc@mail.ifas.ufl.edu

Chittick, Angela

E-mail: achittick@csu.edu.au

Cother, Eric

E-mail: ric.cother@agric.nsw.gov.au

Hurrell, Geoff

E-mail: geoff.hurrell@agresearch.co.nz

Kiss, Levente

E-mail: lkiss@nki.hu

Lamoureaux, Shona

E-mail: shona.lamoureaux@agresearch.co.nz

Lennox, Cheryl

E-mail: vredcl@plant3.agric.za

Lo, Chaur-Tsuen
E-mail: ctlo@wufeng.tari.gov.tw

Lueth, Peter
E-mail: plueth@prophyta.com

Mills, Dallice
E-mail: millsd@science.oregonstate.edu

Morin, Louise
E-mail: louise.morin@ento.csiro.au

Neal, Joseph
E-mail: joe_neal@ncsu.edu

Pottinger, Brenda
E-mail: pottinb1@lincoln.ac.nz

Roskopf, Erin
E-mail: eroskopf@ushrl.ars.usda.gov

Shamoun, Simon
E-mail: sshamoun@pfc.forestry.ca

Singh, S.
E-mail: upcsr@sancharnet.in

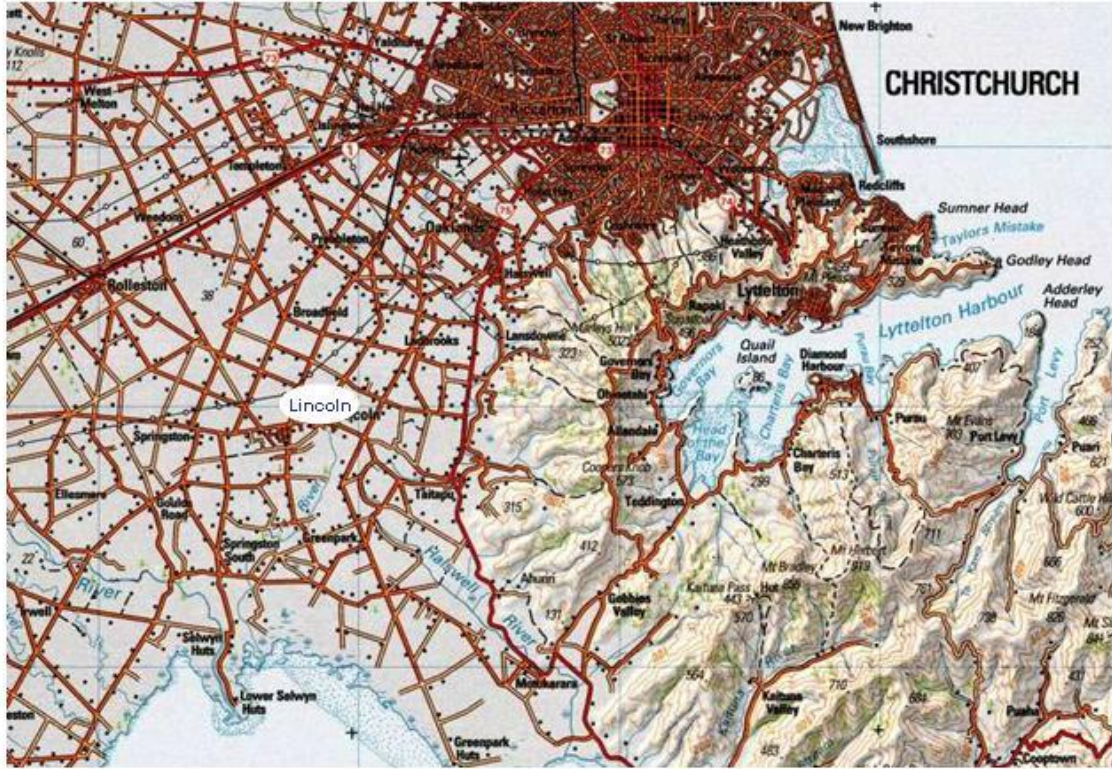
Stewart-Wade, Sally
E-mail: smsw@unimelb.edu.au

Ueng, Peter
E-mail: pueng@asrr.arsusda.gov

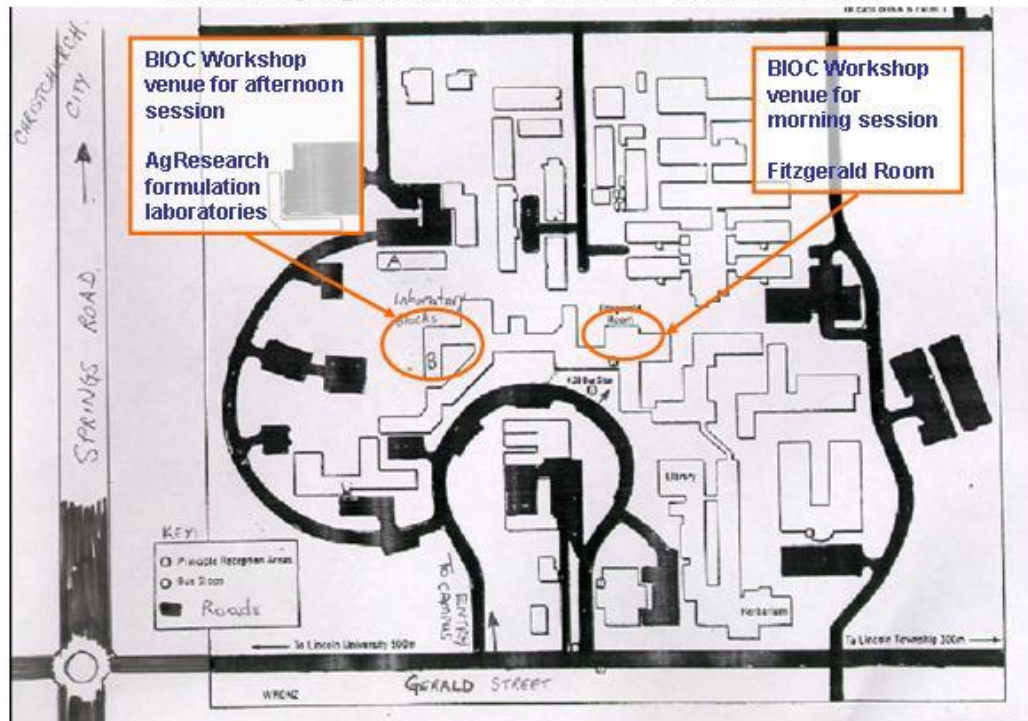
Waipara, Nick
E-mail: nickwaipara@hotmail.com

Widmer, Timothy
E-mail: tlwidmer@ars-ebcl.org

Location of Lincoln south-west of Christchurch City



Canterbury Agriculture and Science Centre, Lincoln



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Chemicals	Skin, damage, respiratory, eye damage
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Biological organisms	Infection, skin irritation
Hot water scald	Minor skin damage
Acids (incl. lactic acid)	Minor skin damage
Fungicides, antibiotics	Minor skin irritation, poisoning
Broken glassware	Cuts lacerations
Trips, falls and slips	Injury
Alcohol : Mist/Aerosol	Damage to internal organs and absorption
Wastes : Discarded experiments which are sterilised	Infection, cuts
Solvents : flammable	Fire, absorption
Trip hazard - cords etc	Abrasions, break bones
Computers	OOS
Inadequate lighting	Sight defects
High shelf storage	Injury from falling objects

Programme

8:20-8:30am Introduction (Dr Graeme Bourdôt)		
Paper Session (Chair: Dr Raghavan Charudattun)		
8:30-8:45am	A mycoherbicide for water hyacinth	Fen Beed <i>Int. Inst. Trop. Agric., West Africa</i>
8:45-9:00am	Development and registration of <i>Chondrostereum purpureum</i> for management of weedy hardwood species, and a brief overview of other mycoherbicide projects currently in progress	Simon Shamoun <i>Pacific Forestry Centre, Victoria, Canada</i>
9:00-9:15 am	Evaluating <i>Fusarium tumidum</i> and <i>Chondrostereum purpureum</i> as mycoherbicides for gorse	Jane Barton, contractor to <i>Landcare Research, New Zealand</i>
9:15 – 9:30am	Developing <i>Rhynchosporium alismatis</i> for biological control of aquatic weeds of rice in Australia – past, present and future.	Gavin Ash <i>Charles Sturt University, NSW, Australia</i>
9:30-9:45am	<i>Phyllachora</i> epidemic on common ragweed (<i>Ambrosia artemisiifolia</i>): a unique natural control phenomenon in Hungary in 1999	Levente Kiss <i>Hungarian Academy of Sciences, Budapest, Hungary</i>
9:45-10:00am	Biological control of the noxious weed <i>Solanum viarum</i> by Tobacco Mild Green Mosaic <i>Tobamovirus</i>	Raghavan Charudattun <i>University of Florida, USA.</i>
10:00-10:30am Coffee Break		
10:30-10:45am	Classical biological control of <i>Acacia saligna</i> with <i>Uromycladium tepperianum</i> in South Africa: A success story	Cheryl Lennox <i>ARC-PPRI, Stellenbosch, South Africa</i>
10:45-11:00am	Biological control of alien invasive plant species in South Africa and conflict of interest	Cheryl Lennox
11:00-11:15am	Removal of approved biological control candidates from a containment greenhouse environment	William Bruckart <i>USDA, Ft. Detrick, USA</i>
11:15-11:30am	Defining safety zones for bioherbicides based on plurivorous pathogens using models of spore escape and dispersion	Graeme Bourdôt <i>AgResearch, New Zealand</i>
11:30-11:45am	Risks and benefits of introducing exotic pathogens for biological control of weeds	Louise Morin <i>CSIRO, Canberra, Australia</i>
11:45-12:00pm Chairman's Summary		
Poster Session (Chair: Dr Jane Barton)		
12:00-12:05pm	Evaluation of <i>Phoma exigua</i> var. <i>exigua</i> as a biocontrol against Californian thistle (<i>Cirsium arvense</i>).	Nick Waipara <i>Landcare Research, New Zealand</i>
12:05-12:10pm	Determining the key pathogenicity factors of a <i>Sclerotinia sclerotiorum</i> -based mycoherbicide	Brenda Pottinger <i>Lincoln University, New Zealand</i>
12:10-12:15pm	<i>Sclerotinia sclerotiorum</i> as a mycoherbicide for giant buttercup (<i>Ranunculus acris</i>)	Michelle Verkaaik <i>AgResearch, New Zealand</i>
12:15-12:20pm	A novel bioherbicide for control of grassy weeds	Dalice Mills, <i>Oregon State University, USA</i>
12:20-1:30pm Poster Viewing and Lunch		
1:30-3:30pm	Formulation and application technologies - laboratory and field demonstrations (Leaders: Von Johnson, Geoff Hurrell, Graeme Bourdôt)	
3:30-4:00pm Coffee Break		
4:00-4:30pm Summing Up (Dr Jane Barton)		
7:00pm- Workshop Dinner		

Continuity person: Hugh Gourlay

A mycoherbicide for water hyacinth

Fen Beed

International Institute of Tropical Agriculture, Cotonou, Republic of Benin, West Africa

Abstract

This presentation concerns the theoretical approach and results of IMPECCA: International Mycoherbicide programme for *Eichhornia crassipes* ((Mart.) Solms) control in Africa, with particular emphasis on activities in West Africa.

Introduction

The aim of this programme was to develop a mycoherbicide from a fungal pathogen showing virulence and host specificity for water hyacinth. Furthermore, for regulatory purposes the intention was for a pathogen to be selected from those indigenous to Africa. At the onset of this programme there were published reports showing that such a pathogen existed, namely, *Alternaria eichhorniae* (Shabana *et al.*, 1995).

Water hyacinth is widely recognised as the world's most obnoxious weed. Since its introduction into Africa 100 years ago it has infested waterways across the contrasting environments of the continent. Its competitive ability is high in Africa because the waterways are eutrophic, permitting the plants regenerative potential to be realised. It threatens the survival of human communities based around watercourses by compromising transport, commerce, fishing, biodiversity and irrigation and by increasing vectors of diseases such as malaria, bilharzia and river blindness. It also affects other communities by reducing the supply of goods and hydroelectric power. Previous control programmes have been based upon (1) physical removal either manually or mechanically, (2) chemical herbicides such as glyphosate and 2,4-D and (3) classical biological control using herbivorous insects. All have been ineffective due either to high cost or as a result of an absence of sustained efficacy.

Material, methods and results

Field Surveys - The intention of surveys was to monitor the status of water hyacinth infestations and to map the distribution of fungal pathogens across the range of environments found in Africa. Dramatic differences exist in climate between dry and wet seasons for sites so visits for each were performed across seasons. Field surveys provided information on the growth (increase in biomass) and development (change in plant form such as flowering or ramet production) of water hyacinth and how this is influenced by changes in its environment. Furthermore, survey data was important to show possible relationships between the incidence and severity of fungal pathogens with environmental conditions, insect attack or the physiological status of water hyacinth. Such observations showed the limitations of control by each fungal pathogen under natural conditions. These were used to identify the required conditions to be produced through the formulation of a mycoherbicide or through appropriate application in terms of day, season and the growth and development of water hyacinth. The fungal pathogens showing the greatest control of water hyacinth at the sites visited were *Acremonium zonatum*, *Alternaria eichhorniae*, *Cercospora piaropi*, *Myrothecium roridum* and *Rhizoctonia solani*.

Testing of Isolates - Methods were developed for the isolation, culture and production of spores for those isolates of fungal pathogens collected on the surveys. Pathogenicity was confirmed through satisfaction of Koch's Postulates and isolates were characterised, stored and their details were entered into a database. Following the development of techniques to achieve inoculation for each species, virulence was assessed. Assessments of disease severity were made on inoculated leaves but also on non-inoculated leaves and those produced after inoculation in order to show dispersal of the disease. In addition, the rate of production of new leaves, flowers and ramets following inoculation was determined to show how plant regeneration was affected by disease and to predict the likelihood of control using each fungal pathogen. Host specificity was then determined against related members of the same family (Pontederiaceae) and crop plants, especially those inhabiting environments infested by water hyacinth i.e. rice and Niger grass.

Formulations were developed using aqueous spore suspensions and mycelial fragments in oil emulsions to increase levels of virulence above those experienced under natural conditions.

Mesocosm Trials - Water hyacinth exhibits dramatic phenotypic plasticity as determined by environmental conditions. Therefore, methodologies were developed in order to produce uniform plants of the same size and development stage that were suitable for experimentation. Observations and measurements from field surveys showed that the nutrient content of water affected the growth and development of water hyacinth. Experiments were designed to investigate the effect of these changes on tolerance to disease due to fungal pathogens. The effect of nutrients, and in particular the form of nitrogen, on water hyacinth growth, development and disease severity was investigated. Results from these experiments helped to identify the concentration of nutrients in water that permit control. Sites and seasons were then identified that had appropriate nutrient levels for mycoherbicide applications. Efforts were made to produce experimental conditions that equated with natural environments and were developed using buckets, basins and eventually tanks. The intention of tank trials was to show that populations of plants could be controlled using a fungal pathogen under controlled conditions. When this objective was not fulfilled the limitations responsible were identified. These were then used to further develop application techniques such as mycoherbicide formulations, climatic conditions or the optimal physiological state of the host.

Discussion

Based on the two years of completed research a summary will be provided to show the potential of using a mycoherbicide to control water hyacinth.

Recommendations will be made for future activities based on results from Africa and observations from Brazil, the reported origin for water hyacinth.

An overview will be given of the effect of IMPECCA in providing educating people of the problem and its possible solution, in disseminating information and developing collaborative and training networks across Africa.

References

Shabana YM, Charudattan R, Elwakil MA, 1995. Identification, pathogenicity and safety of *Alternaria eichhorniae* from Egypt as a bioherbicide agent for water hyacinth. *Biological Control*, **5**, 123-135.

Development and registration of *Chondrostereum purpureum* for management of weedy hardwood species, and a brief overview of other mycoherbicide projects currently in progress

Simon F. Shamoun

Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria, BC V8Z 1M5 Canada

Abstract

In Canada, research effort has led to development and registration of the first mycoherbicide based on formulated product of *Chondrostereum purpureum* as “Myco-Tech™ Paste” for control of weedy hardwood species, while “Chontrol™” is undergoing the registration process in North America. Development of several mycoherbicides for forest weeds is in progress.

Introduction

Management of forest weeds takes many forms, including mechanical, manual cutting or chemical herbicides, but these methods are expensive and have non-target effects that are of environmental concern. The discovery and development of potential biological control agents to suppress forest weeds is receiving increased attention in the management of conifer regeneration sites and utility rights-of-way (Shamoun, 2000). Among the forest weeds targeted are several weedy hardwood species, salal (*Gaultheria shallon*), bramble (*Rubus* spp.) and dwarf mistletoes (*Arceuthobium* spp.) (Evans *et al.*, 2001). To date, application of *Chondrostereum purpureum* onto cut stumps of weedy hardwood species prevents sprouting (Shamoun & Wall, 1996). Treatments of *Rubus* spp. with *Fusarium avenaceum* and other foliar pathogens, caused severe damage to target weed (Shamoun, 2000). In addition, the potential use of *Valdensinia heterodoxa* for control of salal, is showing promise (Vogelgsang & Shamoun, 2002). The utilization of *Colletotrichum gloeosporioides* and *Neonectria neomacrospora* rapidly destroys the seeds, shoots and the endophytic system of dwarf mistletoes (Shamoun & DeWald, 2002). These pathosystems will be presented and discussed in detail.

Materials and Methods

Fungal plant pathogens were isolated from selected target weeds, identified, and tested for their potential use as biocontrol agents, following routine Plant Pathology methods and latest techniques in formulation and delivery technologies in Biological Control.

Results and Discussion

Case study I. Chondrostereum purpureum – weedy hardwood species:

C. purpureum was tested against red alder (*Alnus rubra*). Two formulated *C. purpureum* isolates (PFC 2139 and PFC 2140), were compared with control formulation treatment, two chemical herbicide treatments, and manual cutting (slash). At two years post-treatment, stumps treated with PFC 2139 and with one of the chemical herbicides had a 100% stump mortality. Substantial research effort has recently led to registration and commercialization of *C. purpureum* - “Myco-Tech™ Paste” as the first biocontrol agent for forest vegetation management in eastern rocky mountains of Canada (PMRA, 2001,). A collaborative research

agreement between CFS-PFC and MycoLogic Inc. was initiated in an effort to register *C. purpureum* as “Chontrol™” in North America (Shamoun & Hintz, 1998).

Case study II. Fusarium avenaceum & other foliar pathogens- Rubus spp. pathosystem: *Fusarium avenaceum* is a promising biocontrol agent when formulated on rice grains and the inoculum combined with 0.4% Silwet L-77® enhanced greater foliar damage. One or two applications cause substantial *Rubus* spp. defoliation (Shamoun, 2000). Evaluation of other candidate fungi for their potential as biocontrol agents is in progress.

Case study III. Valdensinia heterodoxa- Salal (Gaultheria shallon) pathosystem:

An agar-based medium that allowed sufficient production of inoculum was developed, and the effects of various abiotic factors on growth, sporulation, and conidia discharge of *V. heterodoxa* were determined. In addition, a solid substrate method was established to assess virulence on intact salal plants (Vogelgsang *et al.*, 2001; Vogelgsang & Shamoun, 2002).

Case study IV. Colletotrichum gloeosporioides & Neonectria neomacrospora- Dwarf Mistletoes (Arceuthobium spp.) pathosystems:

Colletotrichum gloeosporioides & Neonectria neomacrospora are showing promise as potential biocontrol agents for lodgepole pine and hemlock dwarf mistletoes, respectively. Under field situation, *C. gloeosporioides* was evaluated for control of *A. americanum*. The number of mistletoe fruit production compared to the controls was reduced, suggesting that *C. gloeosporioides* did interfere with the life cycle of *A. americanum*. Enhancing the efficacy of *C. gloeosporioides* may be increased through the selection of a more virulent isolate, increase inoculum potential and time of inoculation (Shamoun & DeWald, 2002).

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

G. A. Hurrell^a, J. Barton^b (née Fröhlich), G. W. Bourdôt^a & A. Gianotti^b

^a AgResearch, PO Box 60, Lincoln 8152, New Zealand

^b Landcare Research, Private Bag 92170, Auckland, New Zealand

Abstract

Field studies in New Zealand revealed that the fungus *Chondrostereum purpureum* applied as mycelium on agar blocks to decapitated stems of gorse (*Ulex europaeus*) significantly reduced the number of live regrowth shoots. The addition of an invert emulsion, with or without *Fusarium tumidum* conidia, further reduced regrowth to almost nil.

Introduction

In studies in New Zealand, the foliage of gorse plants has been sprayed with a suspension of *F. tumidum* conidia in an invert (water in oil) emulsion (Fröhlich *et al.*, 2000 and 2001). Gorse seedlings and young tissues on established gorse plants were found to be susceptible to the fungus, while woody tissues were not. In glasshouse studies, the wound pathogen *C. purpureum*, a wood rotting fungus that occurs naturally on gorse in New Zealand, was able to infect gorse stems after application to cut surfaces on woody stem sections (unpublished data). In order to test the efficacy of these pathogens alone and in combination, field studies were set up in two contrasting localities in New Zealand (Canterbury in the South Is. and Auckland in the North Is) (Fröhlich *et al.*, 2001). Further details and some initial results of the South Is. work are presented here.

Materials and Methods

In one experiment individual gorse bushes were treated with *C. purpureum* each month from May 2001 to April 2002. The four treatments were two isolates of *C. purpureum* mycelium on agar, agar alone and an untreated control, and each treatment was applied to two freshly decapitated woody stems per bush. The *C. purpureum* isolates were applied as colonised agar discs (15mm diam.) placed with the mycelium in contact with the cut stem surface and wrapped with Parafilm. The agar-only treatment was also applied and wrapped as described, while on the control bushes the cut stems were left uncovered. The Parafilm was removed after one month and the whole bushes and treated stems were examined for symptoms of infection monthly from June 2001 onwards. At each monthly sampling date, all of the adventitious shoots within 10 cm of the cut end of the stems were counted, measured and classified as healthy, diseased or dead. In a second experiment the combined effects of *C. purpureum* and *F. tumidum* were examined. The *C. purpureum* treatments were the same as in the first experiment but applied only once (in May 2001) to cut gorse stems as described previously. 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 emulsion, the invert emulsion without conidia and water alone as a control. Application was by pressurised sprayer. These bushes and stems were also assessed monthly. The data presented here, for both experiments, are the numbers of live shoots present in May 2002, square-root transformed for analysis.

Results and Discussion

In the first experiment, the number of live shoots in May 2002, averaged over treatment months of May to November 2001, was three times lower when treated with either of the two *C. purpureum* isolates, than when not treated (Table 1). There was no difference in the number of live shoots between the two *C. purpureum* isolates. Bushes treated from December 2001 onwards had few regrowth shoots, and none were dead, so data from these treatment months were excluded from the analysis.

Table 1. Experiment 1: Average numbers of live adventitious shoots on decapitated gorse stems when assessed in May 2002 (averaged over the seven treatment occasions, May to November 2001). Numbers given are square roots and back-transformed means (in parentheses).

Treatment	Number of live shoots	
Control	1.57	(2.5)
Agar	1.47	(2.1)
<i>C. purpureum</i> A	0.88	(0.8)
<i>C. purpureum</i> B	0.84	(0.7)
LSD (5%)	0.49	
Control/Agar vs <i>C. purpureum</i> isolates	*** (0.1% sig.)	

In the second experiment both *C. purpureum* isolates, in the absence of *F. tumidum* and/or the invert emulsion, resulted in significantly fewer live shoots in May 2002 than in the control (Table 2). There was no difference in number of live shoots between the two isolates of *C. purpureum*. These effects were similar to those in the first experiment. Almost no live shoots remained after they had been treated with *C. purpureum* plus *F. tumidum* conidia in the invert emulsion, or the invert emulsion without the conidia (Table 2). This indicates that the emulsion alone was extremely phytotoxic to the young gorse shoots. The effects evident in the two experiments in Canterbury were not apparent in the results from the Auckland site where naturally occurring parasites including lemon-tree borer (*Oemona hirta*) and various opportunistic pathogens have caused high levels of stem and whole bush mortality.

Table 2. Experiment 2: Average numbers of live adventitious shoots on decapitated gorse stems when assessed in May 2002 after treatment with *C. purpureum* in May 2001 and with *F. tumidum* in November 2001. Numbers given are square roots and back-transformed means (in parentheses).

<i>C. purpureum</i> treatment	<i>F. tumidum</i>		
	Control	Invert emulsion	<i>F. tumidum</i> in invert emulsion
Control	2.04 (4.15)	1.05 (1.10)	0.28 (0.08)
Agar	1.17 (1.38)	0.40 (0.16)	1.03 (1.07)
<i>C. purpureum</i> A	0.69 (0.48)	0.28 (0.08)	0.00 (0.00)
<i>C. purpureum</i> B	0.95 (0.89)	0.00 (0.00)	0.20 (0.04)
LSD 5%	0.98		

Acknowledgements

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Developing *Rhynchosporium alismatis* for biological control of aquatic weeds of rice in Australia – past, present and future

Gavin J. Ash^a, E.J. Cother^b, F.G. Jahromi^a, W. Pitt^a, V.M. Lanoiselet^a and S. Cliquet^c

^a Farrer Centre, School of Agriculture, Charles Sturt University, Wagga Wagga, NSW 2678 Australia

^b NSW Agriculture, Agricultural Institute Orange NSW 2800, Australia

^c IUP, Université de Bretagne Occidentale, Pôle Universitaire Creach Gwenn 29000, Quimper, France

Abstract

Glasshouse and field competition experiments have demonstrated that the fungus *Rhynchosporium alismatis* can reduce the impact of the weed *Damasonium minus* on rice growth. In field trials in 2001/02 the combination of low herbicide application rates in conjunction with the fungus reduced the weed's biomass by 75%. Results from field trials being conducted 2002/03 will be presented.

Introduction

Eighty five percent of Australia's annual rice production of 1.2 million tonnes is exported. Australia's rice production is comparatively pest and disease free. However, aquatic weeds have the potential to compete strongly with direct seeded rice under Australian conditions. Plant species in the Family Alismataceae including *Damasonium minus*, *Alisma lanceolatum* and *A. plantago-aquatica* are significant weeds of rice in Australia. Of these species, *Damasonium minus* or starfruit 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.

An endemic fungus, *Rhynchosporium alismatis*, was first observed causing necrotic leaf spots on *D. minus*, *A. lanceolatum* and *A. plantago-aquatica* in 1992 (Cother *et al.*, 1994) and its potential as an inundative biological control agent was identified (Cother & Gilbert, 1994a; b). As part of host range studies, Cother (1999) reported that the fungus could cause symptoms on a range of aquatic species as well as crops such as cucumber, rockmelon, soybean and tomato under extremely severe conditions. However, even under these conditions the pathogen did not appear to have an effect on plant development. On *D. minus* the fungus causes dark necrotic lesions on the leaves, stems and petioles (Cother *et al.* 1994) which may lead to reduction in seed production and viability (Fox *et al.*, 1999). Later studies by Jahromi *et al.* (2001) have also shown that the fungus may act as a mycoherbistat (Crump *et al.*, 1999) by reducing plant growth without actually causing lesions on juvenile plants of *D. minus*.

Methods and Results

The fungus produces both conidia and chlamydospores (Lanoiselet *et al.*, 2001). Early studies have shown that the media used has a marked affect on the number and infectivity of spores produced by the fungus (Jahromi, *et al.*, 1998; Cother & van de Ven, 1999) with Lima Bean Agar proving to be the best medium for conidia production. Nitrate nitrogen is involved in the formation of chlamydospores (Cliquet *et al.*, 2002). Conidia germinate and penetrate the leaves of the weed using appressoria within four hours of inoculation (Jahromi *et al.*, 2002)

with the optimum temperature occurring between 25 and 30 °C. As the disease occurs in an aquatic environment, high humidity and free water are invariably present. Penetration through stomata occurs at low frequency and appears to be a random event. Secondary conidial formation occurs within 48 hours of inoculation. On species of Alismataceae considered to be non-hosts of *R. alismatis*, spore germination occurred but the rate of penetration and appressorium production was reduced.

The genetic diversity within *R. alismatis* as determined by ERIC-, REP- and ISSR PCR was found to be low within and between populations of the fungus from south eastern Australia. Similarly, the genetic diversity within *D. minus* was found to be low. However, the genetic diversity within *Alisma lanceolatum* (an introduced species) was higher between populations within the rice growing area in Australia. This may reflect a number of introductions of the weed, which could harbour resistance to the pathogen.

In most experiments, simple aqueous suspensions of the conidia of the fungus have been used. Disease was maximised when spore concentrations of 1×10^6 conidia per mL were applied. A synergistic effect between low levels of Londax (< 1.5% of recommended rate) and the fungus has been noted. This occurs when the synthetic herbicide is applied prior to the fungus. Granular and powder formulations of the mycoherbistat have been trialed in the glasshouse, but activity and shelf life have been disappointing.

Glasshouse and field competition experiments have demonstrated that the fungus can reduce the impact of the weed on rice growth.

Acknowledgements

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Phyllachora epidemic on common ragweed (*Ambrosia artemisiifolia*): a unique natural control phenomenon in Hungary in 1999

L. Kiss¹, L. Vajna¹, Gy. Bohár², K. Varga³, U. Paksiri⁴, S. Takamatsu⁵ & D. Magyar¹

¹Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

²BIOVÉD Bt., Szigetszentmiklós, Hungary

³Central Service for Plant Protection and Soil Conservation, Budapest, Hungary

⁴Faculty of Agriculture, Chiang Mai University, Thailand

⁵Faculty of Bioresources, Mie University, Tsu, Japan

Abstract

The main lesson from two natural epidemics is that biological control using fungal pathogens could be a possibility to suppress ragweed (*Ambrosia artemisiifolia*) populations in Hungary. The most promising candidates appear to be North American *Puccinia* spp.

Introduction

Since the early 1990s, common ragweed (*Ambrosia artemisiifolia*) has become the most widespread and most important allergenic weed in Hungary. Our annual surveys of fungal diseases of ragweed revealed that this plant, introduced to Europe from North America, is one of the healthiest weeds in the Carpathian basin. Until the mid-nineties, only the following fungal pathogens were found on ragweed in Hungary: *Entyloma polysporum* (Vanky *et al.*, 1988), *Albugo tragopogonis*, *Plasmopara halstedii*, *Verticillium dahliae*, *Botrytis cinerea*, *Rhizoctonia solani*, *Macrophomina phaseolina* (Bohar & Vajna, 1996), *Sclerotinia sclerotiorum* (Bohar & Kiss, 1999) and *Septoria epambrosiae* (Bohar & Schwarczinger, 1999; Farr & Castlebury, 2001). These fungi caused only minor infections in the field between 1995-1999.

In summer 1999, a serious epidemic developed on ragweed caused by *Phyllachora ambrosiae*. This holobiotrophic pathogen has not been reported from Europe prior to 1999 (Vajna *et al.*, 2000). The identification of the pathogen was based on both morphological and molecular data. The morphology of the perithecia, asci and ascospores were similar to those which were examined in two North American herbarium specimens (BPI 636220 and BPI 636225) borrowed from the U.S. National Fungus Collection. Molecular identification of the pathogen was based on rDNA ITS sequences determined in a total of four samples collected in 1999 and 2002, respectively.

Methods and Results

From mid-September, all plants examined in all regions of the country were infected with *P. ambrosiae* and exhibited dead leaves and inflorescences. Thus, *P. ambrosiae* reduced the fitness of ragweed and also the period of production of the allergenic pollen in Hungary in 1999. According to the data of the pollen monitoring service in Budapest, much less ragweed pollen occurred in the air in September and October 1999 than in the previous ten years. This natural epidemic clearly demonstrated the capacity of a pathogenic fungus to reduce the harmful effects of a noxious weed. It is interesting to note that a recent downy mildew epidemic on common ragweed caused by *Plasmopara halstedii* was also correlated with a significant decrease in pollen concentration in the air in Hungary (Vajna, 2002).

The main lesson of these two natural epidemics is that biological control using fungal pathogens could be a possibility to suppress ragweed populations in Hungary. The most promising candidates would probably be North American *Puccinia* spp. (Bohar, 1996; Batra, 1981).

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Biological control of the noxious weed *Solanum viarum* by Tobacco mild green mosaic *tobamovirus*

R. Charudattan, M. Elliott, J.T. DeValerio, E. Hiebert, & M.E. Pettersen

Plant Pathology Department, University of Florida, 1453 Fifield Hall, Gainesville, FL 32611-0680, USA

Abstract

Tobacco mild green mosaic *tobamovirus* causes a systemic, lethal, hypersensitive response in tropical soda apple (*Solanum viarum*; TSA) plants. When manually inoculated with the virus, TSA plants of all ages develop foliar lesions, systemic necrosis of petioles and stem tips, and systemic wilting in rapid succession, beginning 7-14 days after inoculation. TSA is also susceptible to Tomato mosaic *tobamovirus* and Tobacco mosaic *tobamovirus* (strain U1), but these viruses induce only nonlethal mosaic symptoms. Among 31 solanaceous plants screened against TMGMV, only *Capsicum annuum* (most of the 23 cultivars tested), a known host to this virus, developed a 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 size and age categories. Attempts are underway to develop and register TMGMV as the first virus-based bioherbicide.

Introduction

Tropical soda apple (*Solanum viarum* Dunal; TSA) is an exotic weed of South American origin that has become a serious invasive weed in seven south-eastern states in USA including Florida (Mullahey *et al.*, 1996; Byrd & Bryson, 1996; Patterson, 1996; Westerbrook & Miller, 1996). It is a thorny annual that can overwinter and perennate in some areas of the southern USA following mild winters. It has been designated as a noxious weed and is therefore a prohibited plant under both the Federal and the Florida noxious weed statutes.

Cattle, birds, and wildlife that consume TSA fruit, composted cow manure sold for home gardens, and contaminated turf and grass seeds produced in TSA-infested areas are the primary means of dispersal of this weed. Neither temperature nor photoperiod is likely to limit the spread of TSA beyond Florida and the south-eastern USA (Patterson, 1996). The potential range of this weed in the continental United States is therefore quite extensive.

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, lethal, hypersensitive response and kills seedling and mature TSA plants following manual inoculation (Pettersen *et al.*, 2000). Typically, hypersensitive reaction to virus infection is expressed as necrotic foliar spots (i.e., local lesions); lethal hypersensitive reaction is uncommon and usually seen only in seedlings. Thus, TMGMV has the unique capacity to kill TSA plants of all ages and therefore can be used as a highly effective biological control for this weed.

Materials and Methods

Standard virology techniques were used. TMGMV and other tobamoviruses tested were multiplied in susceptible tobacco (*Nicotiana tabacum*) cv. Turkish Samsun. The viruses were manually inoculated on TSA and other test plants. Visual symptoms, back inoculations on

systemic and local-lesion hosts, serodiagnosis using SDS-gel-diffusion plates, and ELISA tests were used to confirm infection as well as viral identity. TMGMV inoculum for greenhouse and field trials was produced in susceptible tobacco. Infected tobacco leaves were harvested and ground in a buffer solution. The leaf extract was filtered to remove large debris and kept frozen at -80°C. Appropriate dilutions of the viral extract were prepared in buffer solution and mixed at desired proportions with tap water. Field applications of the virus were made with different types of tools.

Results and Discussion

Inoculated TSA plants developed foliar local lesions, systemic necrosis of leaves and petioles, and systemic wilting in 7-14 days after inoculation. TMGMV was reisolated from inoculated TSA plants and confirmed by immunodiffusion and ELISA tests. TSA was also susceptible to *Tomato mosaic virus* and *Tobacco mosaic virus* strain U1, but these tobamoviruses induced only mosaic and/or mottle symptoms. Among 32 solanaceous plants screened against TMGMV in a greenhouse, *Solanum macrocarpon* and *S. spinosissimum* developed mild mosaic and mottling symptoms; *S. nigrum* and *S. rostratum* were asymptotically infected; and *S. gilo*, *S. nodiflorum*, *S. americanum*, *S. sessilifolium*, and *S. pseudocapsicum* developed hypersensitive local lesions. Only *Capsicum annuum* (Jalapeño and California Wonder) developed hypersensitive systemic necrosis comparable to that seen on TSA. TMGMV caused 100% mortality of TSA plants at 18°C and 32/22°C cycles but plants at continuous 32°C were asymptomatic. These asymptomatic plants, when transferred 17 days later to a greenhouse at 25-29°C, developed systemic stem necrosis and gradual leaf mortality. In field trials done in three different locations in Florida, TMGMV caused 83 to 97% mortality of TSA plants of various size and age categories. Thus, TMGMV has the unique capacity to induce systemic hypersensitive mortality in TSA. We propose to identify and characterize the viral elicitor of the wilting response in TSA. An understanding of the molecular basis of this system might lead us toward a novel, biologically based template or templates for herbicidal mode(s) of action.

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Classical biological control of *Acacia saligna* with *Uromycladium tepperianum* in South Africa: A success story

C. L. Lennox, C. van Rooi, A. R. Wood, A. Den Breeÿen, M. Serdani, J.L. Markram & G. Samuels

ARC-PPRI Weeds Research Division, Private Bag X5017 Stellenbosch 7599, South Africa

Abstract

The gall-rust fungus *Uromycladium tepperianum* was introduced into South Africa in 1987 as a classical biological control agent for control of invasive *Acacia saligna*. By 1993, almost 100% of trees at release sites monitored were infected. In 2001, tree density at all monitored sites was reduced to between 55.5 and 3.32% of the 1991 density. Although flowers are still being produced on infected trees, there has been a general decline in soil seed numbers. In biological control terms *A. saligna* is under complete control.

Introduction

Acacia saligna (Labill.) H.L. Wendl. (Port Jackson willow), a small willow-like evergreen shrub or tree was introduced into South Africa from south-western Australia in the mid 1800s to stabilize sand dunes in coastal areas. This tree has become a serious environmental weed, invading fynbos, woodlands, coastal dunes, roadsides and watercourses. MacDonald & Jarman (1984) regarded *A. saligna* as the most troublesome invasive alien weed in the Cape Floristic Region of South Africa. According to the March 2001 amendment to ‘The Conservation of Agricultural Resources Act’ (Act No. 43 of 1983), *A. saligna* is classified as a declared invader (category 2). Category 2 plants may not occur on any land or inland water surface other than a demarcated area or a biological control reserve.

The gall-forming rust, *Uromycladium tepperianum* (Sacc.) McAlp. which is highly destructive to *A. saligna* in south-western Australia, was selected as a potential biological control agent and extensively tested for host specificity (Morris, 1987). Once it was established that the *A. saligna* genotype of *U. tepperianum* was suitably specific for use as a biological control agent in South Africa (Morris, 1987), permission for release was approved and the first release took place in 1987 (Morris, 1991). By 1997, the pathogen had become established at nearly 200 sites where it had been released, and wind had dispersed the fungus throughout the range of the weed (Morris, 1997).

Materials and Methods

The effect of the pathogen on *A. saligna* populations and changes in the population levels of the pathogen were measured annually from 1991 to 2001 at eight of the sites inoculated during 1988 and 1989. Four transects per site and 100 trees per transect were evaluated.

Results and Discussion

Disease severity, shown by the mean numbers of galls per tree, was relatively low in 1991 but increased rapidly thereafter at most sites. By 2001 tree density at most sites were reduced to between 55.5 and 3.32% of the 1991 density. New, regenerating seedlings were included in these counts. Most of the old trees were killed and many of the remaining ones are new seedlings, which are now also infected. The number of living trees in the smaller size classes

declined more rapidly than in the larger size classes, but trees of all ages eventually died. In 2001, the mean percentage trees infected per site ranged from 16.8 to 100%, with a mean of 81.35%. The mean number of galls per infected tree in the largest tree size increased from 21.05 in 1991 to 169.34 in 2001. The number of seeds recovered from soil samples varied greatly depending on the history of the site. During the period 1991-1995, mean seed numbers per site increased from 37 497 to 47 386 seeds/m². Thereafter, soil seed numbers tended to decrease. The mean seed number in 2001 was 25 554 seeds/m². At certain sites the occurrence of fires was seen to reduce the seed number considerably.

In conclusion, the gall rust has had a major impact on *A. saligna* populations in South Africa. Although seeds are still being produced, the numbers are now considerably reduced and new emerging seedlings rapidly become infected. In many areas fynbos, other weeds and grasses are replacing dead *A. saligna* trees. The challenge now is to ensure that these areas are not simply recolonized by other weeds. In biological control terms the vegetative part of the weed has been brought under complete control (Morris, 1999). Due to the continued seed production in infected stands, the introduction of seed-destroying agents would further enhance the biocontrol programme. *Melantarius compactus* (Order: Coleoptera, Family: Curculionidae), which feed on seeds of *A. saligna* has recently been released in South Africa.

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Biological control of alien invasive plant species in South Africa and conflict of interest

C. L. Lennox

ARC-PPRI Weeds Research Division, Private Bag X5017 Stellenbosch 7599, South Africa

Abstract

In investigating the potential of fungal agents for the biocontrol of invasive plants it is important that sufficient attention be given to possible conflicts of interest before implementation of a biocontrol strategy. In this paper the strategies employed in dealing with conflict of interest situations in the biological control of two invasive plants in South Africa, namely *Acacia mearnsii* (black wattle) and *Prosopis species* (mesquite) will be discussed.

Introduction

During this past decade researchers in the Weeds Pathology Unit of ARC-PPRI have been involved in a number of projects on the biological control of alien invasive plant species in South Africa through the use of fungal agents (Morris *et al.*, 1999). Target environmental weeds have been *Acacia mearnsii*, *A. pycnantha*, *A. saligna*, *A. cyclops*, *Chromolaena odorata*, *Eichhornia crassipes*, *Hakea sericea*, *Lantana camara*, *Myriophyllum aquaticum*, *Prosopis* spp. and *Rubus cuneifolius*

All of these plant species were actively brought into South Africa for their agricultural, agro-forestry, or horticultural properties, and have become naturalized, often displacing native vegetation and using excessive quantities of water. Because of the length of time many of these plants have been in the country, communities (both commercial and subsistence) have become dependant on them for their livelihoods. In investigating the potential of fungal agents for the biocontrol of a target weed it has been important that sufficient attention be given to possible conflicts of interest before implementation of a biocontrol strategy for each of the weeds. Fungal biocontrol agents have the potential to impact on the livelihoods of both commercial and subsistence communities, and as such these stakeholders are entitled to a say in whether a biocontrol strategy should be implemented or not.

In this paper strategies employed in dealing with conflict of interest situations in the biological control of two of the above-mentioned invasive plant species, namely *Acacia mearnsii* (black wattle) and *Prosopis species* (mesquite) will be discussed.

Acacia mearnsii:

Acacia mearnsii (black wattle), an evergreen tree from south-east Australia and Tasmania, is an important component in the agro-forestry industry of South Africa. This plant invades grassland, forest gaps, roadsides, and watercourses throughout its range (Henderson, 2001). The economic importance of *A. mearnsii* as a timber crop in southern Africa has meant that only locally occurring fungi could be considered as biocontrol agents of this plant. As the target would be the plant where it had escaped its commercial boundaries, we would have to give some assurance that the plant in commercial stands would not be targeted. Clearing of invasive *A. mearnsii* involves felling the trees and because cut stumps coppice, it is essential that the stumps be treated with an herbicide. These herbicides, often in diesel as a carrier, pose an environmental risk in water-courses and catchment areas where *A. mearnsii* infestations

commonly occur. The biocontrol strategy option we had was the development of a fungal inoculant that would kill cut stumps. The result was the development and formulation of the product Stumpout®. The biocontrol agent in this product is living basidiospores of the wood-decay fungus *Cylindrobasidium* • *nfil* which was originally isolated from dead stumps of *A. mearnsii* in South Africa. The product has no effect on • nfilled trees and such, poses no threat to commercial *A. mearnsii* production.

Invasive Prosopis species:

Prosopis glandulosa var. *torreyana* and *P. velutina*, commonly known as honey mesquite and velvet mesquite respectively, are native to North and South America. These two *Prosopis* species were repeatedly introduced into South Africa in the late 1800's as fodder for livestock and have become serious invaders in the arid interior of South Africa and Namibia. Mechanical and chemical control is both difficult, due to the tree's thorny multi-stemmed nature, and expensive in an area of low land value. Long-term, economically viable management of prosopis will probably only be achieved through biological control. As the plant has some useful attributes (fodder, firewood, charcoal, and wood for flooring), biological control has, until recently, been restricted to the use of seed-feeding beetles that reduce the reproductive capacity of the plant without diminishing its usefulness. The extent of the area invaded by prosopis, and recent estimates of water usage by this plant have lent government support for research on the use of fungi as agents of biological control. Both classical and mycoherbicide approaches are being investigated.

In September/October 2001, a survey was made of pathogens on prosopis in Mexico and Texas. Pod anthracnose, caused by an as yet unidentified fungus and characterized by black/grey acervuli, flattening of the pods and seed decay, was found in both Mexico and Texas. Gall rust (*Ravenelia arizonica*) was recorded on young and mature trees in both countries. Leaflet rust (*Ravenelia holwayi*) was regularly found in Texas. Signs of this rust are black telia and an associated leaflet drop. *Diplodia*, *Phoma* and *Sphaeropsis* species were predominantly isolated from material collected in both countries. In November 2001, a decision was taken by a national workshop on the status and long-term management of prosopis in South Africa to limit biological control agents to those targeting flowers, pods and seeds. Based on this, and preliminary tests, the potential of the pod anthracnose causal organism as a biological control agent of prosopis is being further investigated.

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Removal of approved biological control candidates from a containment greenhouse environment

W. L. Bruckart, III

USDA-ARS-FDWSRU, 1301 Ditto Ave., Ft. Detrick, MD 21702, USA

Abstract

Options are described for disinfecting leaf surfaces in order to remove *Puccinia jaceae* from a containment greenhouse. It is important to verify that the pathogen, a candidate for biological control of yellow star thistle (*Centaurea solstitialis*), is not contaminated with other crop pathogens also under study in the same containment facility.

Introduction

Purity of product is a major objective during the introduction and release of a foreign biological control agent. Considerable effort was made to insure that urediniospores of *Puccinia chondrillina* for rush skeleton weed (*Chondrilla juncea*) in Australia were not contaminated with other, potentially dangerous, unwanted pathogens (Hasan, 1974 and 1981). Since approval is expected for the use of *Puccinia jaceae* for biological control of yellow star thistle (YST, *Centaurea solstitialis*) in the United States (US), similar consideration is now given to the removal of this pathogen from a containment greenhouse facility. The major issue in this case is that several crop pathogens of potential importance to the US are under evaluation in the same facility. The goal of this research is to develop a procedure that will verify that only *P. jaceae* is being removed from the containment facility.

Materials and Methods

YST plants, 4-6 wk old, were inoculated with urediniospores of *P. jaceae*, given two dew period treatments (20°C and dark, for 16-20 hr each), and grown in a containment greenhouse for 5 days after inoculation. At this time, various treatments have been applied in attempt to sterilize leaf surfaces and facilitate pustule development in an aseptic environment. To date, two approaches have been taken following studies by Bonde, *et al.* (2003): 1) direct treatment of urediniospores with 1 or 3% bleach (5% NaOCl) or acidified electrolyzed water (AEW) for up to 30 minutes, and 2) treatment of detached leaves with 10 or 20% bleach for 5 or 10 minutes. After treatment, spores or leaves were sampled for sterility by size-selective sieving through 54 µ, 20 µ, and 10 µ screens and plating objects collected on each filter on water agar (Peterson *et al.*, 2000). Suspensions that passed through the 10 µ filter were plated on acidified potato dextrose (APDA) and nutrient broth yeast extract (NBY) agar media. Objects on water agar were identified, catalogued, and examined for germination. The number of colonies was counted on APDA and NBY. Part of the suspension trapped on the 20 µ filter was used to inoculate YST plants.

Results and Discussion

The plan with the strategy described above is to allow time after inoculation for the infection to take place (for 5 days after inoculation), then clean or sterilize the leaf surface, and facilitate pustule development from monoxenic leaves. Thus far, regardless of the sterilant or what was treated (spores or leaves), it has not been possible to achieve absolute sterility; there are always bacteria and facultative fungi that survive the treatments. Leaf washes also had *P.*

jaceae uredinia that germinated, even after bleach treatments, and YST inoculated with material from the 20 μ screen became infected. In a study to remove unwanted contaminants of *P. graminis* urediniospores for a physiological investigation, Searles and French (1964) used an iodine solution with good, but not absolute results; bacterial colonies developed several days after treatment, and there was a reduction in viability of the spores. Urediniospores of *P. jaceae* not only survived treatments, but germination was stimulated by 10 or 15 min. treatments with AEW or 1 or 5 min. bleach treatments. Similar effect has been shown following treatment of *Tilletia indica* teliospores with AEW and bleach (Bonde *et al.*, 1999).

Pathogens of greatest concern as potential contaminants of YST leaves from our containment facility are *T. indica* (the karnal bunt organism) and *Phakopsora pachyrhizi* (the soybean rust pathogen). Each is a basidiomycete and cannot be controlled by fungicides or separated from *P. jaceae* urediniospores by size-selective screening. Also, procedures that stimulate germination of spores, as in the case with *T. indica* (Bonde *et al.*, 1999), should be avoided unless this inoculum can be kept away from susceptible crop host plants.

Other options are under consideration, including isolation of inoculated YST plants, harvesting only from individual pustules, single-sporing of *P. jaceae*, tests of other sterilants, and possibly aseptic growth of plants (Hasan, 1974 and 1981).

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Defining safety zones for bioherbicides based on plurivorous pathogens using models of spore escape and dispersion

G. W. Bourdôt^a, G. A. Hurrell^a & M.D. De Jong^b

^a AgResearch Limited, PO Box 60, Lincoln 8152, New Zealand

^b Dept. Biological Farming Systems, Wageningen University, Marijkeweg 22, 6709 PG Wageningen, The Netherlands

Abstract

Plurivorous plant pathogens that produce airborne spores may be used as bioherbicides without unacceptable increases in the disease risk in neighbouring crops providing a safety distance is observed. The necessary width of a safety zone for a particular bioherbicide depends upon the ratio of “added” to “natural” spores of the pathogen that is considered to be acceptable in the vicinity of the crop. The spatial pattern in this accepted ratio beyond a biocontrol site cannot easily be determined empirically but may be simulated by linking validated models of spore escape and dispersion from natural and biocontrol sources.

Introduction

Plurivorous plant pathogens that are air-dispersed may often have qualities (e.g. high pathogenicity and ease of culture, scale-up and storage) that make them good candidates for development as bioherbicides. However, such pathogens may be needlessly rejected by bioherbicide researchers because of the perceived risk of added disease occurring in susceptible crops downwind of biocontrol site. This additional crop disease risk may be defined as the ratio of “added” to “natural” inoculum in the crop environment (de Jong *et al.*, 1999) and its evaluation in space enables estimation of a safety zone around a biocontrol site (de Jong *et al.*, 1999). Here we discuss a modelling approach using a New Zealand case study in which safety zones for market-garden cropping land were estimated for pastures treated with the plant pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary. The models, suitably parameterised, may be applied to other aerially dispersed pathogens intended as bioherbicides

Materials and Methods

The Gaussian plume model is a valuable tool in predicting the atmospheric transport of fungal spores (Spijkerboer *et al.*, 2002), and was used, as implemented in the air quality management computer programme PC STACKS (Erbrink, 1995), to estimate the atmospheric concentration, C , of *S. sclerotiorum* ascospores at distances, x (m) downwind of a hypothetical 100 m x 100 m biocontrol pasture source (added inoculum) and within a hypothetical market garden area of 49 adjacent 100 m x 100 m sources (natural inoculum) by

$$C_{(x,y,z,H)} = \frac{PQ}{2\bar{p}u_s y_s z_s} * \left[e^{-\frac{(z-H)^2}{2\sigma_z^2}} + e^{-\frac{(z+H)^2}{2\sigma_z^2}} \right] * e^{-\frac{y^2}{2\sigma_y^2}} * C_{ls}$$

where y is horizontal distance (m) from the plume axis, z is height (m) above ground, H is source height (m), P is the inversion layer penetration fraction, Q is the emission rate of the source (spores s^{-1}), \bar{u} is mean wind speed ($m s^{-1}$), C_{ls} is a reflection term and σ_y and σ_z are respectively horizontal (cross-wind) and vertical dispersion terms and are functions of atmospheric stability and x . A plume was calculated for every hour from 1 Sept 1996 - 30 Nov 1996, the time of year when sporulation occurs in pasture (Bourdôt *et al.*, 2001), using 1996

Canterbury weather records to estimate P , \bar{u} , σ_y and σ_z . Using all plumes (2,184 for pasture and 107,016 for market garden), contour plots of 91-day average spore concentrations within and beyond the pasture and within the market garden area were calculated. The contours (distances) beyond the biocontrol site equal to, or 10% of, the median concentration for the market garden area defined safety zones of increasing risk averseness (Fig. 1). The source term was calculated as $Q = R_{spor} \times a \times E_v$, where R_{spor} is the release rate of ascospores from apothecia at the source (spores m^{-2} ground s^{-1}), a is the area of the source ($10,000 m^2$), and E_v is the proportion of the released spores vertically escaping the pasture or market garden crop canopy. R_{spor} was calculated as $R_{spor} = S \times A \times f$, where S is density of sclerotia ($\# m^{-2}$) in the soil in the autumn, A is the size of the sporulating apothecial disc surface (mm^2 sclerotium $^{-1}$) and f is the flux of ascospores from the apothecia (spores mm^{-2} disc surface s^{-1}). S was set to 125 for the biocontrol and 8.8 for the market garden sources (Bourdôt *et al.*, 2000) and A was varied with time (see Fig. 5d in Bourdôt *et al.*, 2001). f followed a diurnal pattern differing between frosty and frostless days according to the data in Fig. 9 of Bourdôt *et al.* (2001). E_v was a mathematically derived function of mean wind speed and pasture leaf area index, LAI (leaf area/ground area), $E_v = \exp[-bLAI/\sqrt{u}]$, with $b=0.934$, while LAI was set to 1.0 in the market garden area but varied in the biocontrol pasture according to data from Canterbury sheep and dairy pastures (mean LAI s of 2.9 and 4.8 respectively) (de Jong *et al.*, 2002).

Results and Discussion

Assuming that a 1:1 ratio of added to naturally present spores is acceptable, no safety zone was necessary around either of the modelled 1 ha pasture sources since the spore concentration contours representing the 1:1 ratios were, in each case, contained within the 1 ha sources (Fig. 1). By contrast, the ten-fold ratio (1:10 added to natural) necessitated safety zones of ca.300 and 150 m for the sheep (·····) and dairy (—) pasture respectively. The zone was narrower for dairy pasture because of its overall higher LAI and hence higher spore trapping ability.

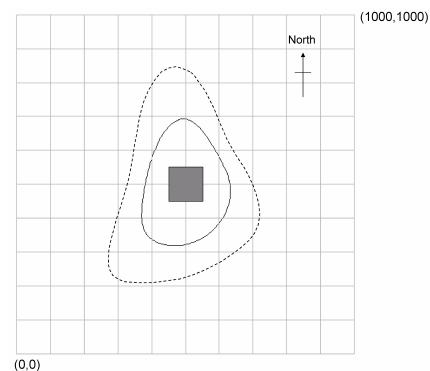


Figure 1

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Risks and benefits of introducing exotic pathogens for biological control of weeds

Louise Morin

CSIRO Entomology, CRC for Australian Weed Management, GPO Box 1700, Canberra, ACT 2601, Australia

Abstract

There are risks of undesirable direct and indirect effects on non-target species associated with the release of exotic pathogens for the classical biological control of weeds. However in most biological control programs the benefits derived from controlling the weed with an introduced pathogen far outweigh these risks.

Introduction

Since the 1970s, exotic plant pathogens (primarily rust fungi) have been increasingly used for the classical biological control of weeds (Evans, 2000). This approach involves the introduction, establishment and self-sustenance of a pathogen from the native range of the target weed into an area where the weed has naturalised and become a problem. Its aim is to gradually reduce the weed population to below the economic or ecological damage threshold. There are examples from Australia, Hawaii and South Africa of spectacular suppression of weed infestations following the release of exotic pathogens. Although there are great benefits to be gained from this weed control method, the deliberate introduction of an exotic pathogen to a new environment inevitably involves risks that have to be assessed before any release is made. This paper will highlight some of the possible risks associated with the introduction of exotic plant pathogens, as well as the benefits to be expected from a successful biological control program.

Risks

The risks of direct effects on non-target plants posed by an exotic pathogen considered for weed biological control are assessed using results from host-specificity tests. The determination of the likely host-range of a pathogen is not an easy process. We cannot test every single plant species occurring on a continent. The choice of the plant species to be tested depends largely on taxonomic relationship, a procedure that is often referred to as “centrifugal phylogenetic sequence testing” (Briese, *in press*). This procedure basically relies on concentrating testing on plants closely related to the normal host of the pathogen. The more distant the relationship, the fewer species tested, as they are less likely to be at risk.

There are also risks of undesirable indirect effects that have to be considered prior to the release of a pathogen in a new environment. Conflicts of interest may arise because the target weed for biological control has economic value for certain groups in the community (e.g. for honey production) (Delfosse, 1990). The successful control of a weed by a pathogen may also open up niches for other undesirable plant species or resistant biotypes of the target weed to invade the area (e.g. skeleton weed), thus creating another problem (Burdon *et al.*, 1981). This can be avoided by integrating classical biological control within an overall land management approach, combining techniques such as herbicides, fertilisers and revegetation.

Like other living organisms, pathogens introduced for weed control will use sexual and/or asexual processes to generate new variants or recombinant progeny to adapt to a changing environment or exploit new niches (hosts). However, there are generally no greater risks associated with the natural evolution process of these exotic pathogens than for the endemic pathogens. There are only a few examples in the literature of rust fungi that have extended their recorded host ranges when a host and/or a fungus have been introduced accidentally to a new region (Walker, 1996). Hybridization between a newly introduced pathogen and a related endemic species to generate a progeny with an increased virulence or a wider host range is also a possibility, as recently demonstrated by the Dutch elm disease pathosystem (Brasier, 2001).

Weed species are not evolutionarily static targets and can respond to environmental change, such as exposure to an aggressive pathogen, by shifts in their genetic composition. The existence of geographical variation in the virulence of pathogen populations and in the susceptibility of their host populations in the native range of the weed makes it reasonable to expect that resistance would similarly evolve following introduction to a new country (Espiau *et al.*, 1998). Although changes are likely to occur, they may remain

undetected for a long period of time in such biological control systems, because the pathogen has the capability of rapid evolutionary responses that counterbalance evolutionary change in the weedy host plant.

Benefits

Classical biological control is an economical and 'environmentally-friendly' approach to suppress weeds, in particular in habitats of low economic and/or high conservation value. There have been some spectacular successes with this method when exotic pathogens have been used, such as the highly successful introduction of *Entyloma ageratinae* against mist flower in Hawaii (Trujillo, 1985) and that of *Uromycladium tepperianum* to control *Acacia saligna* in South Africa (Morris, 1997). The collective gain from such control programs generally far exceeded their implementation cost. For example the biocontrol of the narrow-leaved form of skeleton weed with *Puccinia chondrillina* in Australia is rated as one of the most successful biocontrol program with a benefit-cost ratio of 112:1 (Marsden *et al.*, 1980).

Once a pathogen is introduced for classical weed biological control it is freely available to everyone, irrespective of whether they have contributed or not to the costs of the program. Following the successful establishment of the pathogen, a redistribution program may be required for some fungi to enhance their natural capabilities to disperse. However, rust fungi generally do not require redistribution because they are effectively dispersed by wind. Because of their ability to multiply rapidly and develop severe epidemics, pathogens can exert dramatic pressure on the target weed populations and significantly impact on the ability of the weed to regenerate and sustain itself.

Conclusion

For the last three decades, exotic pathogens have been used for the biological control of weeds and the safety record is unblemished, indicating that the scientific guidelines followed prior to introduction were appropriate. The spectacular successes of some of these pathogens in reducing the impact of weeds remind us that there are major benefits to be gained from this method of weed control. However vigilance in assessing risks and monitoring for unexpected impact on non-target plants must continue.

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Evaluation of *Phoma exigua* var. *exigua* as a biocontrol against Californian thistle (*Cirsium arvense*)

N.W.Waipara

Manaaki Whenua Landcare Research, Mt Albert Research Centre, Auckland, New Zealand

Abstract

Field trials undertaken over two seasons showed that *Phoma exigua* var *exigua* was a highly variable pathogen when inoculated as a conidial spray application onto Californian thistle plants. Infection was present at all field sites, however, disease levels and disease spread ranged from very low to moderately high both within and between trial site properties. The fungus was successfully re-isolated from host leaf and stem tissue but was absent from plant root samples. Disease levels at some sites showed the fungus was capable of inhibiting thistle growth and seeding but more research is needed to improve field efficacy and reduce variability of pathogenicity. Potential improvements to this pathogen's efficacy are being investigated in the current 2002/2003 growing season.

Introduction

A Coelomycetous fungus *Phoma exigua* var *exigua* Desm., was isolated from diseased Californian thistle (*Cirsium arvense* L. Scop.) plants at pastoral farming sites in several regions of New Zealand. The fungus was subsequently the subject of both laboratory experiments and field trials to determine its pathogenic potential against this agriculturally and economically significant weed host. Laboratory trials have ascertained New Zealand strains to be primary leaf pathogens that initially cause superficial necrotic leaf lesions that then systemically infect all plant shoot tissues (Waipara *et al.*, 1991,1997; Bithell & Stewart, 2001). A preliminary series of field based inoculations undertaken in 2000/2001, across four climatic regions established infection of the fungus on thistles at these sites, but efficacy and mortality was variable or undetermined for the majority of plots. Therefore to elucidate the pathogenic potential of this fungus, field based inoculations were again conducted at the Waikato and Coromandel sites during the 2001/2002 season.

Materials and Methods

Inoculum Production - The fungus was streaked onto Potato Dextrose Agar plates, incubated until pycnidial production was induced, and plate cultures expressing copious spore production were then harvested into solution. Spore solutions were filtered through 100 mm polyester mesh and centrifuged at 500 x g for 5 min. Spore counts were undertaken using Haemocytometer slide and all suspensions adjusted to 1×10^6 spores/ml by diluting with a spray solution (sterile distilled water, Tween 80, phosphate buffer)

Field Trial - Four 1m x 1m plots in thistle-infested pastures were pegged at six dairy farm properties. Inoculum (spore solutions, 1L per plot) was applied with a spray applicator, and two applications were made in late spring (Nov) and in summer (March). Treated field plots were visually assessed for the presence of infection and disease symptoms on all shoots. Total shoot counts and percentage thistle coverage in each plot (pre and post fungal application) was undertaken to assess mortality rate and disease levels at each site. Shoots with yellowing and superficial leaf spot lesions were evaluated to have a "low level" of disease, whilst plants

with leaf lesions, leaf death as well as stem lesions and wilting were evaluated as having a systemic or “high level” of disease. A sample of shoots exhibiting both types of symptoms, were then removed to confirm infection by *P. exigua* var *exigua*. DNA was extracted from fungal field isolates and PCR molecular based methodologies were additionally undertaken to provide a rapid diagnostic tool.

Results and Discussion

Phoma infection was successfully established at all field sites, however, pathogenicity of the fungus against Californian thistle was found to be significantly variable across all application sites. Variability with disease development was observed within the same site as well as between sites, and shoots mortality was generally low at all sites. Environmental factors could have influenced the pathogenicity of this fungus as both low and high levels of disease infection, severity and spread were found. The unusually wet summer during the 2001/2002 may have favoured thistle growth at the expense of the fungus, as healthy plants are generally more resistant and resilient to infection by pathogens. Despite the fungus failing to initiate high disease levels at many field sites, there were also significant “hot-spots” where the fungus was found to cause significant reduction of plant growth and development through systemic leaf and stem disease (see accompanying poster for graphs and figures).

The variable 2000-2002 field results reported here are further supported by a series of separate experiments undertaken at Lincoln University (Bithell & Stewart, 2001) which ascertained that the pathogenic potential *P. exigua* var *exigua* was also highly variable between strains on both detached leaves and whole plants, however wounding host tissue increased both infection and pathogenicity of the fungus. Subsequent laboratory based research undertaken has additionally found that age of the host leaf and plant was a critical factor of infection and pathogenicity of *P. exigua* var *exigua* on Californian thistle. Therefore it is likely that field infection and disease could be increased significantly with a prior wounding treatment before fungal inoculation and an earlier field inoculation when thistle rosettes first emerge in the pasture swards, these amendments are currently being investigated with this years field experiments.

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Determining the key pathogenicity factors of a *Sclerotinia sclerotiorum*-based mycoherbicide

Brenda Pottinger¹, Hayley Ridgway¹, Graeme Bourdôt², Geoff Hurrell², Ian Harvey³, and Alison Stewart¹

¹ Soil, Plant and Ecological Sciences Division, PO Box 84 Lincoln University, Canterbury

² AgResearch Ltd., PO Box 60, Lincoln

³ PLANTwise Services, PO Box 8915, Christchurch

Abstract

This poster paper presents the rationale for the senior author's PhD study on the infection processes in *Sclerotinia sclerotiorum*.

Background to *Sclerotinia sclerotiorum* as a mycoherbicide

A mycelial/whole wheat grain preparation of *Sclerotinia sclerotiorum* has been tested against *Cirsium arvense* (Californian thistle) and *Ranunculus acris* (giant buttercup) in New Zealand. An extensive three year study was done to evaluate the effect of application time on the efficacy of the mycoherbicide formulation for control of *C. arvense*. Applications made during October, November and December reduced the ground cover of *C. arvense* for 67, 67 and 44%, respectively of these applications. Reduced ground covers ranged from 38 to 81% of the cover on untreated plots. Late summer and autumn applications were less effective (Hurrell *et al.*, 2001). Other studies measured a 57% reduction in *R. acris* density and cover (Cornwallis, 1998). To date, field trial results indicate that control of targeted pasture weeds using the current mycoherbicide formulation is highly variable.

Studies indicate that resistance of *R. acris* to infection by *S. sclerotiorum* is related to morphological features of the crown: i.e., thickened peripheral cortex, deposits of lignified material at the margin of infected tissue, a wound response, and resistance of the crown's dense network of vascular tissue (Green *et al.*, 1998).

Current limitations of *S. sclerotiorum* as a mycoherbicide

- Applications have resulted in highly variable infection.
- Desiccation of the formulation can prevent host infection.

Objective

- To understand the key processes regulating the germination, infection, growth and *in vitro* survival of *S. sclerotiorum*.
- Information gained from these studies will be used as prerequisites to enhance the storage and field performance of the fungus as a mycoherbicide for control of giant buttercup.

Proposed research

1. *Pathogenicity experiments* To use both ascospores and mycelia inoculum to investigate pre- and post-infection processes and host resistance factors under different nutritional/environmental conditions. Image analysis will be used to determine the pathogenicity of *S. sclerotiorum* isolates.

2. *Longterm storage and maintenance of mycoherbicide isolates* Ascospores, mycelium and sclerotial inoculum will be tested under various storage conditions to determine which is most favourable for *S. sclerotiorum*. To obtain sufficient numbers of ascospores for experimentation, a reproducible method for stimulation of carpogenic germination in *S. sclerotiorum* will be developed.
3. *Production of pathogenesis related (PR) enzymes/compounds* To characterise key PR enzymes/compounds produced by *S. sclerotiorum* during ascospore versus mycelial infection of *R. acris* and compare the expression of these pathogenicity factors under different biotic/abiotic conditions.
4. *Gene expression during the fungus-host interaction* The above biochemical studies will be complemented by molecular studies looking at expression of key genes involved in enzyme production and plant defence responses during aggressive/non aggressive infections. This will lead to studies looking at manipulation of factors that regulate enzyme production (e.g., pH, nitrogen and carbon) in the application mixture in order to increase the reliability of the mycoherbicide.

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Controlling giant buttercup with *Sclerotinia sclerotiorum*

M. L. Verkaarik, G. A. Hurrell, V. W. Johnson and G. W. Bourdôt

AgResearch Limited, PO Box 60, Lincoln 8152, New Zealand

Abstract

Giant buttercup (*Ranunculus acris* L.) is a serious weed of dairy pastures in many regions of New Zealand. The emergence of resistance to the herbicides historically used to control this weed has led to the need for alternative options for control. In a recent laboratory study novel biopolymer formulations of the fungus *Sclerotinia sclerotiorum* exhibited high infection rates on excised giant buttercup leaves.

Introduction

Giant buttercup is an important weed of dairy pastures in Waikato, Taranaki, southern Hawkes Bay, northern Wairarapa, Horowhenua and Golden Bay where, at its peak it can occupy over 50% of the pasture area (Popay *et al.*, 1989). Giant buttercup is unpalatable to cattle and avoided to the extent where pasture plants in the immediate vicinity are also avoided (Popay *et al.*, 1989). The national annual revenue loss incurred by New Zealand dairy farmers from current infestations of giant buttercup is estimated to be \$118m (Graeme Bourdôt pers. comm.).

Continual use of MCPA, has resulted in giant buttercup populations that are resistant to this herbicide (Bourdôt *et al.*, 1989). Added to this problem is the desire of many farmers to either reduce their use of herbicides or to convert to organic milk production.

Previous work investigated the potential of *S. sclerotiorum* as a mycoherbicide against both Californian thistle (*Cirsium arvense*) (Hurrell *et al.*, 2001) and giant buttercup (Cornwallis *et al.*, 1999). This paper reports on a continuation of this developmental work in which we compare the pathogenicity of different formulations of *S. sclerotiorum*.

Materials and Methods

Cultures of *S. sclerotiorum* were grown on agar media in Petri dishes using an isolate that was collected from a natural infestation on Californian thistles. Plugs of mycelium-on-agar were then used to inoculate a potato dextrose broth. After several days in a shaking incubator the broths were homogenised and poured over sterilised kibbled wheat. The wheat was then incubated at 27°C for 4 days to allow the mycelium to grow through the substrate. The wheat was then dried in a forced-air oven to prevent further growth of the fungus. At this stage the formulation can be stored at low temperature for extended periods. The dried wheat was then ground and sieved to a final consistency of 1-3mm diameter particles. This basic formulation has been named WH1.

Variations on this basic formulation were also made in an attempt to increase the efficacy of the fungus and enhance its ability to withstand adverse conditions in the field. Newly developed formulations of *S. sclerotiorum* are based on the novel bioformulation technologies recently developed at AgResearch (Johnson & Pearson, 2001). These new formulations are named T425 and T425N.

The pathogenicity of the three different formulations was compared by bioassay on giant buttercup leaves kept under high humidity in the laboratory. Fifty-four leaves were

inoculated for each treatment. The size of the *S. sclerotiorum* lesions was measured at 24, 48 and 72 hours after application.

Results and Discussion

All but one of the inoculated leaves became infected and produced lesions which increased rapidly in size after 24 hours (Table 1). After 48 hours the mean lesion diameters were slightly larger for T425 and T425N than for WH1 while after 72 hours both T425 and T425N had produced significantly ($P < 0.05$) larger lesions compared to WH1, indicating that the two novel biopolymer formulations were more pathogenic towards giant buttercup than their rudimentary counterpart.

Evaluation of the three formulations on mature giant buttercup, in grazed dairy pasture under field conditions, is underway in field trials in Golden Bay this summer 2002/2003.

Table 1: Comparison of mean lesion size for the three *S. sclerotiorum* formulations. Lesion growth measured on *R. acris* leaves in the laboratory, in mm (# denotes too many zeros in data for analysis).

Formulation	Time after application		
	24 hours	48 hours	72 hours
T425	2.2	21.2	40.7
T425N	0.3	20.1	40.3
WH1	1.1	18.0	35.9
LSD ($P < 0.05$)	#	3.0	3.5

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A novel bioherbicide for control of grassy weeds

Dalice Mills^a, Donald Armstrong^a, Mark Azevedo^b and Gary Banowetz^b

^a Department of Botany and Plant Pathology, Oregon State University and ^bUSDA –ARS NFSPRC, Corvallis, Oregon, USA

Pseudomonas spp. were isolated from the rhizosphere of *Poa*, *Triticale*, *Triticum*, *Hordeum*, and *Lolium* species. These bacterial isolates were initially screened for the ability of live cultures to cause stunting of the roots and shoots of young seedlings of the grassy weed known as annual bluegrass (*Poa annua*). Isolates selected in this manner were evaluated further to determine whether live cultures arrested the germination of *P. annua* seeds. Twelve isolates were active in this test, and culture filtrates prepared from these isolates were also active in arresting *P. annua* seed germination. When culture filtrates of these twelve isolates were diluted to 25% of their original concentration, filtrates from five isolates still provided complete arrest of germination. We concluded that these isolates produced and secreted a putative Germination-Arrest Factor (GAF). Five of the most active isolates (WH6, E34, AD31, AH4 and WH19), identified as *Pseudomonas fluorescens* or *P. putida* with fatty acid methyl ester analysis, were selected for further study.

Bacteria-free culture filtrates of *P. fluorescens* isolates WH6 and E34 irreversibly arrest the germination of seeds of *P. annua* at a stage immediately following emergence of the coleorhiza and plumule. Successive dilutions of the culture filtrates elicit a decreasing array of developmental disturbances that can be scored and used to estimate the relative concentration of GAF responsible for these effects. GAF was shown to be both developmentally- and species-specific. Foliar applications of GAF extracts or root immersion in GAF solutions had little or no effect on growth at later stages of seedling development. The effect of GAF treatment on seed germination is irreversible in a relatively short period of time with permanent arrest achieved in less than 24 hours. The species-specificity of the respective GAF activities present in culture filtrates from isolates WH6 and E34 were evaluated in tests of the seeds from 14 species of graminaceous weeds and crop plants. The GAF activity from both isolates arrested the germination of seeds of *Aegilops cylindrica* (jointed goatgrass), *Bromus tectorum* (downey brome or cheat grass), *Vulpia myuros* (rattail fescue), and six perennial and annual species of *Poa*. Seed germination was arrested in tall fescue and perennial ryegrass regardless of whether the grass cultivars used were infected with the fungal endophytes *Neotyphodium coenophialum* or *N. loliae* (e.g., cultivars Titan and Cutter) or free of these endophytes (e.g., cultivars A.U. Triumph and Linn). Seed germination of spring wheat, barley, maize, clover and *Arabidopsis* was unaffected by GAF treatment.

The discovery of genes that control production or secretion of GAF was accomplished by transposon mutagenesis. Transposon Tn5, which confers tetracycline resistance, was introduced into *P. fluorescens* wild-type strain WH6 on plasmid pUTmini-Tn5gfp (Tn5gfp). Of 1,214 tetracycline-resistant transformants screened, three lacked GAF activity in culture filtrates. Fragments containing Tn5 were cloned from each of the three mutants and used as molecular probes to identify wild-type fragments by sequence homology. These wild-type fragments were cloned into pBlueScript for sequencing and into pME4510 for genetic complementation studies to identify genes that would restore GAF activity in the respective Tn5 mutants. The three Tn5 mutants, designated *gaf1*, *gaf2* and *gaf3*, were complemented

with wild-type DNA fragments. The nucleotide sequences of all three genes have varying degrees of homology with genes already sequenced and entered into the genome data bases, and their putative activities can be tentatively inferred. The complemented mutants exhibit restored GAF activity, establishing that the DNA sequences in question actually code for products essential for the expression of GAF activity in the bacterial culture filtrate.

GAF produced by strain WH6 is a hydrophilic molecule that is insoluble, or sparingly soluble, in all organic solvents tested to date. It has a molecular weight that is less than 3,000 daltons and it contains at least one ionizable group. GAF activity associated with culture filtrates appears to be stable when stored under sterile conditions at 4°C or at room temperature. GAF activity is not measurably affected by heating to temperatures up to 55°C for periods of at least 1 hour, but some loss of activity is evident at 75°C, and activity is destroyed in boiling water (100°C) or by autoclaving. GAF activity from WH6 culture filtrates is not affected by exposure to acid pH (down to pH 2) for periods of up to 3 hours and activity does not appear to be affected by exposure to weakly alkaline pH (up to pH 9) for 3 hours, but exposure to more extreme alkaline pH values (pH 11 and above) results in some loss of activity. GAF reacts with ninhydrin, and it can be visualized as a ninhydrin-positive band that chromatographs with a characteristic R_f value in defined thin-layer chromatography systems. This band is produced by the five isolates that initially exhibited GAF activity. Positive identification of GAF as the compound responsible for the specific ninhydrin-positive band in question has been accomplished by mutational genetic analysis. *gaf1*, *gaf2* and *gaf3* mutants fail to produce the band defined by thin layer chromatography, while the band is restored in each mutant transformed with the respective wild type gene.

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