

# **3rd International Biofumigation Symposium**

Dr John Kirkegaard  
CSIRO Plant Industry

Project Number: VG07191

## **VG07191**

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

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ISBN 0 7341 1898 8

Published and distributed by:

Horticulture Australia Ltd

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Sydney NSW 2000

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## Final Report HAL Project for VG07191

# Third International Biofumigation Symposium

21-25 July 2008 • Canberra ACT

*Biofumigation – from the fundamentals to the farming system*



[www.pi.csiro.au/biofumigation2008/index.htm](http://www.pi.csiro.au/biofumigation2008/index.htm)

Project Title: 3rd International Biofumigation Symposium  
Project No: VG07191  
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**Acknowledgements:**

The project team acknowledge the support of HAL and other Government and private sponsors along with the Australian and International Committee members who contributed in-kind to the development and conduct of the meeting.

31 October 2008



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## Media Summary

Biofumigation refers to the use of bioactive *Brassica* plants or plant products as an alternative to synthetic soil fumigants for pest, disease and weed control in agriculture and horticulture. These green manures or seed meal products release bioactive isothiocyanates (ITCs) – the compounds that give mustard and radish their “hot” flavour and are also the active ingredient in metham sodium a popular synthetic soil fumigant. The idea has gained increasing interest in recent years due to the phase-out of synthetic soil fumigants and a general interest in more environmentally conscious plant production systems worldwide. International scientists and Industry representatives have met at two previous Symposia in Florence (2004) and Idaho (2006) to discuss the development and application of biofumigation, and resolved to meet again in Australia in 2008.

We convened the 3<sup>rd</sup> *International Symposium on Biofumigation* in Canberra from 21 to 25 September, 2008 where 85 delegates from 22 countries met to showcase and exchange ideas from around the world on fundamental research in aspects of biofumigation, as well as product development and practical applications within the farming system. The program and its theme “*Biofumigation – from the fundamentals to the farming system*” allowed for interaction between the underpinning science, Industry partners in product development and farmers applying the technology to progress the successful adoption of the concept in agriculture.

The major conclusions arising from the meeting were (1) biofumigation will not be a “silver bullet” for MeBr replacement in high intensity industries (e.g. virus-free strawberry runners), but it ranked among the top non-chemical control alternatives for soil fumigants in less intensive production systems (2) fine-tuning of species selection and management is required to resolve the variability in control of multiple pests of some cropping systems (e.g. different effects on nematodes and fungi in potatoes) (3) growers reported multiple “system” benefits from biofumigation apart from disease suppression including improved soil health, structure and water infiltration, reduced erosion and higher crop yield. Points (2) and (3) provide the focus of further research and a network of international groups was proposed as a vehicle to co-ordinate this research, and a 4<sup>th</sup> meeting has been planned in Denmark in 2010.

## Evaluation of effectiveness

The effectiveness of the project can be assessed in relation to the stated objectives outlined in the project proposal

*Objective 1. To organise and conduct an International Symposium in Australia as a venue to showcase to Australian growers, Industry specialists and scientists the latest developments in the science and application of biofumigation technology from around the world.*

The 3<sup>rd</sup> International Biofumigation Symposium was held in Canberra from July 21 to 25, where 84 delegates from 22 countries met to discuss the latest developments and applications of biofumigation technology from around the world. The 85 delegates who attended matched the numbers attending the two previous meetings in Florence (2004) and Idaho (2006) which is a reflection of the quality of the program developed by the Committee and reinforces the strong position of Australian researchers in this field worldwide. The meeting proceeded on time and on budget, with no major interruptions, and the feedback from a survey of delegates indicated a high level of satisfaction with all aspects of the meeting by a vast majority of delegates (see feedback survey report this section – Table 1). The funding provided by HAL was specifically used to bring leading Australian vegetable growers and consultants with experience in using biofumigation at a commercial level to the meeting. Presentations by these growers was a feature of the Industry Forum Day which concentrated on practical applications and commercialisation of the biofumigation concept. These grower and consultants (from Tasmania, Victoria, Western Australia and South Australia) were considered to be Industry leaders who would be active in promoting information from the meeting within their own Industries upon their return

*Objective 2 To provide opportunities for Australian scientists, Industry representatives and growers to meet and generate productive and lasting collaborations with International delegates involved in the development and application of biofumigation technologies in sustainable horticultural systems less reliant on soil fumigants.*

Of the 85 Symposium delegates around 38 (~40%) were Australian delegates and represented researchers (CSIRO, Universities, Departments of Agriculture from all States), as well as Industry specialists, company representatives involved in commercialisation of biofumigant products and individual growers. A similar cross-section of different backgrounds was represented in the majority of international visitors from 22 different countries. The program provided significant opportunities to showcase and exchange ideas from around the world on fundamental research in aspects of biofumigation as well as product development and practical applications. The decision to create a program where this mix of fundamental and applied topics appeared to be appreciated by delegates based on responses to our evaluation survey (Tables 1 and 2). Significant communication and interaction has occurred since the meeting and plans for the next meeting in Denmark in 2010 are underway.

*Objective 3. To generate economic benefits to Australian vegetable producers by enhancing and facilitating Industry adoption of biofumigation technologies as part of integrated pest control in modern plant production systems.*

To some extent it is difficult to assess the level of economic benefit arising directly from the meeting as it will take time for these to accrue as ideas and techniques highlighted at the meeting are adopted and adapted by individuals within different industries. However some assessment of likely benefits can be gauged from the results presented by various delegates, and to the feedback from the survey results. An important message from the outset was that biofumigation will have a role in some, but not all farming systems – it will not replace the need for fumigation in highly intensive systems such as the virus-free strawberry runner businesses based in Victoria. However in certain production system (for examples vegetables such as potatoes) there were several examples of successful integration of biofumigation as part for integrated pest management with measureable economic benefit. A common feature of many presentations was the incremental improvements in soil health and crop performance in systems where biofumigation had been adopted as part of an overall management system, even when the diseases initially affecting crops were apparently still present in the soil. In addition to disease suppression and increased yield, several examples of multiple benefits in the system were presented including benefits to soil structure and soil health, reduced pesticide useage, reduced energy costs, and reduced exposure of operators to pesticides.

**Table 1 – Summary of results from evaluation survey**

	<b>1 Poor</b>	<b>2 Needs Improve</b>	<b>3 Adequate</b>	<b>4 Good</b>	<b>5 Excellent</b>
Pre-conference publicity/information		2	7	11	10
Conference registration (efficiency and friendliness of staff)				5	25
Lodging arrangements		2	5	8	12
Food services			1	8	20
Meeting facilities, comfort, acoustics,			1	4	24
Quality of presentations			13	21	5
Adherence of the program to its publicized content			1	15	14
Relevance of presentations to you		1	3	16	8
Overall organization of the program				7	23
Your overall rating of the conference/activity as compared to others you have attended			1	12	17
Length of the entire program			3	13	14
Length of the individual sessions				17	13
Amount of relaxation/social time		2	2	10	16
Opportunity for discussion			4	8	8
Opportunity to have questions answered			1	13	11
Please evaluate the keynote presentations			4	9	16
Overall evaluation of the Conference			1	12	16
<b>Responses from 30 Program Evaluations received</b>		<b>7</b>	<b>47</b>	<b>169</b>	<b>252</b>



**Table 2. Specific comments of attendees (Note – the aim of mixing science, industry and growers appeared to be appreciated)**

<b>Like most</b>	<b>Like least</b>	<b>Suggestions for next conf</b>
<b>Combination of scientific talks and growers experiences</b>	Over-representation of topics	International network approach
Interaction between delegates	Too many sweets	Include analytical techniques to measure GLS and ITC
Place of mtg and facilities close by		
<b>Communicating a lot with other researchers/growers</b>	Nematodes dominated – more info about soil-borne diseases	
<b>Mix of technical and practical discussion</b>		
Personal interaction		
	English a problem for some speakers	Break up talks more. Difficult to sit and listen for so long.
Evolution of biofumigation to additional benefits & management systems	More social time	
<b>Diversity of people attending – growers, scientists, consultants</b>	Lack of computers for internet	Better computer facilities
The warmth and camaraderie of the participants	How the participants presented their papers (oral)	Hope it will be held in Canada
<b>Well organised; good interaction; more knowledge about biofumigation + varieties</b>	Understanding accents. Researchers not presenting yield data. Reducing pest with biofumigation should be related to crop performance	Measurements of soil health before and after growing and biofumigation & measuring affect on crop
Broad scope of conference highlighting some of the good science which I was not aware of	A session where growers and scientists were able to develop a 'best management' practice to promote further	Development of a management guide that can explain in plain English for growers the excellent information which is out there
Organisation and timekeeping	Some content not applicable	No
<b>Scientific content and current relevance to industry problems</b>	Need to focus more on \$ returns/benefits to farmers	Need for equal mix of scientific/commercial content
<b>Being able to network, access to info from others</b>	From a growers level, the detail was over done in first day	Need to target an outcome of (i) varieties, incorporation, general target for growers to work from
Talk to the world's specialists on biofumigation and hear their most recent results and opinions	Accommodated delegates together as the best discussions and getting to know each other happens during breakfast and dinner	Accommodate most delegates in the same location. Organise a workshop in order to start new cooperative projects, written down new ideas, etc.
Farmers' experiences and general overview talks	One long talk wasn't quite 'tight' enough in terms of timing	
Information and discussion –	Cold temperature outside	It to another (integrated pest

contact to 'real' farmer The exchange of information; interest also in post harvest treatment	The little economic data and expansion on what growers need to adopt biofumigation as part of their strategy to manage pests	management, crop health ....) Combine with Biocontrol conference – Pilkington, NSWDPI
Learning from other researchers is my main interest in coming and also to present and share our work in biofumigation	The cool weather because I am not used to it	
Discussion and ability to meet informally	Apart from dinner – no weak points	Don't tinker too much – relaxing conference. Keep the practical focus.
Size, casual atmosphere, topic coverage (ie science – farming)	A few speakers were very difficult to follow	The venue should be similar to this one.
Interactions with others	Not enough microphones to use in Q & A sessions	To decide publication of proceedings ahead of time
Participation of people from different areas of expertise including growers experience and relaxed atmosphere	The growers session was rushed to make time for the field trip, not allowing sufficient question time for discussion	
The organisation of the program	The pre-conference information	
The variability in the program and the chance to speak with colleagues	A few long talks	Case studies/success stories using a standard format for growers (ie what crops, ITC levels, soil health varieties, etc). Limit to 10 slides
The size of the group and the common interest of the delegates. The general discussion about common assessments in a 'network'. The easy way to discuss together during sessions or during breaks.	There were many nematologists and maybe not enough agronomists and pathologists	Maybe a round table on common assessments everybody should collect (growing-crushing-soil health parameters.

## Key Outcomes

### *(1) A successful meeting.*

The major objective was to organise and run a successful meeting and to attract high quality International researchers as well as Australian growers and Industry representatives to interact, exchange ideas and form lasting collaborations. Indications from the significant amount of post-Symposium communications indicates this was achieved.

A website with all abstracts as well as pdfs of powerpoint presentations of oral presentations and pdfs of selected posters is available at the symposium website on [www.pi.csiro.au/biofumigation2008/index.htm](http://www.pi.csiro.au/biofumigation2008/index.htm)

### *(2) Biofumigation is not a silver bullet*

As far as replacing methyl bromide and other synthetic pesticides, biofumigation and associated cultural controls have been shown (by an international analysis of potential alternatives) to offer significant promise in certain cropping systems, but at present fall short of what can be achieved with other synthetic pesticides (Dr Ian Porter - keynote presentation). The important message here is that the technology should not be oversold, but will have applications in appropriate cropping systems.

### *(3) Biofumigants provide multiple benefits*

Biofumigant green manures can provide other benefits to soil improvement, which over time has allowed some growers to discontinue or greatly reduce synthetic chemical inputs, improve soil and maintain or improve yields. It was often these non-disease related benefits which growers come to value as much as, if not more than the disease suppression benefits. This point was emphasised by several of the Australian and International growers who presented their experiences during the Industry Forum. Thus while the pest control benefits related to the ITC release by brassicas has been the focus of the disease suppressive effects to date, research is broadening to understand and quantify the many benefits derived from other soil processes.

### *(4) Differential effects of biofumigants on different pest classes*

As biofumigant crops can sometimes host important pests of the main crop, it is important to evaluate carefully the types of species used and their management. This is particularly true for nematodes as some brassicas can host important nematodes (and some insect pests) of the commercial crop (e.g. grapevines). In other cases the biofumigant may be effective at suppressing one nematode group (eg root knot) but host another group (eg *Pratylenchus*). Thus growers must evaluate which biofumigant crop (or other green manure) best fits the purpose and seek as much information as possible before testing the concept with small-scale local testing before adopting the approach broadly.

### *(5) Biofumigant products need to be differentiated and protected in the market*

In Australia, the early success of the VinEco lines developed and distributed by Seedco indicates the potential for the biofumigation concept in vineyards, however other companies released cheaper generic products which were inferior, but reduced market share and confidence in overall concept. These issues are separate to the effectiveness of the biofumigants themselves, but are important to consider in terms of establishing and maintaining a market for the products.

## Recommendations

### *(1) An International network to provide consistent and reliable recommendations.*

The Symposium delegates highlighted the variability in the results presented at the meeting for various biofumigant species, various target pests and different soils, climate and management. This is to be expected for cultural control methods in contrast to the more predictable efficacy of synthetic chemical approaches. A recommendation was made to maintain the International network that has grown from the Symposium series and to plan specific actions ahead of the next meeting (Denmark or Mexico 2010). The group resolved to target either a specific pest (e.g. root knot nematode) or a specific crop (e.g. potato) as a focus to combine knowledge on biofumigation and draw out consistent responses into a “best-bet” management approach. No specific organisation or institution has the resources nor mandate to co-ordinate such an exercise involving scientists, Industry and commercial interests and growers, but if on-going research were co-ordinated in that way ahead of the next meeting (see (2)) progress in the application of the technology to agriculture would be advanced.

We recommend HAL take this into consideration when contemplating further funding in this area, so that limited Australian research \$\$\$ can be leveraged with access to the wider International research community. Australia has been an International leader in this field and has contributed significantly to the existing International networks. We would be in a position to participate further in this perhaps initially with a focus on specific crops (e.g. potato) in which ACIAR and HAL have both had previous and on-going projects related to biofumigants, and in which there is also significant activity in UK, USA, Netherlands and Germany.

A group of scientists lead by Dr Hoong Pung, Peracto, and including SARDI and CSIRO has been preparing a new funding submission to HAL to focus on further developing biofumigation for on-farm adoption in intensive vegetable production systems taking account of the Outcomes of this meeting.

### *(2) 4th International meeting*

Groups based in Spain and Denmark have both offered to host the next meeting in 2010 which will provide an opportunity to maintain and strengthen the networks established at the Canberra meeting.

## Budget Summary

Table 3. Budget income and expenditure at time of writing. HAL funds were used for advertising targeted at growers, and for travel costs of growers from all southern Australian States to attend and present at the meeting.

<b>Income</b>		<b>Expenses</b>	
<b>Item</b>	<b>\$AUs</b>	<b>Item</b>	<b>\$AUs</b>
<b><i>Sponsors</i></b>		Venue hire and food	14,812
HAL (AUS)	6,000	Buses	3,285
ACIAR (AUS)	5,000	Sponsor Dinner	2,524
CSIRO (ASI)	5,000	Conference Dinner	2,640
CSANR (USA)	1,000	Abstract booklet and CD	1,104
Terraprotect (GER)	1,000	Website design, photography	5,005
High Performance Seeds (USA)	1,000	Poster prize	105
Triumph Italia Spa (Italy)	1,000	PA Hire	346
SeedCo (AUS)	500	Grower accommodation	2,110
Mustclean® (AUS)	(in kind)	Travel 6 farmer guests(HAL)	5,000
<b><i>Registrations</i></b>		Advertising (2 ads) HAL	1,000
Registrations* (60)	20,040	Printing posters	210
Field tour (25)	650		
<b>Total Income</b>	<b>41,190</b>	<b>Total cost to date</b>	<b>38,142</b>

## Communications summary

The meeting was advertised widely throughout the existing network of scientists, industry specialists and growers in Australia and worldwide and a website was live 12 months prior to the meeting with information, contacts and registration material. In Australia we also advertised the meeting and in particular the Industry Forum in the months leading up to the meeting with advertisement in *Good Fruit and Vegetables* and *SA grower* as well as *Veg Tech* website.

During the meeting a number of delegates were interviewed by local Canberra ABC Rural reporter Sarina Locke and other interviews from interstate radio and media were conducted in a media room at the Discovery centre. Following the meeting articles appeared in several newspapers and magazines and Dr Kirkegaard was also interviewed at ABC studios by Marnie Chesterton from Radio Nederland indicating the International reach of the meeting (Table 3)

Table 3 – A summary of media coverage of the meeting made known to organisers.

Topic or Headline	Date of Story	Media
Radio Interview with John Kirkegaard	28-Jul-08	ABC Radio Mildura, Swan Hill News
Radio Interview with John Kirkegaard	23-Jul-08	ABC Radio Goldfields WA
Radio Interview with John Kirkegaard	25-Jul-08	ABC Radio North and West SA (Port Pirie)
Radio interview with Bryan Robertson	24-Jul-08	ABC Rural Radio Canberra
Radio interview with Dale Gies (USA)	23-Jul-08	ABC Rural Radio, Canberra
Various radio interviews with delegates	22-24-Jul-08	ABC reporter Sarina Locke
Radio interview with John Kirkegaard	12-Aug-08	Radio Nederland
Biofumigation to reduce soil-borne diseases	28-Aug-08	Queensland Country Life
Green mulching mustard a help to fight soils diseases	28-Aug-08	Farm Weekly
Biofumigation still has potential in vineyards (Bianca Turner)	September Issue	Australian and NZ Grapegrower and Winemaker



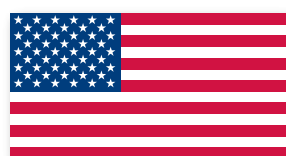
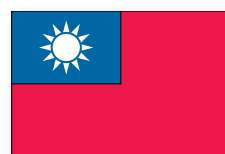
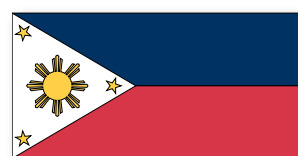
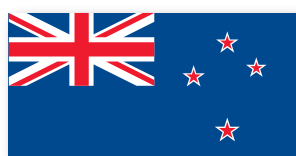
# Third International Biofumigation Symposium

CSIRO Discovery Centre, Canberra, Australia  
21 – 25 July 2008

## PROGRAM AND ABSTRACTS



## Countries represented





# Foreword

Biofumigation is a term originally coined to describe the use of bioactive brassicaceous plant products for pest, disease and weed control in agriculture and horticulture. The phenomenon has been recognised for centuries, but has gained increasing interest in recent years due to the phase-out of synthetic soil fumigants and a general interest in more environmentally conscious plant production systems worldwide. The concept is based upon capturing benefits from the bioactive products of the glucosinolate-myrosinase system in plants, which originally evolved as part of the plant's own defence system. Recent fundamental research has provided significant advances in the understanding of the soil and plant mechanisms which underpin biofumigation. At the same time, advances in product development and farming systems research has better identified the most promising opportunities to apply this knowledge to generate economic benefits in practical farming systems. We have gathered as a focussed group of International scientists and Industry representatives previously in Florence (2004) and Idaho (2006), and following these successful meetings resolved to meet again in Australia in 2008.

At this *3rd International Biofumigation Symposium* our aim is to provide a venue to showcase and exchange ideas from around the world on fundamental research in aspects of Biofumigation, as well as product development and practical applications within the farming system. The integration of knowledge across these disciplines will be essential if biofumigation is to be refined and adopted in the most reliable and relevant ways. Our theme "*Biofumigation – from the fundamentals to the farming system*" reflects our desire to enhance the links between excellent underpinning science with the Industry partners for product development and provision so essential for the successful adoption of the concept in Agriculture.

**John Kirkegaard**

A handwritten signature in black ink, appearing to read 'John Kirkegaard', with a stylized, flowing script.

Chair, Organising Committee

# Committees

The 3rd International Biofumigation Symposium has been planned in consultation with an experienced network of scientists and Industry representatives both in Australia and from countries throughout the world. The committee members have direct experience in research, development and education in the use of bioactive brassicaceous plant products and many were involved in the previous meetings in Florence (2004) and Idaho (2006).

## International Advisory Committee

John Kirkegaard	CSIRO, Canberra, Australia (Chair)
Matthew J Morra	University of Idaho, Moscow, USA
John Halbrendt	Penn Sate University, Bilgerville, USA
Anne Louise Gimsing	KVL, Copenhagen, Denmark
Luca Lazzeri	ISCI, Bolgna, Italy
Jaw-Fen Wang	AVRDC - The World Vegetable Center, Tainan, Taiwan
Reinette Gouws	ARC, Roodeplant, South Africa
Stuart Gowers	CRI, New Zealand

## Australian Organising Committee

John Kirkegaard	CSIRO Plant Industry, Canberra (ACT)
John Matthiessen	CSIRO (Ret.), Perth (WA)
Robin Harding	SARDI, Lenswood (SA)
David Nehl	NSW DPI, Camden (NSW)
Graham Stirling	Biological Crop Protection, Brisbane (QLD)
Ian Porter	Department of Primary Industries, Victoria, Melbourne (Vic)
Hoong Pung	Peracto, Devonport (TAS)

## Local organisation and website

Val Oliver	CSIRO Plant Industry, Canberra (Registration, enquiries)
Siobhan Duffy	CSIRO Plant Industry, Canberra (Website design & management)



# Third International Biofumigation Symposium

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21 – 25 July 2008

## PROGRAM AND ABSTRACTS

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# PROGRAM

**Monday 21 July – Welcome Reception & Registration****4.00 – 6.00 pm Welcome Reception and Registration (CSIRO Discovery Centre)**

An informal reception with light refreshments on Monday afternoon will allow travellers to arrive, register and settle into Canberra and to locate and familiarize themselves with the venue for the meeting.

**Tuesday 22 July**

8.00 – 9.00 am      Registration (CSIRO Discovery Centre)

**Session 1. Plenary session (Chair Professor Matt Morra)**

- 9.00 – 9.15      Welcome and opening remarks  
*Dr John Kirkegaard, Chair Organising Committee*
- 9.15 – 9.55      Soil biofumigation – a strategy for the new world or a complexity too hard to get right?  
*Dr Ian Porter (Australia)*
- 9.55 – 10.30      Fate of glucosinolates and their hydrolysis products in soil  
*Dr Anne Louise Gimsing (Denmark)*
- 10.30 – 11.00      Coffee

**Session 2. Root glucosinolates and non-ITC effects of biofumigants (Chair Robin Harding)**

- 11.00 – 11.20      The distribution of glucosinolates and sulphur-rich cells in roots of field-grown canola (*Brassica napus*)  
*Margaret McCully (Australia)*
- 11.20 – 11.40      Myrosinase immobilized on Ca-polygalacturonate hydrogel: enzyme efficiency as a function of soil characteristics  
*Onofrio Leoni (Italy)*
- 11.40 – 12.00      Analysing disease progress curves to understand the effects of biofumigation on *Rhizoctonia* root rot of sugar beet  
*Natacha Motisi (France)*
- 12.00 – 12.20      Evaluation of *Brassica* accessions as potential biocidal green manure to control tomato bacterial wilt  
*Jaw-Fen Wang (Taiwan)*
- 12.20 – 12.35      Discussion
- 12.35 – 1.30      Lunch (Discovery Centre)

**Session 3. Seed meals, green manures and post harvest applications (Chair Luca Lazzeri)**

- 1.30 – 1.50      Ionic thiocyanates (SCN-) production, fate and phytotoxicity in soil amended with Brassicaceae seed meals  
*Matthew J Morra (USA)*
- 1.50 – 2.10      The effects of *Brassica* seed meal amendments on *Meloidogyne* hapla viability in laboratory bioassays  
*John M Halbrendt (USA)*
- 2.10 – 2.30      Understanding the mechanisms of plant pest suppression using *Brassica* green manures  
*John Kirkegaard (Australia)*

- 2.30 – 2.50 Five years of research to evaluate the potential utilisation of isothiocyanates to control postharvest fruits and vegetable losses by fungi infections in Mexico  
*Martin-Ernesto Tiznado-hernandez (Mexico)*
- 2.50 – 3.05 Discussion
- 3.05 – 3.30 Coffee

#### **Session 4. Beyond biofumigants (Chair Scott Mattner)**

- 3.30 – 4.00 Biorefinery - Brassicaceae plants as more than biofumigants!  
*Luca Lazzeri (Italy)*
- 4.00 – 4.30 Discovery and development of *Muscodor* species for mycofumigation  
*Gary Strobel (USA)*
- 4.30 – 4.45 Discussion
- 4.45 – 6.30 Poster Session 1 (with drinks, nibbles) – All posters on display

#### **Free evening for Symposium delegates**

#### **Sponsor and Committee Dinner, University House**

### **Wednesday 23 July – Industry Forum Day**

A focus on application and adoption of biofumigation including product development and demonstrations and grower experiences in utilizing biofumigants

- 8.00 – 9.00 Registration for Industry Forum Day

#### **Session 5. Applying biofumigation for disease control worldwide (Chair Brad Wells, HAL)**

- 9.00 – 9.20 Development of biofumigation as a non-chemical control method against plant parasitic nematodes and soil-borne diseases in temperate climates  
*Michalela Schlathölter (Germany)*
- 9.20 – 9.40 Green manure and biofumigation for root lesion nematode and verticillium wilt management in strawberry production  
*Guy Belair (Canada)*
- 9.40 – 10.00 The use of biofumigant green manure crops for soil-borne disease management in Tasmania, Australia  
*Hoong Pung (Australia)*
- 10.00 – 10.20 Innovative approach for producing high value products in non food agro-industrial chains  
*Giampiero Patalano (Italy)*
- 10.30 -11.00 Coffee

#### **Session 6. Industry and grower experiences 1 (Chair Les Baxter, ACIAR)**

- 11.00 – 11.20 Development and utilisation of mustard cover crops in the USA  
*Dale Gies (USA)*
- 11.20-11.40 Specialised Australian cover crops – the Seedco Experience  
*Bryan Robertson (Australia)*

#### **Farmer experiences**

- 11.40 – 12.00 *Keith Taylor (Western Australia)* (assisted by J Matthiessen)
- 12.00 – 12.20 *Darren Long/Peter Aird (Tasmania)* (assisted by H Pung)
- 12.20 – 12.40 *Tim Widdison (South Australia)* (assisted by R Harding)
- 12.40 – 1.00 *George Weda/Darren Schreurs (Victoria)* (assisted by I Porter)

## PROGRAM

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- 1.00 – 1.30 Lunch on bus en route to CSIRO Ginninderra Experiment Station
- 1.30 – 3.00 Visit to CSIRO Farm to view field plots, discuss biofumigant products and incorporation techniques. Discuss of field applications and farmer experiences.  
[Speakers re varieties on view – Dale Gies, Michaela Schlathoelter, Luca Lazzeri, David Jacobs, Bryan Robertson, John Kirkegaard, Stuart Gowers]  
[Afternoon tea at farm]

### **Session 7. Utilising Brassicas and other green manures (Chair John Matthiessen)**

- 3.30 – 3.50 Utilising green manures in irrigated agriculture – brassicas vs other options  
*David Nehl (Australia)*
- 3.50 – 4.10 Soil biodisinfection for the management of soil-borne pathogens and weeds  
*Antonio Bello (Spain)*
- 4.10 – 4.30 Farmers' experiences of biofumigation for bacterial wilt management in solanaceous crops in the Philippines  
*Valeriana Justo (Philippines)*
- 4.30 – 5.30 Discussion Forum – Questions and Answers with expert panel  
*Moderator - Dr Ian Porter*
- 5.30 – 6.30 Optional poster viewing for day Registrants
- 6.30 – 7.15 Koomurri – Aboriginal Dance Performance (Discovery Lecture Theatre)

### **7.30 pm Conference Dinner (CSIRO Discovery Centre)**

## Thursday 24 July

### **Session 8. Integrating biofumigants for disease control and other benefits (Chair Jaw-Fen Wang)**

- 9.00 – 9.20 Strategies for enhancing brassicas multipurpose attributes in managing nematode parasitism complexes  
*Haddish Melakeberhan (USA)*
- 9.20 – 9.40 Biofumigation and management of soil-borne organisms in sandy soil crops in Almeria (Spain)  
*Miguel A. Diez-Rojo (Spain)*
- 9.40 – 10.00 Evaluation of biofumigation crops in the control of *P. penetrans* and *V. dahliae*  
*Gerard Korthals (Netherlands)*
- 10.00 – 10.20 The use of by-products from the wine industry in the control of plant parasitic nematodes  
*J.A. Lopez-Perez (Spain)*
- 10.20 – 10.40 Alterbromide, a European programme for the dissemination of alternatives to methyl bromide  
*Gael du Fretay (France)*
- 10.40 – 11.00 Discussion
- 11.00 – 11.30 Coffee

### **Session 9. General Discussion and awards**

- 11.30 – 12.30 General Discussion, Poster competition award announcement and closing Remarks
- 12.30 – 1.30 Lunch and removal of Posters
- 1.30 – 2.30 Committee meeting re Conference Proceedings

### **Free afternoon for group meetings/Canberra sightseeing or travel**



## Friday 25th July – Optional Field Tour

An optional one day field tour will be organised by John Kirkegaard to the area west of Canberra. The area has historic significance as site of original gold rushes, settlement by squatters producing merino fine wool, terrorised by bushrangers during the 1800s and is now the heartland of Australia's famous wheat/sheep belt. Closer to Canberra cool climate wineries and fine food production has also expanded and we will sample some local produce during the day.

The day will include:

- visit to Yandilla mustard enterprise – (food as well as biofumigant products)
- a biofumigant seed producer
- a visit to a typical Australian farm (sheep shearing etc) with Aussie BBQ lunch
- a local glass artisan who supplies his wares to the English Royal Family
- a visit to a local winery where we can sample some local food

### Proposed tour schedule

8.00 am	Depart Canberra via Yass to Wallendbeen Commentary on bus of exploration, settlement, land use, agriculture, in the region
10.00 am	Arrive David Jacobs farm View sheep dogs in action, shearing Morning tea and description of typical Australian mixed farm Discussion of biofumigant seed production
12.00 noon	Depart Farm
12.15 pm	Arrive Yandilla Mustard Enterprise Wallendbeen Inspection of facilities, history of mustard industry Lunch
2.00 pm	Depart Yandilla Visit Young Visit John Gilbert Grave (Bushranger) Binalong Visit Cooma cottage
3.30 pm	Arrive Jeir Creek winery for winery tour, for wine tasting and gourmet local food
5.30 pm	Depart winery
6.00 pm	Arrive Canberra 6.00 pm

Note: Exact plans may change a little depending on numbers and specific interests of those involved as there are alternative attractions along the route.

## Poster List

### Disease control

#### Nematodes

- POS-101 Influence of soil biofumigation in the control of *Meloidogyne javanica*, *Mesocriconea xenoplax* and free living nematodes in different soil layers using castor cake and cabbage  
*Cesar Bauer Gomes (Brazil)*
- POS-102 Management of *Meloidogyne incognita* (Kofoid et White) Chitw. in organic horticulture  
*Giovanna Curto (Italy)*
- POS-103 Effect of biofumigation on different plant parasitic nematodes  
*Michaela Schlathöelter (Germany)*
- POS-104 Biofumigation for the control of root-knot nematodes on flower crops  
*Giuseppe De Mastro (Italy)*
- POS-105 Effectiveness of biofumigating *Brassica* treatments for the control of root-knot nematode *Meloidogyne incognita* on melon and watermelon  
*Giuseppe De Mastro (Italy)*
- POS-106 Biofumigation in greenhouse for the control of root-knot nematodes  
*Giuseppe De Mastro (Italy)*
- POS-107 Control of carrot root-knot and cyst nematodes by biofumigating treatments  
*Giuseppe De Mastro (Italy)*
- NO POSTER** Assessing bionematicide effect of vegetable extracts on *Meloidogyne javanica* hatch  
*Seddigheh Fatemy (Iran)*
- NO POSTER** Potential impact of green manure crops on the sustainable sugar beet cyst nematode management in Idaho, USA  
*Saad L. Hafez (USA)*
- POS-108 Biofumigation for control of *Globodera pallida* in UK potato fields  
*James Lord (UK)*
- POS-109 Effectiveness of biofumigation technique to control the southern root-knot nematode (*Meloidogyne incognita*) in Sicily  
*Giuseppe Marano (Italy)*
- POS-110 Nematode suppressive effect of two biofumigation crops (*Brassica juncea* and *Cleome hussleriana*) evaluated by laboratory and greenhouse experiments  
*Tomoko Mitsuho (Japan)*
- POS-111 Organic amendments and host resistance as components of integrated disease management strategy for root-knot nematode *Meloidogyne incognita* in lettuce  
*Nordalyn Pedroche (Philippines)*
- POS-112 Optimizing biofumigation varieties and blends for non chemical control method against plant parasitic nematodes and soil born diseases in temperate climates  
*Michaela Schlathöelter (Germany)*
- POS-113 Cover crops in the year before planting potatoes – a key factors in the spraing disease management strategy  
*Sanja Manduric (Sweden)*
- POS-114 Expression of a 70 KDa heat shock protein (HSP70) in *Caenorhabditis elegans*, as an indicator of allyl isothiocyanate toxicity in mustard  
*Akal Rachna K. Saini (Canada)*
- POS-115 Behavioral response of *Meloidogyne incognita* to benzyl isothiocyanate  
*Inga Zasada (USA)*

#### Fungi

- POS-116 Biofumigant activity of Brassicaceae against soilborne fungi  
*Francesco Faretra (Italy)*
- POS-117 Effects of soil Sulphur and Nitrogen on Isothiocyanate production within Brassica species and subsequent mycelial inhibition of *Rhizoctonia solani*  
*Robin Harding (Australia)*
- POS-118 Introduction of fumigation crop research against soil-borne diseases and nematode in Japan  
*Ken Hashizume (Japan)*
- POS-119 Biofumigation to control *Verticillium* wilt influenced by plant species and soil types  
*Luca Lazzeri (Italy)*

- POS-120 Biofumigation against soilborne pathogens and weeds of strawberry  
*Scott Mattner (Australia)*
- POS-121 Growing crops of *Brassica juncea* and then incorporating their residues give complementary control of *Rhizoctonia* root rot of sugar beet  
*Natacha Motisi (France)*
- POS-122 Biofumigation for soil-borne disease management in flower bulb culture  
*Gera van Os (The Netherlands)*
- POS-123 Evaluating biofumigant amendments for the management of diseases caused by sclerotial pathogens in vegetable crops  
*Oscar Villalta (Australia)*
- POS-124 Biosolarisation as an alternative to methyl bromide in protected pepper crops in Spain  
*Miguel Díez-Rojo (Spain)*
- POS-125 The use of vinasses in the management of soil-borne pathogens  
*José Antonio López Pérez (Spain)*
- POS-126 Isolation of cDNAs upregulated in *Alternaria alternata* tolerant to lethal concentrations of 2-propenyl isothiocyanate  
*Maria Elene Baez Flores (Mexico)*
- POS-137 Incorporation of *Brassica nigra* and *Diplotaxis tenuifolia* residues and incubation under different soil conditions affects the survival of *Rhizoctonia solani* AG2-1 (ZG5), the causal agent of damping off of canola differently  
*Titiek Yulianti (Indonesia)*

**Bacteria**

- POS-127 Evaluating biofumigation for soil-borne disease management in white potato  
*Fe Abragan (Philippines)*
- POS-138 Potency of Brassica residues as biofumigation for control of bacterial wilt of tobacco in Indonesia  
*Titiek Yulianti (Indonesia)*

**Insects**

- POS-128 *A Folsomia candida* (Collembola: Isotomidae) bioassay to investigate biofumigation process by *Brassica carinata* seed meals  
*Lorenzo D'Avino (Italy)*

**Soil and plant processes**

- POS-129 Potentially mineralizable nitrogen is soil amended with biocidal and non-biocidal plant materials  
*Luca Lazzeri (Italy)*
- POS-130 An alternative pathway for glucosinolate degradation in soil  
*Onofrio Leoni (Italy)*
- POS-131 Isolation and technological characteristics of *B. carinata* myrosinase  
*Onofrio Leoni (Italy)*
- POS-132 Myrosinases from *Crambe abyssinica* seeds: a new tool for biofumigation?  
*Onofrio Leoni (Italy)*
- POS-133 A long-term green manure trial within intensive cropping in Tasmania  
*Leigh Sparrow (Australia)*
- POS-134 Selection of *B. napus* and *B. rapa* lines for biofumigation potential  
*Stuart Gowers (New Zealand)*
- POS-135 Bioprospecting for endophytes from Australian flora with mycofumigation potential  
*Ross Mann (Australia)*
- POS-136 Travelling the green route to soil fumigation  
*Sue Hockland (UK)*
- POS-139 New products based on Brassicaceae materials: a liquid formulation with fertilizing and biocidal effects for application in drip irrigation  
*Luca Lazzeri (Italy)*
- POS-140 A simple analytical method for dhurrin content evaluation in cyanogenic plants for their application as biofumigant  
*Luca Lazzeri (Italy)*
- NO POSTER** Effect of rape/wheat strip intercropping on disease resistance, yield and agronomic characters of winter wheat  
*Chunlei Zhang (China)*
- NO POSTER** Effects on Paddy field Environment and Rice Growth  
*Xiu-fu Zhang (China)*

## Field Trip – CSIRO Ginninderra Experiment Station

Wednesday 23 July, 1.00pm – 3.00pm

### Background

CSIRO Plant Industry maintains the Ginninderra Experiment Station (GES) near Canberra to conduct a range of field research on crop, pasture and animal science. The farm is a total of 700 ha, with 100 arable, 80 irrigated and the remaining area supports a flock of 3000 fine-wool merino sheep and some fat lambs on native and improved pastures. CSIRO used the farm for its mustard breeding program (Rex Oram), initially for condiment types, and then canola-quality mustard. We have conducted a number of research projects on biofumigation at GES since 1995 including;

1. Screening of 85 different species/varieties for biofumigation potential  
(*Kirkegaard and Sarwar 1998, Plant and Soil*)
2. Studies on take-all suppression by canola and mustard rotation crops  
(*Kirkegaard et al 2000; Smith et al 2004; Australian Journal Agricultural Research*)
3. Inheritance of root glucosinolates in canola  
(*Kirkegaard et al 2001, Australian Journal Agricultural Research*)
4. Release of ITCs in soil following *Brassica* green manures  
(*Morra and Kirkegaard, 2002; Gimsing and Kirkegaard 2006; Soil Biology Biochemistry*)

### Biofumigant species and varieties on display

A range of different species and varieties which have been used as biofumigants or cover crops in different parts of the world have been sown so that delegates can see the variety of Brassicaceous plants utilised for Biofumigation. The collection is not exhaustive, but includes most of the species currently used, and some specific varieties which are either in use or have been used previously as cover crops or to produce seed meals or other products. Representatives who provided the seed will give some background to the development and characteristics of the varieties and how they are currently marketed or utilised.

Brief notes and a field map are shown on the following page indicating those who have developed or marketed the varieties and the key characteristics.

### Incorporation strategies

CSIRO researchers, along with visitors from USA (Matt Morra) and Denmark (Anne Louise Gimsing) have also conducted several experiments to investigate the effect of different incorporation strategies on isothiocyanate release in soils. We have demonstrated the importance of significant tissue disruption, rapid incorporation and sufficient water in increasing ITC release from incorporated tissues. The benefits of maximising ITC release must be considered against potential damage from excessive cultivation and the non-ITC benefits of the green manure which are significant in many systems.

We will inspect the approaches used and discuss the relative merits of different incorporation strategies used by different growers and groups from around the world.

## Field plan and species/variety notes

Species/Variety	Use/Origin/Company/Representative
Idagold ( <i>Sinapis alba</i> )	Incorporation demonstration area
Fumus ( <i>Brassica juncea</i> )	
BioQure-Mulch ( <i>Brassica napus/campestris</i> )	
<b>Nemat</b> ( <i>Eruca sativus</i> )	<b>Biofumigant</b> Italy/Triumph Italia Spa/Patalano-Lazzeri
<b>Adagio</b> – Fodder raddish ( <i>Raphanus sativus</i> )	<b>Nematode resistant cover-crop</b> Germany/PH Petersen /Schlathölter
<b>Maxima-Plus</b> - Rape ( <i>Brassica napus</i> )	<b>Fodder rape – very low GSL</b> Kirkegaard
<b>BioQure-Mulch</b> – Rape-turnip ( <i>Brassica napus/campestris</i> )	<b>Biofumigant</b> NZ/ Wrightson PGG/Gowers
<b>Rex</b> – Indian mustard ( <i>Brassica juncea</i> )	<b>Condiment</b> Australia/Jacobs
<b>Idagold</b> – White mustard ( <i>Sinapis alba</i> )	<b>Oilseed (meal for biofumigant)</b> USA/Uni Idaho/Morra
<b>CT 207</b> ( <i>Brassica carinata</i> )	<b>Biofumigant</b> Italy/Triumph Italia Spa/Patalano-Lazzeri
<b>ISCI 7</b> ( <i>Brassica carinata</i> )	<b>Biofumigant</b> Italy/Triumph Italia Spa/Patalano-Lazzeri
<b>Arid</b> ( <i>Brassica juncea</i> )	<b>Low-GSL oilseed mustard</b> Canada/Kirkegaard
<b>Nemfix</b> ( <i>Brassica juncea</i> )	<b>Biofumigant</b> Australia/Seedmark/Robertson
<b>Fumus</b> ( <i>Brassica juncea</i> )	<b>Biofumigant</b> Australia/Agseeds Research/Kirkegaard
<b>Mustclean</b> ( <i>Brassica juncea</i> )	<b>Biofumigant</b> Australia/Jacobs
<b>Katy</b> ( <i>Brassica juncea</i> )	<b>Condiment oilseed</b> Australia/Jacobs
<b>ISCI 99</b> ( <i>Brassica juncea</i> )	<b>Biofumigant</b> Italy/Triumph Italia Spa/Patalano/Lazzeri
<b>ISCI 61</b> ( <i>Brassica juncea</i> )	<b>Biofumigant</b> Italy/Triumph Italia Spa/Patalano/Lazzeri
<b>ISCI 20</b> ( <i>Brassica juncea</i> )	<b>Biofumigant</b> Italy/Triumph Italia Spa/Patalano/Lazzeri

## Small plot nursery

Turnip	Turnip	Turnip	Collard	B carinata	Radish
Radish	Radish	Radish	Radish	Radish	Radish
Radish	Radish	Fodder rape	Fodder rape	Fodder rape	Fodder rape
Hakuran x swede	Swede x rape	Swede	Bulb juncea	Bulb juncea	Bulb juncea
Bonchoi (B juncea)	Mustard	Mustard	Mustard	Mustard	Kale



# ORAL ABSTRACTS

## **Soil Biofumigation – a strategy for the new world or a complexity too hard to get right?**

**Ian Porter<sup>1</sup>, Scott Mattner<sup>1</sup> and George Lazarovits<sup>2</sup>**

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The phase-out of methyl bromide for soil fumigation under the Montreal Protocol indicates to the world that pesticides that damage the environment will no longer be tolerated in agriculture. The phase-out has been a major turning point for science, as it stimulated a massive research effort to find a 'silver bullet' replacement. Scientists considered that new technologies could be developed and adopted by farmers because finally the western world was ready to adopt sustainable production practices. Similarly it was anticipated that growers would readily adopt practices which conserved the biodiversity values and principles of biological equilibrium. In reality this has not happened. A decade later, most uses of methyl bromide have been replaced by other fumigant chemicals, as other technologies have not yet provided the same advantages afforded by soil fumigation, (i.e. a high level of pest and disease control, high quality, high yields and most importantly large profits and low risk). However, a bigger set of factors is now influencing crop production and crop protection; water is becoming scarce, demand for inorganic fertilisers has dramatically increased their costs, fumigant pesticides are facing further restrictions because of tropospheric pollution, and concerns over climate change and soil health are finally being recognised.

It seems likely that future crop protection will therefore rely more on sustainable practices which utilise the principles of more natural pathogen reduction such as biofumigation, or more broadly, biodisinfestation. However, there must be acknowledgement that grower profit is paramount for selection of crop protection methods. While the agronomy and economics of some cropping systems may accommodate the use of biofumigant rotation crops (e.g. broad-acre cereals), other high-value/short-rotation crops (e.g. vegetables) may require the use of biofumigant amendments (e.g. isothiocyanate generating or high-N products) or biofumigant-derived pesticides (e.g. allium thiosulfinates, dimethyl disulphide). Many papers also show that many biofumigant products suppress nematodes more effectively than fungal pathogens and obligate pathogens more so than facultative pathogens which can utilise organic matter as a food source. Suppression by biofumigants may also require persistent efforts to modify the system, as ITC levels in biofumigant crops are still generally low (ie. 10 to 50 times less than that with chemical fumigants) and the effect may be short-lived in soils. However, many of the technologies that are being tested still lack sufficient understanding for delivery of optimal disease control activity. Biofumigation needs to be considered as a broader concept which accounts for all the physical, chemical and biological changes that occur in soil to achieve crop production and improved soil health. Models must further be expanded to elucidate which chemical and biological changes predominate during biofumigation or biodisinfestation (eg. ITCs, nitriles,  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ , nitrous acids, C/N ratios, cation levels (Ca), volatile fatty acids, *Streptomyces* or other microbial antagonists, etc.) and which are most involved in disease suppression. Further challenges for adoption of biofumigation will be discussed during the presentation.



## Fate of glucosinolates and their hydrolysis products in soil

Anne Louise Gimsing<sup>1</sup>, John A. Kirkegaard,<sup>2</sup> Bjarne W. Strobel<sup>1</sup> and Hans Christian Bruun Hansen<sup>1</sup>

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The fate of glucosinolates (GSLs) and their hydrolysis products in soil determines both the efficacy and environmental impact of biofumigation. Knowledge of the processes by which these compounds are sorbed, degraded or otherwise lost from the soil is fundamental to developing effective, but environmentally benign biofumigation strategies. Effective biofumigation relies on maximum hydrolysis of the GSLs in the plant tissue to generate high isothiocyanate (ITC) concentrations in the soil after incorporation. This is favoured by maximum cell disruption, by addition of water, and a high soil temperature. Residual GSLs are very weakly sorbed, readily leached and are microbially degraded and mineralised in soil. GSL degradation usually follows logistic kinetics with typical half-lives around 1 day in top soils and 8 – 9 days in sub-soils. Degradation is faster in clayey soils than in sandy soils probably because of an association between myrosinase activity and the clay fraction. In contrast, ITCs are strongly sorbed by the organic matter in soil, react strongly with nucleophilic groups present in soil, and are prone to volatilization losses in addition to microbial degradation and mineralisation. ITC degradation follows first-order kinetics with half-lives from hours to a few days. These loss processes are influenced by soil type, water content and temperature. A leaching study using intact soil columns has demonstrated that ITCs can leach to 1 m following simulated biofumigation, although at low concentrations (0.1 – 1  $\mu\text{M}$ ).

Using appropriate incorporation strategies, sufficiently high ITC concentrations ( $>100 \text{ nmol g}^{-1}$ ) can be achieved in soil using biofumigation for effective suppression of susceptible pests on light-textured soils. The relatively rapid sorption and degradation of the ITCs in the period of days after incorporation minimizes the risks of persistence in the environment or leaching.

## The distribution of glucosinolates and sulphur-rich cells in roots of field-grown Canola (*Brassica napus*)

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In canola, the predominant glucosinolate (GSL) is 2-phenylethyl glucosinolate (2-PE GSL) whose hydrolosate is the very biotoxic 2-PE ITC. The effect of 2-PE GSL in the roots of canola on parasitic nematodes invading the root and on the inoculum of cereal pathogens in the rhizosphere has been investigated. The mechanisms for release of the biocidal ITC from the intact root systems are uncertain. To investigate any specific role played by the distribution pattern of GSLs in root systems in the release of biocides to the rhizosphere, we have, for the first time, localized GSLs to particular regions and cells in healthy roots of field-grown canola. Secondarily-thickened taproots and branch roots with diameters > 300 µm comprise ca. 97 % of the dry weight of canola root systems at early flowering, when total GSL content is highest. HPLC analysis of the inner and outer regions of these roots, separated longitudinally by fracture at their cambial region, revealed up to 5X the GSL concentration (ca 90% 2-PE GSL) in the outer tissues than in the cores. Cell-specific measurements of sulphur concentrations were made in roots in which the water soluble components, including GSLs, were retained *in situ* by cryo-fixation and cryo-microscopy. By extrapolation from the sulphur concentrations we have determined that the highest GSL concentrations in individual cells (up to > 100 mM) are in two cell layers just under the surface of the secondarily thickened roots. These cells die and are renewed as root width increases, continuously releasing GSLs to the rhizosphere. Myrosin cells in these roots are sparsely located in deeper-lying tissues that are not shed during normal root development. The implication is that myrosinase, endogenous in the soil, releases ITCs from the shed GSLs thus accounting for the earlier finding of 2-PE ITC in the rhizosphere of healthy canola roots. The release of ITCs to the rhizosphere as a result of normal root development supplies biocide protection for the large diameter components of the root system. These roots are crucial, not only as the pipelines to the shoots for the nutrients and water acquired by the very fine, ephemeral roots, but also as the site of fine root development. The fine roots themselves contain little GSL but their continued renewal depends on protection of the outer secondary tissues of the wide roots from which they continuously develop.

## Myrosinase immobilized on Ca-polygalacturonate hydrogel: enzyme efficiency as a function of soil characteristics

Carlo E. Gessa<sup>1</sup>, Ilaria Braschi<sup>1</sup>, Antonio E. Faleo<sup>1</sup>, Susanna Cinti<sup>2</sup>, **Onofrio Leoni<sup>2</sup>**,  
and Sandro Palmieri<sup>2</sup>

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Myrosinase (MYR) from *Sinapis alba* (EC 3.2.1.147) was immobilized on a network of Ca-polygalacturonate hydrogel, which shows a composition and morphology similar to the mucigel present at the soil-root interface. The influence of pH, temperature, and time of storage on immobilized enzyme activity was investigated using sinigrin as a substrate. The immobilized MYR shows an activity which is 40% lower than the free enzyme, an optimum temperature at 55°C and an optimum pH in the range 5-7, similarly to the free enzyme. We observed that Ca-polygalacturonate preserved the activity of the immobilized enzyme for more than two months. The Michaelis-Menten parameters,  $V_{\max}$  and  $K_m$ , were determined for the free and immobilized MYR. The  $V_{\max}$  increased from 60 U mg<sup>-1</sup> protein in free form to 127 U mg<sup>-1</sup> protein in immobilized form, whereas the  $K_m$  was increased even more: from 0.17 mM (for the free enzyme) up to 1.55 mM. Similarly to the behaviour of other immobilized enzymes, the kinetic parameters of MYR immobilization induced a higher specific activity together with a lower substrate affinity. MYR activity was also studied as a function of different substrates: sinigrin, gluconasturtin, sinalbin, and *epi*-progoitrin. It is important to mention that the highest activity of immobilized enzyme on the mucigel network was recorded using as a substrate the gluconasturtin, the glucosinolate found in roots of *Brassicaceae*.

The activity of immobilized enzyme was also tested as a function of soil water potential in three soils with different organic matter content. At the field capacity (2.0 pF), the MYR activity was better preserved in soils low in organic matter for two weeks, whereas, at the wilting point (4.2 pF), the activity was very quickly lost in all soils. The immobilization of MYR on Ca-polygalacturonate hydrogel preserved the enzymatic activity with time only in soils with low organic matter content. These results indicate not only that the soil water status is an important factor for MYR protection, but also that mucigel maintains the enzyme in the hydrated form at the soil-root interface. On the other hand, the soil organic matter content, owing to its hydrophilic properties, strongly competes with the mucigel for soil water content thus reducing the maintenance of the enzymatic activity.

## Analysing disease progress curves to understand the effects of biofumigation on rhizoctonia root-rot of sugar beet

Natacha Motisi<sup>1</sup>, Doug J. Bailey<sup>1</sup>, Joao A.N. Filipe<sup>3</sup>, Philippe Lucas<sup>1</sup>, Thierry Doré<sup>2</sup>  
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With biofumigant crops being increasingly viewed as a good alternative to synthetic chemicals for the control of soil-borne disease, the number of studies concerning the biofumigant effect of *Brassica* species on soilborne pathogens has increased. However, results remain variable with inconsistent levels of control from one work to the next. In this study we use an epidemiological approach to identify the mechanisms that are affected by biofumigation and that might, in part, be responsible for the variable efficacy of disease control observed in the field. The biofumigant effect of a *Brassica juncea* catch crop is considered to occur in two phases; the first phase concerns the effect of the growing *B. juncea* crop, the second phase concerns the effect of *B. juncea* debris following its crushing and incorporation into the soil. During 2007, a field study was carried out with differing management regimes of a *Brassica juncea* (mustard) catch crop, grown within a sugar beet–winter wheat rotation, to analyse its action on sugar beet root rot caused by *Rhizoctonia solani*. The study involved three treatments (each with four replicates); mustard pulled out at flowering (MP) and representing the first phase, mustard crushed at flowering and incorporated into soil (MC) representing the first and second phases, and bare soil (BS) as a control. The development of epidemics was followed non-destructively by counting the number of plants for which disease symptoms were visibly detectable (infected) on the above-ground parts. B-sigmoidal increase in the number of diseased plants over time combined with the presence of visible mycelium on the soil surface, the proximity of neighbouring plants and the potential (published) growth rates of *Rhizoctonia* suggest that the epidemics included both primary and secondary infection. Hence, disease data were analysed using an epidemiological model including terms for primary infection, secondary infection and inoculum decay. Biofumigation mainly affected the parameters associated with primary infection resulting in a net reduction in the primary phase of the epidemic where control by MC was greater than that of MP. However, a significant, albeit much reduced effect was detected for parameters associated with secondary infection that resulted in a net increase in the intrinsic growth parameter of secondary infection of MP and MC treatments compared to control. This study, by its epidemiological approach, provides a first insight into the underlying mechanisms that are most likely to be affected by biofumigation. Moreover, the contrasting effects on the primary and secondary phase of the epidemic may explain, in part, the variable success of biofumigation in the field. The assumptions of the model and the implications of these results on the use of a biofumigant as a method for disease control at the field scale are discussed.

## Evaluation of Brassica accessions as potential biocidal green manure to control tomato bacterial wilt

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Reduction of initial inoculum of *Ralstonia solanacearum* can be an effective approach in controlling tomato bacterial wilt. The potential of *Brassica* green manure for disease control efficacy was investigated. A total of 26 *Brassica* accessions from Southeast and South Asia were evaluated for their biofumigation potential using a freeze/thaw bioassay with a field soil from the AVRDC farm (clay 12%; organic matter: 0.74%). Of these, 8 accessions of *B. juncea* were shown to have significant biocidal effect on *R. solanacearum*, reducing the pathogen density from  $10^7$  to  $10^3$  cfu/g soil or undetectable levels when amended at the rate of 5%. Accessions “Daulat” from Bangladesh and “TB 574” from Indonesia were the most biocidal and were similar to “Nemfix”, a commercial Indian mustard variety with a demonstrated biofumigation effect. In order to assess potential hosting, the 8 selected accessions were inoculated with strain Pss4 in the greenhouse both with and without root wounding. The mean percentage of wilting was 0 - 12.5% in unwounded plants and 75 - 96% when the roots were wounded prior to inoculation. This indicated when growing Indian mustard in infested fields, root damage should be avoided. The control efficacy of variety “TB 675” as a green manure was evaluated in pot and field trials. Results of preliminary pot trials showed that amendment using chopped tissues with a 28-day incubation period or amending macerated tissues with a three-day incubation resulted in a population density of *R. solanacearum* of 10 or 100 cell per g soil compared with  $10^6$  cell per g soil in the control. This suggests both short-term biofumigation and longer-term organic matter effects can suppress the pathogen population. Preliminary field trial results showed a TB 574 green manure could delay disease development. Growing Indian mustard as a green manure to control tomato bacterial wilt can be a component of an integrated management program. However, this can only be achieved by producing sufficient biomass of the green manure and understanding of other factors that could affect the control efficacy, such as soil type and moisture content.

## **Ionic Thiocyanate (SCN<sup>-</sup>) production, fate, and phytotoxicity in soil amended with Brassicaceae seed meals**

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Meals remaining after oil extraction from seed of Brassicaceae species contain glucosinolates, which in themselves have limited biological activity. After hydration of the seed meals however, glucosinolates are hydrolyzed enzymatically by thioglucoside glucohydrolase (EC 3.2.1.147) (myrosinase) to form a number of allelochemicals including ionic thiocyanate (SCN<sup>-</sup>), a bioherbicidal compound. We determined the fate of SCN<sup>-</sup> in a field soil amended with seed meals of *Sinapis alba*, *Brassica juncea*, and *Brassica napus*, and quantified crop phytotoxicity by monitoring carrot (*Daucus carota*) emergence. Meals were applied at 1 or 2 t ha<sup>-1</sup> and soils were sampled to 35 cm for SCN<sup>-</sup>. Maximum SCN<sup>-</sup> (211 µmol kg<sup>-1</sup> soil) was measured at 5 d in 0-5 cm samples from plots amended with *S. alba* meal at 2 t ha<sup>-1</sup>. At the second sampling date 8 d after meal amendment, we measured a 58% decrease in SCN<sup>-</sup> at a depth of 0-5 cm (123 µmol kg<sup>-1</sup>) and a three-fold increase in SCN<sup>-</sup> at 5-10 cm (108 µmol kg<sup>-1</sup>). Soil concentrations of SCN<sup>-</sup> in the *B. napus* and *B. juncea* 2 t ha<sup>-1</sup> treatments followed an overall pattern analogous to that found for *S. alba* treatments. Less than 30 µmol SCN<sup>-</sup> kg<sup>-1</sup> soil was measured at soil depths below 15 cm. At 44 d, SCN<sup>-</sup> was less than 15 µmol kg<sup>-1</sup> soil in all treatments. Emergence inhibition of carrots seeded 15 to 36 d after meal amendment was found only in *S. alba* treatments. The rapid decrease of SCN<sup>-</sup> concentrations in Brassicaceae meal-amended soil indicates limited potential for off-site environmental impacts.

## The effects of *Brassica* seed meal amendments on *Meloidogyne hapla* viability in laboratory bioassays

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The effect of six *Brassica* seed meal amendments on the viability of northern root-knot nematode (*Meloidogyne hapla*) juveniles (J2) was determined in laboratory bioassays. Seed meals from *Brassica napus* cultivars 'Dwarf Essex', 'Sterling', 'Sunrise Spring', and 'Hyola 401'; *B. juncea* cv 'Pacific Gold' and *Sinapis alba* cv 'Ida Gold' were evaluated at rates of 0, 1, 2, and 4 mg per cm<sup>3</sup> soil. Seeds were heated to 100°C for 24 hr prior to pressing and pressed seed meals were stored frozen. Soil bioassays were conducted in 1 cm<sup>3</sup> soil in 2 cm<sup>3</sup> capacity vials. Approximately 100 *M. hapla* J2 were added to vials in 250 µl aqueous suspension and myrosinase (Sigma Chemical Co.) was added at the rate of 1 unit per cm<sup>3</sup> soil in an additional 25 µl. One cm<sup>3</sup> pasteurized sandy loam field soil amended with seed meal treatments was added, and vials were capped and held at ambient temperature for 48 hr. Soil was removed and nematodes were recovered using a pie pan extraction for 72 hr. The numbers of viable *M. hapla* J2 differed ( $P = 0.0001$ ) between seed meal treatments, and nematode recovery as a percent of the control was 7, 16, 33, 61, 83, and 94% for the high rates of Dwarf Essex, Pacific Gold, Sterling, Ida Gold, Sunrise Spring, and Hyola 401, respectively. In factorial experiments, Dwarf Essex seed meals were heated to 100°C for 24 hr to denature enzymes such as myrosinase or not treated, and then myrosinase was added to bioassay vials or not. Meals were added to bioassay vials at rates of 0, 2, 4, and 8 mg per cm<sup>3</sup> as before, and nematodes counted. Recovery of viable *M. hapla* J2 differed ( $P = 0.0001$ ) for different seed meal rates (40.1, 31.3, 18.4, and 7.5 J2 for 0, 2, 4, and 8 mg per cm<sup>3</sup> soil, respectively). The seed heat treatment did not affect experimental results, however, the addition of myrosinase to soil resulted in fewer viable nematodes recovered ( $P = 0.02$ ). These results indicate that certain de-oiled seed meals were more efficacious against root-knot nematodes and that this activity was not affected by heat treating seed prior to pressing. While not necessary for activity, the addition of myrosinase to bioassay soils resulted in a slight decrease in numbers of viable juveniles recovered.



## Evaluation of isothiocyanates to control postharvest fruit and vegetable losses by fungal infections in Mexico

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Mexico is a large producer of fruits and vegetables which represents an important source of revenue for the country. Postharvest fruit and vegetable losses due to fungal infections are an important problem. The utilization of synthetic chemicals is linked to environment and food contamination as well as the development of resistance. Isothiocyanates (ITCs) are defense compound from Brassicas which do not have known negative effects on human health. Therefore, ITCs represent environmentally friendly compounds which can be used in an ecologically sustainable manner. We began by testing *in vitro* the fungicide activity of volatiles from broccoli (*Brassica oleracea*), cauliflower (*B. oleracea*) and cabbage (*B. oleracea capitata* group) on *Alternaria alternata*, *Botrytis cinerea*, *Aspergillus spp*, *Fusarium oxysporum*, *Geotrichum candidum*, *Penicillium spp.*, *Phytophthora capsici* and *Rhizopus, ssp.*, finding 100% inhibition in most of them. Solid phase microextraction (SPMS) followed by gas chromatography-mass spectrometry (GC-MS) of these volatiles showed the presence of allyl-isothiocyanate (AITC) and benzyl-isothiocyanate (BITC). Experiments *in vitro* to test the effect of AITC and BITC on *A. alternata*, *P. capsici*, *G. candidum*, *B. cinerea* and *F. oxysporum* showed a 100% growth inhibition at very low concentrations. Based on those results, we tested the effect of BITC on *A. alternata* infecting tomato fruit packaged in low density polyethylene film (LDPF), at concentrations of 0.28 and 0.56 mg·ml<sup>-1</sup>. We found 85% reduction in the tomato fruit infection by *A. alternata* without any negative effects on fruit quality and ripening physiology. Analysis of cabbage volatiles by SPMS and GC-MS showed allyl, benzyl, 2-phenylethyl and phenyl-ITCs were present in a ratio of 1:3.5:5:3:9.6. A mixture of those ITCs (MITC) in the same proportion was tested against *A. alternata* growing *in vitro* and infecting bell pepper fruit (*Capsicum annuum*). We found that MITC showed a much higher fungicide effect than allyl, benzyl, 2-phenylethyl and phenyl-ITCs tested separately, in such a way that 0.03 mg·ml<sup>-1</sup> of the MITC was enough to inhibit 100% *A. alternata* growth *in vitro*. In addition, 0.56 mg·ml<sup>-1</sup> of the MITC in combination with LDPF reduced the fungi infection in the bell pepper fruit better than a commercial fungicide (Captan®) without any detrimental effects on fruit quality. Further investigations sought evidence that the ITCs can induce fungi mutant strains. We exposed *A. alternata* to a BITC and AITC, in which the concentration of the compounds were gradually increased until reaching lethal concentrations. Analysis of alterations in the inter simple sequence repeats (ISSR) were carried out in the tolerant strains. We found that upon elimination of the selection pressure imposed by the ITC treatment, the *A. alternata* tolerant strain lost the ability to resist the lethal concentrations of ITC, suggesting an environmental adaptation rather than a genetic one. Genome analysis of the tolerant strains showed alterations in the ISSR as compared with the isogenic and untreated fungi strains. We conclude that ITCs represent an environmentally friendly alternative to reduce the postharvest fruit and vegetable losses by fungal infections in Mexico.



## Understanding the mechanisms of plant pest suppression using *Brassica* green manures

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A five-year study (2001-2006) was conducted to investigate the potential of biofumigation for the control of control of bacterial wilt (*Ralstonia solanacearum*) and root-knot nematodes (*Meloidogyne spp.*) in tropical vegetable production systems. During the course of the experimental program, several experimental outcomes provided evidence for both ITC-related and non-ITC related pest suppression.

Root knot nematodes were generally suppressed by the incorporation of any organic material whether *Brassica* or not, and suppression was often unrelated to GSL content. *Brassica* tissues rarely out-performed other forms of green manure unless experimental conditions favoured the impacts of volatile compounds – such as in sealed containers. An experiment in which the solid, liquid and vapour phases were separated and applied individually or in combination showed all of these components had activity against nematodes. A significant outcome of the nematode work was the revelation that among brassicas only some radish lines (*Raphanus sativus*) were poor hosts of the nematodes and during their growth could reduce populations to a similar extent as traditional sorghum green manures.

The suppression of bacterial wilt in soil resulted from two separate mechanisms. The first mechanism was related to the “Biofumigation” as usually defined – the short-term release of isothiocyanates. It was most obvious on sandy soils with low organic matter content, and could be enhanced using strategies to increase ITC release and persistence in the soil. The second mechanism occurred over 3-4 weeks and was unrelated to GSL content of the added tissue but was related to the added organic matter. Further experiments utilising additions of pure C and N sources confirmed that the N components of the amendments were most active in suppression and N sources alone could reproduce the suppressive effects. Investigations using T-RFLP suggested this suppression was not related to a general shift in the soil biological community but to more specific changes in the microbial community.

The results highlight the need to understand the mechanisms of pest suppression using GSL-containing plants in order to maximise the impact on the target organism. Significant non-GSL related suppression may often be operating, and strategies to enhance these effects may not necessarily be optimised using strategies which maximise ITC release.

## **Biorefinery – Brassicaceous plants as more than biofumigants!**

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Biofumigation using Brassicaceous plants has been widely studied and now applied at a commercial level in several countries in both organic and conventional farming systems. The interest in Brassicaceous plants and products derives from their allelopathic and biocidal effects on several soil-borne pathogens and pests, as a 100% organic alternative to chemical fumigants and pesticides. The total or partial substitution of chemicals clearly results in some advantages for the environment and for the health of the consumers and farmers. These aspects are important when considering a full evaluation of the technique, by public and political institutions. But the benefits derived from a wider application of biofumigation are not only limited to this fundamental property of the materials.

Starting from the chemical characterization of the plants and pellets, we find clear evidence of interesting fertilizing properties that are significant given the recent rapid increase in the economic and environmental costs of chemical nitrogen fertilizer in this last year. These relate to the high organic matter content. In addition, the oil derived from the defatted seed of Brassicaceous plants is also an interesting industrial co-product. This oil, rich in erucic acid, is of no interest for food purposes, but has found several different applications in Italy including the production of biofuel and energy. It can also be applied as a basic material for biolubricants (e.g. in tannery industry) or to produce, after saponification, the erucic acid that can represent a building block for organic synthesis for the production of plastics and polymers. These considerations make the production chain of biofumigant pellets based on *B. carinata* defatted seed meals a model for a new industrial development system or based on vegetable materials that integrate chemical, physical or microbiological conversion processes for a full and integrated utilization of the biomass to produce not only energy, but chemical materials with a high added value. This new proposal, strongly encouraged from the European Community in the 7<sup>th</sup> Framework Projects, is termed “Biorefinery” and represents an alternative production system to the conventional petrochemical refinery.

## Discovery and development of *Muscodor* species for mycofumigation

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We have found a novel fungal genus that produces extremely bioactive volatile organic compounds (VOC's). This fungal isolate was initially discovered as an endophyte in *Cinnamomum zeylanicum* in a botanical garden in Honduras. This endophytic fungus, *Muscodor albus*, produces a mixture of VOC's that are lethal to a wide variety of plant and human pathogenic fungi and bacteria. It also is effective against nematodes and certain insects. The mixture of VOC's has been analyzed using GC/MS and consists primarily of various alcohols, acids, esters, ketones, and lipids. Final verification of the identity of the VOC's was carried out by using artificial mixtures of the putatively identified compounds and showing that the artificial mixture possessed the identical retention times and mass spectral qualities as those of the fungal derived substances. Artificial mixtures of the VOC's nicely mimicked the biological effects of the fungal VOC's when tested against a wide range of fungal and bacterial pathogens.

Recently, a number of other isolates of this fungus have been discovered in the wet tropical forests of Australia, Peru, Bolivia, Indonesia, Thailand and Malaysia. Each *Muscodor* sp. isolate is chemically unique but all are closely related by virtue of their 18s rDNA sequences.

Potential applications for "mycofumigation" by *M. albus* are currently being investigated and include uses for treating various plant parts, and human wastes. Another promising option includes its use to replace methyl bromide fumigation as a means to control soil-borne plant diseases. Several muscodor products are already in the marketplace.

## Development of biofumigation as a non-chemical control method against plant parasitic nematodes and soil-borne diseases in temperate climates

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In Germany a national project was initiated in 2007 to improve the efficiency and sufficiency of biofumigation under temperate conditions. The goals of the project are (1) to determine and improve the composition and concentration of glucosinolates in crucifers, (2) to maximize the amount of glucosinolates effectively produced per hectare through agronomic practice, (3) to investigate the efficiency of biofumigation against *Meloidogyne hapla*, *Pratylenchus* spp., *Ditylenchus dipsaci*, *Heterodera schachtii* and other economic important plant parasitic nematodes, as well as the soil-borne fungus *Rhizoctonia solani*, (4) to transfer the gained knowledge to farmers. Information about the effect of biofumigation on plant-parasitic nematodes and *R. solani* is complemented with information about the concentration and composition of the glucosinolates in the biofumigation crops, which are analysed by HPLC technique. The effect of selected biofumigation plants on target organisms are tested *in vitro*, in the greenhouse and in field trials. In field trials the biofumigation is applied with different varieties of three crucifer species namely *Brassica juncea*, *Raphanus sativus*, *Sinapis alba* and the seed mixes Terraprotect RB (*B. juncea* and *R. sativus*) and Terraprotect MB (*B. juncea* and *S. alba*). In the following year the effect of biofumigation on yield and possible damage of a cash crop (e.g. sugar beet) is assessed. To record the effect of biofumigation on nematode population densities, soil samples are taken before (Pi) and two to four weeks after (Pf) the biofumigation as well as before and after the cash crop. The selection of plants which are suitable for the specific arable or horticultural production system with a focus on high concentration of glucosinolates is provided by the breeder. First results from *in vitro* tests and field trials of all project partners will be presented and discussed.

## Green manure and biofumigation for root lesion nematode and verticillium wilt management in strawberry production

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From 2004 and 2006, a field experiment was conducted to assess the efficacy of a 2-year crop rotation and biofumigation on *Pratylenchus penetrans* densities and Verticillium wilt damage (*Verticillium dahliae*) in strawberry in Quebec, Canada. Crops tested as rotation were: canola followed white mustard (biofumigation), corn, forage pearl millet cv. CFPM 101, forage pearl millet cv. Tifleaf, and oats as the standard control. For the biofumigation treatment, canola was ploughed under at late bloom (end of July) followed by white mustard which was ploughed at full bloom (October). Nematode population densities were significantly lower after biofumigation and both pearl millet cultivars than after corn and oats. In 2006, both pearl millets and biofumigation significantly reduced by an average of 21% the incidence of Verticillium wilt and allowed a 54% average increase in the development of strawberry plants cv. Jewel. In 2006 and 2007, two field trials were set out to investigate the potential of improving the efficacy of biofumigation by incorporating poultry manure (15 t/ha) at canola-crop ploughing time, with and without the addition of a plastic mulch. In 2006, no significant improvement of biofumigation was observed by the addition of poultry manure and soil tarped with a transparent plastic much for 18 days. In 2007, plastic mulch significantly improved biofumigation with a 95% reduction in the number of *P. penetrans* densities when compared canola ploughed with poultry manure but no plastic mulch.

## The use of biofumigant green manure crops for soil-borne disease management in Tasmania

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Four field studies were conducted to determine the feasibility of incorporating *Brassica* and non-*Brassica* green manure crops in between vegetable crops for *Sclerotinia minor* disease management. *Brassica* plant materials were collected and analysed for the types and levels of isothiocyanates (ITCs). Soil compaction and pathogen levels in soils were also assessed. The studies indicated that *Brassica* green manure plants that produce high plant biomass and high concentrations of biofumigants may offer advantages over non-*Brassica* green manure plants for *Sclerotinia* disease control. *Brassica* green manures did not eliminate *S. minor* inoculum in soil, and short-term disease suppression is believed to be the mode of action against *Sclerotinia*. *Brassica rapa* or rape (BQ-Mulch™), which produces high levels of isothiocyanates (ITCs) in roots, was found to be more effective for *Sclerotinia* control than *Brassica juncea* or mustard (Fumus™), which produces high levels of ITCs in its foliage. The effectiveness of BQ-Mulch may be related to the high level of the non-volatile, but more persistent and toxic, 2-phenylethyl-ITC produced in its root tissues. The studies also showed that the high plant biomass and deep tap root systems of *Brassica* green manures helped reduce soil crusting, improved infiltration, increased organic matter and reduced sub-soil compaction. These overall soil improvement effects are likely to increase soil fertility and improve soil structural properties, thereby contributing to improved crop health and disease control.

## Innovative approach for producing high value products in non food agro-industrial chains

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The natural bio-fumigation is widely acknowledged as a technique that is able to improve the soil fertility, to get plants more healthy, permitting a significant reduction of pesticide use.

In this context, the studies on the glucosinolates – myrosinases system, typical self-defence of some species of Brassica's family, gave the opportunity to produce a wide range of products with good performances.

In order to start the industrial formulation of products for bio-fumigation, it has become necessary to create a new non food chain, differently shaped in comparison with conventional production of oils for energy.

The display describes the experience of the last five years of an Italian enterprise (Triumph Italia), which, starting from a breeding program specific for the selection of new Brassica carinata germoplasm with an improved glucosinolate content, has created a non food chain between the producers of quality oilseeds and the firms involved in biomass conversion processes into high value natural products for biofumigation.

In the process fine-tuned, the whole plant looks as a bio-refinery: the oil with an erucic acid content of 47% minimum, goes to further transformation process in order to produce bio-energy, biopolymers, bio-lubricants and intermediate products for the lipo-chemical industry. Moreover, from Brassica's homogeneous grains it is possible to obtain - by means of a checked process innovation - defatted oilcakes with a high stability of composition that make them as the basic material for the production of soil organic amendments and liquid formulations containing meals.

This bio-refinery, integrated on the territory, represent a sort of model for an environmental friendly production.

**Key-words:** *Bio-refinery, Brassica carinata, glucosinolate-myrosinase system.*

## Commercial development and utilisation of *Brassica* green manures

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*Brassica* species, mainly radish and white mustard, have been used as a nematode trap crop for Sugar Beet Cyst Nematode on a limited basis in the USA since the early 1980's. In 1992, commercial trials began in Washington State to utilize *Brassica* species for a green manure to improve soil physical conditions. Cultural techniques were developed that allowed large biomass production in short growing periods with minimal inputs. Early field trials also showed disease suppression in some fields. Subsequent grower trials in cooperation with Washington State University tested species and varieties from around the world for their adaptability and biomass production. The ability of this green manure technique to reduce wind and water erosion, increase organic matter, and improve the overall soil biology has been verified by researchers throughout the USA.

From the success realized by some Washington potato growers, interest spread to other regions and commodity groups. Most growers were interested in the "biofumigation" properties to solve issues with disease or nematodes that intensive cropping and short rotations made increasingly difficult to control with chemicals. Organic growers quickly realized the benefits of this technique. Publications of the "Biofumigation Update" from CSIRO kept growers and researchers aware of the potential uses for these plants, but biofumigation was not the "silver bullet" many were hoping for. Seed companies made varieties available that were not well suited or tested for green manure/biofumigation use and poor field performance resulted. Replicated trials by University researchers showed that some of these varieties reduced yield and quality, or hosted various nematode species of concern to growers. The lack of performance due to poor variety selection and technique led to a decline in interest from growers. The evidence from continued success in some field and research trials gave the concept merit, but the commercial biofumigation acreage declined.

It was critical for commercial success to have varieties available that were adapted to varied climatic conditions and rapidly produced large amounts of biomass without producing adverse effects on the following crop. Elevated *Pythium*, *Fusarium*, *Sclerotinia*, nematode, and wireworm levels had been observed in crops following green manure incorporation of some *Brassica* varieties. The Italian research institute, ISCI, had identified the properties of individual glucosinolates and developed varieties that maximized the biofumigation potential of these compounds. These varieties performed consistently without negative side effects in many areas of the world. The right variety combined with proper production techniques were both key in making this a tool that growers rely on today to reduce pest pressure and increase crop yield and soil quality. Growers have successfully adapted this to organic and conventional cropping systems. Understanding how to use biofumigation as a positive part of an Integrated Pest Management (IPM) program puts this practice to use on thousands of hectares every year.



## Specialised Australian cover crops – the Seedco experience

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In January 1994, Seedco (Seedmark's parent company) began work on a Grape & Wine R&D Corporation (GWRDC) funded project to look at the development of specialized cover crops for Australian vineyards. Richard Porter was the leader for the three year project which specifically looked at finding management solutions to increased soil salinity, deterioration of soil structure and texture, declining soil fertility and a proliferation of herbicide "escape" weeds. As a result of Richard's work, Seedco released the following specialized cover crop products - Biomax (green manuring), Weedcheck (Fodder Radish), Rebound (annual swards), Bt Assist (insectory habitat mix), Nemfix (soil fumigation of nematodes) and Permagreen (perennial swards) under the brand name "VinEco".

These VinEco products were widely promoted to the wine industry through seminars, grower meetings, field days, printed brochures, magazine and journal articles, conference presentations and direct grower contact. The products were enthusiastically taken up by industry with sales outstripping supply. Feedback for the industry was very positive and grape production responses were very promising with major benefits being seen in subsequent years. With this national success, Seedco's competitors began developing their own cover crop mixes based on cheaper components and trading off against the VinEco brand name. The advertised benefits of these competitor mixes were untested and in many instances, they were inferior in performance and purely cosmetic. The only benefit to the end user was that they were cheaper to purchase. This competition led to a major reduction in market share of the VinEco products and coupled with a decrease in the amount of money earned by the wine industry due to a surplus of wine worldwide, the demand for cover crop mixes decreased across Australia.

As increased costs in wine production began to bite, the wine industry looked for savings and as a result, generic cover crop mixes became the norm. Today there is very little use of specialised cover crop mixes in vineyards with many mixes used, based purely on low priced components. Today, Seedmark sells next to no VinEco products as there is very low industry demand due to poor economic conditions and returns.

The take home message on specialized cover crops is that even though you have an excellent product that provides major economic solutions to specific industry needs, and the products are widely accepted, there still needs to be an awareness that when industry is economically constrained, the products need to be continually promoted as to their economic benefit. Seedco failed to promote the differences between their specialized products and those of their competitors. As a result, the VinEco products lost market share and industry lost focus on why they should plant specialized cover crops in vineyards given decreasing dollar returns.

## **Biofumigation in potato crops in south-western Australia – a potato grower's perspective**

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Lack of reliable *Brassica* seed supplies is the main factor holding back wider use of biofumigation on the Taylor properties south of Perth in Western Australia.

Dramatic improvements in yield and quality in potato crops using commercial formulations of the fumigant metham sodium eventually led us into becoming involved in CSIRO projects on biofumigation for several years. We are firmly of the opinion that with a little further development biofumigation can replace or at least dramatically reduce rates of metham sodium use.

This presentation will detail how we learned to establish, grow and process *Brassica* crops to maximize biofumigation potential, and to fit in with our farming system.

## The successful integration of biofumigant crops into farming systems for soil improvement and soil-borne disease management in Tasmania, Australia

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In north-west Tasmania, a major temperate horticultural production region in Australia, *Brassica* green manure or biofumigant crops are increasingly being integrated into farming systems for soil-borne disease management as well as for soil health and other agronomic benefits. This presentation will discuss how *Brassica* green manure crops are integrated into a potato production system. The early and on-going experiences of more than 10 years from a farm in Sheffield have led the way for many growers in evaluating and integrating the use of *Brassica* green manure in large scale commercial farm practices. On the farm at Sheffield, an overall soil management system, including *Brassica* green manure crops, has been developed to reduce soil-borne diseases and improve the profitability of fresh market potatoes. The major soil-borne disease of potato tubers - powdery scab (*Spongospora subterranea*), common scab (*Streptomyces scabies*) and black scurf (*Rhizoctonia solani*) are serious threats to the tuber quality and hence profitability. The potato production system involves improving soil health by planting short term pasture and biofumigant crops, reducing tillage, reducing the number of volunteer potatoes present as weeds in between potato crops in order to reduce disease carry-over and growing new disease tolerant potato varieties. Early investigations in comparing “Fumus” (Indian mustard) and “BQ Mulch” (fodder rape/turnip) against a bare ground fallow indicated a substantial reduction in black scurf and powdery scab in the following potato crop. *Rhizoctonia* lesions were reduced from 75% in the bare ground fallow to 0-2% in the biofumigant treated areas and powdery scab lesions were reduced from 15% in the bare ground fallow to 2-5% in the biofumigants treated areas. The use of “Fumus” and “BQ Mulch” has many other benefits in addition to biofumigation. Soil health benefits from an extensive fibrous and deep tap root system, and nutrients released from their rapid breakdown after incorporation into soil has helped to increase potato yields. When sown in autumn and winter under cooler conditions, excellent germination, high growth rates, low disease and insect pressure make them an easy crop to manage and ideal for a break crop between other horticultural crops in spring and summer in Tasmania.

## Effects of different pre planting soil preparations and cover crop rotations on the incidence of the disease “Potato Early Dying”

Tim Widdison

Kalangadoo, South Australia

Tim Widdison is a South Australia potato grower, located approximately 400 km, South East of Adelaide. He contract grows potatoes for the local processing factory owned by McCain Foods.

In 2003 and 2004, Tim noticed several sections within different potato fields that had died off earlier than the main crop. Pathology tests indicated that a disease complex known as “Potato Early Dying” (PED), caused by the interaction of the fungi *Verticillium dahliae* and nematode *Pratylenchus* sp was responsible.

In November 2007, Tim decided to plant a 36ha trial (approx 500m x 700m) within the paddock he first noticed problems. The main aim was to determine if different soil treatments and/or cover crops reduced the incidence of PED.

The paddock was divided into two main soil types, one being well drained and the other poorly drained. Main plots were 50m x 700m, running in a North – South direction and were either, left uncultivated, ripped to 40cm depth, ripped to 40cm depth then ploughed or plough only. Sub plots ranging from 15m x 50m were sown to cover crops in an East – West direction. Individual cover crops consisted of Revolution sorghum 25kg/ha, Barley 120kg/ha or the Brassica cultivars Rangi Rape, Fumus Mustard at 5, 10 and 20kg/ha, Komatsum Mustard 14kg/ha and Sovereign Kale at 5kg/ha. Control sub plots comprise of grass based pasture. Cover crops of Kale and Rape required replanting in mid December due to severe insect damage. Both Mustard crops were incorporated as green manures in mid January and the summer weeds “Fat Hen” (*Chenopodium album*) has not been observed within these treatments. In early March, lambs were placed on both the Rape and Barley treatments for 2 months.

Full results of this trial will not be known until early 2009, when the main crop of potatoes has been harvest. However Tim will be discussing the many agronomic issues that he has encountered and some of his ideas whilst conducting this trial.

## Experiences of Victorian horticultural growers with biofumigation

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Past and present research and technology transfer programs have built a strong awareness of biofumigation practices amongst Victorian horticultural growers, and the potential benefits they offer for management of soil-borne pathogens and pests. Despite this, biofumigation is still not widely practiced in horticultural production systems in Victoria, and there is potential for greater adoption within individual industries. In this presentation, growers from two Victorian horticultural industries (certified strawberry nursery and vegetable industries) will share their experiences with the use of biofumigants in their production systems. This discussion will highlight some of the possible barriers to the adoption of biofumigants in horticulture and reveal potential opportunities for future research and technology transfer activities to address these issues.

## Utilising green manures in irrigated agriculture: Brassicas and other options

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The concept of biofumigation involves planting a crop that releases compounds that are toxic to pests or pathogens in the soil. Biofumigation offers a safe, self-generating method of distributing a 'natural' fumigant throughout the soil profile. Conventional soil fumigation is not a practical option for broadacre crops, such as cotton. Hairy vetch (*Vicia villosa*) has been used successfully as a biofumigation crop against black root rot, caused by *Thielaviopsis basicola*, in cotton in the USA. This pathogen is cosmopolitan and is a serious pathogen of cotton in Australia. In soils used to grow cotton in Australia, green manure crops with hairy vetch and *Brassica juncea*, demonstrated useful biofumigation potential against *T. basicola* and, in contrast, enhancement of other soilborne pathogens. Biofumigation experiments were conducted in several fields that were infested with *T. basicola*, over three seasons (Table 1). The results varied and this appeared to be related to the dry matter production in the biofumigant crop and the success of its incorporation. The population density of *T. basicola* in the soil was variable within plots and experiments, adding errors to the spore population data and masking treatment effects. However, biofumigation clearly has potential to reduce the severity of black root rot (Table 1).

**Table 1.** Summary of results of biofumigation field experiments using Indian mustard and woolly pod vetch for control of black root rot in cotton in NSW.

Trial	Crop	Difference from control (%)			
		Disease severity	Spore numbers	Cotton growth	Crop maturity
Narrabri 98/99	Vetch	-61	-59	NS	ND
Narrabri 98/99	Mustard	-56	ND	NS	ND
Moree 99/00	Mustard	-34	NS	26	26
Narrabri 99/00	Mustard	-31	-65	22	ND
Narrabri 99/00	Vetch	-23 NS	NS	NS	ND
Walgett 99/00	Vetch	ND	-24	ND	ND
Warren 99/00	Mustard	-29	-21	47	26
Warren 99/00	Mustard	-70	-88	19	19
Warren 99/00	Vetch	-38	NS	NS	1NS
Narrabri 00/01	Vetch	-17NS	NS	14	ND
Narrabri 00/01	Mustard	-28	NS	19	ND
Moree 00/01	Vetch	-24	ND	54	ND

NS = Not statistically significant. ND = Not determined

In contrast, incorporation of woolly pod vetch and Indian mustard as green manure crops in a field infested with *Fusarium oxysporum* f.sp. *vasinfectum*, increased the severity of Fusarium wilt in the subsequent cotton crop. Furthermore, 'late' incorporation of vetch crops (10 days before sowing cotton) increased the severity of damping off of cotton, caused by *Rhizoctonia* and *Pythium*. Effective deployment of biofumigation crops clearly requires an integrated approach with evaluation of the complete pathosystem, including the biology of the pathogens.

## Soil biodisinfection for the management of soil-borne pathogens and weeds

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The biodisinfection of soils should be based on agro-ecological criteria, taking into account the integration of the crops in the agro-ecosystem, and in the use of local resources, to reduce transport costs of the biodisinfection materials. The elimination of the ozone depleting soil fumigant methyl bromide (MB), has pushed the development of new non-chemical alternatives in the control of soil-borne pathogens, weeds and replant problems, such as biofumigation, biosolarization, biodisinfestation or biological soil disinfestation (BSD), which are based on chemical, physical and biological, or even soil ecology concepts. These alternatives are generally employed in an isolated manner, aiming to have the same model as MB. The biodisinfection of soil aims to harmonise the available non-chemical alternatives by including agronomic aspects to design production systems with ecological criteria. Soil is a live entity and for this reason the term biodisinfection is proposed, which is fundamental for the BSD process to occur. The application of organic material as a biodisinfectant should be a dynamic concept and not only include the gases released during tissue decomposition (biofumigation), the effect of which can be complemented in some areas with higher temperatures (biosolarization). The use of crop or agro-industrial residues allow the transformation of by-products through biodisinfestation alternatives, which has an important future in the agro-fuel industry, and closing nutrient cycles. The production aspects should not be the only consideration in plant protection but also the improvement in soil fertility and global soil characteristics. Biodisinfestation can be applied in extensive production systems, including fruit trees, strawberries and vineyards, as well as in protected crop systems such as vegetables, cut flowers and ornamentals. This alternative which is based on the management of organic material is not difficult for producers as they have the experience in the application of organic manure, although the doses, time period and method of application should be defined, taking into account regions, crop and the specific phyto-pathological problem being addressed..

## **Farmers' experiences on biofumigation for bacterial wilt management in solanaceous crops in the Philippines**

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In the highlands of southern provinces of Bukidnon, Davao del Sur and South Cotabato, Philippines, vegetable farmers mainly grow potato and tomato. Both solanaceous crops were, most of the time, afflicted with bacterial wilt (*Ralstonia solanacearum*). After the introduction of biofumigation into these provinces in 2003 through the ACIAR-CSIRO project, bacterial wilt incidence was reduced by as much as 30-60%. Biofumigation entails the use of clean and healthy potato seeds, crop rotation and incorporation of radish as green manure crops. Some of the problems encountered by farmers were the following: a) large volume requirement of brassicas for incorporation, b) laborious chopping of brassicas, c) if radish is plowed under during cultivation, the tissues are inadequately broken, d) and lack of irrigation facilities particularly during dry months in the highlands. Several farmers who were successful in reducing bacterial wilt in their own fields through biofumigation promote the technology to other farmers. At present, farmers are making their own trials using locally available brassicas and plants within their respective areas such as sunflower. They have now increased their production and reduced bacterial wilt incidence in their fields.



## Strategies for enhancing Brassicas' multipurpose attributes in managing nematode parasitism complexities

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Nematode management is a major challenge in many cropping systems and more so with the increasing demands for environmentally sustainable food and fiber production. Lack of broad spectrum and “one-size-fits-all” management options, plant-parasitic nematodes' multi-taxa infestations and species, as in root-lesion (RLN, *Pratylenchus*), and sub-species genetic variability, as in root-knot (RKN, *Meloidogyne*) and cyst (*Heterodera*) nematodes, are among the major limitations. Many brassicas like oilseed radish (OSR, *Raphanus sativus*) and arugula (*Eruca sativa*) have potential for use as sustainable nematode management options. For example, some brassicas may be used as a cover crop and/or a biofumigant when incorporated into soil while others may potentially be used as trap (dead-end host), cover, or vegetable crops. A host can also be a trap crop if it is destroyed before the nematode in question completes a life cycle. Because many brassicas are hosts to many nematodes or are commonly used for one purpose (e.g. trap or cover), their multi-purpose use potentials may not be fully realized. Using OSR and arugula and *M. hapla* models, the objective of this presentation is to discuss three intertwined strategies designed to enhance brassicas' multi-purpose uses. First, the relationships between nematode life cycle and host range to determine the best use of a nematode susceptible OSR as a trap or as a cover crop against *M. hapla*. Nematodes complete a life cycle in approximately 450-500 degree-days (DD, base 10°C) in different soil types. Under these circumstances, an OSR cultivar that does not produce the necessary vegetative biomass for either biofumigation or cover crop purposes before the nematode completes a life cycle may not be an effective trap crop. The second strategy is to integrate arugula's nematode trapping ability with agronomic and commercial uses. As long as there is no physiological cost associated with trapping nematodes, arugula can be used as a trap and a vegetable crop and parts that are not consumed may be used for biofumigation. It can be grown in a rotation system within a vegetable farm or non-vegetable lands with nematode problems on short-term (seasonal) lease basis. Hence, creating potential opportunities for entrepreneurship. The third strategy is potential application of a modified fertilizer use efficiency (FUE) model to assess and enhance efficiency of brassicas multi-purpose uses when managing nematodes. Defined as increase in host productivity and/or decrease in plant-parasitic nematode population density in response to a given fertilizer treatment, the FUE model identifies four categories of interactions that account for agronomic, economic, ecological and environmental and pest management needs. Thus, creating bridges for multi-disciplinary and sustained applications of brassicas multi-purpose attributes.

## Biofumigation and management of soil-borne organisms of crops on sandy soils in Almería (Spain)

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The effects of biofumigation and the use of organic matter combined with solarization (biosolarization) in sandy soils (“*enarenados*”) are studied in the most representative areas of Almería. In this area there are no effective control methods to reduce the losses caused by soil-borne organisms and nematodes, mainly of the genus *Meloidogyne* in integrated and organic production systems. Biosolarization combined with other management alternatives is proposed, including the use of resistant varieties which have been previously studied in relation to distinct biotypes of the genus *Meloidogyne*, and other cultural practices, selecting those which allow the closing of nutrient cycles, and based on the use of local resources. The effectiveness of biosolarization fundamentally depends on the method of application and not exclusively of individual factors such as doses, composition or distribution of the organic matter in the soil, determining the costs for the method employed, aiming to reduce doses and transport costs. The importance of traditional methods in the control of nematodes and other soil-borne organisms in sandy soils was analyzed, including the distribution to the depth of the root systems of the crops, which is a limiting factor in the design of a system of agronomical management adapted to each circumstance.

## **Evaluation of biofumigation crops in the control of *P. penetrans* and *V. dahliae***

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This research project concentrates on Biofumigation, as an alternative for chemical soil disinfestation against soil pathogens like nematodes and fungi. Biofumigation is a new technique, by growing specific (green manure) crops (mainly crucifers) and incorporating these crops into the soil. By incorporating the crops, the contents (among others some breakdown products) diffuse through the soil and can help to improve soil health. Within this project 16 different crops are tested in a field experiment. The impact of the crops on nematodes (*Pratylenchus penetrans*), fungi (*Verticilium dahliae*) as well as the cash crop (potato) grown afterwards are tested in a randomized block design. Until now it seems that most crops can be seen as a host plant for nematodes, and that the supposed population decrease due to the biofumigation effect does not compensate this negative effect. However, on the quantity and quality of the potatoes, there was an overall positive effect of most biofumigation crops. If the biofumigation crops and the techniques to grow and to incorporate them improve, biofumigation can become a new alternative to improve soil health.

## The use of by-products from the wine industry in the control of plant parasitic nematodes

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Different concentrations of vinases from by-products of the wine industry were studied in cultivated soils, selecting those that have major agronomic viability, and to optimize the application technique. The fundamental objectives are: (1) to study the influence of these products on soil nematode populations, in particular the control of *Xiphinema index*, the nematode vector of the grape fanleaf virus (GFLV) which is one of the principle reason that disinfection of soils before vineyard replantation is obligatory in the EU member states. The effects on the root-knot nematode *Meloidogyne arenaria* will also be studied, as well as being parasites of grapevines they are also one of the principle problems causing losses in vegetable crops; (2) to determine the effects of these products on fertility using vineyard and vegetable crop soils as a reference, and to know possible phytotoxic and environmental effects, and (3) to use fundamental agronomical criteria for the utilisation of agro-industrial by-products, determining optimal dosage, time, methods and adequate machinery for the application. The problems of grapevine fanleaf virus and its nematode vector *X. index* in Spanish vineyards are highlighted, analyzing current control and disinfection methods. The use of these sub-products as biofumigants is analysed as an alternative to the use of chemical disinfection in soils with the aim of reducing economic and environmental costs. The nematode *X. index* was controlled *in vitro* with vinases from the alcohol industry, and controlling populations of *M. arenaria*. The saprophytic nematodes of the group rhabditids and enquitreids which belong to the oligochetes group which are fundamental in the decomposition of organic materials increased in biofumigated soils, especially when solid sub-products are used. It is concluded that biofumigation should be applied to the whole field to avoid the appearance of infected patches of parasitic nematodes. The use of biofumigation could reduce the costs of disinfection of soils destined for vineyard replanting, acting as an improver of soil characteristics, increasing fertility, biodiversity, and improving physical properties, at the same time contributing to the search for solutions for by-products generated by distilleries in our country.

## ***Alterbromide* – A European programme for the dissemination of alternatives to methyl bromide**

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Methyl bromide (MB) is a significant ozone depleting substance which was added to the Montreal Protocol in 1992. The Regulations EC2037/00 & EC3093/94 required reductions in MB consumption and particularly a prohibition for the majority of its uses in EU during 2008. *Alterbromide* programme (2006 - 2009) will be set up and implement a coordination framework improving the delivery of sustainable MB alternatives.

The consortium is composed of 11 partners from 7 countries: France, Italy, Spain & Greece (using high quantities of MB), Belgium and Netherlands (where MB has been continuously decreasing) and Romania. They represent different types of organisations (technical transfer organizations, research institutes, experimentation centres, national agencies and associations). A.R.E.F.L.H, European association in fruits & vegetables is the coordinator, CRITT INNOPHYT coordinate the work package including the dissemination of alternatives.

*Alterbromide* will produce the following three types of deliverables:

- an overview of the reports which indicate the reasons why the adoption of MB alternatives in Europe is delaying.
- Some good practices guides: technical alternative descriptions with the essential know-how to improve the use of MB alternatives by the end-users in Europe
- a coordination platform of MB alternatives including electronic site where MB users can find information for alternatives.

Suitable alternatives listed by the consortium are listed in the following table

<b>Chemical alternatives</b>	<b>Non chemical alternatives</b>
<b><u>In soil fumigation:</u></b> Existing Chemicals: Chloropicline, Dazomet, Dichloropropene, Metam Sodium, Potassium in staight & in combination New Chemical: dimethyl disulfide Other new Chemical: Ethadinytrile, Methyl iodide	<b><u>In soil fumigation:</u></b> Physical alternatives: Solarization, Steam, Biofumigation, Grafting & resistant cv Other alternatives: Biological alternaives, Integrated Pest Management, Soil less culture
<b><u>In post harvest:</u></b> Exiting Chemicals: Phosphine & Contact Insecticides New Chemicals: Sulfuryl fluoride	<b><u>In post harvest:</u></b> Physical alternatives: Heat

Biofumigation originally refers to the use of *Brassica* species containing biocidal sulfur-compounds, principally isothiocyanates (ITC), as green manure or rotation crops to control soil-borne pests and diseases (Angus 1994). The term 'biofumigation' has been extended to the use of *Allium* species producing compounds derived from sulfur amino-acids. The primary active compounds produced in crushed *Allium spp* are thiosulfinates but they are rapidly transformed into active disulfides, particularly dimethyl disulfide (DMDS) which is also produced by Brassicaceae (Auger and Thibout, 2005). A lot of other plants have shown interesting activities against nematodes and fungi (Djian-Caporalino *et al*, 2005).



# POSTER ABSTRACTS

## **Influence of soil biofumigation in the control of *Meloidogyne javanica*, *Mesocriconema xenoplax* and free living nematodes in different soil layers using castor cake and cabbage**

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The effect of soil solarization and biofumigation in the control of phytoparasitic and free living nematodes was investigated under field conditions. The experiment was conducted in microplots in an area naturally infested with *Meloidogyne javanica* and *Mesocriconema xenoplax* during the summer of 2007. Pots containing 20 kg of soil infested with nematodes were amended with castor cake and cabbage leaves. Thereafter, the soil was wet and covered with a 100µm thick transparent plastic film. Pots containing soil without amendments and not covered with the film were used as controls. After 50 days, the nematode control was evaluated at different soil layers. The soil solarization and biofumigation using castor cake were more efficient in the control of *M. xenoplax*, *M. javanica*, reducing both nematodes at 76-93%. All the treatments had less impact on free living nematodes although the solarization or biofumigation with castor cake caused higher suppression of these nematodes in the soil (50-60%). The reduction of all nematodes was more pronounced in the first 15cm soil layer and interaction between each nematode and the treatments were evident.



## Management of *Meloidogyne incognita* (Kofoid et White) Chitw. in organic horticulture

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The effectiveness of different crop systems in organic farming was checked, from 2005 to 2007, in a coastal area of Northern Italy, on a sandy soil naturally infested by Southern Root-Knot Nematode (*Meloidogyne incognita*). Eight treatments were compared as combination of crop rotation (either sorghum or tomato in 2005 followed by melon in 2006 and 2007), biocidal intercropping (*Eruca sativa* cv. Nemat) and biofumigant treatments (pellet based on formulated defatted seed meals of *Brassica carinata*), with the aim of verifying their action in nematode control. The results showed that sorghum cultivation as a non host crop halved the nematode population in the soil, while the tomato host crop increased *M. incognita* infestation (reproduction factor as  $R = 1.33$ ). The biofumigant treatment in spring 2006, caused a strong decrease in galling index (G.I. based on a scale from 0 to 5) on the roots of the following melon crop in rotation (G.I. = 0.3), significantly lower if compared with the untreated control (G.I. = 4.2). These positive results were further confirmed in 2007, when an important increase in quality and quantity was recorded in the yield of melon fruits. The autumn cultivation of *E. sativa* cv. Nemat and its incorporation into the soil, brought a good amount of organic matter, with positive effects on melon yield.

The intrinsic characteristics of the pellet based on *B. carinata* defatted seed-meals in rotation with *E. sativa* highlighted an excellent biofumigant effect, fully comparable to chemical nematicides, and a noticeable contribution in both organic matter and nitrogen that played an important fertilising effect. The full effectiveness of pellet in decreasing substantially the nematode population, allows us to suggest its application in alternate years with *Eruca* green manure, in the presence of low larval infestations in the soil.

## Effect of biofumigation on different plant parasitic nematodes

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Plant-parasitic nematodes are major pathogens of many crops in conventional as well as organic farming systems. Although economic damage in most cases is caused by only one dominant species, the crops themselves are infested by a broad spectrum of different nematode taxa. If the main nematode species is controlled, other species might step in and cause plant damage. Therefore, it is most desirable to find a control strategy covering the broad spectrum of plant-parasitic nematodes. One non-chemical alternative meeting those objectives is seen in biofumigation. In 2007, we tested the control efficacy of ten biofumigation crops towards different nematode taxa on an organic farm in the northern part of Germany. The field was naturally infested with *Meloidogyne hapla*, *Pratylenchus penetrans*, *P. neglectus*, *Tylenchorhynchus dubius* and *Heterodera goettingiana*. The biofumigation treatments included three cultivars each of *Brassica juncea* and *Raphanus sativus*, two cultivars of *Sinapis alba* and two species mixes namely Terraprotect RB (*B. juncea* cv Energy x *R. sativus* cv Defender) and Terraprotect MB (*B. juncea* cv Energy x *S. alba* cv Luna). The biofumigation crops were seeded in mid summer (July 24) and chopped and incorporated into the soil at flowering stage on Sept. 14<sup>th</sup>, 2007. Initial nematode population (Pi) was recorded on July 24<sup>th</sup> and final population density (Pf) on Oct. 24<sup>th</sup>. In addition, total glucosinolate production was calculated based on plant dry biomass and glucosinolate concentration. For the latter, maximum glucosinolate production was 14.71 kg/ha sinigrin for *B. juncea* cv Energy, 19.8 kg/ha sinalbin for *S. alba* cv Luna and 9.6 kg/ha 4-Methylthiobutenyl for *R. sativus* cv Defender. The biofumigation treatments had little effects on *M. hapla* and *T. dubius* but reduced *Pratylenchus* spp. The effect on *H. goettingiana* varied between the crop species; no effect by *B. juncea* and *S. alba* but population reduction by *R. sativus*. Non plant-parasitic nematode densities increased following treatment with *R. sativus* but were not affected by *B. juncea* and *S. alba*. Although the chosen biofumigation crops did not provide broad nematode control, results produced very promising data for optimisation of the biofumigation strategy under temperate conditions.

## Biofumigation for the control of root-knot nematodes on flower crops

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Flower and ornamental plant cropping systems play an economically relevant role in the province of Bari, southern Italy, though their yield is constantly at risk due to soil-borne pathogens and infestations of root-knot nematodes (*Meloidogyne* species). Phase-out of methyl bromide enhanced the problems raised by these pests and requires technically effective and environmentally sustainable alternatives. The aim of this study was to investigate the suppression by cruciferous biofumigant green manures and plant-derived commercial formulations on the root-knot nematode *M. incognita* on *Lisianthus* and *Aster* flower crops in the greenhouse. Soil biofumigation with green manures of *Brassica juncea* or *Eruca sativa*, and a *Brassica* seed meal commercial pellet, alone or combined with *E. sativa* manure, was compared with treatments of quillay or tagetes formulations, the chemical nematicide fenamiphos and an untreated control.

In the greenhouse cultivated with *Lisianthus russellianus*, nematode population densities in plots treated with *B. juncea* and *E. sativa* green manures were reduced by 50 – 60% compared to untreated soil and resulted in significantly lower populations than the quillay treatment. Combination of *E. sativa* green manure with a reduced dose of the pellet treatment improved, though not significantly, the nematicidal effect of single green manure or pellet treatments. Moreover, *E. sativa* green manure, alone or combined with pellet, resulted in the lowest root gall indices. Compared to untreated soil, all treatments resulted in a significant increase of stem length and diameter and in a higher number of flowers per stem. In the following *Aster* crop the highest suppression of *M. incognita* population and the lowest gall formation on *Aster* roots were achieved after *E. sativa* green manure and pellet, alone or in combination, which was also significantly lower than fenamiphos. A consistent but lower reduction of nematode population was found after *B. juncea* green manure. Control plots showed significantly lower stem length and diameter, number of flowers per stem and dry matter compared to soil treated with *E. sativa* and *B. juncea* green manures or pellet.

## Effectiveness of biofumigating *Brassica* treatments for the control of root-knot nematode *Meloidogyne incognita* on melon and watermelon

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Root-knot nematode (*Meloidogyne* spp.) infestations may cause heavy yield loss in the intensive horticultural cropping systems of Salento region, southern Italy, due to favourable climate and highly susceptible crops. Among these crops, melon and watermelon are cultivated in large areas either for export or the national market. Phase-out of methyl bromide and other chemical nematicides raised many technical problems for the control of root-knot nematode attacks. Among the available control strategies, apart from some synthetic nematicides, commercial formulations of plant extracts and nematicidal plant green manures may represent an effective and environmentally sustainable alternative. Use of *Brassica* green manure as a source of nematicidal active principles is well known, although some technical aspects of their application are still to be resolved, especially the most appropriate period to incorporate a green manure within the intensive horticultural crop rotations.

The aim of this study was to investigate the technical feasibility and effectiveness of *Brassica* green manure, i.e. biofumigation, for the control of root-knot nematode on melon and watermelon crops. *Brassica juncea* and *Eruca sativa* green manures were compared with a commercial amendment from *Brassica* meals, alone or combined with *E. sativa* green manure, and with commercial formulations of aqueous extracts of quillay and tagetes, and the nematicide cadusaphos. Untreated soil was used as a control. Sowing and cultivation of green manure crops were scheduled in different periods. The duration of *Brassica* crops was limited to 70 days for the late crop cycles, allowing an easier inclusion in crop rotations, but reducing *Brassica* plant biomass potential and thus its fumigating potential. Before the early crops, green manure brassicas were cultivated for about four months, allowing an increase of biomass.

In the melon crop cycle *E. sativa* green manure resulted in a nematicidal effect statistically similar to cadusaphos and higher than *B. juncea* amendment. *B. juncea* amendment effects were not improved by combination with pellet. Melon yield recorded for chemical treatment and *B. Juncea* were 79.2 and 70.8 t ha<sup>-1</sup>, respectively, and were 3 times higher than untreated control.

In the following watermelon crop the lowest population densities of *M. incognita* were recorded after the combined treatment with *E. sativa* green manure and pellet, and after cadusaphos, followed by *E. sativa* green manure alone and at a significantly higher values, by *B. juncea*. Nematicidal effects of quillay formulation, though significantly higher than untreated control, was consistently lower than biofumigating treatments. Most effective treatments for nematode control provided the highest watermelon yields, 119.0 and 85.7 t ha<sup>-1</sup> for *E. sativa* manure plus pellet and cadusaphos, respectively.

## Biofumigation in greenhouse for the control of root-knot nematodes

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Intensive vegetable cropping systems enhance infestations of root-knot nematodes (*Meloidogyne* spp.) and related damage in the Salento region, southern Italy. Sustainable alternatives to chemicals for the control of these pests may be represented by nematicidal green manures or plant-derived formulations. Biofumigant green manures of *Brassica* plants may be limited by the need to insert them within intensive rotations with short intervals among the crops. Technical feasibility and effectiveness of *Brassica* green manure biofumigation for the control of root-knot nematodes were investigated in a greenhouse experiments on a tomato - melon succession. Green manures of *Brassica juncea* and *Eruca sativa* were compared with a *Brassica* seed meal commercial pellet, alone or combined with *E. sativa* manure, two commercial formulations of quillay or tagetes, chemical nematicide cadusaphos and an untreated soil.

In the tomato crop, green manure of *E. sativa* resulted in the lowest *M. incognita* population densities and gall formation, as its results were not significantly different from chemical treatment but were lower than *B. juncea* green manure, the pellet and quillay formulation. This last treatment was statistically similar to the untreated control. *E. sativa* green manure, either alone or combined with the pellet, provided a tomato yield two-three times higher than control, whereas *B. juncea* and cadusaphos almost doubled the yield of untreated soil.

In the following melon crop the lowest nematode densities were recorded in soil treated with cadusaphos or the commercial pellet. Green manure of *E. sativa*, alone or combined with the pellet, resulted in a higher suppressivity than *B. juncea*, whereas a limited nematicidal activity emerged for the quillay formulation. The highest melon yield was provided by the seed meal pellet, followed by the combination of *E. sativa* green manure and pellet and by both single green manures.

## Control of carrot root-knot and cyst nematodes by biofumigating treatments

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Root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* spp.) nematodes cause heavy yield losses to vegetable crops, such as carrot, onion, tomato and potato, in the coastal sandy soils of the Province of Foggia, southern Italy, due to the intensive cropping systems and the absence of suitable crop rotations. The technical applicability and effectiveness for the control of the root-knot nematode *Meloidogyne incognita* of soil biofumigation treatments with *Brassica juncea* and *Eruca sativa* green manures, *B. juncea* hay, and a *Brassica* seed meal commercial pellet were investigated over two years in field experiments on a carrot – tomato rotation. Biofumigation treatments were compared with the standard nematicide fenamiphos, a quillay-derived nonchemical formulation and an untreated control. At the end of the first cycle, soil population density of *M. incognita* were significantly lower in the plots treated with green manure or hay of *B. juncea* or with green manure of *E. sativa*, either alone or combined with seed meal pellet, compared with untreated control. Nematicidal effects of biofumigation treatments did not differ from that of fenamiphos. Moreover, commercial pellet and *B. juncea* green manure and hay resulted also in carrot yield almost double the control, whereas lower increases were provided by *E. sativa* green manure, either alone or combined with the pellet.

In the following tomato crop the lowest *M. incognita* densities were recorded in soil treated with *B. juncea* hay or with *E. sativa* green manure plus pellet. Hay and green manure of *B. juncea* and integrated treatment with *E. sativa* green manure and pellet resulted in the highest tomato yields, always significantly higher than fenamiphos.

In a second field infested by the carrot cyst nematode *Heterodera carotae* Jones, biofumigation with *B. juncea* hay or *Brassica* seed meal pellet was compared with commercial liquid nematicidal formulations of quillay, tagetes or neem, and with the chemical fenamiphos and an untreated control. At the end of the first year, soil populations of *H. carotae* was significantly suppressed by all the treatments, though neem and fenamiphos provided the lowest nematode populations and the highest carrot yield. At the end of carrot cycle in second year, *H. carotae* population did not increase in plots treated with hay or fenamiphos, whereas pellet and quillay resulted in significantly higher, though lower than untreated control, densities. Pellet and fenamiphos provided the highest total yield and percentage of commercial tap roots.

## Biofumigation for control of *Globodera pallida* in UK potato fields

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Potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* occur in at least 64% of potato fields in England and Wales and cause losses of approximately £43 million per annum. Growers depend on integrated control based on rotation plus chemical control and *G. rostochiensis* resistant cultivars where they are appropriate. A crisis is approaching for growers as nematicides are withdrawn under EU regulations and supermarkets and NGOs press for production without chemical control. Biofumigation can at least partially replace synthetic fumigants such as methyl bromide but this potential is yet to be fully realised, especially in the UK. This project aims to develop an understanding of the factors that influence the efficacy of biofumigation for control of *Globodera* and other soil pests in UK potato fields in order that these factors can be manipulated to maximise efficacy. The initial aim was to identify compounds and plants with efficacy against *G. pallida*.

A toxicity assay was developed in which *G. pallida* second stage juveniles (J2s) were exposed to test substances for 24 h then transferred to sand columns, which separate mobile and immobile J2s. Five commercially available isothiocyanates were tested at a range of concentrations and dose-response curves were generated. Based on these results and those reported in the literature, brassicas containing effective or untested compounds were selected. Dried, powdered leaf and root tissues were rehydrated at 1.8 mg tissue per ml water for three hours to allow glucosinolate hydrolysis and filtrates were used in toxicity assays. The percentages of suppression caused by tissues of 22 cultivars from 15 species of Brassica were determined.

The relative order of toxicity of the pure isothiocyanates was benzyl > phenethyl > 2-propenyl > 3-(methylthio)propyl > 2-methylbutyl, with ED<sub>50</sub> values ranging from 4 to 23 µM. The plants causing greatest suppression were, in ascending order, *B. juncea* cv. Fumus, *Sisymbrium austriacum*, *Brassica juncea* cv. Nemfix, *Nasturtium officinale* and *Raphanus sativus* cv. Weedcheck, causing from 50 to 90% suppression under the test conditions.

Work is currently underway to quantify total glucosinolate in the test plant tissues and to determine the efficacy of these materials in suppressing *Rhizoctonia solani*.



## Effectiveness of biofumigation technique to control the southern root-knot nematode (*Meloidogyne Incognita*) in Sicily

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The authors report the results of field trials undertaken during the last two years in an unheated plastic-house in the southern part of Sicily (Italy) in order to verify the effectiveness of biofumigation strategy in managing nematode *Meloidogyne incognita* in tomato crops.

In the first trial, two accessions of mustard species of the *Brassicaceae* family were selected for their glucosinolate content (*Brassica juncea* sel. ISCI 99 and *Eruca sativa* cv Nemat). These were rotated to ascertain their effectiveness against the root-knot nematode in comparison to the addition of pellets derived from the *Brassicaceae* species. Each species was planted in a plastic-house and grown until flowering; the green mass was then cut up and incorporated into the soil. After, a 15 days soil solarization was effected on all the trial fields. In the second trial the tomato crop was treated with oil extract of *Brassicaceae* species, distributed by drip irrigation when the maximum development of nematode population was registered. At the end of each crop cycle, the yield was recorded and nematode infestation on tomato roots and in the soil was estimated.

All techniques succeeded in reducing the juvenile nematode population in the soil and gall index in the roots at the end of the tomato's growth cycle. All the techniques produced a tomato yield significantly higher than with untreated soil. In particular, *Brassica juncea* sel. ISCI 99 acted mainly as a biofumigant while *Eruca sativa* cv Nemat revealed an interesting trap crop effect. Also the use of pellets derived from *Brassicaceae* species, incorporated into the soil before the transplanting has shown a lower galls index in the roots. The oil extract of *Brassicaceae* species reduced the juvenile nematode population in the soil during the whole tomato cultivation cycle.



## Nematode suppressive effect of two biofumigation crops (*Brassica juncea* and *Cleome hussleriana*) evaluated by Laboratory and Greenhouse Experiments

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To develop environmentally harmonious measures to manage harmful plant-parasitic nematodes and soil-borne diseases, and to supply low-chemical-applied agricultural products required by consumers, we have developed some graminaceous and leguminous plants (green manure crop) which suppress those pests in rotation cropping. In 2006 we started a 5-year project entitled “Development and utilization of Biofumigation Crops” which is supported by Japanese Ministry of Agriculture, Forestry and Fisheries. We developed some new lines of brown mustard (*Brassica juncea*) and cleome (*Cleome hussleriana*), and evaluated their suppressive effects against *Meloidogyne incognita*, *Pratylenchus penetrans*, *Heterodera glycines* and *Helicotylenchus dihystra* by laboratory and greenhouse experiments. Results obtained are as follows:

1. A peak of isothiocyanate (ITC) concentration in soil was detected two hours after incorporation of biofumigation crops in pot trials. On the other hand, the amount of glucosinolate (GSL) in incorporated plant tissues markedly decreased at the same periods and after one week nothing was detected in soil and tissues of fumigation crops incorporated.
2. Nematode suppressive effects depended on amounts of the crops incorporated into soil and incorporation equivalent to 80 FW ton / ha was required to obtain about 80% reduction of the nematode populations.
3. The nematode suppressive effects was maximum about 14 days after incorporation into soil at 25°C.
4. Suppressive effects of these crops differed among nematode species. *P. penetrans* was more susceptible than *M. incognita*.
5. The nematode suppressive effects of these crops were greater in conditions of higher soil moisture contents.

We hope to develop fumigation crops with much higher GSL content and more effective and practical methods to utilize such crops in this project.

## Organic amendments and host resistance as components of integrated disease management strategy for root-knot nematode *Meloidogyne incognita* in lettuce

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The influence of organic amendments applied as green manures and composts were tested against root-knot nematode *Meloidogyne incognita* in resistant and susceptible lettuce cultivars (*Lactuca sativa*). This experiment was conducted in modified microplots under greenhouse conditions at Benguet State University, La Trinidad, Benguet, Philippines. Chopped leaves, stems and roots of six plants namely, broccoli and cauliflower (*Brassica oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), mustard (*B. juncea*), radish (*Raphanus sativus*), and *Tithonia diversifolia*, including processed chicken manure were incorporated into the soil previously inoculated with 10,000 second stage juveniles of root-knot nematode. The amendments were applied at 20g per kg of soil. A treatment using fallow for 3 weeks was included as control. Significant increase ( $P \leq 0.05$ ) in plant height and top weight were recorded in the two lettuce cultivars incorporated with organic amendments. Highest top weight and plant height were noted in broccoli- and cabbage-amended soil. Root weight was highest in broccoli- and cauliflower-amended soil. This was significantly different from the other treatments. Significantly lower number of galls was recorded in resistant cultivar Great Lakes as compared to that of susceptible cultivar Tyrol. Highest number of galls was observed in fallow and processed chicken manure which was significantly different from the rest of the treatments. No egg masses were produced in cultivar Great lakes while in cultivar Tyrol, fallow gave the highest number of egg masses significantly different from the amended soil. Significant reductions in second stage juveniles in the soil were found in mustard and radish amendment but comparable with the other treatments. A relatively low number of *Helicotylenchus* sp. and *Pratylenchus* sp. were also found in the soil. The number of free-living nematodes was significantly higher in cauliflower-amended soil but comparable with the other treatments which could probably be an indication of their dynamic interaction in the soil. These results suggest that there is a notable difference in using organic amendments in suppressing the population of root-knot nematodes in both resistant and susceptible lettuce cultivars.

## Optimizing biofumigation varieties and blends for non-chemical control method against plant parasitic nematodes and soil-borne diseases in temperate climates

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In order to optimize the Biofumigation technique for the practical use in temperate climates in Germany a national project was initiated in 2007. One goal of the project is presentation of the Biofumigation technique to the farmer and to provide the corresponding technical information to the practical grower. Therefore adaptation in agronomical parameters (seeding time, seeding density, composition of the blends, fertilizer) have been tested with a basic collection of different varieties of three crucifer species *Brassica juncea*, *Raphanus sativus*, *Sinapis alba* and seed mixes Terraprotect RB (*B. juncea* and *R. sativus*) and Terraprotect MB (*B. juncea* and *S. alba*). The field of possible use is widely spread and therefore special solutions for the individual cultivation have to be found. Each solution forms a part of the general scheme for the use of Biofumigation.

Another goal of the project is the evaluation, testing and selection of suitable plants for Biofumigation. Special emphasis is given to material with resistance against nematodes such as *Heterodera schachtii*, *Meloidogyne* spp., *Pratylenchus* spp., *Ditylenchus dipsaci* and economically important plant diseases. The following results were gathered from field trials and selection which were conducted in the frame of the project

## **Cover crops in the year before planting potatoes – a key factor in a spraing disease management strategy**

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Potato spraing causing brown corky arcs and spots in tuber flesh, has become a severe constraint to potato production in Scandinavia. Field studies were carried out during 2006-2007 to appraise brassicaceous amendments against trichodorid nematodes, the vectors of tobacco rattle virus (TRV) that causes spraing disease. The trials were conducted in a small grain-potato rotation in Central Sweden. Three cover crops, Caliente mustard (*Sinapis alba* and *Brassica juncea* blend, cv. Brandt 119), oil raddish (*Raphanus sativus*, cv. Colonel) and arugula (*Eruca sativa* cv. Nemat) were tested and compared to a bare soil. The cover crops reduced nematode populations at all different locations although the suppression level differed among locations and cover crop species used. Substantial yield improvements were also recorded.

## Expression of a 70 KDa heat shock protein (HSP70) in *Caenorhabditis elegans*, as an indicator of allyl isothiocyanate toxicity in mustard

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Members of the 70 KDa heat shock protein (HSP70) family are involved in preventing protein aggregation and refolding of denatured proteins and are produced in response to cellular stress caused by an insult. We developed an assay for testing AITC toxicity using HSP 70 expression in *Caenorhabditis elegans*. *C. elegans* strain N2 was exposed to different concentrations (0 to 10  $\mu$ M) of allyl isothiocyanate (AITC) for 2 h at room temperature. Western blotting with anti-HSP70 antibody showed a marked increase in the expression of HSP70 protein in a dose-dependent manner. Further, the expression of HSP70A mRNA was investigated by quantitative real time reverse transcriptase PCR (RtPCR). The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference housekeeping gene and relative expression levels were calculated after correction for expression of GAPDH. Although, no significant change in the expression of HSP70A mRNA was observed at low concentrations of AITC (< 0.1  $\mu$ M) treatments with higher concentrations (>10  $\mu$ M) resulted in 4 to 5 fold increased expression of HSP70A mRNA over the control. Therefore, it may be concluded that AITC induces stress responses in *C. elegans*. Further, to understand if mustard toxicity is contributed by AITC alone or other compounds in mustard meal affected HSP70 transcript production, *C. elegans* were exposed to AITC (0-10  $\mu$ M) and/or *Brassica juncea* cv. *Arid* meal (*Arid* is a mustard variety with low levels - < 3 micromoles of sinigrin per gram of seed). To confirm maturation of HSP70 transcript, ELISA was conducted. Similar to RtPCR ELISA revealed increased expression of HSP70 protein in *C. elegans* treated with AITC + mustard meal but the levels of protein was less than those observed with AITC alone. These results indicate that mustard meal toxicity is contributed primarily by AITC and some meal components antagonize AITC toxicity in *C. elegans*.

## Behavioral response of *Meloidogyne incognita* to benzyl isothiocyanate

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One reported mechanism of plant-parasitic nematode suppression by brassicaceous cover crops is the production of isothiocyanates (ITC) in soil after biomass incorporation. While plant-parasitic nematode mortality is the objective when using these cover crops for biofumigation, very little is known about how ITCs influence nematode behavior (i.e. movement, infectivity, gene regulation). Experiments were conducted to determine the effects of sub-lethal doses of benzyl ITC (BITC) on *Meloidogyne incognita* juvenile (J2) behavior. *Meloidogyne incognita* J2 responded rapidly to *in vitro* exposure to  $\mu$ M doses of BITC, in DMSO/water preparations, by altering their movement behavior and movement frequency. The responses of the J2 suggested that such behaviors may affect locomotion. In subsequent experiments *M. incognita* J2 were exposed to the BITC concentrations found to be effective from the *in vitro* studies, and then inoculated onto tomato to evaluate infectivity. In related experiments, a subset of these treated nematodes was examined for the production of heat-shock proteins (HSPs). A survey of HSP70 among *M. incognita*, *Heterodera glycines*, and *Xiphinema americanum* revealed significant differences in constitutive levels of HSP70, suggesting responses to stress, may vary among plant-parasitic nematodes. Understanding how BITC modifies nematode behavior may ultimately provide insight as to how better to manage brassicaceous cover crops for plant-parasitic nematode management.

## Biofumigant activity of *Brassicaceae* against soil-borne fungi

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Plants of the *Brassicaceae* family are well known for their ability to accumulate glucosinolates that, in the presence of myrosinase enzyme, release substances with antifungal activity. The biological activity of crude extracts of *Brassica carinata* L., *Brassica juncea* L., *Brassica rapa* L., *Brassica napus* L., *Crambe abyssinica* L., *Raphanus sativus* L. and *Sinapis alba* L., and of their hydrolyzed glucosinolate derivatives was evaluated *in vitro* against the soilborne pathogens *Sclerotinia sclerotiorum* (Lib.) de Bary, *Verticillium dahliae* Kleb., *Fusarium oxysporum* f.sp. *lycopersici* (Wr. et Rg.) Schlecht., *Phytophthora* sp., *Pythium* sp., *Pyrenochaeta lycopersici* Schneider et Gerlach and *Rhizoctonia solani* Kühn, as well as the microbial antagonists *Trichoderma asperellum* Samuels, Lieckf. Nirenberg and *Trichoderma viride* Pers.. Crude extracts generally showed a low biological activity, even though appreciable inhibition of *V. dahliae* and *F. oxysporum* f.sp. *lycopersici*. was observed. *S. alba* was the less active species, while *B. rapa* and *B. napus* were the most effective, followed by *B. carinata* and *B. juncea*. All hydrolyzed glucosinolate products inhibited the growth of all tested microorganisms. Biofumigant activity of fresh, dry and lyophilized *B. juncea* plants and of pellets of *B. carinata* (Biofence, Triumph Italia, Livorno, Italy) was evaluated on pot-grown tomato plants under controlled conditions. The two above species were mixed with soil artificially infested by *V. dahliae* or *F. oxysporum*. Both treatments consistently improved the growth of tomato plants and fruit yield. Under all tested conditions, a strong reduction of propagule viability was observed. *V. dahliae* was totally devitalized either by fresh or dried material of *B. juncea*, and it was reduced up to 47% also when lyophilized *B. juncea* or *B. carinata* pellets were applied. *B. juncea* totally prevented the growth of *F. oxysporum*; less than 3% of the pathogen propagules survived in the presence of *B. carinata* pellets. These results, even though preliminary and requiring further investigations, open the possibility of using *B. juncea* and *B. carinata* in greenhouses and in the field as biofumigants for their activity against soil-born fungal pathogens.

## Effects of soil Sulphur and Nitrogen on Isothiocyanate production within *Brassica* species and subsequent mycelial inhibition of *Rhizoctonia solani*

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The aim of this study was to determine if soil treatments of Sulphur and Nitrogen induce higher production of glucosinolates (GLS) that are isothiocyanate (ITC) liberating within five plant species belonging to the family Brassicaceae, and if ITCs released from the roots of plants grown within these treatments inhibit the growth of the soil borne pathogen *Rhizoctonia solani*.

*Brassica napus* (2809), *B. napus* (4063), *B. campestris*, *B. juncea* and *Raphanus sativus* were grown under five different Sulphur regimes: low Sulphur, medium Sulphur, high Sulphur, low Sulphur + Nitrogen post sowing and medium Sulphur + Nitrogen at sowing. Five plants per replicate were removed at 10% flowering and freeze-dried for 7 – 9 days. Samples were separated into 3 categories of stem (S), leaf (L), root (R) tissue and analysed for isothiocyanates using gas chromatography.

Results showed that levels of soil Sulphur varied significantly in their effect on mean GLS and individual GLS concentrations between species and individual plant parts within species. Analysis of the mean GLS from combined “LRS” tissue showed that the highest concentration of 86.8  $\mu\text{mol g}^{-1}$  was released from *B. napus* (4063) grown in soil containing a high Sulphur content (30ppm) and the lowest (24.5  $\mu\text{mol g}^{-1}$ ) from *B. napus* (2809) grown in soil containing a medium Sulphur content (15ppm) and ammonium nitrate applied at sowing. Analysis of root tissues showed higher total GSLs levels than within leaf or stem tissue tissues from the same plants except in *B. juncea* where GLSs were lower in root tissue than leaf. The glucosinolate profiles within leaf, root and stem tissues of all species were dominated by aliphatic GLS except in *B. campestris* where aromatic GLS were more dominant.

Volatiles released from the root tissue of *Brassica napus* (2809), *B. napus* (4063), *B. campestris* and *B. juncea* but not *Raphanus sativus* were inhibitory *in vitro* to the growth of *Rhizoctonia solani* AG3. Whilst mycelial growth was reduced by most treatments only *B. napus* (2809), *B. napus* (4063) and *B. campestris* completely inhibited growth. None of the individual GLS showed a significant correlation between concentration per gram of tissue and inhibition of *R. solani*.



## Introduction of fumigation crop research against soil-borne diseases and nematode in Japan

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We began to develop high potential fumigation crops, brown mustard (*Brassica juncea*) and cleome (*Cleome spinosa*) for control of soil-borne disease and nematode, which is supported by MAFF project (No.18039) from 2006 to 2010. The following results were obtained in the two years of research in 2006 and 2007:

1. We obtained high glucosinolate (GSL) containing brown mustard lines as green manures after cultivation of winter wheat using HPLC maternal selection, whose GSL content is about 50% higher than parents (Sakuma). We also selected a cleome line containing high GSL for green house farmers using recurrent selection with HPLC with a level about twice as parents (Hashizume).
2. Control value of sugar beet root rot caused by *Rhizoctonia solani* in pot trials after cultivation of brown mustard (Exp.Y-008) was as high as 90, which is the same as wild oats (Sakuma). Three types of ITC (Allyl, Ethyl, Phenyl) inhibited germination of oospore and encysted zoospore of *Phytophthora sojae*, causal agent of soybean *Phytophthora* stem and root rot, and the same effect was shown by treatment of brown mustard (Kondo). After cultivation of brown mustard, damage of radish *Verticillium* black spot caused by *Verticillium dahliae* was reduced, but it is not clear whether this is due to ITC gas effect (Fujine and Sumino). Freeze-dried powder of a higher GSL plant that was selected in this program reduced tomato bacterial wilt by *Ralstonia solanacearum* more than lower GSL lines (Maeda). We got healthy spinach after cultivation of brown mustard and cleome in infested soil with *Fusarium oxysporum* f. sp. *spinaciae* (Mitsuhori).
3. After mustard and cleome fumigation of 40 or 80 FW ton /ha, 80% of *Meloidogyne incognita* and *Pratylenchus penetrans* populations were reduced (Mitsuhori and Sano).
4. While a high DM production of cleome was obtained when sown on May 23<sup>rd</sup> 2006, GSL content was high when sown on July 25<sup>th</sup>, which is the best time for cultivation after winter wheat in Hokkaido area. High nitrogen and sulfur supply increase GSL content (Ubukata).

## Biofumigation to control *Verticillium* wilt influenced by plant species and soil types

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Biofumigation control of *Verticillium dahliae*, causal agent of *Verticillium* wilt, was tested in several pot and field trials at the Research Center Conthey and in on-farm trials. The impact on the microsclerotia i.e., the surviving structures of *V. dahliae* in the soil, was measured to assess the impact of biofumigation independently from the crop. After brown mustard (*Brassica juncea* sel. ISCI-99 and ISCI-20) green manure, the mean reduction compared to no plant was of 77 and 44% respectively. This result reflects the difference between the two brown mustard cultivars; ISCI-99 has a quite higher glucosinolate content than ISCI-20. The reduction of microsclerotia after a low-glucosinolate canola (*Brassica napus*) cultivar (Talent) green manure was 35%, indicating that next to the biofumigation effect other factors are most probably involved in the effect of cruciferous plants on the survival of *V. dahliae* in soil. When the brown mustard ISCI-99 was tested in two soil types, an 85% reduction of *V. dahliae* microsclerotia occurred in a loamy soil. In contrast, no significant effect was measured in a highly sandy soil (> 80% sand). The role of the soil type on the efficacy of biofumigation to control *Verticillium* wilt will be further studied.

## Biofumigation against soilborne pathogens and weeds of strawberry

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Our research aimed to identify factors that improve and / or limit the efficacy of biofumigants for soil disinfestation against strawberry pathogens and weeds. In laboratory bioassays, the volatiles released from macerated roots of a *B. rapa* / *B. napus* mixture were six times more effective at suppressing the growth of the strawberry pathogen *Rhizoctonia fragariae* than shoots. The toxicity of the volatiles increased as plant developmental stage progressed. These results related to the release of higher quantities and a greater diversity of isothiocyanates from the roots of mature biofumigant plants than from their shoots. Separate bioassays showed the volatiles from the biofumigant crop suppressed the growth of six soil-borne pathogens of strawberry and seven clover weed species. Suppression of clovers did not relate to their seed size, but might relate to their hard-seededness or genotype. Freeze dried root meal of the *Brassica* crop (8 tonnes/ha) released 10 molar equivalents less of ITCs into soil (64 mol/ha) than the commercial soil fumigant, metam sodium (736 mol/ha, applied at 106 kg ai/ha). Currently, the relatively low amounts of ITCs released by biofumigant crops into soils are important limitations to their ability to control soilborne pests to the levels of synthetic fumigants.

In the field, rotary incorporation of the biofumigant crop did not produce detectable levels of ITCs in soil; reduce the survival of the strawberry pathogens, *Phytophthora cactorum* and *Cylindrocarpon destructans*; or affect populations of culturable soil microflora. Yet, it suppressed the growth of emerging weeds by 40% and the growth of *P. cactorum* by 20%. We hypothesise that allelochemicals other than ITCs, such as nitriles, or other biological mechanisms might also play a role in biofumigation. A key to improving the efficacy of biofumigation in the field seems to lie in the development of application technologies that can macerate and incorporate biofumigants evenly in soils, in addition to incorporating biofumigants under optimal edaphic conditions for release of ITCs.

## **Growing crops of *Brassica juncea* and then incorporating their residues give complementary control of *Rhizoctonia* root rot of sugar beet**

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*Brassica* species are nowadays increasingly used as catch crops with the aim of suppressing soil-borne pathogens, though effectiveness of this practice is not always demonstrated. To understand the mechanisms responsible for the irregular efficacy observed in the field, we proposed to decompose the catch crop cultivation phase by assuming that the *Brassica* crop, during its growth phase, can have a negative effect on soilborne pathogens and that this effect is enhanced after crushing the crop at flowering and incorporating the residues into the soil. To test this, two field studies were carried out in 2006 and 2007 with different management of a *Brassica juncea* (brown mustard) catch crop, in a sugar beet–winter wheat rotation, to analyse its action on sugar beet root rot caused by *Rhizoctonia solani*. Three treatments, mustard pulled out at flowering (MP), mustard crushed at flowering and incorporated into soil (MC) and bare soil (BS) as control were set up and assessed for their effect on root rot incidence and severity measured at harvest. The incidence mean adjusted to the effect of year was significantly the highest in BS plots and was significantly higher in MP plots than in MC plots. In addition, MC treatment significantly reduced the mean severity compared to MP and BS treatments. These results suggest that both above- and below-ground parts of mustard can reduce *Rhizoctonia* root rot and that growing mustard alone has a suppressive effect on disease incidence while growing mustard and incorporating the residues could give further control by reducing disease severity. These findings further raised the question on how mustard management could act on the epidemiological mechanisms of *Rhizoctonia* root rot.

## Biofumigation for soil-borne disease management in flower bulb culture

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Effects of biofumigation against *Pythium* root rot were investigated. *Pythium spp.* cause root rot in bulb crops like hyacinth, crocus and iris. Broad-spectrum soil fumigants, e.g. methylbromide, dichloropropene and metam sodium, are not allowed for environmental reasons. Only one specific fungicide (metalaxyl) is available for application in flower bulb culture. The effects of this fungicide are variable. Furthermore using only one fungicide leads to a high risk for resistance development. In order to develop an effective method to suppress *Pythium* and to reduce the dependence on this fungicide, non-chemical control methods have been tested. Biofumigation is such an alternative method, suppressing soil borne diseases by biocidal compounds, released from Brassicaceous green manure crops when glucosinolates are hydrolysed via myrosinase after cell disruption.

In a field experiment with hyacinth and crocus, treatment with *Brassica juncea* ISCI20 was compared to *Raphanus sativus* and untreated (fallow). Incorporation of both *B. juncea* and *R. sativus* resulted in increase of bulb yield compared to the fallow treatment. Moreover, yield losses caused by *Pythium* root rot were diminished. In infested field soil, no reduction of yield loss was observed compared to the non-infested control, whereas in untreated soil more than 20% yield loss occurred due to *Pythium* root rot. Based on these promising results, participatory experiments were performed with bulb growers on naturally infested fields.

## Evaluating biofumigant amendments for the management of diseases caused by sclerotial pathogens in vegetable crops

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The soilborne pathogens *Sclerotium cepivorum*, *Sclerotinia minor* and *S. sclerotiorum* cause significant yield losses in vegetable production in Australia. *S. cepivorum* causes white rot in Allium crops and *Sclerotinia* species cause diseases in a range of important vegetable crops including lettuce (lettuce drop) and green beans (white mould). These pathogens survive in soil as sclerotia which are difficult to eradicate from soil and control with chemical treatments. Previous work has shown that volatile compounds released from some cruciferous plant residues can reduce the viability and survival of these sclerotial pathogens. Our research is therefore aimed at quantifying the ability of biofumigant treatments for reducing the amount of sclerotia in soil and disease incidence in susceptible vegetable crops. The research also aims to determine the potential of integrating biofumigants with other chemical and non-chemical control measures to improve disease management. The biofumigants being evaluated include residues of *Brassica* green manure crops (e.g. B.Q. Mulch) and two biofumigant soil amendments (a *Brassica juncea* seed meal, Fumafert® and an oil amendment, Voom®).

Preliminary *in-vitro* bioassays have shown that volatile compounds released from Voom effectively reduce the viability of sclerotia of *S. cepivorum*. A preliminary field trial, however, showed that when Voom™ was shank-injected into a sandy soil (raised beds), infested with a natural population of *S. cepivorum*, it was not as effective as the fumigant Basamid® (dazomet) in decreasing the amount of sclerotia in soil and subsequently the incidence of white rot in a spring onion crop. Preliminary results from laboratory experiments and field trials are reported.

## Biosolarisation as an alternative to methyl bromide in protected pepper crops in Spain

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In the southeast of Spain, 11,700 ha of peppers are produced under greenhouse conditions, and in the provinces of Murcia and Alicante 2,200 ha have used methyl bromide (MB) every year since 1988 for the control of *Phytophthora* spp., *Meloidogyne incognita* and to minimize the effects of soil fatigue due to repeated monocropping. In 1997, biofumigation and biosolarization experiments were initiated using local organic amendments (sheep and chicken manure) to look for non chemical alternatives equally efficient as MB, studying the timing and number of applications, doses, soil type, environmental conditions and the combination effects with resistant root stocks. To determine the efficacy of the disinfection, the incidence of fungal and nematode infection, the control of weeds and the effects on plant development and production were measured. Biofumigation with manure was not effective. The biosolarization had a positive effect on the control of *Phytophthora* when carried out in the middle of September. When applied for a second year the control of *Meloidogyne* was random but was improved when repeated applications were used. The same effect was observed with weeds. The development and production of the plants were comparable with MB treatment in the second year. Non pathogenic fungi were not affected, physical and chemical properties of the soil were improved and lixiviates were not produced. This technique is currently being used on 12% of the surface area dedicated to pepper production.

## The use of vinasses in the management of soil-borne pathogens

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The purpose of this research was to study the biocidal effect of three agroindustrial by products, including sugar beet, sugar cane and wine vinasse. Results from *in vitro* testing demonstrated that wine vinasse can suppress fungal growth 100% with concentrations between 5% and 7% for *Fusarium oxysporum* f.sp. *melonis* race 0 and 1, *Sclerotinia sclerotiorum*, *Pythium aphanidermatum* and *Phytophthora parasitica*, and 10-15% for *Fusarium oxysporum* f.sp. *radicis-cucumerinum*. Sugar beet vinasse showed approximately 100% suppression of fungal growth for only some of the phytopathogens tested: *S. sclerotiorum* (15%), *P. aphanidermatum* (7%), *P. parasitica* (15%) and *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (15%). On the other hand, sugar cane vinasse did not produce any significant effect on fungi. The efficacy on the control of plant-parasitic nematodes was also studied, confirming that they have a similar efficacy to the conventional nematicides in the control of the root-knot nematodes (*Meloidogyne*) and the virus transmitting nematode *Xiphinema index*, depending on how and when the vinasse is applied. The three vinasses studied also increased the bacteria, fungi microbiota, and saprophytic nematodes, improving soil quality.



## Isolation of cDNAs upregulated in *Alternaria alternata* tolerant to lethal concentrations of 2-propenyl-isothiocyanate

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The development of resistant fungi to synthetic fungicides is a serious problem in agriculture which suggests the importance of studying alternative compounds with antifungal activity. Among these compounds, isothiocyanates (ITCs) represents a good choice. Even though ITCs have a potent fungicide activity, in our lab we had observed that *Alternaria alternata* can tolerate lethal concentrations of 2-propenyl-isothiocyanate (2-PITC) after a gradual and prolonged exposition. The possibility of fungal tolerance appearance against ITC makes important to elucidate the tolerance mechanism involved in these phenomena. The objective of the present work was to identify cDNAs upregulated in response to the presence of 2-PITC. Using a protocol for subtractive suppressive hybridization, we isolated several differentially expressed DNA fragments from a 2-PITC tolerant *Alternaria alternata* strain. The differentially expressed fragments were cloned into pGEM-T vector and *E. coli* JM109 cells were used for transformation. The DNA fragments were sequenced and analyzed using BLAST program and Blastn, Blastx and Tblastx algorithms and also it was done the assembly of DNA fragments to uncover redundancy in the library. At the nucleotide level we have found clones of 2-pITC treated *A. alternata* with significant similarity to genes induced during the interaction of *A. brassicicola* with *Brassica oleracea*, and to clones isolated of *A. brassicicola* under nitrogen starvation conditions. At protein level we found clones with significant similarity to genes encoding opsins, transcription factor C6, nucleic acids binding proteins, glyoxal oxidase, amino acid aminotransferases, and amino acid permeases type Agp2 of several fungi species. Moreover we found clones similar to PMR1, pmrA, Ca and Mg type ATPases and to hypothetical proteins of different fungi. In the sequence assembly, we found one domain similar to a transmembrane domain of ABC transporters present in several fungi groups. The nucleotide sequences analyzed to date present similarity to genes coding for proteins involved in transport cellular processes, secretion routes and antibiotic resistance in diverse fungal species. The fragments isolated and analyzed coding for proteins not studied to date, could imply the discovery of unknown genes in *Alternaria alternata* that are playing a role in the tolerance mechanism of this fungus to the toxic effect of ITCs. The sequencing process continues and when finish, we expect to generate scientific evidences to help in the elucidation the molecular basis of the tolerance mechanism of *A. alternate* to the 2-PITC toxic effect.

## **Incorporation of *Brassica nigra* and *Diplotaxis tenuifolia* residues and incubation under different soil conditions affects the survival of *Rhizoctonia solani* AG2-1 (ZG5), the causal agent of damping off of canola differently**

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Incorporation of residues of *Brassica nigra* and *Diplotaxis tenuifolia* containing high glucosinolates were tested for their potential to reduce the saprophytic survival in soil of *Rhizoctonia solani* AG2-1 (ZG5), the causal agent of damping off of canola seedlings. The major ITC released from *B. nigra* tissues was allyl (2-propenyl) ITC (AITC) while 3-butenyl and 4-methylthiobutyl ITC were the major compounds released from *D. tenuifolia* tissues. Residues of both species were able to inhibit the growth of *R. solani* when high concentrations (5-10%) of fresh residues were incorporated into moist soil, but failed to reduce the saprophytic growth of the fungus when the soil was hot and dry. This indicated that glucosinolates from *B. nigra* and *D. tenuifolia* macerated tissue were hydrolyzed to form isothiocyanates in the presence of water resulting in the reduction of the inoculum of *R. solani*. Concentrations of ITCs in soil reached their peaks 3-5 days after incorporation but were gradually reduced after 7 days. The population of the pathogen progressively declined and was undetectable one month after incorporation, while the populations of soil microorganisms (bacteria, nematodes and *Arthrobrotys*) in the same treatments increased. This indicated that the reduction of population of *R. solani* may be associated with levels of both ITCs and soil microorganisms. Hot and dry soil conditions retarded both the hydrolysis of glucosinolates and the activities of other soil microorganisms. This treatment therefore failed to suppress soil survival of *R. solani*, which is known to have high competitive saprophytic ability, especially in the presence of fresh organic matter.

## Potency of Brassica residues as biofumigation for control of bacterial wilt of tobacco in Indonesia

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Preliminary experiments have been conducted to evaluate the effectiveness of Brassica residues as biofumigants to control bacterial wilt of tobacco (*Ralstonia solanacearum*) in laboratory and glass house of The Research Institute for Tobacco and Fibre Crops, Indonesia. The species of Brassicas used in this trial were common commercial vegetables in Indonesia including cabbage, broccoli, cauliflower, white Chinese cabbage (WCC), green Chinese cabbage (GCC) and weed (wild *Brassica nigra*). *In vitro* test using water extract of the six Brassica residues showed the population and growth of *R. solanacearum* in CPG medium were inhibited. There was no bacteria grew in medium added with broccoli, or cauliflower extracts. A number of small colonies of the pathogen appeared in medium added with water extract of WCC 3 days after inoculation (dai), GCC or weed (4 dai) , and cabbage 7 dai. The colonies grew rapidly in the following days. *In vivo* test used fresh and chopped residues of six Brassicas residues. The residues were individually incorporated to infested soil at a rate of 10% (w/w) and incubated for a month before susceptible tobacco seedlings were transplanted. The water condition was maintained by covering the container with plastic wrap during incubation. The disease severity and growth of tobacco plants were monitored 30 days after transplanting (dat). Tobacco plants wilted within 3 dat and died rapidly in untreated soil. No disease symptoms appeared in soil added with broccoli or cauliflower residues. The disease symptoms in soil added with residue of WCC or weed appeared 14 dat with severity reduced by 10%. The height and dry mass of plant were higher and heavier in treated soil compared to untreated soil. Total population of bacteria, actinomycetes, and fungi increased significantly in treated soil. Result from this preliminary experiment indicated that incorporation of residue of Brassicas, especially broccoli and cauliflower offer potential control for bacterial wilt of tobacco and source of nutrition for the plant. The field trial is conducting to confirm the effectiveness of brassica residues in *R. solanacearum* natural infested soil.

## Evaluating biofumigation for soil-borne disease management in white potato

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The project aimed to evaluate the potential of biofumigation technique by which soil-borne diseases and pathogens of white potato are suppressed using the locally grown *Brassica* crops such as broccoli, radish, cabbage and mixed crucifers as biofumigants. The study was conducted at the Northern Mindanao Agricultural Research Center (NOMIARC) in Malaybalay City and in on-farm potato sites at Lantapan, Bukidnon.

Results showed that the application of different crucifers has decreased the population of the pathogen *Ralstonia solanacearum* from the initial count of 81,050 colony-forming units per gram (cfu/g). The mean population densities in terms of cfu/g showed significant difference in plots applied with chopped broccoli (45,342), chopped cabbage (46,776), shredded broccoli (46,962), shredded cabbage (48,064) and chopped radish (54,605). On the other hand, nematode population has decreased significantly with mixed crucifers by 87% while an increase of 17.2% in nematode population is observed in untreated plots.

In terms of marketable tubers, treatments with chopped and shredded brassicas under on-station trial showed no significant difference with yield ranging from 7.81-8.75 t/ha but differed significantly with the control of 4.07 t/ha. On-farm trial using mixed brassicas has posted superior economic advantage with the highest ROI of 192%.

Generally, the project has given a positive economic benefit to potato farmers by reducing pathogen and nematode populations in the soil and thereby increases productivity and income.

## **A *Folsomia candida* (Collembola: Isotomidae) bioassay to investigate biofumigation process by *Brassica carinata* seed meals**

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Biofumigation by pellets based on *Brassica carinata* defatted seed meals is now widely applied in plant defence at commercial scales as a soil amendment. Starting from the clear biological activity in soil of this compound, the aim of this study was to test the effect of these meals on springtail *Folsomia candida* (Isotomidae), a non target insect widespread in soil and frequently used in laboratory ecotoxicological tests. For this purpose a quantitative bioassay was defined and optimised as an acute test relatively easy to carry out that consists of a 24 h exposure of adults to the tested product. As a substrate a common artificial soil has been used.

The toxicity curve of a biocidal seed meal was calculated and compared with the toxicity curve of Vapam®, a chemical widely applied in crop protection. The results showed a high toxicity for both products, even if meal was clearly less toxic than Vapam®: the concentration that kills 50% of the tested organisms (LC50 value) was, respectively, 132 and 3.41 mg kg<sup>-1</sup> even if the LC50 of the active compounds released from meals (Allylisothiocyanate) and vapam (Methylisothiocyanate) was of the same order of magnitude.

The bioassay was applied on meals derived from different batch productions and mixtures of meals with the aim to compare the toxicity of similar materials characterised by different glucosinolates (GLS) contents. The results showed a good correlation ( $R^2=0.991$ ) between insect mortality and GLS contents, measured for each tested substance, when a sufficient amount of the enzyme Myrosinase (MYR) was present. In fact, after a complete deactivation of MYR by an autoclave treatment mortality was related to intake of exogenous purified MYR.

Our results show how the proposed bioassay is a practical tool to evaluate biofumigation biological effects and in particular to compare acute toxicity of meals with different GLS content.

## Potentially mineralizable nitrogen in soil amended with biocidal and non-biocidal plant materials

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In recent years *Brassica* defatted meals have been studied for their biofumigant effect on soil-borne pathogens and pests. However, when incorporated, these materials also supply the soil with nutrients, which may interact with crop nutrition. Potentially mineralizable N (PMN) is a measure of soil ability to release inorganic N useful for crop N uptake. The aim of this study was to compare the PMN of soil amended with *Brassica* meals, with that of soil after incorporation of other plant materials or inorganic N.

Treatments were: *Brassica carinata* meal, thermally deactivated *B. carinata* meal, non-biocidal sunflower (*Helianthus annuus* L.) defatted meal, *Brassica juncea* green manure (GM), *B. carinata* meal + *B. juncea* GM, control. Inorganic N, alone or in combination with *B. carinata* meal and *B. carinata* meal + *B. juncea* GM, was also included in the comparison in order to simulate soil fertilization with inorganic N sources, for a total of 9 treatments. Plant material and/or N fertilizer were mixed into a silty clay soil in pots at incorporation time,  $t_{inc}$ . After 1 week at room temperature, soil was distributed in vials for the determination of PMN. The inorganic (nitrate + ammonium) N content in soil was determined 28 ( $t_1$ ) and 91 ( $t_2$ ) days after the start of the incubation, at 30°C and 75% of plant-available water. The PMN was calculated as the difference of inorganic N content in soil between time  $t$  ( $t_1$  or  $t_2$ ) and time  $t_{inc}$  (3 replications for each treatment, at each time).

At time  $t_1$  the soil amended with *B. carinata* + fertilizer N showed the highest PMN; soil green manured with *B. juncea* was not significantly different from the control, whereas the PMN for the other treatments was higher than the control, without any significant difference between treatments. At time  $t_2$  the PMN decreased as follows: *B. carinata* meal + *B. juncea* GM + Fertilizer N > *B. carinata* meal + Fertilizer N > *B. carinata* meal + *B. juncea* GM and Fertilizer N > *B. carinata* meal, *B. carinata* meal deactivated, *B. juncea* and Sunflower meal > Control. The lack of significant differences between biocidal and thermally deactivated *Brassica* meals shows that the PMN was not affected by the presence of biocidal substances. Green manuring with *B. juncea* produced a delayed, but remarkable increase of PMN. The simultaneous supply of inorganic N and organic matter (whether meal or GM) gave rise to higher PMN values.

## An alternative pathway for glucosinolate degradation in soil

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Soil disinfection by incorporation of *Brassicaceae* plants materials is based on the exploitation of the glucosinolate-myrosinase system which releases inhibitory compounds such as isothiocyanate into the soil. However, the biocontrol effectiveness of this practice is often lower than expected, possibly due to microbial interaction with the bioactive compounds. In order to identify the possible function of microorganisms in this process, their interaction with glucosinolates and glucosinolate-derived products was investigated.

We report the ability of a soil isolate of *Aspergillus flavus* to grow in liquid culture containing 2-propenyl and 2-phenylethyl glucosinolate or their desulfo-derivatives, and convert them to nitriles. This finding would suggest the existence in *A. flavus* of an arylsulfatase and a  $\beta$ -thio-glucosidase, different from myrosinase, whose combined action could direct glucosinolate conversion in soil towards nitriles rather than isothiocyanates. Experiments are in progress to confirm in soil this pathway, which could at least partially explain the low concentrations of isothiocyanates recovered after biofumigation.

## Isolation and technological characteristics of *B. carinata* myrosinase

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To optimize the use of Brassicaceae defatted seed meals as biofumigants, it is important to control the kinetics of the glucosinolate-myrosinase system, which is responsible of the production of a number of active compounds. This parameter is related to the efficiency of soil biofumigation and depends on different applications/targets. This kinetics is affected not only by environmental conditions in which the myrosinase-catalyzed hydrolysis of glucosinolates takes place, but also by physico-chemical properties of the enzyme. The aim of this work is to study the characteristics of *B. carinata* seedmeal myrosinase and its behaviour in the various phases of preparation and application of the amendment formulation containing the glucosinolate-myrosinase system. According to the experimental procedures used to purify mustard and crambe myrosinases, this enzyme was isolated and characterized for the most important molecular properties involved in the biofumigation activity. We established that myrosinase in *B. carinata* seedmeals was present in a soluble and an insoluble form. The soluble form of myrosinase, was purified about 300 fold starting from the raw extract and appears to be homogeneous in SDS PAGE, showing a molecular weight of about 140 kD. The enzyme activity reaches a maximum value at pH of about 6 and at 70°C. Although it appears similar to the mustard myrosinase for many aspects, this enzyme seems to be more resistant to high temperature. Comparable characteristics have been observed for the insoluble form, which is certainly an important item that should be considered in the amendment formulation groundwork process. To understand the behaviour of this myrosinase in the defatted meals, namely when it is working in its natural conditions, a number of experiments are in progress to evaluate the substrate specificity and effectors of the two isolated enzymatic forms.



## **Myrosinases from *Crambe abyssinica* seeds: a new tool for biofumigation?**

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*Crambe* (*crambe abyssinica*) has been used since mid 1960s as an oil and feed source. As in many other cruciferous oilseeds, the glucosinolates-myrosinase (GLs-MYR) system contained in *crambe* seedmeals actually limits their use as a feed. The MYR-catalyzed hydrolysis of *epi*-progoitrin (*epi*-PG) contained in the meals produces, in specific reaction conditions, the cyanohydrin (2S)-1-cyano-2-hydroxy-3-butene (crambene). The insecticidal fumigation toxicity of several natural and synthetic cyanohydrins was recently evaluated against store-product pests. Among these compounds, crambene was found a weak fumigant against the adult housefly and the lesser grain borer, being about 70 times less effective than chloropicrin. Nevertheless, crambene could be easily transformed into 1-cyano-1-hydroxy-2-propene, which is as effective as chloropicrin or methyl bromide, with the difference that it is fully biodegradable and much less hazardous to mammals than conventional fumigants. Thus, the knowledge of the *crambe* MYR-GLs system appears to be important for a selective production of bioactive *epi*-PG breakdown products, but also for producing useful intermediates for synthesizing new effective and safe fumigants. In this work, we have investigated the most important properties of the *crambe* MYR system of the cv. Mario. The crude extract and the Con-A affinity chromatography were performed according to Bernardi R. et al (2003). After Con-A chromatography, the collected fractions were tested for protein concentration and activity with *epi*-PG and sinigrin as substrates. The chromatographic profile of the recovered glycosylated proteins appeared quite complex, and the overall yield appeared to be comparable to that of the other known plant MYRs (>90%). The proteins were concentrated and analyzed by native PAGE and gel filtration. The results indicates the presence of two MYRs: a soluble form of ca. 140 kDa and an insoluble one of ca. 470 kDa. The latter is the most abundant and is present only in the fractions containing the less glycosylated proteins, while the enzyme of 140 kDa is present in both fraction groups. With this study, we confirmed that the insoluble MYR is almost 10 times more specific for *epi*-PG than for sinigrin, whereas the new recovered MYR isoenzyme shows a conventional MW and appeared to be more specific for sinigrin.

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## **A long-term green manure trial within intensive cropping in Tasmania**

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Irrigated cropping of vegetables and poppies is an important farming system in north-west Tasmania. Green manure crops are often grown between cash crops to provide ground cover and organic matter and to retrieve subsoil nitrogen. Ryegrass is a popular green manure because it can be grazed to fatten animals and its fibrous root system is thought to improve soil structure. However, soil-borne diseases are also of concern in this region, and *Brassica* biofumigants also deserve consideration as green manures. A trial was therefore started in 2007 at Forthside Research and Demonstration Station near Devonport to assess the long-term effects of 3 autumn-winter land uses: fallow, annual ryegrass and *Brassica* (BioQure BQ Mulch™) green manures. These 3 treatments were established in a randomized block design from May to October 2007, and barley was grown over the whole site from October 2007 to March 2008, when the green manures were re-established on the same plots as for 2007. The annual cycle of green manures – cash crop will be continued and the trial will therefore assess the progressive impact of repeated growth of the green manures. Cash crops will include onions, carrots, *Brassica* vegetables, poppies and potatoes.

Trial plots are about 3000 m<sup>2</sup>, allowing for them to be split in the future. The first split will be in 2009/10 when half of each plot will be planted to potatoes. This will be 3 years after a potato crop was grown over the whole site in 2006/07, and will be followed by another whole-site potato crop in 2012/13, 6 years after the 2006/07 potatoes. The aim is to compare the effect of the green manures on potatoes grown in 3- and 6-year rotations, because these represent rotation lengths which are short and average for potatoes in the region, and because the soil-borne potato diseases powdery scab, common scab and *Rhizoctonia* are common. We hope to continue the trial for at least 12 years or 2 full cycles of the 6-year rotation.

As well as assessing the effect of the green manures on soil-borne diseases and crop production, the trial will enable changes in carbon and other soil quality measures to be monitored and modelled. The measures include DNA of the pathogens associated with the potato diseases mentioned above. Data from the barley crop are still being analysed, but there were no obvious responses to green manures at harvest.

## Selection of *B. napus* and *B. rapa* lines for biofumigation potential

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A range of *B. napus* and *B. rapa* material was available from a forage *Brassica* breeding programme for testing for biofumigation. This material was initially screened for glucosinolates, with particular interest in lines which had been discarded because of poor grazing acceptability. From laboratory and field studies carried out at that time, 2-phenylethyl (2PE) isothiocyanate appeared to be the most promising compound in brassicas for biofumigation. Several *B. napus* lines had high levels of 2PE glucosinolate, and these were selected and intercrossed to produce breeding lines. Three rape lines and a leafy turnip line were combined together to produce two mixtures which were sold by Wrightsons under the tradenames BQ Graze and BQ Mulch.

## Bioprospecting for endophytes from Australian flora with mycofumigation potential

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Soil mycofumigation is the process of using volatile allelochemicals produced by fungi to control soilborne phytopathogenic fungi, bacteria, nematodes, weeds and insect pests. Endophytic fungi inhabit plant tissues without causing disease, and many have formed symbiotic relationships with their hosts. For example, some endophytes protect their hosts from pest and pathogen infection through the production of allelochemicals. Our preliminary research aimed at bioprospecting for endophytic fungi from native Australian flora that produce volatile allelochemicals, with potential for use as mycofumigants against soilborne pathogens of horticultural crops.

By direct culturing, a total of 66 endophytic fungi were isolated from 12 Australian native plants in a cool temperate rainforest in Victoria (Sherbrooke Forest, Dandenong Ranges). *In vitro* bioassays showed that two isolates, from *Lomatia fraseri* and *Olearia argophylla*, produced volatile allelochemicals that suppressed the growth of the fungal pathogens *Rhizoctonia fragariae*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Verticillium dahliae*, and *Colletotrichum acutatum* by between 50–100%. Molecular (amplification and sequencing of the ITS region of the rRNA) and morphological identification placed the two isolates in the same undescribed species of *Nodulisporium* (teleomorph *Hypoxyton*, Xylariaceae). The volatile compounds (characterised by SPME GC/MS) produced by the two isolates varied, but mostly included a range of terpenes and alcohols. We hypothesise that some of these compounds are allelochemicals with potential for use as mycofumigants. Our future research aims to: (1) screen the individual volatile compounds produced by the *Nodulisporium* isolates for their activity against phytopathogens, weeds and pests; (2) determine the potential of the isolates for soil mycofumigation against pathogens of vegetable crops, and (3) determine the potential to integrate mycofumigation with other treatments (eg biofumigation, biological control) to offer growers more sustainable soil disinfestation systems than the use of synthetic pesticides.

This is the first report of volatile allelochemicals from *Nodulisporium* spp. having antifungal activity against phytopathogens.

## Travelling the green route to soil fumigation

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The control of weed seeds and soil-borne pests and diseases remains one of the biggest challenges for farmers and growers trying to reduce their reliance on synthetic pesticides. The soil-conditioning role of green manure and biofumigant crops may also help to improve the sustainability of pathogen control. By 2005 a number of biofumigant crops were already on the UK market but there had still been little independent assessment of their effectiveness. This project assessed two biocidal crops, namely Caliente Brands 99 and 119, each different blends of mustard, *Brassica juncea*. As green manures, both mustards appeared effective at nitrogen capture and by producing a total ground cover canopy in three weeks they suppressed weeds well. However, neither of the mustards produced a significant overall reduction of plant-parasitic nematodes; chopping and incorporation of the crop did reduce nematode numbers compared with the pre-incorporation populations, but the mustards themselves appeared to have stimulated nematode multiplication, thus reducing the benefit of their biofumigant action. The effect of the crops on *Pythium* species was insignificant throughout the trial. There was evidence that site-specific factors, perhaps soil type or management techniques, appear to be overriding factors in nematode management. This needs more investigation but illustrates the importance of individual farm assessments in the development of sustainable pathogen control strategies. Long-term studies in the regular use of biofumigant crops might produce a different picture.

## **New products based on Brassicaceae materials: a liquid formulation with fertilizing and biocidal effects for application in drip irrigation**

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In Italy, in recent years, some new materials for biofumigation based on vegetable tissues have been derived and are currently begin utilized commercially. In addition to the organic soil amendment coming from biocidal green manure plants or pellets, recent research has been strongly directed to the definition and optimization of new liquid formulations based on a vegetable oil emulsion containing small amounts of defatted meals. The first patented liquid formulation was essentially focused on the plant epigeal treatment for the control of some fungi and insect and some applications were presented at the Second Biofumigation Symposium (Idaho 2006), while at this meeting, characteristics of a new liquid formulation to apply in drip irrigation will be presented and discussed. As for the epigeal formulation, the hypogeal formulation is also based on an oil emulsion in which, a limited amount of a defatted meal is dispersed just before distribution. The meal has to be properly ground at a specific size for an easier filtration and formulated with vegetable materials to achieve glucosinolate hydrolysis in less than 30 minutes after water addition. The formulation is suitable for drip application and presents a significant fertilizing effect due to the presence of an organic nitrogen extract.

The kinetics of the release of the glucosinolate degradation products after water addition to the oil fraction showed the time required for reaching the maximum glucosinolate degradation rate which was around 80% on the theoretical total yield. In this way, it is possible to filter away the solid fraction after glucosinolate hydrolysis and to distribute by drip irrigation only a liquid fraction containing the biofumigant compounds. The optimised formulation was used in some toxicity tests in laboratory trials on springtail *Folsomia candida* (Isotomidae), a non target insect widespread in soil and frequently used in ecotoxicological tests. The formulation was tested at different concentrations also evaluating the toxicity of each main component. In addition, the effect of the new formulation has been verified in pot trials towards the southern root-knot nematode, *Meloidogyne incognita*; a dose-effect correlation has been observed, confirming the central role of the glucosinolate system in the biocidal properties of the formulation.

## A simple analytical method for dhurrin content evaluation in cyanogenic plants for their application as biofumigant

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Cyanogenic plants appears as a possible alternative to Brassicaceae plants in biofumigation technique to control some soil borne pathogens and pests. In fact, in these last years, several experiences on their application as green manure have been made with positive results. In addition, their use would contribute to improve biodiversity and to amend soil with different active compounds, permitting to delay the development of resistance of some pathogens to glucosinolate degradation products.

Among cyanogenic plants, Sorghum and Sudangrass are considered at moment the more interesting crops for their agronomic and chemical characteristics. In fact, these plants can be cultivated easily even in dry conditions with good yield and they contain the cyanoglucoside dhurrin (4-hydroxymandelonitrile- $\beta$ -D-glucoside) which is hydrolyzed by an endogenous  $\beta$ -glucosidase (dhurrinase) to produce glucose and *p*-hydroxymandelonitrile. This latter compound is unstable and so is quickly converted by the endogenous enzyme  $\alpha$ -hydroxynitrile lyase to free cyanidric acid and *p*-hydroxybenzaldehyde. In plant tissue cells, as for glucosinolates and myrosinase in Brassica plants, dhurrin and hydrolytic enzymes are separated and they get in contact only when plant tissues are lesioned or disrupted producing the bioactive compounds.

With the aim of characterizing a large number of genotypes and tissues of Sorghum and Sudangrass plants for their dhurrin content, a simple analytical procedure has been defined and optimized.

Freeze-dried, finely grounded plant tissue was extracted in the presence of activated carbon in MeOH by sonication at room temperature. The sample, after centrifugation was analyzed by the application of a modified reverse phase HPLC procedure developed previously by Johansen et al. (2007). Dhurrin was quantified by comparing its peak area to standard curve. The reproducibility of the optimized analytical procedure and its applicability at our plant materials by recovery tests has been evaluated. Using this technique it was possible to quantify the dhurrin content on different varieties cultivated in agronomic trials realized last year in Italy and also to evaluate the dhurrin distribution in roots and stems during cultivation. The results will be reported and discussed

Johansen H. et al. Rate of hydrolysis and degradation of the cyanogenic glycoside – dhurrin – in soil  
*Chemosphere* 67 (2007) 259-266

**- NO POSTER -**

**Assessing bionematicide effect of vegetable extracts  
on *Meloidogyne Javanica* hatch**

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Root knot nematodes are major pests in many greenhouses and crops of agronomic and horticulture importance in Iran. Recent global awareness of pesticides deleterious effects on environment and health has diverted attention to more research on natural means of nematode control. An experiment was conducted to examine nematicidal effects of some vegetables/herbs which are consumed fresh and cooked. Plant extracts were obtained by chopping fresh leaves of vegetables with distilled water in a blender and passing them through filter papers. Fifty eggs of *Meloidogyne javanica* were placed in each well of tissue culture plates to which 1 ml of each solution was added, distilled water was assigned as control, three replicates allocated per treatment and number of emerged juveniles were estimated after two weeks. Compared with the control, tarragon, coriander, mint, parsley, garlic, onion, chives and fenugreek trigonella prevented egg hatch by average 80% and basil and bay leaves extract controlled hatch by average 60%.



**- NO POSTER -****Potential impact of green manure crops on the sustainable sugar beet cyst nematode management in Idaho, USA****Saad L. Hafez** and P. Sundararaj

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Sugar beet cyst nematode (SBCN), *Heterodera schachtii*, is one of the most serious nematode pests causing significant yield loss on sugar beet in Idaho, USA. Following the major breakthrough in 1985 that oil radish variety RSO1841 significantly reduced SBCN population, field studies were conducted with Pagletta, Nemex and R184 and more reduction in nematode population was achieved. Higher quality of sugarbeet crop was generally produced on soils planted with oilseed radish or white mustard residues than with the regular management practices. In the recent green house studies it was found that two new oil radish varieties Defender and Comet significantly reduced the population of *H.schachtii* (95%). In the first experiment, mustard 'Concerta' produced 35% more above ground biomass than radish 'Colonel' and the viable cysts declined 29% and 19% in oil radish and mustard treatments, respectively. In the second experiment, radish 'Adagio' produced significantly more above ground biomass than mustard 'Metex'. In the third experiment, biomass (top, root and total) production of oilradish 'Dacapo' was significantly higher than mustard 'Metex'. A fourth experiment indicated that maximum beet yield (T/A) was with the Luna (37.0) planted plots followed by the Defender (36.0) plots. Recent studys in 2007 proved that among six varieties of green manure crops, maximum beet yield (T/A) was from Defender (36.8) Colonel (36.1) and Arugula (36.4) planted pots. At present Defender is the most economical variety highly suitable for the SBCN management.

**- NO POSTER -**

**Effect of rape/wheat strip intercropping on disease resistance,  
yield and agronomic characters of winter wheat**

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The effect of Rape/Wheat strip intercropping with different strip width on disease resistance, yield and agronomic characters of winter wheat were studied in field experiments conducted in Xiangfan city, Hubei Province, China. Our results indicated that suitable intercropping width of rapeseed/wheat could improve not only disease resistance but also yield of winter wheat. Compared with control, incidence of wheat disease was reduced by 3.5% and 8.7% respectively after artificial inoculation with root-rot and take-all. The spike number per plant, spike length and yield increased by 6.5 to 11.5%, 4.5 to 7.5% and 6.1 to 13.3% respectively, but spikelet rate decreased by 1.0 to 2.4%. Intercropping width should not more than 8m. Soil analysis and results of a laboratory experiment may explain these differences to some extent. Analysis on soil nutrients after harvesting showed that N, P and K content in intercropping strip were higher than control. The laboratory experiment showed that glucosinolate and their breakdown products which excreted from rapeseed have a significant inhibition on wheat root rot and take-all disease.

## - NO POSTER -

### Effects on paddy field environment and rice growth using winter rape as green manure

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Winter rape (*Brassica napus*) is one of the important oil crops in China, and due to its high biomass; can also be used as green manure. Glucosinolates (GSLs) are rich in rape plant, and hydrolysis by the myrosinase enzyme yields toxic isothiocyanates (ITCs) and nitriles, which can be used as biofumigants in controlling pests, such as nematodes, fungi and bacteria. A long term field experiment conducted since 2003 has investigated the effects winter rape green manure on soil fertility, rice yield and quality, and plant senescence. The winter rape and rice cultivar was “gaoyou 605” and “No6 guodao” respectively. The results in year 2007 showed that returning rape as green manure had the following impacts:

1. Increased soil fertility - the content of soil organic matter, total N, available P, available K in the treatment were increased by 7%, 23%, 39% and 15% above the control respectively. The activity of the soil proteinase, catalase and phosphatase was increased by 38%, 11% and 12% above the control.
2. Postponed the senescence of rice. During the grain filling periods, the content of the soluble protein, the activity of POD and the activity of SOD was 7%, 14% and 41% higher than that of the control respectively; The yellow leaf ratio of control was 37%, while in the treatment was 27%.
3. Increased the rice biomass accumulation, yield and quality. Compared with control, the biomass during the heading period, LAI and yield were increased 15%, 11% and 38% respectively. The grain milling and appearance quality traits were improved.

The mechanism of these effects are worthy of further study and the following investigations are underway, including studies investigating:

1. Soil microorganism, especially those microorganisms related with nutrition utilization.
2. The dynamic change of soil silicon content after returning rape as green manure.
3. The toxic effects of ITCs and nitriles on soil organisms.
4. The effect on rice growth, especially the senescence of rice root using rape plant as green manure.

The eutrophication of nitrogen in rice soil were obvious in recently years, and because of the greenhouse effect, pests cause more serious damage to crops, biological control and nutrient regulation has become one of the most important issues for sustainable rice production. The technique of direct seeding rape and returning to the paddy field as green manure after flowering can save labour and time, is low cost; has the effect of improving paddy field fertility, postponing rice senescence, and improving rice yield and quality, and was of great value in sustainable rice production in south China. Further research into the ecological mechanisms underlying the response to the GSLs and its hydrolysates will assist to refine the strategy.



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