

**Attendance at 2nd
International
Biofumigation
Symposium, June
2006**

John Matthiessen
CSIRO Entomology

Project Number: VG05013

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Conference & Travel Report

**John N Matthiessen
CSIRO Entomology, Perth**

2nd International Biofumigation Symposium

Moscow, Idaho, USA

25-29 June 2006

A report for HAL Project VG05013

to:

Horticulture Australia Ltd

CSIRO Entomology

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Summary

This document reports on attendance by John Matthiessen, CSIRO Entomology, Perth, at the 2nd International Biofumigation Symposium, held at the University of Idaho, Moscow, Idaho, USA 25-29 June, 2006.

Attendance at the Symposium related to several HAL-funded research projects of which I was the Principal Investigator during the period 1994-2003 on the development of *Brassica*-based biofumigation for the suppression of soil-borne pests and disease in horticultural production systems.

I presented an oral paper at the Symposium (copy appended). The presentation related to our findings of considerable environmental effects on the biological activity of isothiocyanates, the biologically-active compounds that biofumigation seeks to harness from brassicas to enhance the pest suppressive properties of *Brassica* green manures and rotation or break crops. The environmental effects related principally to the depression of isothiocyanate biological activity in soil types rich in organic matter and the strong effect of low temperature in depressing activity, particularly for aromatic isothiocyanates.

This work, which has been published in the scientific literature, is unique and of major practical benefit. It showed that previous literature, based on *in vitro* laboratory studies, which indicated that aromatic isothiocyanates (common in *Brassica* roots) were more toxic than aliphatic isothiocyanates (more common in *Brassica* shoots and stems), was not the case when the compounds were in the presence of soil. It also showed that the biological activity of aromatic isothiocyanates was depressed by low temperature much more so than that of aliphatic isothiocyanates, and that more complex longer-chain isothiocyanate molecules had their activity compromised much more than simpler short-chain variants by the presence of soil and lower temperature.

The work gave a timely reminder that great caution should be exercised when extrapolating the results of *in vitro* laboratory studies to the field situation. More importantly, from a practical application point of view, it turned on its head previous notions that root-derived isothiocyanates would be the most appropriate to target for enhancing biofumigation effects. It is now clear that greater biofumigation effects under field conditions are likely to be achieved from brassicas high in short-chain aliphatic isothiocyanates, such as above-ground biomass-rich mustards.

The coverage of topics at the 2nd International Biofumigation Symposium related more to applied aspects of biofumigation, in contrast to the dominance of basic chemistry and laboratory studies at the 1st International Biofumigation Symposium held in Italy in 2004. This was a welcome shift that was given considerable impetus by me and colleague John Kirkegaard of CSIRO Plant Industry in Canberra during the period between the two symposia. We believe that much of the basic work now needs to be tested or validated in the field before much more 'fine-tuning' basic work is carried out, in order to be sure that the basic research is on the right track to deliver practical benefits.

My paper vindicated our strong approach in this regard. We achieved this broad change in approach through direct influence as members of the organising committee of the symposia, through recent work and publications, and particularly through the recent publication of a comprehensive review of the topic (copy include in this report). That review article formed the basis of a forum discussion at the Symposium.

Introduction

The 2nd International Biofumigation Symposium held at the University of Idaho, Moscow, Idaho, USA 25-29 June 2006 was a highly relevant and timely Symposium to attend as it has continued the momentum originally established with an Australasian Biofumigation Workshop organised by me in 2002. That Workshop coincided with the visit, on a CSIRO McMaster Fellowship, of Dr Sandro Palmieri, director of the internationally-recognised Industrial Crops Research Institute in Bologna, Italy. This provided the impetus for the 1st International Biofumigation Symposium in Florence, Italy in 2004 and, in turn, the 2nd Symposium in 2006. It is anticipated that the 3rd International biofumigation Symposium will be held in Australia in 2008.

This report gives an overview of the scope and content of the Symposium. A copy of the program, my presentation and the abstract are included.

2nd International Biofumigation Symposium

A copy of the Symposium program and the paper and poster abstracts is included, as is the abstract of my presented paper and my oral presentation is embedded as a .pdf file in the electronic version of this report or in hardcopy in the printed version. Similarly, a copy of the recent review of biofumigation (and the related enhanced biodegradation of soil-applied pesticides authored by me and John Kirkegaard is embedded as a .pdf file in the electronic version and as hardcopy in the printed version, of this report.

The number of delegates attending the Symposium was around 85.

The format of the Symposium was of invited and contributed papers and poster sessions. I presented a paper giving details of a very extensive range of studies carried out largely during and subsequent to HAL Project VX00013 on establishing the effect of contrasting pure and plant-derived isothiocyanates in the presence of soil and varying temperatures on a pest organism. That work was published in 2005 (Matthiessen, J. N. and Shackleton, M. A. (2005). Biofumigation: environmental impacts on the biological activity of diverse pure and plant-derived isothiocyanates. *Pest Management Science* 61: 1043-1051).

It is significant that in the Pacific North-west of the USA, in the Idaho/Washington region, that about 20,000 ha is put under *Brassica* rotation crops, notably high-isothiocyanate-producing mustard cultivars. The predominant use is in potato production. While much of this use is based on the excellent general rotation crop and green manure attributes of the brassicas through effects on soil structure, water infiltration, nitrogen benefits that particularly suit the production system on the light sandy soils of that region, growers are adopting and modifying practices to capture or enhance biofumigation benefits. These are based on the plant pulverisation and watering practices developed in Australia in Project VX00013 aimed at maximising the release of isothiocyanates from above-ground plant parts and to ensure their rapid dispersion into the soil.

This usage has spawned very active seed merchandising business in the region, which is a key requirement to ensure critical mass that enables new practices to be widely adopted. Unfortunately, in Australia, we appear to be having difficulty achieving that critical mass for biofumigant *Brassica* seed production that in turn will spawn greater usage. It is the classic

Catch-22 that can often occur in Australia because of the small market. Nevertheless, it is at least personally very gratifying that our Australian research, sponsored by HAL, has had major influence on the practices of producers where there is sufficient scale for a dedicated seed industry to develop.

Another area where biofumigation is finding very useful niche is in small-holding vegetable production in developing countries in Asia, particularly for suppression of bacterial wilt. The causative organism of bacterial wilt is very susceptible to suppression by brassicas grown even as green manures, but the isothiocyanates provide an extra element of suppression that ensures a high level of control of the pathogen. The use of biofumigation is of particular value in such situations where small-scale growers in developing countries cannot afford costly soil fumigants. It is gratifying to see the adoption of the technique, and the value put on it, by the users. This work has been part of an ACIAR-funded project carried out by my colleague John Kirkegaard with researchers and extension agents in the Philippines.

Outcomes

The overseas travel reported here was extremely valuable in maintaining my expertise gaining broader insights into the many complex issues and the applications and opportunities related to biofumigation internationally. It was also very valuable in maintaining international contacts previously made with world-class researchers in the field, developing new contacts and relationships, and gaining better understanding of the significant issues related to use of biofumigation in other parts of the world. Many of these aspects are intangible, but they are diffused into the local industry and research realm in reports such as this, commentary at various forums, and general interaction with industry.

The Australian research has been highly influential in setting a highly applied and pragmatic course for biofumigation and it is gratifying to see adoption and uptake accelerating in various situations around the world. This can only be beneficial in improving the process through user innovation. Unfortunately, it is difficult to gauge the extent of adoption in Australia because such things are not usually documented. At this stage, it would seem that adoption in Australia is scattered and it may be restricted by the limited availability of suitable seed. Hopefully, this situation will gradually resolve itself as more growers seek to apply the technique. It is evident from the presentations that there are benefits to be achieved in appropriate production systems.

It was particularly gratifying to be able to present what was recognised as a significant basic science research result at a key international meeting coming from what was, in world terms, a relatively small research project that was sponsored by the Australian horticultural industries.

Opportunities to attend such meetings overseas, and visit other research institutions, offer intangible and non-immediate outcomes for industry, but I trust that this report offers sufficient to demonstrate that those intangibles can contribute to real benefits over time.

Acknowledgements

I am grateful to HAL and AUSVEG for providing the funding to enable attendance at the Pesticide Microbiology Symposium and the visit to the University of Tennessee.

John N Matthiessen

14 July 2006

Appendices

Appendix 1: The program and oral and poster abstracts of the 2nd International Biofumigation Symposium.

Appendix 2: Copy of the abstract of my paper to the Symposium.

Appendix 3: Embedded .pdf file (electronic version of this report) or printout (hardcopy version of this report) of my oral presentation at the Symposium.

Appendix 4: Embedded .pdf file (electronic version of this report) or reprint (hardcopy version of this report) of a recently-published review of biofumigation (and the related topic of enhanced biodegradation of soil-applied pesticides).

Appendix 1

The program and oral and poster abstracts of the 2nd International Biofumigation Symposium.

Second International Biofumigation Symposium

Biofumigation: Pest Control for the Future

June 25-29, 2006
Moscow, Idaho, USA
University of Idaho

Sponsored by:

Department of Plant, Soil
& Entomological Sciences

PROGRAM

Sunday, 25 June 2006

6:00-7:30 PM Welcome reception and registration, Iddings Agricultural Science Foyer

Monday, 26 June 2006

(All oral and poster presentation will be in the University Commons)

8:00-9:30 AM Registration, University Commons

9:30-9:45 AM Welcome and opening remarks, M.J. Morra, Univ. of Idaho, USA

9:45-10:45 AM Keynote Address 1, Glucosinolates and Chronic Disease Prevention. J.W. Fahey, Johns Hopkins Univ., USA.

10:45-11:45 AM Keynote Address 2, Evolution of Biofumigation Materials: From Plants Towards Biochemical Compounds. S. Palmieri, Italian Agricultural Research Council, Italy.

11:45 AM-12:00 PM Discussion

12:00-1:00 PM Lunch

Oral Presentation Session 1, Glucosinolate/Myrosinase Biochemistry, Session Chair: H. Sørensen, The Royal Veterinary and Agricultural Univ., Denmark.

1:00-1:20 PM Effect of Ascorbic Acid and Glutathione on the Production of Nitriles by Myrosinase. N. Bellostas*, J.C. Sørensen, H. Sørensen, The Royal Veterinary and Agricultural Univ., Denmark.

1:20-1:40 PM Comparative Nematicidal Impact of Two Isothiocyanates: MITC and 2-Phenylethyl on Different States of Two Dreadful Nematodes, *Meloidogyne graminicola* and *Heterodera Sacchari*, H. Nabil*, G. Reversat, ENFI-ESB, Morocco; IRD-LEST, France.

1:40-2:00 PM Environmental Impacts on the Biological Activity of Contrasting Isothiocyanates. J Matthiessen, CSIRO, Australia.

2:00-2:20 PM Glucosinolate and Isothiocyanate Concentrations in Soil Following Biofumigation. A.L. Gimsing*, J.A. Kirkegaard, The Royal Veterinary and Agricultural Univ., Denmark; CSIRO, Australia.

2:20-2:40 PM Antifungal Volatiles Emitted from *Brassica juncea*-amended Soils Differing in Water Content. T. Takehara*, H. Kanno, N. Obara, Y. Fujii, National Agricultural Research Center for Western Region, Japan; Miyagi

Prefectural Ishinomaki Agricultural Extension Center, Japan; National Institute for Agro-Environmental Sciences, Japan.

2:40-2:55 PM Discussion

2:55-3:10 PM Break

Oral Presentation Session 2, Pest Control with Green Manures, Session Chair: Carl E. Sams, Univ. of Tennessee, USA.

3:10-3:30 PM Separating Glucosinolate-related Suppression from Other Impacts of Mustard Biofumigants. J.A. Kirkegaard*, A.L. Gimsing, J.F. Wang, S. Akiew, A. Young, CSIRO, Australia; The Royal Veterinary and Agricultural Univ., Denmark; The World Vegetable Center, Taiwan; Dept. of Primary Industries and Fisheries, Australia.

3:30-3:50 PM Effects of *Brassica* Biofumigants on Celery and Onion Production Under Temperate Climate. M. Ngouajio*, G. Wang, K.S. Charles, Michigan State University, USA.

3:50-4:10 PM Biofumigants in Commercial Onion Production to Control Weed, Nematode and Disease Pests. B. Geary*, B. Brown, C. Ransom, S. Hafez, Brigham Young Univ., USA; Utah State Univ., USA; Univ. of Idaho, USA.

4:10-6:00 PM Poster Session 1 and social hour

6:30-7:30 PM Dinner, University Commons

7:30-9:00 PM Musical entertainment, Charles Houston Shattuck Arboretum

Tuesday, 27 June 2006

Oral Presentation Session 3, Nematode and Insect Control, Session Chair: Ekaterini Riga, Washington State Univ., USA.

8:20-8:40 AM Comparison of Five Crops Used as Biofumigants to Manage Root-knot Nematodes in Tomato. A.T. Ploeg*, J.A. Lopez-Perez, T. Roubtsova, S. Edwards, Univ. of California Riverside, USA.

8:40-9:00 AM Two Decades of Green Manure Crop Research in Idaho for Nematode Management – An Overview. S. Hafez*, P. Sundararaj, Univ. of Idaho, USA.

9:00-9:20 AM Strategies for Using Green Manure Crops to Suppress Columbia Rootknot Nematode. R.E. Ingham*, N.L. David, D.A. Horneck, M. Dillon, J. Delgado, Oregon State Univ., USA; Colorado State Univ., USA; USDAARS, USA.

9:20-9:40 AM Effectiveness of Crop Rotation with Brassicaceae Species for the Management of the Southern Root-knot Nematode *Meloidogyne incognita*. G. Curto*, L. Lazzeri, E. Dallavalle, R. Santi, L. Malaguti, Plant Protection Service, Italy; CRA-Research Institute for Industrial Crops, Italy.

9:40-10:00 AM Evaluation of Field Pennycress as an Overwinter Green Manure Crop in Corn for Suppression of Western Corn Rootworm. S.F. Vaughn*, T.A. Isbell, R. Stessman, R.W. Behle, USDA-ARS, USA.

10:00-10:20 AM Break

Oral Presentation Session 4, Pathogen Control, Session Chair: Mark Mazzola, USDA-ARS, USA.

10:20-10:40 AM Management of the Intercrop Period to Reduce Take-all Disease. S. Ennaïfar, P Lucas*, J-M Meynard, D Makowski, UMR d'Agonomie INRA/INA P-G, France; UMR BIO3P INRA-Agrocampus, France; SADAPT, France.

10:40-11:00 AM Biofumigation for Controlling Soilborne Diseases of Tomato, Potato and Olive. L. Tsrer (Lahkim)*, S. Lebiush, M. Meshulam, E. Matan, L. Lazzeri, Agricultural Research Organization (ARO), Israel; R & D B'sor Experiment Station, Israel; CRA- Research Institute for Industrial Crops, Italy.

11:00-11:20 AM Can Biofumigation Effectively Control Common Scab of Potato Under Field Conditions? R Gouws*, F. C. Wehner, Agricultural Research Center, South Africa; Univ. of Pretoria, South Africa.

11:20-11:40 AM The Impact of Mustard Cover Crop Rotations with Lettuce on the Incidence of Lettuce Drop Caused by *Sclerotinia minor*. R.F. Smith*, K.V. Subbarao, S.T. Koike, T. Bensen, Univ. of California, USA.

11:40AM-12:00 PM Soil Fumigation with *Allium* Sulfur Volatiles and *Allium* Byproducts. L.A. L.Arnould, G.F. G. du Fretay*, F.V. F.Vey, A.T. A.Tissier, C.F. C. Fleurance, CRITT INNOPHYT, France; LNPV UFPS, France; SELT, France.

12:00-1:00 PM Lunch

Oral Presentation Session 5, Waste Materials and Seed Meals as Biopesticides, Session

Chair: John Kirkegaard, CSIRO, Australia.

1:00-1:20 PM Biofumigation for Soil-Borne Disease Management in Small-scale Vegetable Production of Asia. V. Justo*, J. Kirkegaard, L.A. Lando, F. Abragan, J.J. Dangan, National Crop Protection Center, Univ. of the Philippines, Philippines; CSIRO, Australia.

1:20-1:40 PM Herbicidal and Crop Phytotoxicity of Brassicaceae Seed Meals on Strawberry Transplants and Established Crops. J. Brown*, M. Hamilton, J. Davis, D. Brown, L. Seip, Univ. of Idaho, USA.

1:40-2:00 PM Contribution of Resident Soil Microorganisms to Brassicaceae Seed Meal-induced Disease and Weed Suppression. M. Mazzola*, L. Hoagland, L. Carpenter-Boggs, R. Abi Ghanem, M. F. Cohen, USDA-ARS Tree Fruit Research Lab, USA; Washington State Univ., USA; Sonoma State Univ., USA.

2:00-2:20 PM Brassicaceae Seed Meal Top-dressing - An Alternative to Bluegrass Burning? J. Davis*, C. Seamons, J. Brown, D. Brown, L. Seip, Univ. of Idaho, USA.

2:20-2:40 PM Cruciferous Materials in Biofumigation and Not Only....L. Lazzeri*, L. Malaguti, CRA Research Institute for Industrial Crops, Italy.

2:40-2:55 PM Discussion

2:55-3:10 PM Break

Oral Presentation Session 6, Commercialization and Technology Transfer, Session Chair: Luca Lazzeri, CRA Research Institute for Industrial Crops, Italy.

3:10-3:30 PM Developing Designer Biofumigation *Brassica* Crops Through Interspecific Hybridization. L. Seip*, D. Brown, N. Baker, J. Brown, J. Davis, Univ. of Idaho, USA

3:30-3:50 PM Nematode Suppression with Stoller Root Feed: An Alternative to Methyl Bromide. A. Liptay*, J.H. Stoller, R.A. Salzman, Stoller Enterprises Inc, USA.

3:50-4:10 PM Extraction of High Erucic Oils, Proteins and Glucosinolates from Cruciferous Oilseeds. L. Ugolini, S. Palmieri*, Italian Agricultural Research Council, Italy; Research Institute for Industrial Crops, Italy.

4:10-6:00 PM Poster Session 2 and social hour

6:30-7:30 PM Salmon Dinner, Administration Lawn

7:30-9:00 PM Native American entertainment, Administration Lawn

Wednesday, 28 June 2006

Field tour/Rapeseed and Canola Field Day, Univ. of Idaho Parker Farm

Thursday, 29 June 2006

9:00 AM-12:00 PM Workshop: Glucosinolates/Myrosinase: Analytical Techniques

10:20-10:40 AM Break

9:00 AM-12:00 PM Roundtable Discussion: Opportunities and Limitations of Biofumigation

10:20-10:40 AM Break

To Be Arranged Tour: Seed Crushing and Biodiesel Production Facilities

12:00-1:00 PM Lunch and closing remarks

Monday, 26 June 2006, Poster Session 1

4:10-6:00 PM University Commons

1. Sorption and Degradation of Benzyl and 2-Propenyl Isothiocyanates in Soil. A.L. Gimsing*, B.W. Strobel, H.C.B. Hansen, The Royal Veterinary and Agricultural Univ., Denmark.

2. Potentially Mineralizable Nitrogen in Soils Amended with Biocidal Crops. R. Marchetti, L. Lazzeri*, L. Malaguti, A. Orsi, L. Sghedoni. Agronomical Research Institute, Italy; Research Institute for Industrial Crops, Italy.

3. Microbial Community Structure of Biofumigated Soils. M.T. Lyons, C.E. Sams*, C.S. Charron, A.D. Peacock, D.C. White. Univ. of Tennessee, USA.

4. Effect of Mustard Green Manure on Soil Microbiology in a Potato Cropping System. J. Nunez*, M. Davis. University of California Cooperative Extension, USA.

5. Extraction of Glucosinolates Released in Soil. J.C. Sørensen*, A.L. Gimsing, H.C.B. Hansen, H. Sørensen. Department of Natural Science, Denmark.

6. Preliminary Study on the Biofumigant Effects of *Brassica juncea* as Green Manure on *Rhizoctonia solani* and *Gaeumannomyces graminis* var. tritici. F. Montfort*, F. Limache, N. Motisi. INRA (National Institute of agronomic research), France.

7. The Use of Arugula on its Own and in Combination with Synthetic Nematicides Against Plant Parasitic Nematodes of Potatoes. E. Riga*, F. Pierce, H. Collins. Washington State Univ., USA.

8. *Brassica* Rotations for Managing Soilborne Potato Diseases in the Northeast. R.P. Larkin R. Lynch*, T.S. Griffin, C.W. Honeycutt. USDA-ARS, New England Plant, Soil, and Water Laboratory, USA.

9. Effect of Mustard Green Manure Amendment on the Infectivity and Mortality of Two Entomopathogenic Nematode Genera, *Steinernema* and *Heterorhabditis*. D. Henderson*, R. Ramirez*, J. Brown, E. Riga, W.E. Snyder. Washington State Univ., USA; Univ. of Idaho, USA.
10. Potential Role of Brassicaceae Plant as Biofumigation Agent in Suppression of Potato Cyst Nematodes (*Globodera rostochiensis* cv. Woll). A. Aires, E. Rosa, R. Carvalho, C. Rodrigues. Univ. of Trás-os-Montes e Alto Douro, Portugal.
11. Mustard-derived Biofumigation for Lettuce in Coastal California. O. Daugovish, A.J. Downer*, M.J. Mochizuki. Univ. of California Cooperative Extension, USA.
12. Glucosinolate Content of Selected Brassicaceae Grown in the Salinas Valley, California. R.F. Smith, T.A. Bensen*, K.V. Subbarao, S.T. Koike. Univ. of California Cooperative Extension, USA; Univ. of California, USA.
13. Composition and Content of Glucosinolates in Rapeseed and In Vitro Inhibition of *Botrytis cinerea* Persoon. C.L. Zhang*, F. Li, G.M. Li. Institute of Oil Crops Research, CAAS, China.
14. Intersatellite DNA Polymorphism in *A. alternata* Following Exposure to Lethal Concentrations of Isothiocyanates. Maria-Elena Baez-Flores*, R. Troncoso-Rojas, B. Pryor, H.S. Garcia, M.E. Tiznado-Hernandez. Centro de Investigación en Alimentación y Desarrollo, Mexico; Univ. of Arizona, USA.
15. Myrosinase Immobilized by Plant Root-mucigel: Effect of Soil Water Status on the Enzyme Efficiency. C.E. Gessa, I. Braschi*, S. Cinti, O. Leoni, S. Palmieri. Bologna Univ., Italy; Research Institute for Industrial Crops (ISCI-CRA), Italy.
16. Molecular Marker Associated with High Level of Sinigrin Content in Brown Mustard (*Brassica juncea*). E. Lionneton, G. Aubert, S. Ochatt, O. Merah*. INRA, URGAP, France.
17. *Brassica juncea* Seed Meal for Fungus Gnats Control Associated with Houseplants with Consideration of Phytotoxicity to Specific Plant Species. J.P. McCaffrey*, M.J. Morra, M.E. Main. Univ. of Idaho, USA.
18. Biofumigation as Alternative Method for Management Root-knot Nematode *Meloidogyne incognita* Affecting Tomato under Field Conditions. H.H. Ameen, M.M.M. Mohamed*. National Research Center, Egypt.
19. Control of *Rhizoctonia solani* in Lily by Biofumigation with *Brassica* Seed Meal. G.J. Van Os*, L. Lazzeri. Applied Plant Research, The Netherlands; CRA-ISCI, Italy.
20. Application of Brassicaceae Seed Meals for the Management of Apple Replant Disease. M. Mazzola*, R. A. Ghanem. Washington State Univ., USA.

Tuesday, 27 June 2006, Poster Session 2

4:10-6:00 PM University Commons

21. Efficacy of *Brassica* Biofumigation in Tomato Production. S.G. Harvey, C. E. Sams*. Georgia Southwestern State Univ., USA; Univ. of Tennessee, USA.
22. Enhanced Biofumigation and Composting Systems for Strawberry. D. Deyton, T. Karpinets, M. Lyons, J. Cummins, C. Sams*. Univ. of Tennessee, USA.
23. A Liquid Formulation as Biopesticide for the Control of California Red Scale (*Aonidiella aurantii* Maskell). D. Rongai, L. Lazzeri*, C. Cerato, S. Palmieri, G. Patalano. Research Institute for Industrial Crops, Italy; Triumph Italia, Italy.
24. Production of Allylisothiocyanate from Different Source and its Persistence in Soil. L. D'Avino, L. Lazzeri*, C. Gaggi. CRA- Research Institute for Industrial Crops, Italy; Univ. of Siena, Italy.
25. Glucosinolate Degradation in Soil. A.L. Gimsing*, J.C. Sørensen, B.W. Strobel, B.A. Halkier, H.C.B. Hansen. The Royal Veterinary and Agricultural University, Denmark.
26. Toxicity of Benzyl Glucosinolate and Benzyl Isothiocyanate to the Soil Dwelling Springtail *Folsomia fimetaria*. B. Styrihave, A.L. Gimsing*, J. Jensen, H.C.B. Hansen. The National Environmental Research Institute, Denmark; The Royal Veterinary and Agricultural Univ., Denmark.
27. Effect of Mustard Green Manure on Nematodes. J. Nunez*, University of California Cooperative Extension, USA.
28. In Vitro Screening of the Effect of Three Glucosinolate Derived Nitriles on Soilborne Fungi. N. Bellostas*, E. Casanova, J.M. Garcia-Mina, J.C. Sørensen, H. Sørensen. The Royal Veterinary and Agricultural University, Denmark; Inabonos, Group Roullier, Spain.
29. Proposal for an Analytical Method for the Quantitative Determination of Residues of Iso-thiocyanates in Preserved-Refrigerated Fruit. A. Bovolenta, M. Morelli. Arpa Emilia-Romagna sez. di Ferrara - Eccellenza Fitofarmaci, Italy.
30. Biofumigant Response to Phosphorus. B. Brown*, B. Geary, M. Morra, V. Borek. Univ. of Idaho, USA.
31. Terra Protect – Biofumigation and Soil Protection with *Brassica* Blends. M. Schlathoelter*. Terra Protect Seeds, Germany.
32. Advancing Biofumigation Research - The Need for Minimum Data Sets. J.A. Kirkegaard*, J.N. Matthiessen. CSIRO, Australia.

33. Evaluation of Alternative Strategies for the Control of Root-knot Nematode *Meloidogyne incognita* (Kofoid et White) Chitw. on Tomato Crop, in Plastic Greenhouse. G. Curto*, L. Lazzeri, R. Santi, E. Dallavalle. Plant Protection Service, Emilia-Romagna Region. Italy; CRA-Research Institute for Industrial Crops, Italy.
34. *Trichoderma* Strains Tolerant to *Brassica* Meals for a Combined Use in Biofumigation. S. Galletti*, E. Sala, P. Burzi, S. Marinello, C. Cerato. Centro di Ricerca per le Coltura Industriali, Italy.
35. Glucosinolate-containing Seedmeals With Bioherbicidal Activity. S.F. Vaughn*, D.E. Palmquist, M.A. Berhow. USDA-ARS, NCAUR, USA.
36. Large Scale Biofumigation of Fruit with Allyl-isothiocyanate from *Brassica* Meals for Controlling Postharvest Pathogens: Preliminary Results. O. Leoni*, M. Mari, R. Bernardi, L. Casalini, S. Cinti, N. Aliano, S. Palmieri. Research Institute for Industrial Crops (ISCI-CRA), Italy; Univ. of Bologna, Italy.
37. Weed Suppression in Potting Soil Amended with Dried Distillers Grains. R.A.Boydston*, S.F. Vaughn, H.P. Collins. USDA-ARS, IAREC, USA.
38. The Effect of Glucosinolate Type and Quantity on Weed Seed Germination and Growth in Glasshouse Studies. D. Brown *, J. Brown, M. Hamilton, J. Davis, L. Seip. Univ. of Idaho, USA.
39. Brassicaceae Seed Meals as a Nitrogen Source in Carrot and Strawberry Production. A.S. Snyder *, J.L. Johnson-Maynard, M.J. Morra. Univ. of Idaho, USA.

Abstracts of presentations – 2nd International Biofumigation Symposium.

Session Theme: Additional Biopesticidal Crops and Plants

Title: SOIL FUMIGATION WITH ALLIUM SULFUR VOLATILES AND ALLIUM BY PRODUCTS

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Keywords: Allium, disulfides, soil fumigation

Presentation Type: Oral

Abstract:

Like Brassicaceae spp, Allium spp present biofumigation properties which are attributed to sulphur components and mainly three disulfides: dimethyl disulfide (DMDS), dipropyl disulfide (DPDS) and diallyl disulfide (DADS). Their fungicidal activity was studied on nine soil pathogenic fungi and DMDS was the most efficient*. The 3 objectives of this study are:

- evaluation of the biofumigant activity of onion and leek by-products in vitro, with an experimental model, and in vivo, with field experiments
- investigation of the behaviour of these fumigants in soil
- evaluation of disulfides on others soil pests, especially nematodes, in comparison with isothiocyanates.

The experimental model consists of a host-pathogen system: cucumber-Pythium ultimum. The results of the bioassay show that cucumber planted in compost containing the pathogen and onion or leek by-products present a better vegetative growth compared to those of the inoculated control.

The soil biodesinfection with onion by-products in asparagus crops lead to an intermediate yield between the untreated soil modality and the methyl bromide treatment modality. In addition, the strawberry plants with onion by-products soil fumigation present a more vigorous root system than the plants seedling in untreated soil.

Actually, DPDS released in soil by onion and leek by-products have a sufficient life-span to produce the disinfectant observed effect.

* J. AUGER, I. ARNAULT, S. DIWO-ALLAIN, M. RAVIER, F.MOLIA, M.PETTITI

Insecticidal and fungicidal potential of Allium Substances as biofumigants.

Agroindustria, 2004, 3(3), 367-370

Session Theme: Additional Biopesticidal Crops and Plants

Title: COMPARATIVE NEMATICIDAL IMPACT OF TWO ISOTHIOCYANATES: MITC AND 2-PHENYLETHYL ON DIFFERENT STAGES OF TWO DREADFUL NEMATODES, MELOIDOGYNE GRAMINICOLA AND HETERODERA SACCHARI

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Keywords: Isothiocyanates, nematicides, soil fumigation

Presentation Type: Oral

Abstract:

Two pure isothiocyanates 2-phenylethyl and methylethyl isothiocyanate were tested in vapour exposure tests for nematicidal activity against different stages of two nematodes. Two species were studied in vitro at 25 C: the cyst nematode *Heterodera sacchari* and the root knot nematode *Meloidogyne gramminicola*. The purpose was to determine the lethal concentration of the two products on the various stages of the two nematodes, and to estimate the effect of the natural physical barriers such as roots, egg shell or cyst cuticle.

Suppressive effect on larval stages was comparable between MITC and PEITC. The MITC was more effective on eggs, cyst and galls than PEITC. The results indicate that the thicker the physical barrier, the higher the concentration requested to reach 100% suppressive effect on the resistant nematode stage.

Session Theme: Commercialization and Technology Transfer

Title: Extraction of High Erucic Oils, Proteins and Glucosinolates from Cruciferous Oilseeds

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Keywords: Extraction, Glucosinolates, Oils

Presentation Type: Oral

Abstract:

Cruciferous oil seeds are important sources not only of oil and proteins but also of some bioactive secondary metabolites such as glucosinolates (GLs). The great potential use of GLs in fine chemistry, food technology, and, in particular, in crop protection has been well reported. At present however, GLs are not commercially offered at industrial scale, although this aspect appears to be fundamental for their exploitation in agriculture. In this view the importance of a sustainable cost-effective extraction technology for isolating high erucic acid oils together with GLs at industrial scale is evident. This extraction procedure has been explored and evaluated with an holistic approach, making emphasis to the process-product potential offered by “water in oil” technology for isolating some high value molecules, such as GLs, myrosinase, enzyme effectors, etc., besides conventional co-products such as oil and proteinic meal.

Session Theme: Commercialization and Technology Transfer

Title: Terra Protect – Biofumigation and soil protection with brassica blends

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Keywords: Brassica blend, breeding, benefits

Presentation Type: Poster

Abstract:

Terra Protect are special blends of brassicas for biofumigation and soil protection. They are developed to have maximum biological active phytochemicals (Isothiocyanates) and high biomass production. The most effective plants species for biofumigation were found in the family of the Brassicaceae which are Raphanus sativus (Oil Radish), Sinapis alba (White Mustard) and Brassica juncea (Indian or Oriental Mustard). Research and commercial use of biofumigation with Terra Protect Blends in agricultural, vegetable and flower bulb crop rotation are showing increased main crop quality and yield. Research has been carried out for controlling nematodes, verticillium wilt, silver scurf, rhizoctonia and sclerotinia. Additional benefits of utilizing biofumigation can be noticed in improving soil health and structure. A close relation between science, breeding, crop adviser and farmers is helpful to maximize the benefits and thus the farmer's returns.

Session Theme: Commercialization and Technology Transfer

Title: Nematode suppression with Stoller Root Feed: an alternative to methyl bromide

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Keywords: nematode, suppression, yield

Presentation Type: Oral

Abstract:

Stoller serendipitously discovered that their product Root Feed™, containing compounds naturally occurring in plants, when applied exogenously to crops under Stoller recommended practices, not only enhanced crop yield and quality (the original Stoller objective) but also strongly suppressed nematodes resulting in much larger and more nutritious crops.

A study done by a graduate student on grapes in Peru indicated that Root Knot nematode was suppressed resulting in healthy whitish roots compared to brownish roots in untreated plots. Studies were then done at UC Davis on grapes, broccoli and sugar beets, at Auburn University with Cucumbers and Peppers, at the University of Florida with tomatoes. In all cases crop canopy was increased, root mass was increased and nematodes were reduced to varying degrees but always with very desirable yields and crop quality. This nematode suppression phenomenon was related to an entomologist and he suggested that if nematode damage is overcome, then the Stoller Root Feed™ should also overcome Phylloxera in grapes (the pest that devastated European grapes). An initial study in California in 2005 confirmed the very good grape recovery from Phylloxera damage to grapes. Stoller is currently doing physiological and gene regulation studies to understand Root Feed™ mode of action of overcoming nematode damage to crops.

Session Theme: Experience and Observations from the Field

Title: Efficacy of Brassica Biofumigation in Tomato Production

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Keywords: Biofumigation, Tomato, isothiocyanates,

Presentation Type: Poster

Abstract:

Allyl isothiocyanate (AITC) inhibits mycelial growth and sclerotial germination of *Sclerotium rolfsii*, a common soilborne pathogen of tomatoes. Volatiles from macerated Brassica spp (which included AITC) were more suppressive than AITC alone. The objective of this study was to determine the efficacy of biofumigation utilizing Brassica spp. in field, with tomato as the indicator crop. Cover crops, including Brassica spp., were over wintered and then incorporated into the soil three weeks prior to tomato transplanting. Number and total weight of tomato fruit were measured. The experiment was conducted for three seasons. The number and total weight of marketable fruit differed significantly among the Brassica treatments and the control ($P < 0.01$) by as much as 45 percent in one season. However, year to year variability was high and problems with equipment, weather, and timing raised issues that would need to be addressed prior to field implementation on a commercial scale.

Session Theme: Experience and Observations from the Field

Title: Effect of Mustard Green Manure on Soil Microbiology in a Potato Cropping System

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Keywords: fallow, greenmanure, metam

Presentation Type: Poster

Abstract:

We have shown that mustards can be grown in the San Joaquin Valley successfully and produce significant biomass for use as a green manure. Over 8000 lbs dry weight of green manure was produced per acre. The addition of the biomass to the soil did have a direct effect on the soil microbiology. We showed that the addition of green manure stimulated the activity of the soil microbes in comparison to the fallow treatments that received no green manure and especially in comparison to the metam/fallow plots that besides not receiving green manure was fumigated. The addition of mustard green manure increased the levels of aerobic bacteria, Pseudomonads, and nitrogen fixing bacteria. Keeping the soil fallow or fallow plus fumigating with metam lowered the level of aerobic bacteria, Pseudomonad, and nitrogen fixing bacteria while metam/fallow increased total fungi and anaerobic bacteria levels in the soil. Soil microbiology is significantly altered when abundant mustard green manure is added to the soil. Potato yields can be increased with the use mustard green manures to the same level as fields treated with metam.

Session Theme: Experience and Observations from the Field

Title: Glucosinolate Content of Selected Brassicaceae Grown in the Salinas Valley, California

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Keywords: mustard cover crops, isothiocyanate, soil-borne disease

Presentation Type: Poster

Abstract:

Plants of the Brassicaceae are well known as a rotation or cover crop with potential biofumigant effects. In the Salinas Valley of coastal central California, Brassica juncea is a mustard cover crop used widely to build soil health and to potentially suppress the soil-borne fungal pathogen, Sclerotinia minor, and various weed species. In field trials, however, we found low to no suppression of S. minor or weeds through the use of this or another mustard, Sinapis alba. Such minimal effects are most likely due to a low glucosinolate content of the two cultivars we used; total glucosinolates in our samples averaged 15.7 $\mu\text{moles/gram}$, which is equivalent to 15.9 to 21.5 liters/hectare of the commercial fumigant, Vapam. In contrast, Vapam is labeled for rates between 351 to 701 liters/hectare. These previous results led us to evaluate the performance of six other mustard cover crop cultivars since glucosinolate concentrations vary considerably across this diverse plant family and among cultivars. We tested six cultivars of three Brassica species and Sinapis alba at four different fields with the goal of identifying cultivars with higher biofumigation potential than those tested previously. For these cover crops to be effective biofumigants, glucosinolate contents must be elevated by greater than 20 times the levels observed thus far.

Session Theme: Experience and Observations from the Field

Title: Management of the intercrop period to reduce take-all disease

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Keywords: intercrop period management, take-all, wheat

Presentation Type: Oral

Abstract:

Crop rotation is the oldest and certainly the most efficient cultural practice in reducing take-all disease on wheat but is more driven by economics than by soil health considerations. When analyzing the role of successive crops on soil-borne diseases, attention barely focused on the intercrop period. The effects on take-all of winter wheat of five summer-crops plus a bare soil situation, in interaction with tillage (till vs no-till) were investigated in a five site-year experiment. Summer-crops were oat, rape, white mustard, rye-grass and wheat volunteers. Take-all incidence and severity were significantly higher on winter wheat following wheat volunteers, while maintaining bare soil provided the lowest level of disease, although not acceptable for environmental reasons. Rape did not show any significant effect on take-all incidence. The best candidates appeared to be mustard, oat and rye-grass.

These summer-crops decreased disease only when associated to conventional tillage. Summer-crops did not alter take-all decline as does a break crop after a wheat monoculture. The way the summer-crop canopy residues are managed before wheat sowing seems to be very important. More investigation is needed to understand the effects of intermediate cropping either directly on the fungus, through microbial changes or through allelopathic effects.

Session Theme: Experience and Observations from the Field

Title: Biofumigant Response to Phosphorus

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Keywords: Biofumigants, Phosphorus, Glucosinolates

Presentation Type: Poster

Abstract:

Brassica biofumigant-green manure nutrient and glucosinolate content response to available P has not been widely reported. Field studies conducted in a silt loam over three years at Parma, Idaho measured P effects on condiment yellow mustard (*Synapis alba*, variety Idagold) and oilseed radish (*Raphanus sativa*, variety Colonel). Low P was more limiting to radish biomass, whole plant P concentrations or uptake in some years than it was to the mustard. Higher available P did not generally affect N, K, S, Ca, or Mg concentrations or accumulation other than reducing N and Mg concentrations in one year. Yellow mustard biomass in two of three years exceeded oilseed radish biomass and was generally lower in most macronutrient concentrations. Despite frequent differences in nutrient concentrations, biofumigants did not differ in their nutrient accumulation in tops, except S in two of three years was greater in the mustard. Glucosinolate concentrations identified in tops of mustard (primarily sinalbin and glucotropaeolin) and radish (primarily indolyl-3-Me brassicin, sinigrin, gerusin, and 4-MeO-indolyl-brassicin) were quite variable. Neither glucosinolate concentrations or accumulation in tops were significantly affected by available P. While biofumigants differed in their biomass and P content response to P, glucosinolate content was less affected by available P.

Session Theme: Experience and Observations from the Field

Title: Biofumigation for Soil-Borne Disease Management in Small-scale Vegetable Production of Asia

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Keywords: green manure, bacterial wilt, root knot nematodes

Presentation Type: Oral

Abstract:

Participatory field studies on biofumigation for bacterial wilt and root knot nematode were conducted with vegetable farmers of Pangasinan, Benguet, Bukidnon and Davao del Sur, in The Philippines. Waste materials available after harvest of popular brassicas grown in the area (cabbage, broccoli, cauliflower, radish, and mustard) were evaluated as sources of biofumigant tissues, as well as radish grown as green manure. Significant reductions in bacterial wilt incidence were achieved (50-80%) although impacts of non-Brassica amendments were also recorded. Root knot nematode populations could also be significantly reduced by radish green manure due to its poor hosting and its suppressive green manure effects. Economic analysis suggests potential increase of income in potato production due to biofumigation by 19.5% in Benguet, 4.7% in Bukidnon and 30.6% in Davao del Sur, and integration of biofumigation in disease management has encouraged growers to resume potato production. FAO Regional Programme for Integrated Pest Management (IPM) in Vegetables and NCPC have developed training courses (Farmer's Field Schools and Training of Trainers) and participatory action research on biofumigation for bacterial wilt, club root, root knot nematodes and other soil-borne pathogens for IPM trainers and farmers. We discuss these in the context of small-scale Asian farming systems.

Session Theme: Experience and Observations from the Field

Title: Effects of Brassica biofumigants on celery and onion production under temperate climate

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Keywords: mustard, vegetables, cover crops

Presentation Type: Oral

Abstract:

This research was conducted to determine effects of Brassica cover crops on growth and yield of celery and onion under a temperate climate with short growing season. Field studies were conducted in 2004 and 2005 in a commercial farm for celery and at a research station for onion. Treatments included oilseed radish, oriental mustard, brown mustard, yellow mustard, and bare ground. The cover crops were sown in the fall, and celery or onion the following spring. The Brassica cover crops increased celery yield but had no effect on onion yield. The celery plot was in continuous celery for over ten years while the onion plot was fallow the previous year and had not been planted with onion for many years. Onion stand increased by 8% in the Brassica treatments resulting in smaller bulbs. This suggests the possibility to reduce onion planting density in those treatments. The Brassica cover crops enhanced the population of beneficial microorganisms in the soil at both sites. The population of plant parasitic nematodes was also reduced, but only in the commercial field that was in continuous celery for many years. These findings suggest that celery and onion cropping systems could be improved with Brassica cover crops.

Session Theme: Experience and Observations from the Field

Title: Comparison of five crops used as biofumigants to manage root-knot nematodes in tomato.

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Keywords: root-knot nematode, Meloidogyne, management

Presentation Type: Oral

Abstract:

Laboratory and greenhouse experiments on effects of biofumigation on root-knot nematodes indicated the potential of this method for nematode management. The effects of biofumigation on root-knot nematode populations and crop performance are currently being studied under field conditions. Crops grown during the fall-winter season in a root-knot nematode infested field in the coastal area of Southern California are broccoli, nematode-resistant tomato, carrot, marigold and strawberry (plus fallow control). Crops are mowed, chipped into small pieces, and incorporated in spring, and then covered with plastic for 6 weeks. Tomato is then grown over the entire field during the summer season. Results from the first 2 years indicate that effects of the different crops on soil nematode population levels are minor, but that root-galling on tomato is lowest and yield is highest when following broccoli. We conclude that growing nematode-susceptible crops (carrot, broccoli) during the cool fall-winter season did not result in significant nematode build-up. The fact that broccoli followed by biofumigation appears to have little effect on soil nematode levels but significant effects on tomato root-galling and yields might be due to “early season” protection of the tomato crop.

Session Theme: Experience and Observations from the Field

Title: Microbial Community Structure of Biofumigated Soils

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Keywords: PFLA, Isothiocyanates, Tomato

Presentation Type: Poster

Abstract:

Effective management of the soil microbial ecology is important in sustainable production. During an ongoing study at the University of Tennessee, biofumigation, as an alternative pre-plant treatment had higher tomato yields and lower incidence of Southern Blight than controls. Phospholipid fatty acid (PLFA) analysis of the soil showed that this treatment produced an enhanced microbial biomass and a shift in community composition. High bacteria:fungal ratios in the treated soils correlated with disease suppression.

Session Theme: Genetics and Crop Breeding

Title: Developing designer biofumigation Brassica crops through interspecific

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Keywords: Interspecific hybrid, Breeding, glucosinolates

Presentation Type: Oral

Abstract:

The Brassicaceae breeding group at the University of Idaho has been developing cultivars with allelopathic effects suitable to replace synthetic soil fumigants. Breeding efforts have been directed toward two soil fumigation systems, green manure/plough-down and seed meal that can be used as a soil amendment. A major factor that limits the use of Brassicaceae crops as pesticidal agents is availability of suitable cultivars that have sufficient concentrations of the required allelopathic compounds to be truly effective. Mustard species *Brassica juncea*, *B. carinata*, and *Sinapis alba*, and their weedy relatives *B. nigra* and *S. arvensis* have significantly higher levels of glucosinolates compared to *B. napus* or *B. oleracea*. In addition, the major glucosinolate types in these species (allyl and p-hydroxybenzyl) have shown greater allelopathic effects compared to other glucosinolate types. The University of Idaho breeding group has developed in vitro techniques which has allowed us to extend hybrid cross combinations possible between Brassicaceae species. Some hybrid species combinations show extremely high glucosinolate content and variability of profiles and could have potential as allelopathic plow-down crops.

Session Theme: Genetics and Crop Breeding

Title: Molecular marker associated with high level of sinigrin content in brown mustard (*Brassica juncea*)

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Keywords: *Brassica juncea*, sinigrin content, molecular marker

Presentation Type: Poster

Abstract:

Many reports on *Brassica juncea* have emphasised its interest for biofumigation to eliminate soil pests and diseases. This effect is due to the sinigrin (2-propenyl GSL, SIN). The glucosinolate (GSL) composition of *B. juncea* followed two main patterns characterised by high SIN and high gluconapin (3-butenyl GSL, GNA), respectively. The development of markers associated with SIN content could help in selection program. In our study, an AFLP-based genetic linkage map of *B. juncea* was used for the QTL study. Two of the four QTLs involved in the SIN content were associated with the QTLs for GNA. A candidate gene approach was developed to identify genes or markers linked to the QTLs involved in SIN and GNA content. Oligonucleotide primers were designed from the IPMS-At1 and IPMS-At2 gene sequences identified as candidate to regulate glucosinolate side chain elongation in *Arabidopsis thaliana*. Polymorphisms were detected with these primers in the DH population studied and some of the candidate genes were localised near the mapped QTLs for SIN and GNA. The effectiveness of these markers was tested on other genetic backgrounds and on populations deriving from selection program under process. The interest of these markers for high SIN content genotypes selection is discussed.

Session Theme: Glucosinolate/Mryosinase Chemistry and Biochemistry

Title: Glucosinolate and isothiocyanate concentrations in soil following biofumigation

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Keywords: Soil, Rape, Indian mustard

Presentation Type: Oral

Abstract:

The concentration of glucosinolates (GSLs) and isothiocyanates (ITCs) was monitored in soil following biofumigation with high and low GSL varieties of rape (*Brassica napus*) and Indian mustard (*Brassica juncea*) biofumigant crops. The concentration of GSLs and ITCs in soil was highest 30 min after incorporation and they could be detected for up to 8 and 12 d, respectively. Irrigating with 18 mm of water over 3 h had no effect on GSL or ITC concentrations. The amounts detected were generally related to the amount of GSL added in the incorporated plant tissue. Maximum total GSL concentration detected in the soil was 13.8 nmol/g and 22.8 nmol/g for rape and mustard respectively, representing 7% and 13% of the GSL present in the incorporated tissues. Maximum total ITC concentration was 21.6 nmol/g and 90.6 nmol/g for rape and mustard respectively. Calculated ITC release efficiency was 26% and 56% for high GSL rape and mustard respectively at the time of the highest ITC concentration measured. These results indicate that plant GSL can persist un-hydrolysed in soil for several days following *Brassica* incorporation, and investigations of plant treatment and incorporation methods to maximise ITC release are warranted.

Session Theme: Glucosinolate/Mryosinase Chemistry and Biochemistry

Title: Flourescent gene expressed in response to AITC and KCN

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Keywords: Heat shock protein, Glucosinolate, Isothiocyanate

Presentation Type: Poster

Abstract:

In all living organisms the gene coding for 70 KDa heat shock proteins (HSP-70) is known to increase its expression in response to a variety of stresses. Plants in mustard family (*Brassicaceae*) produce ITC and nitrile containing compounds as defense compounds against herbivory and pathogenic attack. We investigated the chemotoxic effects of AITC and KCN on a transgenic model target nematode (*Caenorhabditis elegans*) and a transgenic non- target organism, zebra fish (*Danio rerio*). Both these organisms contained an enhanced green fluorescent protein (eGFP) driven by the 1.5kb promoter of HSP-70 gene. Greater HSP-70 expression is

induced in response to a variety of stresses including chemotoxicity. AITC and KCN caused expression of the HSP-70-eGFP reporter gene in tissues of live zebra fish including the heart, olfactory area, tail, pectoral fins and epidermis. Toxicity contributed by AITC was comparable with KCN, a known toxic compound. Concentration of AITC as low as 10 μ M caused 40% mortality in zebra fish. Unlike AITC, KCN does not induce eGFP expression in the heart. Further, toxicity / stress caused by AITC was also studied in living transgenic (HSP-70 controlled GFP) nematodes. We are currently investigating to determine if the responses of *C. elegans* are consistent with those observed in *D. rerio*.

Therefore, it may be concluded that zebra fish and nematodes used in the present study can be used as a model system for screening the toxic compounds including glucosinolates, ITC and mustard meal products.

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: Extraction of glucosinolates released in soil

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Keywords: Analysis, glucosinolates, soil

Presentation Type: Poster

Abstract:

Few have addressed the problem of extracting glucosinolates from soil. The prerequisite for evaluating the fate of glucosinolates in soil is acceptable methods for quantitative extraction and analytical tools for subsequent analysis. Methods for analysis of individual intact glucosinolates following extraction from plant materials have been known for some time both as HPLC and HPCE techniques.

Extraction and group separations of glucosinolates from complex matrices including soil, are key steps in proper methods of glucosinolate analyses. Loss of glucosinolates have to be avoided, as it can both be a result of binding or complex formations and a result of degradation resulting from non-enzymatic reactions, microbial activity and/or hydrolysis by plant produced myrosinase isoenzymes (-thioglucosidase; EC 3.2.1.147). To overcome the above mentioned problems, the present studies have comprised evaluations of data obtained by use of alternative procedures. Traditional extraction procedures with initial myrosinase inactivation, and simple liquid extraction followed by centrifugation, have been used and compared with results obtained by use of pressurised solvent extraction (PSE). Three different soil types have been included in the study in order to test the influence coming from different soil types on the extractability of glucosinolate from soil.

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: Proposal for an Analytical Method for the Quantitative Determination of Residues of Iso-thiocyanates in preserved-refrigerated Fruit

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Keywords: Isothiocyanates, SPME, GC/MS

Presentation Type: Poster

Abstract:

In 2002 a plan called 'Valorizzazione della quantità della produzione nazionale di frutta: studio di una nuova tecnologia di conservazione post-raccolta a base di formulati vegetali funzionalizzati (VALE)', (Valorization of the amount of the national production of fruit: study for a new technology of post-collection conservation based on the use of functionalized vegetal formulations) started; this plan has involved some organizations: the Experimental Institute for Industrial Cultivations (ISCI) of Bologna, the Research Center of Vegetables Productions (CRPV) of Cesena, the Pathology Experimental Center for Conservation and Transformation of Fruit and Vegetables (CRIOF) of Bologna, and ARPA Emilia-Romagna district section of Ferrara.

One of the contributions deriving from the scientific collaboration with ARPA Ferrara is the definition of a monitoring system and the determination of the residues of iso-thiocyanates on preserved-refrigerated fruit (peaches, apples and pears).

The analytical method set use the SPME technique (Solid Phase Micro Extraction), for the extraction of the ITC from fruit and consequent their determination on HRGC-MS.

The SPME Technique combined with the HRGC-MS, for being particularly selective and more sensitive, is preferred in comparison with the simple gas-chromatographic technique equipped with flame ionisation detector (FID).

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: IN VITRO SCREENING OF THE EFFECT OF THREE GLUCOSINOLATE DERIVED NITRILES ON SOIL-BORNE FUNGI

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Keywords: nitriles, soil-borne fungi, in vitro screening

Presentation Type: Poster

Abstract:

Glucosinolates are allelochemicals present in all plants of the order Capparales that are hydrolysed by endogenous enzymes (myrosinases) forming a variety of compounds with biological activity. 'Biofumigation' is the term used to describe the effect of these compounds on soil-borne pathogens and it has normally been attributed to isothiocyanates. At acidic pH and in the presence of redox co-factors such as glutathione, glucosinolate hydrolysis yields also nitriles, which are more hydrophilic and stable than isothiocyanates. Three nitriles (allyl-, benzyl- and phenethyl cyanide) were tested against soil borne fungi of economic importance: *Aphanomyces euteiches* var. *pisi*, *Gaeumannomyces graminis* var. *tritici* and *Verticillium dahliae*. The nitriles were initially tested at 1 mM and four additional concentrations were further tested in order to determine LD50.

At 1 mM, allyl cyanide showed in all cases less than 10% inhibition and it did not inhibit fungi growth at higher concentrations. LD50 of benzyl cyanide was 2.5 mM for *Verticillium* and *Aphanomyces*, whereas it was as low

as 0.5 mM for *Gaeumannomyces*. LD50 of phenyl ethyl cyanide was 2.5 mM for *Verticillium*, 1.4 mM *Gaeumannomyces* and 1.25 mM *Aphanomyces*. Although nitriles are generally less toxic than ITCs, their role in biofumigation should not be disregarded.

Session Theme: Glucosinolate/Mryosinase Chemistry and Biochemistry

Title: Intersatellite DNA polymorphism in *A. alternata* Following Exposure to Lethal Concentrations of Isothiocyanates.

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Keywords: *Alternaria alternata*, Isothiocyanates, intersatellite DNA polymorphisms.

Presentation Type: Poster

Abstract:

Isothiocyanates (ITCs) are defense-related compounds synthesized by *Brassica* spp. They have wide biocidal activity, yet have positive effects on human health and have emerged as environmentally friendly compounds to control plant pathogens, particularly fungi. However, the development of fungal resistance to ITCs is a growing concern. The objective of this study was to expose the fungus *A. alternata* to allyl isothiocyanate (AITC) and benzyl isothiocyanate (BITC), and to study potential genomic change through analysis of intersatellite DNA polymorphisms. Strains of *A. alternata* isolated from cabbage and tomato were exposed in vitro to AITC and BITC. Concentrations of ITCs were steadily increased to 0.08 and 0.6 mg/mL of AITC and BITC, respectively, and the fungi appeared to develop resistance. For analysis of intersatellite DNA, two minisatellite primers (M13 and T3B) and two microsatellite primers (ACA5 and GACA4) were used. Following exposure to ITCs, changes in size of amplified intersatellite DNA were detected, particularly in the cabbage strains. Upon elimination of isothiocyanates in vitro, the fungi soon lost the ability to grow in lethal ITC concentrations although the ITC-induced DNA polymorphisms remained. It is concluded that ITC exposure does not induce development of resistance and that changes in intersatellite DNA are a response to ITCs toxic effects.

Session Theme: Glucosinolate/Mryosinase Chemistry and Biochemistry

Title: Evolution of Biofumigation Materials: from Plants towards Biochemical Compounds

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Keywords: Evolution, Biofumigation, Biochemicals

Presentation Type: Oral

Abstract:

Numerous plant species of Brassicaceae family contain molecules, mostly glucosinolates (GLs), which are precursors of strongly bioactive compounds useful also in crop protection. Some of them show physico-chemical properties suitable to be used in biofumigation. The aim of this technique is the control of soil-borne pathogens without using special chemicals as the methyl bromide, which is one of the stronger ozone destroyer in the upper atmosphere layer and, for this reason, banned from 2005. About ten years ago, the first trials of biofumigation were carried out simply by incorporating whole Brassica plants into the soil as green crop. In recent years, however, other materials suitable to be used in biofumigation have been studied and set up. The most significant (in increasing order of GLs content) are: dry plant tissues, defatted meals, and different types of myrosinase-GLs extracts and formulates. As the biofumigation materials are of different type and effect, also their applications could be diverse of that of soil treatment, depending on of their nature and composition. The rationale of this evolution of biofumigation materials and applications will be presented.

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: Production of Allylithiocyanate from different source and its persistence in soil

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Keywords: Biofumigation, Sinigrin, DT50

Presentation Type: Poster

Abstract:

Biofumigation, even if applied by vegetable materials, has to be evaluated even for its environmental aspects, moreover if we consider that synthetic pure Allylithiocyanate (AITC), according to several toxicology tests, is classified (CAS#: 57-06-7, RTECS#: NX8225000) as a toxic compound with a hazard class of 6.1. Environmental toxicity depends also on the released amounts during treatments and their persistence in soil. So, the AITC amounts released in soil after Brassica juncea sel. ISCI20 tissues and from B. carinata sel. ISCI7 seed meals incorporation were estimated and compared with those of Methylithiocyanate released during fumigation with Vapam®, showing a significantly different active compounds release. In addition, persistence of AITC from B. carinata sel. ISCI7 seed meals was assessed in an open system and compared with persistence of synthetic AITC and of AITC from isolated sinigrin and myrosinase. To limit environmental variation, the trials were performed in an artificial standard soil (20% sphagnum peat, 20% kaolinite clay and 70% industrial quartz sand) and AITC level was calculated by GC-FID after extraction by ethyl acetate. The DT50 in all trials resulted very low, with values between 450 to 517 minutes depending on different AITC sources.

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: EFFECT OF ASCORBIC ACID AND GLUTATHIONE ON THE PRODUCTION OF NITRILES BY MYROSINASE

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Keywords: nitriles, redox cofactors, myrosinase

Presentation Type: Oral

Abstract:

Biofumigation is based on the use of glucosinolate-containing plants for the control of soil-borne pest and diseases. Upon tissue damage, glucosinolates are hydrolyzed by endogenous enzymes (myrosinase) and a range of biologically active compounds are formed. Isothiocyanates (ITCs) are the quantitatively dominating products formed at neutral pH. Most of these compounds are volatile and only sparingly soluble in aqueous systems, and depending on the R-group structure and the presence of nucleophiles, further transformation of ITCs occurs. At lower pH and in the presence of certain molecules able to deliver two redox equivalents, the proportion of nitriles increases at the expense of ITC.

The effect of ascorbic acid and glutathione on the production of nitriles at pH 5 was investigated by micellar electrokinetic capillary chromatography (MECC). The presence of 0.25 μmol ascorbic acid increased the production of nitriles although at higher concentrations the proportion of nitriles decreased. Increasing amounts of GSH favored the production of nitriles (40% of the total degradation products were nitriles in the presence of 2 μmol GSH). The oxidation of GSH gives the redox equivalents needed for the liberation of the sulfur from the unstable intermediate of the glucosinolate hydrolysis leading to the formation of the nitrile.

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: Sorption and degradation of benzyl and 2-propenyl isothiocyanates in soil

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Keywords: Sorption, Degradation, Soil

Presentation Type: Poster

Abstract:

Sorption and degradation kinetics of benzyl- and 2-propenyl isothiocyanate (ITC) in top and subsoil from a sandy and a clayey soil profile was investigated in laboratory experiments at a soil:solution ratio of 1:1, at the pH of the soil slurries and room temperature. Both non-sterile and sterilized soil (autoclaved) was used. Sorption isotherms with kaolinite, goethite and humic acid were performed under the same conditions.

In non-sterile soils the ITCs were non-detectable after 5-30 hours. Degradation followed first order kinetics. Degradation was faster in surface than in sub soil, and faster in clayey than in sandy soil. In sterilized soils the concentration declined initially due to sorption to organic matter and evaporation and then remained constant for the duration of the experiment (80 h). Benzyl ITC seems to be stronger sorbed than 2-propenyl ITC, which was confirmed by the isotherms on humic acid (Koc 311 L kg⁻¹ for benzyl, 17 L kg⁻¹ for 2-propenyl). No sorption was detected on the more polar sorbents, kaolinite and goethite.

The results show that ITC is quickly degraded and sorbed in soil, and volatilisation needs to be taken into account. ITCs mainly sorb to organic matter with benzyl-ITC more strongly sorbed than 2-propenyl ITC.

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: Myrosinase immobilized by plant root-mucigel: effect of soil water status on the enzyme efficiency

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Keywords: myrosinase immobilization, plant root mucigel, soil water status

Presentation Type: Poster

Abstract:

Water plays an important role in the native conformation and activity of myrosinase. At the rhizosphere level, water exists in three phases namely, that associated with mucigel, free water in soil solution, and water adsorbed on soil particles. The activity of myrosinase was investigated as a function of soil water status by adding the enzyme to soil either in water solution form or immobilized in the hydrogel of one of the most important root mucigel polysaccharides. At high soil moisture, water and hydrogel-delivered myrosinase activities were comparable whereas in water stressed soil, the activity of myrosinase immobilized on hydrogel was twice as high as that allowed by water delivery. The mucigel-related increase in myrosinase activity could be ascribed to the polarization of water molecules within the hydrogel system, which prevents them from being adsorbed on soil particles and hence available to preserve the enzyme active conformation.

Session Theme: Glucosinolate/Myrosinase Chemistry and Biochemistry

Title: Glucosinolate degradation in soil

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Keywords: Soil, Glucosinolate degradation, Myrosinase

Presentation Type: Poster

Abstract:

The fate of glucosinolates (GSL) in soil was studied in laboratory experiments with pure GSL's. Degradation in a 1:1 soil:water slurry with four different GSL's at 20 C was faster in a clayey soil ($t_{1/2}$ 3.5 – 6.8 hours) than in a sandy soil ($t_{1/2}$ 9.2 – 15.5 hours), and the degradation kinetics was largely independent of the

GSL structure. Degradation was much slower or non-existing in sub soils. In experiments with top soils at 8 oC and 3 – 10 % water content degradation of benzyl GSL was slower with $t_{1/2}$ of 1.7 days and $t_{1/2}$ 16.2 hours for sandy and clayey soils, respectively. None of the GSL's were sorbed in soil. The degradation potential was associated with the clay fraction. Measured myrosinase activity in the soils correlated well with the GSL degradation kinetics and results indicate that extracellular myrosinase is important for GSL degradation in soil. It was possible to retrieve up to 25% of the added benzyl GSL as benzyl isothiocyanate in the top soils. Only small amounts were detected in the sub soils. ¹⁴C-benzyl GSL was synthesized and purified from transgenic *Arabidopsis thaliana* for use in mineralization studies. After 60 days 40-50% of the applied benzyl GSL was mineralized.

Session Theme: Glucosinolates/Myrosinase as Chemoprotectants

Title: Glucosinolates and Chronic Disease Prevention

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Keywords: clinical trials, sulforaphane, broccoli

Presentation Type: Oral

Abstract:

Over 120 glucosinolates have been identified in a large number of edible plant species. Much early research had focused upon the “antinutritional” or goitrogenic properties of some member of this diverse class of water soluble secondary metabolites which are abundant in crucifers and about 15 other plant families. Glucosinolates are enzymatically converted to isothiocyanates by myrosinase, an enzyme which coexists in the plant cells and is released upon damage to the plant tissue which contains them. Although their cancer-preventive potential of these isothiocyanates was initially described as a consequence of their induction of Phase 2 enzymes, they have more recently been shown to possess potent antiproliferative, apoptosis-promoting, redox regulatory and phase 1 enzyme inhibitory roles, as well as being directly bactericidal against the carcinogenic bacterium, *Helicobacter pylori*. Since glucosinolates have potential value as phytochemical components of healthy diets, we have developed methods for enriching, isolating and purifying these compounds. We have also evaluated and developed germplasm enriched in certain glucosinolates. In addition to biochemical and molecular characterization of the mode of action of their active isothiocyanate metabolites, we have engaged in a variety of clinical trials designed to evaluate the protective effect of ingested glucosinolates on a number of diseases.

Session Theme: Glucosinolates/Myrosinase as Chemoprotectants

Title: Composition and content of glucosinolates in rapeseed and in vitro inhibition of *Botrytis Cinerea* Persoon

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Keywords: glucosinolates, biofumigation, Brassica napus

Presentation Type: Poster

Abstract:

The composition and content of glucosinolates in root, stem and leaf of rapeseed (*Brassica napus*) were analyzed. The results shows that within root 4-methoxyglucobrassicin and neoglucobrassicin predominated, while in leaf and root tissue progoitrin and gluconasturtin were the most abundant. From pre-winter stage to final flowering stage, the profile of glucosinolates in root were constant, while the contents of glucosinolates were congruously variable, highest in final flowering stage, lowest in mature stage. There were significant differences between the profile and concentration of glucosinolates in root of 19 rape varieties, which may partially account for differences in inhibition of *B. Cinerea* mycelium growth by hydrolytic compounds release by root of different rape varieties. It can be presumed that *B. Cinerea* is very susceptible to butenyl ITC, and the mycelium growth inhibition of indole ITC was less than that of aromatic ITC.

Session Theme: Pest Control with Green Manures

Title: Separating glucosinolate-related suppression from other impacts of mustard biofumigants.

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Keywords: Bacterial wilt, green manure, biofumigation

Presentation Type: Oral

Abstract:

Mustard (*Brassica juncea*) biofumigants have proven effective in suppressing the bacterial wilt (BW) pathogen (*Ralstonia solanacearum*), one of the world's most significant diseases of bacterial origin. However the role of glucosinolates (GSLs) in this suppression has not been separated from other potential mechanisms of suppression. We used a mustard breeding line containing very low tissue GSLs together with traditional high GSL mustard in laboratory and field experiments to separate GSL-related impacts from those related to other impacts of the incorporated tissue. In the laboratory, suppression of BW in the short term (3 days) was clearly related to GSL hydrolysis products (principally ITCs), while significant longer-term suppression (3 weeks) occurred to a similar extent in both high and low GSL-amended treatments. This was confirmed in a field experiment comparing BW suppression using the same mustard lines, non-*Brassica* amendments and un-amended controls. GSL-related suppression was generally reduced in soils with high clay and organic matter

content, while non-GSL related suppression occurred relatively consistently across soil types. The results indicate that while short-term GSL-related suppression is effective for BW suppression on light textured soils, significant longer-term suppression unrelated to tissue GSL content may contribute to disease suppression on a range of soils.

Session Theme: Pest Control with Green Manures

Title: Mustard-derived biofumigation for lettuce in coastal California

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Keywords: Brassicaceae, glucosinolates, Sclerotinia minor

Presentation Type: Poster

Abstract:

Lettuce has traditionally benefited from rotation with fumigated crops. The biocidal properties of glucosinolate degradation products from plants in the Brassicaceae family offer potential alternatives to commercial fumigants. This study near Santa Paula, California in 2005-2006 evaluated the effects of biofumigation (mulching and subsequent irrigation) with five incorporated cover crops on sclerotia of *Sclerotinia minor* (lettuce drop), seed of five weed species, and yield of romaine lettuce. The cover crops were *Sinapis alba* 'IdaGold,' *Brassica juncea* 'PacificGold,' *Brassica napus* x *Brassica campestris* 'BQ mulch™,' *Vicia faba*, and *Triticum aestivum* x *Secale cereale*. Sclerotia and weed seeds remained viable after burial at 15 cm 14 days after biofumigation with all crops, consistent with previous research. Disease incidence in lettuce was low; however, disease severity was reduced 25% when biomass amendments were doubled, regardless of cover crop. Lettuce heads were 15% larger after 'IdaGold' compared to 'BQ mulch,' which corroborates previous findings that 'IdaGold' resulted in improved agronomic performance of subsequent lettuce. This may be attributed to the presence of benzyl glucosinolate in above-ground tissues and 4-methylthiobutyl and 4-hydroxybenzyl glucosinolates in roots of 'IdaGold' and their absence in the other crops. Soil microbiological activity of treated plots is currently under assessment.

Session Theme: Pest Control with Green Manures

Title: Advancing biofumigation research - the need for minimum data sets

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Keywords: green manure, biofumigation, disease

Presentation Type: Poster

Abstract:

A recent review of the literature reporting the use of GSL-containing plants for pest suppression ("biofumigation") reveals that many studies do not report key background information necessary to interpret the

role of glucosinolates (GSLs) in pest suppression. As a consequence it is difficult to identify opportunities to improve the levels of pest suppression achieved. Few studies report the concentration or profile of GSLs in the tissues at the time of incorporation, the plant growth stage and the amount of biomass incorporated, details of the incorporation strategy used or the important soil characteristics likely to influence suppression levels (e.g. soil water content, clay content or organic matter). As a result it is impossible to compare the effectiveness of suppression achieved using different approaches based on some suitable comparative metric such as the amount of GSL incorporated into the soil as measured in nmol/g soil. Evaluating biofumigation as a credible pest management strategy and comparing the approaches used in different studies will require minimum data sets to facilitate such comparisons.

Session Theme: Pest Control with Green Manures

Title: Effect of mustard green manure amendment on the infectivity and mortality of two entomopathogenic nematode genera, *Steinernema* and *Heterorhabditis*.

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Keywords: Entomopathogenic nematodes, mustard green manure,

Presentation Type: Poster

Abstract:

Effect of mustard green manure amendment on the infectivity and mortality of two entomopathogenic nematode genera, *Steinernema* and *Heterorhabditis*. Henderson, Donna,¹ R. Ramirez,² E. Riga,¹ W. E. Snyder.² Departments of Plant Pathology¹ and Entomological Sciences², Washington State University, Pullman, WA 99164.

The use of mustard green manures has become a common practice of integrated pest management for plant parasitic nematode control in Washington State potato agriculture. Entomopathogenic nematodes (EPNs) are biological control agents used against both insect pests and plant parasitic nematodes. Mustard green manures, used for the control of plant parasitic nematodes, may also be antagonistic to entomopathogenic nematodes. The effect of glucosinolates on the infectivity and mortality of two EPN genera, *Steinernema* and *Heterorhabditis* were examined. Two cultivars of *Brassica juncea* with high or low glucosinolate levels were tested against seven EPN species: *Steinernema carpocapsae*, *S. glaseri*, *S. riobrave*, *S. feltiae*, *Heterorhabditis marelatus*, *H. bacteriophora*, and *H. megidis*. A *Galleria melonella* assay was used to determine the indirect effect of mustard cultivar on EPN infection. A Petri dish bioassay examined the direct effect of mustard cultivar on EPN mortality. These data suggest mustard cultivars with high glucosinolate levels have a negative effect on EPN infection rate and mortality.

Session Theme: Pest Control with Green Manures

Title: Effect of mustard green manure on nematodes

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Keywords: root knot, stubby root, soil microbiology

Presentation Type: Poster

Abstract:

Various pesticides and soil fumigants are used extensively in potato cropping systems to achieve high productivity and quality. However, the cost of these materials and their application significantly impact the potato crop's profitability, may negatively affect environmental quality, and pose human safety issues. Also, restrictions on the use of some materials make it difficult to use pesticides in certain areas at the time and place where they are needed. The application of a mustard green manure in a potato cropping system significantly changed nematode levels in the soil. Root knot nematode and stubby root nematode population levels were reduced after incorporation of mustard green manure. Conversely, the level of free living nematodes increased with the addition of mustard green manure. The level of soil microbes was also significantly changed following green manure incorporation. Bacterial levels increased with incorporation of mustard green manure while fungi levels decreased with fumigation. But most importantly was the increased potato yield of tubers associated with mustard green manure. Marketable and total yield was significantly increased with the use of mustard.

Session Theme: Pest Control with Green Manures

Title: Toxicity of benzyl glucosinolate and benzyl isothiocyanate to the soil dwelling springtail *Folsomia fimetaria*

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Keywords: Ecotoxicology, Soil, Isothiocyanate

Presentation Type: Poster

Abstract:

Survival and reproduction of the soil dwelling springtail, *Folsomia fimetaria*, was investigated during exposure to benzyl-isothiocyanate (BITC) and benzyl-glucosinolate (BGSL).

F. fimetaria were exposed to 6 soil concentrations of BITC and BGSL (0, 10, 50, 100, 250 and 500 mg/kg) for a period of 21 days in microcosm experiments (30 g soil and 20 springtails (10 males, 10 females) in each microcosms, 4 replicates). Soil samples for BITC and BGSL analysis were collected on day 0, 1, 2, 5, 9, 12, 16 and 21.

BITC proved to be highly toxic, as no survival and reproduction was observed after 21 days of exposure at all concentrations above 50 mg/kg. The higher the initial concentration of BITC the more persistent it was. When 10 mg/kg BITC was applied it was detectable for 3 days, at 500 mg/kg it was detectable for 21 days.

BGSL proved to be less toxic. No significant difference in adult survival was observed between control and test concentrations. However, a 71% reduction in reproduction occurred at 500 mg/kg compared to the control.

BGSL was only detectable in the soil for 1-2 days at all initial concentrations.

Toxicity of BITC to springtails is comparable to insecticides such as pyrethroids.

Session Theme: Pest Control with Green Manures

Title: The Impact of Mustard Cover Crop Rotations with Lettuce on the Incidence of Lettuce Drop Caused by *Sclerotinia minor*

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Keywords: Mustard Cover Crops, Lettuce, *Sclerotinia minor*

Presentation Type: Oral

Abstract:

Growers in the Salinas Valley, California, USA are unable to rotate away from lettuce as often as desirable due to economic pressures. Poor rotations aggravate problems with *Sclerotinia minor* infection on lettuce. Mustard cover crops (*Brassica juncea* and *Sinapis alba*) contain glucosinolates that break down to compounds such as isothiocyanates (ITC) which are toxic to fungi. Salinas Valley growers utilize mustard cover crops to provide a short-term rotation to reduce the incidence of *S. minor*. We evaluated the impact of mustard cover crops on *S. minor* for three years. In seven trials, mustard cover crops did not reduce number of soil sclerotia nor the incidence of *S. minor* at harvest. The mustard cover crop species were also susceptible to *S. minor*, which raises the question, does the build up of *S. minor* on the cover crop diminish potential beneficial effects of the biofumigation? We measured the equivalent ITC content of mustard cover crops and found them to be equivalent to 15.9 to 21.5 liters of Vapam per hectares. Vapam is labeled at rates between 351 to 701 liters per hectares. The small amount of biofumigant contained in the mustard cover crops may explain the lack of measurable reductions of *S. minor* infection on lettuce in these studies.

Session Theme: Pest Control with Green Manures

Title: TWO DECADES OF GREEN MANURE CROP RESEARCH IN IDAHO FOR NEMATODE MANAGEMENT– AN OVERVIEW

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Keywords: *Heterodera schachtii*, *Meloidogyne chitwoodi*, overview

Presentation Type: Oral

Abstract:

The use of green manure crop for nematode management in Idaho dates back to 1985 when the first experiment conducted in Parma using the oil radish variety RSO1841 significantly reduced the population of sugar beet cyst nematode, *Heterodera schachtii*. Following this major breakthrough, field studies were conducted with Pagletta, Nemex and R184 and more reduction in nematode population was achieved. During early nineties with the funding from Sugar beet Grower Association in the first field application, it was found that new varieties

reduced nematode population (80%) with significant increase in beet yield. In mid nineties due to funding from Idaho Potato Commission, the program was extended to *Meloidogyne chitwoodi* management on potato and oil radish varieties Colonel, Commodore, and mustard variety Metex were commercially planted in grower's field. In recent field studies it was found that two new oil radish varieties Defender and Comet significantly reduced the population of *H.schachtii* (95%) and *M.chitwoodi* (99%). Recent economic analysis at the University of Idaho confirmed that the growers and the Idaho agricultural community benefited \$52 for every dollar invested on green manure research.

Session Theme: Pest Control with Green Manures

Title: Evaluation of field pennycress as an overwinter green manure crop in corn for suppression of Western corn rootworm

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Keywords: *Thlaspi arvense*, *Diabrotica virgifera*, green manure

Presentation Type: Oral

Abstract:

Field pennycress (FP; *Thlaspi arvense* L.) is a winter annual species of the Brassicaceae which is a native of Europe but has a wide distribution throughout temperate North America. FP tissues contain the glucosinolate sinigrin, and release a mixture of the biocides allyl thiocyanate and allyl isothiocyanate when plant tissues are macerated. Although FP is considered a major agricultural weed, its ability to overwinter in areas with severe winters and its sinigrin content led us to investigate it as a potential biofumigation green manure crop for the control of Western corn rootworm (WCR; *Diabrotica virgifera* LeConte), a leaf beetle whose larvae feed predominantly on corn roots. Plots were seeded on September 15, 2004 with FP seeds and allowed to grow overwinter, with fallow ground maintained between plots as a control. Soil was removed in March and analyzed in the greenhouse for WCR eggs and larval development. Although eggs were more numerous in soil from FP plots, survival was less than in fallow plots. FP plants were incorporated into the soil by mowing and immediate rototillage at three different dates (April 11, 18 and 25, 2005), which corresponded with different developmental stages of the plants. Corn (*Zea mays* L.) was planted one week after the last incorporation date. Corn growth was reduced by FP incorporated at the later two dates but not at the earliest date. Results for corn root damage by WCR and adult emergence did not vary statistically among treatments in the test plots. From these results it does not appear that FP as a green manure will reduce WCR damage to corn cropped the following season.

Session Theme: Pest Control with Green Manures

Title: The use of arugula on its own and in combination with synthetic nematicides against plant parasitic nematodes of potatoes

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Keywords: green manure, nematodes, nematicides

Presentation Type: Poster

Abstract:

Arugula (*Eruca sativa*) has shown great potential for controlling plant parasitic nematodes as, it has a dual role; it is both a green manure and a nematode-trap crop. Control of fungal pathogens and parasitic nematodes is essential to quality potato production in the USA both for the domestic and the international market. Arugula in combination with half the recommended rate of Telone reduced root knot nematode populations, *M. chitwoodi*. Arugula in combination with Temik and Mocap also reduced *M. chitwoodi*. In addition, Arugula did not reduce the beneficial free-living nematode populations and the non-pathogenic *Pseudomonas*. The cost of growing and incorporating Arugula and combining it with ½ rate of Telone and one fungicide was approximately half of the present commercial cost of Telone and fungicide applications.

Session Theme: Pest Control with Green Manures

Title: Antifungal volatiles emitted from Brassica juncea-amended soils differing in water content.

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Keywords: Brassica juncea, reductive soil disinfestation, antifungal volatile compounds

Presentation Type: Oral

Abstract:

Previously, when Brassica juncea (Indian mustard) leaves were incorporated into soils with different water contents, the fungicidal effect against *Fusarium oxysporum* f. sp. *spinaciae* (spinach wilt pathogen) in the soil was much greater at 45-50% water content than at 40%. At 45-50%, the redox potential dropped considerably, indicating a reducing condition in the soil. To investigate the mechanisms involved in this phenomenon, which we have named reductive soil disinfestation, antifungal volatile compounds emitted from the soil were collected using a headspace SPME method and analyzed by GC-MS. At 40% water content, dimethyl sulphide, dimethyl disulphide, dimethyl trisulphide, and allyl isothiocyanate were the main products 3 days after leaf incorporation. At 50% water, cresol, skatole, indole, and phenethyl alcohol were additionally detected as major compounds. In a growth-inhibition assay against *F. oxysporum* on potato sucrose agar, the EC50 for allyl isothiocyanate, skatole, cresol, indole, phenethyl alcohol, dimethyl sulphide, and dimethyl disulphide was estimated at 30, 100, 200, 300, 900, >1000, and >1000 µg/ml, respectively. Although the concentrations of these chemicals in soil have not been determined, bacteria such as *Clostridium* sp. that are known to produce cresol, skatole and indole under anaerobic conditions might be involved in reductive soil disinfestation.

Session Theme: Pest Control with Green Manures

Title: Environmental impacts on the biological activity of contrasting isothiocyanates.

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Keywords: biofumigation, isothiocyanates, brassicas

Presentation Type: Oral

Abstract:

Four pure isothiocyanates (methyl, 2-propenyl, benzyl and 2-phenylethyl), hydrolysing tissue of two brassicas rich in either 2-propenyl or 2-phenylethyl ITC, and the methyl ITC-generating pesticide metam-sodium were tested in vapour exposure tests for biological activity against a model soil insect both in vitro and in the presence of three contrasting soils and under four temperatures from 5-20 °C. The purpose was to develop an understanding of the factors controlling ITC release and maintenance in soil in order to identify advantageous attributes to seek in utilising brassicas for ITC-based biofumigation. Methyl ITC, structurally the simplest and the most volatile was the most biologically active ITC under all conditions. It was less affected by the presence of soil and lower temperature than the longer-chain aliphatic 2-propenyl ITC. The activity of the less volatile aromatic ITCs was reduced much more by soil, with a decline up to many thousand-fold in the presence of soil with high organic matter content at lower temperature. Metam-sodium closely reflected the methyl ITC results. The results indicate that brassicas rich in aliphatic ITCs are more likely to have the potential to exert stronger ITC-based biofumigation effects than those similarly rich in aromatic ITCs.

Session Theme: Pest Control with Green Manures

Title: Biofumigants in Commercial Onion Production to Control Weed, Nematode and Disease Pests

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Keywords: Green manure, Onions, Biofumigation

Presentation Type: Oral

Abstract:

Onions are a high value crop that requires significant inputs of pesticides to grow a high yielding, quality crop. Use of synthetic pesticides continually poses a wide range of problematic issues. Biofumigant crops in onion production have potential for reducing chemical soil inputs. Mustard and oilradish cultivars have been suggested as substitutes for commercial fumigants in controlling soil-borne pests. In this study, a high glucosinolate (Idagold mustard), low glucosinolate (Sunrise canola), oilradish (Colonel), metam sodium and fallow treatment were tested for effectiveness against nematodes, weeds and pink root. Soil samples for root lesion, stunt, spiral and ring nematodes were assayed but no significant differences were observed among treatments. Biofumigants did not positively affect weed control, but reduced onion stand and yield in two of the three years. Canola and

oilradish plants volunteered each year. In low lime plots, pink root severity was significantly lower in metam sodium plots 2 of the 3 years. Mustard had significantly less pink root 1 out of 3 years. Oilradish and canola treatments did not significantly lower pink root. In the high lime plots, metam sodium was significantly lower than the other treatments all 3 years. Mustard and oilradish were not significantly different from the fallow.

Session Theme: Pest Control with Green Manures

Title: Biofumigation for Controlling Soilborne Diseases of Tomato, Potato and Olive

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Keywords: Fusarium, Verticillium, Colletotrichum

Presentation Type: Oral

Abstract:

Soilborne pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Verticillium dahliae*, *Colletotrichum coccodes* and *Pythium* spp. are difficult to control because of their persistence in the soil and their wide host range. Biofumigation was tested as means of controlling these diseases.

We developed a rapid bioassay for screening the biofumigation potential of different plants in which only the volatile compounds' effects are tested. A significant reduction in *R. solani* colony diameter after 3 days of incubation was obtained by amendments of broccoli, roquette and mustard (10% v/v), however for *V. dahliae* and *C. coccodes* higher concentrations were needed.

Biofumigation against *R. solani* was demonstrated in tomato greenhouse experiments: yields were increased and disease was suppressed. Controlling root-knot nematodes by solarization alone was observed, but the combination of solarization with green manure residues resulted in even better disease suppression.

In potato field infested with PED pathogens (*V. dahliae*, *C. coccodes*) incorporation of broccoli, *Brassica juncea* ISCI20 and red giant mustard resulted with higher yields (113, 111, and 107% of the control).

With 10-years old olive trees infected by *V. dahliae*, solarization alone or biofumigation alone were not successful, and the combination of both solarization with biofumigation is currently tested in a field experiment.

Session Theme: Pest Control with Green Manures

Title: Preliminary study on the biofumigant effects of *Brassica juncea* as green manure on *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *tritici*

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Keywords: Indian mustard, Soil-borne pathogens,

Presentation Type: Poster

Abstract:

Management of polyetic epidemics caused by soil-borne fungi such as *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *tritici* needs researches performed at the crops succession scale. A study is being engaged to analyze, in a sugar beet - wheat rotation, the potential effects of mustard (*Brassica juncea*) in controlling these soil-borne pathogens. Researches focused on the mechanisms involved according to the epidemiological attributes of the pathogens. In this preliminary study, the sensitivity of *R. solani* and *G.g. tritici* to *B. juncea* green manure was compared in vitro. Different types of inocula were, during 5 days, incubated in the atmosphere of crushed aerial tissues or submerged in these tissues. In such lab experiments, both fungi are very sensitive and large pieces of inoculum, i.e. colonized barley grains, are less affected than small pieces of diseased roots or small inoculum propagules. The experiments, performed twice with different greenhouse conditions (temperature and photoperiod) showed important variations of the biofumigant effect according to the duration of cultivation of the mustard (time between sowing and flowering stage). These results emphasize the importance, for a better understanding of the processes involved at a field-scale, of taking into account the agronomical factors which influence both inoculum survival in soil, mustard canopy and efficiency of biofumigation.

Session Theme: Pest Control with Green Manures

Title: POTENTIALLY MINERALIZABLE NITROGEN IN SOILS AMENDED WITH BIOCIDAL CROPS

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Keywords: soil nitrogen, brassicaceae, soil texture

Presentation Type: Poster

Abstract:

Biocidal green manure (GM), in addition to reducing the incidence of some soilborne pathogens and pests, may represent a way of integrating N fertilizer in agricultural soils. Two laboratory experiments were performed with the following objectives: (i) to compare the potentially mineralizable N (PMN) of soil added with glucosinolate-containing (GLS+) and non-containing (GLS-) crops; and (ii) to compare the PMN of different types of soils after incorporation of GLS+ crops. In Experiment I, PMN was evaluated in a silty clay soil after incorporation of *Brassica juncea* (GLS+), *Eruca sativa* (GLS+) or winter wheat (GLS-), and compared to the PMN of an untreated soil and a soil fumigated with Metam sodium (Methyl isothiocyanate). In experiment II, PMN after incorporation of *B. juncea* or *E. sativa* was also compared to an untreated control for three soils belonging to contrasting textural classes: silty clay, loam, and loamy sand. The results showed that PMN was higher in soil added with *B. juncea* and *E. sativa* than in soil added with winter wheat. Metam sodium strongly inhibited nitrification, and greatly stimulated the soil endogenous-N mineralization. After a 3-month incubation, the highest amount of inorganic N per unit of soil dry weight was found in the silty clay soil, characterized by the highest content of total N and that had received the highest amount of N from GM. The highest N mineralization rate, expressed as the amount of inorganic N per unit of total N measured in soil after GM incorporation, was

observed in the loamy sand amended with *B. juncea*. Nitrogen availability in soil for crops following green manure incorporation is linked to both green manure and soil type.

Session Theme: Pest Control with Green Manures

Title: Potential role of Brassicaceae plant as biofumigation agent in suppression of potato cyst nematodes (*Globodera rostochiensis* cv. Woll)

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Keywords: Glucosinolates, Brassicaceae, Nematicidal

Presentation Type: Poster

Abstract:

The potato cyst-nematode (*Globodera rostochiensis* cv. Woll) is responsible for large yield losses in the potato crop in Portugal. Soil fumigation with chemicals although efficient to control this nematode for a short period clearly shows a strong negative effect on environment. Plants from the Brassicaceae family have been recognised for their potential use on biofumigation practices, based on the presence of the glucosinolates which after enzyme hydrolysis release active volatile compounds, such as the isothiocyanates. The aim of this study was to evaluate the effect of two concentrations (0.2 and 0.05 $\mu\text{moles} \cdot 100 \text{ g}^{-1}$ dry weight) of total glucosinolates from 6 plants extracts (broccoli, cauliflower, collards of *B. rapa*, collards of kale, Portuguese cabbage and watercress) on the potato cyst nematodes. The results showed a highly significant effect ($P < 0.001$) of the type of plant extract and concentration on the suppression of cyst nematodes. On average, the minor reproductive factor (Rf) was observed for watercress, followed by cauliflower and *Brassica rapa*, based on the levels of 2-phenethyl and 2-propenyl glucosinolates. The results suggest also that the incorporation of plant extracts, simultaneously to the potato cultivation could be an effective strategy to suppress the potato cyst nematode population.

Session Theme: Pest Control with Green Manures

Title: Brassica rotations for managing soilborne potato diseases in the Northeast

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Keywords: soilborne disease, Brassica, crop rotation

Presentation Type: Poster

Abstract:

Numerous soilborne diseases are persistent, recurrent problems in potato production, resulting in reduced plant growth and vigor, and reduced tuber yield and quality. In a series of field experiments conducted on commercial and research farms in the Northeastern U.S., a variety of Brassica crops are being evaluated as rotation, cover, and green manure crops for their potential in managing soilborne diseases and improving potato yields through biofumigation. In the first set of experiments on commercial farms with substantial soilborne disease problems, canola, rapeseed, and oriental mustard incorporated as green manures reduced powdery scab by 15-40% and black scurf by 50-85%, and oriental mustard reduced common scab by 25% in the subsequent potato crop compared to a standard grain rotation. In ongoing research, multiple two-year rotations have been established with up to six different Brassica crops (canola, winter rapeseed, condiment mustard, oriental mustard, oilseed radish, and a white/oriental mustard blend) managed in different ways (as a harvested crop, green manure, with and without addition of a fall rapeseed cover crop), to better determine how to best utilize and incorporate these Brassica crops into potato production systems for maximum efficacy of disease control, tuber yield, and economic viability.

Session Theme: Pest Control with Green Manures

Title: Effectiveness of crop rotation with Brassicaceae species for the management of the southern root-knot nematode *Meloidogyne incognita*

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Keywords: green manure, *Meloidogyne incognita*, biocidal intercropping

Presentation Type: Oral

Abstract:

Four accessions of mustard species (*Brassica juncea* sel. ISCI 99, *Eruca sativa* cv. Nemat, *Crambe abyssinica* cv. Mario, and *Raphanus sativus* cv. Boss), selected for their glucosinolate content, were rotated with host crops of the southern root-knot nematode, *Meloidogyne incognita*, to ascertain their effectiveness in managing the nematode, in comparison with a rotation using a susceptible crop *Vicia villosa* cv. Haymaker plus. The Brassica crops were sown in late summer or in late spring respectively before tomato and carrots cultivation. All the species used significantly suppressed numbers of the nematode juveniles in the soil and in the roots in comparison with a susceptible intercrop. *B. juncea* and *C. abyssinica* acted mainly as biofumigants while *R. sativus* and *E. sativa* revealed a clear catch crop effect. The results showed a significant tomato yield increase after mustard crops, except for the rotation with *B. juncea*. The cause of the lower yield in the plots cultivated with *B. juncea* is still unclear and will be discussed. Significant carrot yield increase was also observed in the plots previously rotated with *E. sativa* cv. Nemat, *B. juncea* and *R. sativus*. The beneficial effect of the rotation with mustard species was greater in biocidal crops sown in late spring rather than in late summer.

Session Theme: Pest Control with Green Manures

Title: Strategies for Using Green Manure Crops to Suppress Columbia Root-knot Nematode.

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Keywords: Meloidogyne chitwoodi, Nematode control, Potato

Presentation Type: Oral

Abstract:

Columbia root-knot nematode, CRKN (*Meloidogyne chitwoodi*) infests potato tubers to cause quality defects that can lead to crop rejection. Green manure crops can assist in management of CRKN provided proper strategies are used. In Oregon, mustard (cv Martigena) and radish (cv Colonel) or rapeseed (cv Jupiter) and sudangrass (cv Trudan 8) had similar nonhost effects on CRKN suppression while these crops were growing. However, mustard and rapeseed had a stronger green manure effect on suppression after incorporation. Green manure crops planted in late summer after grain did not control CRKN alone but were successful when used with nonfumigant nematicides or when planted after cropping sequences with poor host crops. In Colorado, green manure crops must be grown in the summer instead of a grain crop since the season is too short to grow grain and a green manure crop. Nonhost effects were stronger with sudangrass (cv Sordan 79) or canola than with radish (cv Rimbo) or mustard (Calinete 119), but green manure effects were stronger for mustard and radish. Without any nematicides, sudangrass provided best suppression of CRKN, eliminated any external symptoms of CRKN in tubers, and reduced infection the most of any green manure crop tested.

Session Theme: Pest Control with Green Manures

Title: Can biofumigation effectively control common scab of potato under field conditions?

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Keywords: Potatoes, Common scab, Field trial

Presentation Type: Oral

Abstract:

Common scab, caused by *Streptomyces scabiei*, is a major disease of potato in South Africa and most other parts of the world. To determine if volatile compounds released from residues of brassicaceous crops have a reducing effect on the incidence of common scab, field trials were conducted over three seasons with various brassica amendments. The treatments included green manuring, amendment with fresh residue, amendment with dry residue and no amendment (control). Amendment of naturally infested soil in the field with dry residues of cabbage at a rate of 1.56 % (m/v) resulted in a significant reduction in disease. Green manuring with cabbage and amendment with fresh cabbage residue did not result in significant reduction of common scab on potato. No adverse effect on growth or yield of potatoes planted to soil amended with dry brassicaceous material was observed.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: The effect of glucosinolate type and quantity on weed seed germination and growth in glasshouse studies

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Keywords: Brassicaceae seed meal, phytotoxicity, Weed control

Presentation Type: Poster

Abstract:

Many researchers have investigated pesticidal properties of Brassicaceae species. Most studies have investigated plow-down crops for pest control. However, Brassicaceae seed meal can contain up to 10 times higher glucosinolate concentrations than other plant parts. This paper investigates the phytotoxicity of canola, Oriental mustard and yellow mustard seed meal amended potting media in greenhouse conducted experiments on seedling emergence and growth of selected weeds and crops. A mixture of redroot pigweed, wild mustard and wild oat seeds were planted into potting media amended with 0.5 and 1.0 Mt ha⁻¹ canola, Oriental mustard or yellow mustard seed meal, along into untreated potting media, and weed seedling emergence and biomass were recorded. Yellow mustard seed meal amendment reduced wild mustard and redroot pigweed weed seedling emergence by 68% and 70%, respectively, and biomass by 68% and 80%, respectively, compared to the untreated control, but canola and Oriental mustard meal treatments had little effect on the broadleaf weeds. In contrast, Oriental mustard meal reduced wild oat emergence and biomass most effectively. Yellow mustard meal treatments affected not only weed seedling emergence, but seedling growth and mortality. Overall, Oriental and yellow mustard meal treatments had the greatest phytotoxic effect on weed emergence and biomass and have potential herbicidal uses.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Weed suppression in Potting Soil amended with Dried Distillers Grains

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Keywords: Weed control, *Stellaria media*, *Poa annua*

Presentation Type: Poster

Abstract:

Dried distillers grains with solubles (DDGS) is a byproduct of ethanol produced from corn and developing new uses for DDGS could increase the profitability of ethanol production. This research is evaluating DDGS as an amendment to potting soil used in the perennial ornamental industry. Adding DDGS to a commercial pine bark potting mix reduced emergence and growth of annual bluegrass (*Poa annua*) at concentrations of 10 % (wt/wt) or more and common chickweed (*Stellaria media*) at concentrations of 5 % (wt/wt) or greater. *Coreopsis auriculata*, var. 'Nana'; *Rosa hybrid*, var. 'Red Sunblaze'. and *Phlox paniculata*, var. 'Franz Schubert' transplanted into potting soil amended with 20% DDGS (wt/wt) were severely stunted and most plants died. Plants survived

when transplanted into potting soil containing 10% DDGS (wt/wt), but growth was stunted and flowering of roses and coreopsis was reduced. Due to the phytotoxicity observed on ornamentals transplanted into DDGS amended potting soil, current studies are evaluating the potential of DDGS used as a surface applied mulch to suppress weeds. Soil pH, nitrogen release, and soil microbial activity is being characterized in potting soil amended with DDGS and methanol-extracted DDGS is being evaluated for herbicidal activity.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Application of brassicaceae seed meals for the management of apple replant disease

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Keywords: Rhizoctonia solani, Phytophthora, Pythium

Presentation Type: Poster

Abstract:

Field trials were established to evaluate the efficacy of brassicaceae seed meal amendments for control of the soilborne pathogen/parasite complex inciting apple replant disease, and capacity of such treatments to enhance tree growth and yield. Brassica napus seed meal amendment conducted in the fall prior to planting used in conjunction with a post-plant mefenoxam soil drench was as effective as Telone-C17 soil fumigation in controlling replant disease and enhancing tree growth and yield on a site lacking the lesion nematode. Although the treatment provided initial nematode control on a site possessing significant Pratylenchus penetrans numbers, tree growth in seed meal amended soils was inferior to that obtained in response to pre-plant fumigation. This likely resulted from the observed re-infestation of seed meal treated soils by P. penetrans. In subsequent studies, multiple seed meals were evaluated with and without a post-plant mefenoxam drench. Although Brassica juncea seed meal did not stimulate root infection by resident Pythium spp., application of mefenoxam to such treated soils nevertheless stimulated tree growth, apparently resulting from the suppression of root infection by Phytophthora. To date, tree growth in B. juncea seed meal/mefenoxam treated soils has been superior to all other treatments, including pre-plant soil fumigation.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Contribution of resident soil microorganisms to brassicaceae seed meal-induced disease and weed suppression

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Keywords: induced plant resistance, microbial ecology, nitric oxide

Presentation Type: Oral

Abstract:

Brassicaceae seed meal (BSM) amendments effectively control Rhizoctonia root rot of apple and suppress weed growth in orchard soils. Pasteurization of BSM-amended soils prior to pathogen introduction eliminated disease control, suggesting the functional role of a resident biological factor. When BSM amendment, infestation with *R. solani* and planting were conducted simultaneously, application of the nitrification inhibitor nitrapyrin suppressed NO emission and disease control. However, nitrapyrin had no impact on disease control when pathogen infestation and planting of apple were delayed until after peak NO emission. Amplification of resident *Streptomyces* populations was repeatedly observed in response to BSM amendment. Inoculation of pasteurized BSM-amended soils with individual *Streptomyces* isolates restored disease control, and did so via the induction of plant resistance in a manner similar to that of BSM. *Brassica napus* and *Sinapis alba*, but not *B. juncea*, stimulate resident *Pythium* spp. populations and suppress emergence of various plant species. Application of pythiaceous-specific mefenoxam to *B. napus* and *S. alba* treated soils results in significant increases in emergence of wheat, pigweed and hairy vetch. These data suggest that BSM-induced control of specific soilborne pathogens and weeds results, in part, through the capacity to stimulate certain elements of the resident soil microbial community.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: 'Herbicidal and crop phytotoxicity of Brassicaceae seed meals on strawberry transplants and established crops

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Keywords: Brassicaceae seed meal, phytotoxicity, Herbicidal

Presentation Type: Oral

Abstract:

Environmental concerns have restricted registration and use of synthetic soil fumigants considered essential on strawberry production. This has generated interest in biological control of soil-borne pests and diseases. Brassicaceae seed meal contains glucosinolates that breakdown in the soil to substances which have shown pesticidal properties. Field studies were conducted to compare the effects of amending 2 Mt ha⁻¹ of canola (*Brassica napus* L.), Oriental mustard (*B. juncea* L.), and yellow mustard (*Sinapis alba* L.) seed meal into soils on strawberry crop health and weed control, compared to untreated and weed-free controls treatments. Yellow mustard meal-amended soils had significantly greater herbicidal activity with 95% and 88% reduction in weed emergence and biomass compared to the no-treatment control. Oriental mustard and canola treatments both reduced weed counts and biomass significantly compared to the no-treatment control. Strawberry plants showed most visible phytotoxicity when transplanted into Oriental mustard amended soils with 61% more damage than the weed-free control. Fruit yield from Oriental mustard side dress treatment was significantly higher than the weed-free control, and the other meal treatments were not significantly different from the weed-free control. Results showed that seed meal treatments can be used to control weeds in strawberry production without significant yield loss due to crop phytotoxicity.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Brassicaceae seed meal top-dressing - an alternative to bluegrass burning?

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Keywords: Bluegrass, phytotoxicity, seed meal

Presentation Type: Oral

Abstract:

Kentucky bluegrass has traditionally been grown in the Pacific Northwest region of US. Health concerns related to field burning of grass residue, a practice necessary to ensure crop longevity and productivity, has greatly affected production of this crop in the region. Several alternative practices have been investigated as an alternative to field burning, but as yet none have proven as effective for overall productivity and crop management. We found herbicidal activity of Brassica seed meals and designed a study to examine the effects of applying Brassica seed meal on bluegrass productivity. Field trials were conducted at Genesee, ID, in 2003 and at Tensed, ID, in 2004. Twelve treatments were examined: no-treatment control; burn bluegrass straw; rake and remove straw; and apply canola, yellow mustard or oriental mustard seed meal in the fall at 500 lb/acre, 1000 lb/acre and 2000 lb/acre. Bluegrass seed yield from the seed meal treatments were generally as high as the burn control and most were markedly better in yield response than the rake and straw removal treatment. Highest seed yield were obtained from the Lower rates of yellow or oriental mustard. Overall there is strong suggestion that Brassica seed meal application may offer an alternative to burning bluegrass to increase seed yield potential.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Enhanced Biofumigation and Composting Systems for Strawberry

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Keywords: isothiocyanates, plasticulture, mustard meal

Presentation Type: Poster

Abstract:

A three-year study was initiated in 2003 to study the effects of timing, cover and rates of soil incorporation of mustard meal (MM) in combination with mushroom composting (MC) on soilborne diseases and strawberry yield. The strawberry plants were planted into a raised-bed plasticulture system after treatment. The treatments had very little effect the first year on weed populations or yield. >Chandler= plants in beds treated with MM tended to have slightly less anthranose in spring of 2004. Plants on beds treated with 2.24 kg/ha MM had statistically significantly ($P < 0.05$) less incidence of the disease. After a second year of treatment, >Sweet Charlie= plants grown on beds treated with MM had an increase in the total strawberry yield (an average increase was 17%). Plants on beds treated with 4.48 kg/ha and immediately sealed had 27% more yield than plants on untreated beds.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Glucosinolate-containing seedmeals with bioherbicidal activity

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Keywords: seedmeals, glucosinolates, herbicides

Presentation Type: Poster

Abstract:

Seedmeals from fifteen glucosinolate-containing plants were analyzed for herbicidal activity when seedmeals were added to a sandy loam soil at concentrations of 0.1, 0.5 and 1.0% (w/w), using wheat (*Triticum aestivum*) and sicklepod (*Senna obtusifolia*) as bioassay species. For wheat, all of the seedmeals significantly inhibited seedling emergence at the 1.0% concentration. At the 0.1% concentration three of the seedmeals (*Brassica juncea*, *Lunaria annua*, and *Thlaspi arvense*) completely inhibited wheat emergence. For sicklepod emergence, while none were completely inhibitory at the 0.1 % level, eight of the seedmeals (*B. juncea*, *T. arvense*, *Eruca vesicaria*, *Erysimum x allioni*, *Erysimum cheiri*, *Lepidium sativum*, *Lobularia maritima*, and *Matthiola longipetala*) were completely inhibitory at the 1% level. Intact glucosinolates and their corresponding hydrolysis products varied among the seedmeals with the highest activity. Major hydrolysis products produced by the seedmeals with the most phytotoxicity, respectively, included allyl isothiocyanate, allyl thiocyanate, isopropyl isothiocyanate, 3-butenyl isothiocyanate, 4-methylthiobutyl isothiocyanate and 6-methylthiohexyl isothiocyanate. From our data it appears that both the type and concentration of glucosinolates and their hydrolysis products present in the seedmeals affect seed emergence inhibition.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Evaluation of alternative strategies for the control of root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw. on tomato crop, in plastic greenhouse

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Keywords: Biocidal plant, *Meloidogyne incognita*, plant extracts

Presentation Type: Poster

Abstract:

The effectiveness of natural and chemical substances in the control of root-knot nematode *Meloidogyne incognita* was evaluated on a tomato crop in two plastic greenhouses, in sandy soil of Ferrara province (Po Valley, Emilia-Romagna, Italy). In the first one tomato was preceded by a nematocidal green manure of *Eruca sativa* cv Nemat, then, in both greenhouses, three products based on *Quillaja saponaria* extract, *Tagetes erecta* oil

and brassica defatted meal were applied and compared with a chemical control (Cadusafos) and an untreated one. The results showed a statistically significant effectiveness among treatments, as in nematode reproduction factor ($0.9 < R < 1.1$), as in infestation root index (2.4-1.9 in the untreated control and in *Q. saponaria*, 1.3-0.5 in *T. erecta*, 1 in brassica defatted meal and 0.4-0.2 in cadusafos) and in fruit weight (from 3.8-4.2 kg per plant in the control, brassica defatted meals and cadusafos, to 5.7-4.9 in *Q. saponaria* and in *T. erecta*), while the factorial analysis registered a statistical relevance of nematicidal green manure in decreasing the initial nematode charge in the soil till safe levels for the following tomato crop. The lower yield of the two thesis with the best nematode control was due to phytotoxic problems arisen after a too early transplanting.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: A Liquid Formulation as Biopesticide for the Control of California Red Scale (*Aonidiella aurantii* Maskell)

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Keywords: Mineral oil, Epigeal pathogens, Biodegradable

Presentation Type: Poster

Abstract:

In recent years, the wide use of chemicals in crop protection has caused resistance phenomena in plant pathogens, environmental pollution and a severe impact on human and animal health. In the meantime, some very interesting results were obtained in soil biofumigation by biocidal seed meal obtained from Brassicaceae materials (patent N. BO 2002 A 000544). Here the insecticidal effects of a liquid formulation based on 100% vegetable compounds toward some pests of the plant epigeal part will be reported and discussed.

The first innovation was the combination of mineral oils with the “Triumph-Biofence” technology. The mortality of adult red scale on detached orange fruits treated with a mineral oil emulsion activated by Biofence technology was 100%, significantly greater than mineral oil alone (47.5%). With the aim of improving biodegradability and lowering environmental impact of the formulation mineral oils were substituted by vegetable one with no statistical differences. Even this time, vegetable oil alone determined a mortality reached (42.5%) significantly lower than the treatment with new technology (97.5%).

A clear “dose-effect” of liquid formulation was demonstrated. Linear regression between values of mortality and doses showed $R^2 = 0.936$ and $F = 29.01$ on mineral oil, and $R^2 = 0.944$ and $F = 33.9$, on vegetable oil.

These results are encouraging although further trials aimed at assessing the activity towards other insect species and mites are necessary. It would be interesting to evaluate the formulation not only on the adult phase but even on eggs and immature stages.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: LARGE SCALE BIOFUMIGATION OF FRUIT WITH ALLYL-ISOTHIOCYANATE FROM BRASSICA MEALS FOR CONTROLLING POSTHARVEST PATHOGENS: PRELIMINARY RESULTS

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Keywords: Brassica meal, Fruit pathogens, Postharvest

Presentation Type: Poster

Abstract:

Replacement of fungicidal fruit treatments to control post-harvest diseases with microbial antagonists or natural substances with biocidal activity is growing in interest. This is due both for the occurrence of pathogen resistance strains toward existing molecules and the pressure from public opinion to reduce their use applying, more healthy and environmental friendly practice. Among these natural substances, the hydrolysis products via myrosinase of glucosinolates, mainly isothiocyanates, has been evidenced to be effective in controlling some important pathogens of fruit during postharvest phase both in vitro and in vivo test. In addition these compounds also show control of thiabendazole resistant strains (1, 2). Initially, in vivo tests have been carried out in a little cabinet (100L) on a sample of 100 fruits. These trials showed, among the assayed isothiocyanates, allylisothiocyanate from sinigrine as the more interesting to be used in fruits fumigation. On the basis of the encouraging positive results a pre-pilot plant has been built up (3, 4) and we are now realizing large scale trials to control *Monilinia laxa* on stone fruits and *Penicillium expansum* on pears by fumigation of fruit, before storage, with allylisothiocyanate produced in situ from defatted meals of brassica. The preliminary results of these trials are presented and discussed.

References

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- 4) Bernardi R., Mari M., Leoni O., Casalini L., Cinti S. and S. Palmieri (2005) Proceedings of the Annual Meeting of the Association for the Advancement of Industrial Crops: International Conference on Industrial Crops and Rural Development (17-21 September 2005, Murcia, Spain) pp 467-474

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Brassicaceae Seed Meals as a Nitrogen Source in Carrot and Strawberry Production

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Keywords: Brassicaceae seed meal, plant-available nitrogen,

Presentation Type: Poster

Abstract:

Brassicaceae seed meals, by-products of mustard and canola oil production, average 6% nitrogen (N) by weight and have the potential for use as a soil N amendment. Although the efficacy of seed meals as biofumigants has been investigated, information regarding utility as a nitrogen source is scarce. A field study was initiated to investigate the influence of *Brassica juncea*, *Brassica napus*, and *Sinapis alba* meals on soil and plant (carrot and strawberry) parameters. Among 2 t/ha rates and the unamended control, carrot N uptake and yields were not different and across all treatments, averaged 117 kg N/ha and 37 t/ha., respectively. Increases in plant-available N (NO₃⁻ and NH₄⁺) in soil, from pre-amendment to harvest, were 85, 66, 36, and 15 kg N/ha for *B. napus*, *B. juncea*, *S. alba*, and the control, respectively. Preliminary data indicate similar trends in plant-available N in plots planted to strawberry. Strawberries displayed excellent vegetative growth, with prolific runner and daughter-plant establishment. Leaf percent N values were similar among treatments and within the accepted sufficiency range, averaging 2.3% across treatments. All Brassicaceae seed meals increased plant-available N relative to the control, however mineralization rates vary among species possibly due to different glucosinolate types and concentrations in the seed meals.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Control of *Rhizoctonia solani* in lily by biofumigation with Brassica seed meal

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Keywords: Brassica seed meal, biofumigation, *Rhizoctonia solani*

Presentation Type: Poster

Abstract:

Rhizoctonia solani causes bare patch disease in bulb crops like tulip, iris, lily and gladiolus. Broad-spectrum soil fumigants, e.g. methylbromide, dichloropropene and metam sodium, are not allowed for environmental reasons. Only one specific fungicide is available for application in lily, with a high risk for resistance development. In order to reduce the use and dependence on fungicides, non-chemical control methods have to be developed. So far, several alternative measures have been tested, including antagonists, composts, and biofumigation. In a field experiment with lily, application of defatted seed meal from *Brassica carinata* (2.5 ton/ha Biofence, ISCI) resulted in a better crop stand compared to the non-treated control, probably due to increased availability of nitrogen from the meal. Bulb yield was increased with 70%, and total nitrogen uptake was increased with 80% after seed meal application. In infested soil with *Rhizoctonia solani*, application of seed meal resulted in significant control of stem infection in lily. Percentage of healthy plants was 20% in the infested control treatment and 60% in the infested seed meal treatment. The efficacy of seed meal was equal to the chemical treatment with azoxystrobin (6 l/ha Amistar, Syngenta). These are very promising results, that encourage to continue the research.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Biofumigation as alternative method for management root-knot nematode *Meloidogyne incognita* affecting tomato under field conditions

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Keywords: Biofumigation, canola seed meal, root-knot nematode, tomato, biocontrol, organic amendment, root-knot nematode, tomato, field conditions, root-knot nematode, canola seed meal, tomato.

Presentation Type: Poster

Abstract:

Crop losses inflicted by nematodes are becoming one of the major limiting factors affecting plant growth and yield. Tomato is a highly susceptible crop to root-knot disease caused by the nematode *Meloidogyne incognita*. The prolonged and overuse of chemical nematicides has resulted in several hazardous which led to search for safe and more compatible alternatives among which natural products are of first importance. The Brassica plants (containing glucosinolates their break down products serve as anti-nematode components) have received particular attention. Three doses of canola *Brassica napus* seed meal 4, 6 and 8 tons/feddans used as organic amendment incorporating into the soil were evaluated against root-knot nematode *Meloidogyne incognita* inhabiting field planting with tomato Supper strain B. All three doses significantly affect root-knot population in soil, galls formation and yield production which rise to 39% over control for the highest dose.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Brassica juncea seed meal for fungus gnats control associated with houseplants with consideration of phytotoxicity to specific plant species.

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Keywords: fungus gnats, Oriental mustard, Indian Mustard

Presentation Type: Poster

Abstract:

Brassicaceous seed meals may be used to control soil-based insect pests in horticultural systems; however, phytotoxicity is a concern. Bioassays were conducted to assess the potential of *Brassica juncea* L. seed meal for control of fungus gnat larvae and phytotoxicity to four common houseplant species. Two rates of *B. juncea* L., 'Pacific Gold,' seed meal (1.7 g and 4.2 g), no meal, 4.2 g *B. napus* 'Athena' canola seed meal (control), and 10ml/L Gnatrol™ (standard) were applied to plants of each species in 10-cm diameter pots by spreading the meal on the surface of the potting medium. Fungus gnat mortality, plant height, leaf number, and dry shoot and root weights were measured 2 wk post treatment. Fungus gnat larval mortality was significantly higher with the 4.2 g *B. juncea* seed meal treatment than the other treatments. Plants showed significant phytotoxic post-treatment effects with the *B. juncea* seed meal treatments at both doses. *Brassica juncea* seed meal is toxic to fungus gnat larvae, but it may show signs of phytotoxicity in some plant species, suggesting that all target plant species should be evaluated prior to use of this product.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Trichoderma strains tolerant to Brassica meals for a combined use in biofumigation

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Keywords: Trichoderma, Brassica carinata, seed meals

Presentation Type: Poster

Abstract:

Trichoderma are saprophytic fungi living on debris in soil, having characteristics of antagonism towards pathogenic fungi, as well as ability to induce resistance in plants. Thus selected Trichoderma strains are currently utilised as effective biocontrol agents towards many plant pathogens, especially for soil-borne diseases. In biofumigation by means of Brassica meal incorporation into the soil, rebound effects on pathogen population were observed after a first decline, due to the enrichment in organic substrate, which tend to favour pathogen re-growth.

A multiple in vitro screening was conducted among 40 Trichoderma isolates of different species and origin, aiming at identifying some strains i) tolerant to biocidal compounds released by Brassica carinata meals ii) antagonist towards soil-borne pathogens iii) capable to readily colonize Brassica meal, to the detriment of pathogens re-growth.

A large variability was observed among Trichoderma isolates. Two of them, showing the best performances in the laboratory tests, were selected for preliminary trials of joint incorporation with Brassica meal into soil naturally infested or artificially inoculated by Pythium in pots under controlled conditions. The trials are currently under evaluation.

Session Theme: Seed Meals and Dried Tissues as Biopesticides

Title: Cruciferous materials in biofumigation and not only....

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Keywords: Pellet, Atylisothiocyanate, Mineral oils

Presentation Type: Oral

Abstract:

Cruciferous seed meal can give additional applicative perspectives to biocidal green manure technique in biofumigation, but for their efficacy the defatting procedure is fundamental to reach a good isothiocyanate release after hydration. Using the best meal preparation the efficacy of seed meals, even at full field level, was very interesting in the control of several soil borne pathogens such as nematodes and wireworms. Some trials, including a rotation experience in greenhouse, using both green manure and pellet, will be reported and discussed not only for the efficacy on soil borne pest control but even for the yield influence. The opinion of the farmers that applied biofumigation will be also reported. These exciting results and the possibility of producing meals at an industrial level conducted to their commercialisation under the name of Biofence, but the availability of these materials and the possibility of controlling isothiocyanate release opens new perspectives in different application fields. So a new patented proposal of a liquid formulation produced by a Biofence technology is at moment studied for its extremely interesting potentials in controlling some pathogens and pests as a 100% vegetable alternative to mineral oils.

Appendix 2

Copy of the abstract of my paper to the Symposium.

Environmental impacts on the biological activity of contrasting isothiocyanates.

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Abstract

Four pure isothiocyanates (methyl, 2-propenyl, benzyl and 2-phenylethyl), hydrolysing tissue of two brassicas rich in either 2-propenyl or 2-phenylethyl ITC, and the methyl ITC-generating pesticide metam-sodium were tested in vapour exposure tests for biological activity against a model soil insect both *in vitro* and in the presence of three contrasting soils and under four temperatures from 5-20 °C. The purpose was to develop an understanding of the factors controlling ITC release and maintenance in soil in order to identify advantageous attributes to seek in utilising brassicas for ITC-based biofumigation. Methyl ITC, structurally the simplest and the most volatile was the most biologically active ITC under all conditions. It was less affected by the presence of soil and lower temperature than the longer-chain aliphatic 2-propenyl ITC. The activity of the less volatile aromatic ITCs was reduced much more by soil, with a decline up to many thousand-fold in the presence of soil with high organic matter content at lower temperature. Metam-sodium closely reflected the methyl ITC results. The results indicate that brassicas rich in aliphatic ITCs are more likely to have the potential to exert stronger ITC-based biofumigation effects than those similarly rich in aromatic ITCs.

Appendix 3

Embedded .pdf file (electronic version of this report) or printout (hardcopy version of this report) of my oral presentation at the Symposium.



Appendix 4

Embedded .pdf file (electronic version of this report) or reprint (hardcopy version of this report) of a recently-published review of biofumigation (and the related topic of enhanced biodegradation of soil-applied pesticides).

