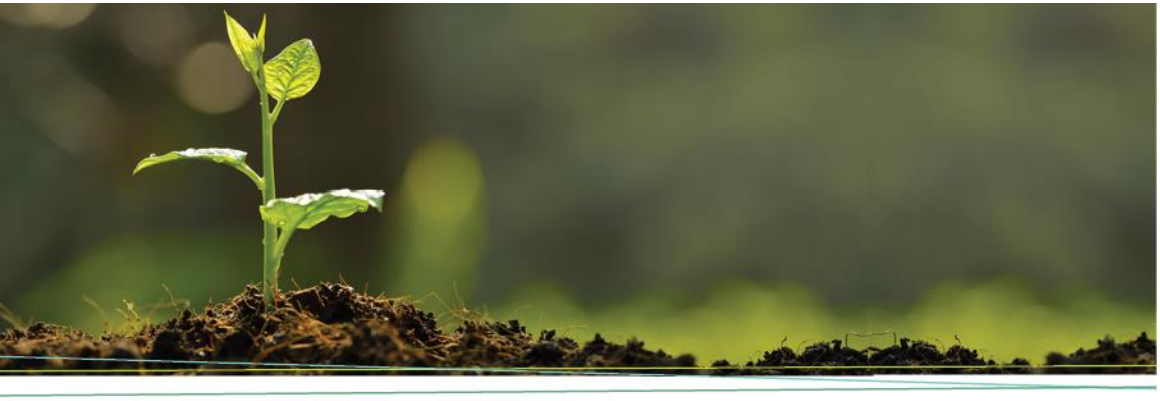

Focus Group SOIL-BORNE DISEASES

Mini-paper - *Biofumigation for the control of soil-borne diseases*

John Clarkson[1], Vincent Michel[2] and Roy Neilson[3]

[1] Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne Campus, Warwick, CV35 9EF, UK.
[2] Federal Department of Economic Affairs, Education and Research EAER, Agroscope, Institute for Plant Production Sciences, Route des Vergers 18, 1964 Conthey, Switzerland
[3] The James Hutton Institute, Dundee, DD2 5DA, Scotland, UK

What is biofumigation?

The term 'biofumigation' was originally coined by J.A. Kirkegaard to describe the process of growing, macerating / incorporating certain *Brassica* or related species into the soil, leading to the release of isothiocyanate compounds (ITCs) through the hydrolysis of glucosinolate (GSL) compounds contained in the plant tissues (Kirkegaard et al., 1993). This can result in a suppressive effect on a range of soil borne pests and diseases. Since then, the term 'biofumigation' has been used rather loosely and incorrectly in some contexts, to describe any beneficial effects derived from the use of green manures, organic amendments and composts. In this mini-paper, biofumigation is considered in its strictest sense as referring to the use of glucosinolate-containing plant material with the intention of enabling ITC-mediated pest and disease suppression. Biofumigation could be considered as a 'natural' alternative to chemical fumigation and there is an analogy with the use of metam sodium which releases methyl-ITC, to control a variety of soilborne diseases.

Mode of action of biofumigant crops

Glucosinolate / isothiocyanate and chemical effects

Many cruciferous species produce significant levels of glucosinolates (GSLs), which are held in plant cells separately from the enzyme myrosinase and are in themselves not fungitoxic (Manici et al., 1997). However, when plant cells are ruptured the GSLs and myrosinase come into contact and are hydrolysed in the presence of water to release various products, including ITCs (Vig et al., 2009; Figure 1). ITCs have a wide range of biocidal characteristics and are acutely toxic to a variety of pests and pathogens (Chew, 1987). GSLs are β-thioglucoside N-hydroxysulfates, with a side group (R) and a sulphur-linked β-d-glucopyranose moiety (Fahey et al., 2001) and are classified as aliphatic, aromatic or indole GSLs according to the type of side chain (Fenwick et al., 1983; Figure 1). The R group is retained in the ITCs and influences its biological activity.

Commonly used biofumigant plants which include brown mustards, white mustards, radishes and rocket species contain different GSLs hence resulting in different ITCs being released (Table 1). Although some biofumigants have a dominant GSL (Table 1), others may contain a mixture. Different cultivars or plant parts may also contain different amounts or profiles of GSLs. For instance, 2 phenylethyl GSL is mainly produced in the roots of *B. napus* (Potter et al., 2000).

funded by European Commission

Table 1: Some commonly used biofumigant crops and their respective GSLs and ITCs

Common name	GSL	ITC
Brown mustard (*Brassica juncea*)	Sinigrin	2-propenyl-ITC (= allyl-ITC)
Black mustard (*Brassica nigra*)	Sinigrin	2-propenyl-ITC (= allyl-ITC)
White mustard (*Sinapsis alba*)	Sinalbin	4-hydroxybenzyl-ITC
Radish (*Raphanus sativus*)	Glucoraphenin	4-methylsulfinyl-3-butenyl--ITC
Rocket (*Eruca sativa*)	Glucoerucin	4-methylthiobutyl-ITC

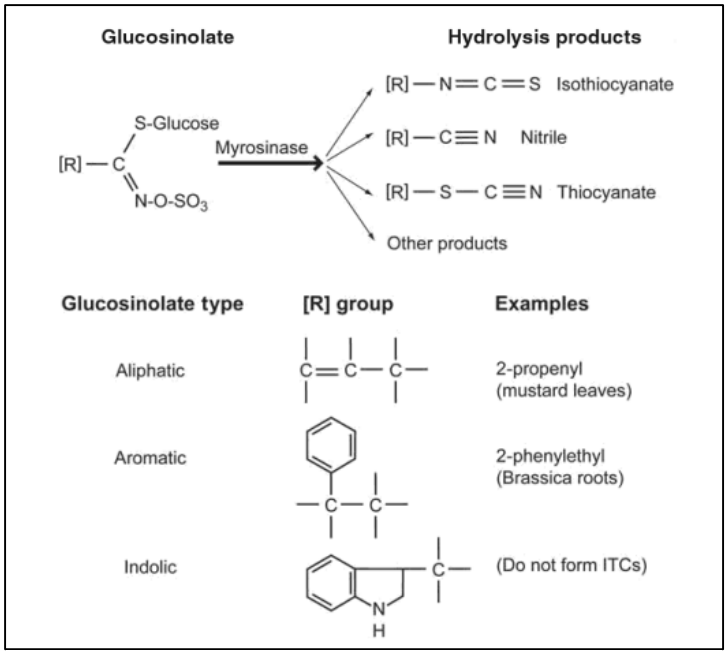

Figure 1: Glucosinolate structure and products of hydrolysis (from Kirkegaard, 2009)

Although ITCs have generally been the focus of biofumigation-related research and are considered the most bioactive of the hydrolysis products, other compounds such as non-glucosinolate sulphur-containing compounds, fatty acids, nitriles and ionic thiocyanates may also affect pest and pathogen populations (Matthiessen & Kirkegaard, 2006) and may explain why some low GSL brassica crops have been shown to have suppressive activity against soilborne diseases.

Other effects

As researchers have been trying to understand, demonstrate and optimise the biofumigation process, and as more studies have now employed quantification of GSLs or ITCs, it has become increasingly apparent that the beneficial effects observed may not always be related to the activity of GSL-based hydrolysis compounds and that other mechanisms may play a complimentary or more dominant role in disease suppression. This is probably as a result of incorporating large amounts of organic matter into the soil potentially resulting in improved soil structure, increased nutrient availability, increased water holding capacity and stimulation of beneficial / pathogen-suppressive microbial communities. However, disentangling the multitude of mechanisms which may operate is a challenge but advances in next generation sequencing to characterise microbial populations associated with the observed

funded by European Commission

disease suppression may provide further insights for optimising ITC and non-ITC benefits of biofumigants.

Biofumigant crops for control of soilborne diseases

There are various reports of soilborne plant disease suppression through the use of biofumigant plants, some of which have been summarised by Matthiessen & Kirkegaard (2006) and Motisi et al., (2010) but some of the important groups of pathogens have been targeted including *Aphanomyces*, *Fusarium*, *Gaumannomyces*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinia* and *Verticillium* as well as species of endoparasitic and semi-endoparasitic nematodes such as *Globodera*, *Meloidogne*, *Pratylenchus* and *Tylenchus*. There has been less emphasis however regarding the effect of biofumigation on free-living nematode species. Overall, research concerning biofumigation for control of soilborne pathogens does not constitute a major area of work and there has been a lack of a consistent experimental approach. Hence, levels of control have varied considerably between different target organisms and different studies which highlights one of the major problems associated with adopting biofumigation commercially. It is clear however from *in vitro* studies that pathogens vary in their sensitivity to different ITCs (e.g. Brown and Morra, 1997; Smith and Kirkegaard, 2002) as do the susceptibility of different life cycle stages and structures such as spores, mycelium and sclerotia. It is clear therefore that different pathogens will require different biofumigants for effective control and further work is required to elucidate the best biofumigant(s) for specific disease problems.

Use of biofumigant crops

Biofumigant crops can be used in a number of different ways for disease control:

Intercropping and rotations with biofumigants

In this case, above-ground plant material is harvested and hence activity against plant pathogens relies on GSLs, ITCs or other compounds released through leaf washings or root exudates. Several studies have detected both GSLs and ITCs in the rhizosphere which have been implicated in the suppression of pests and pathogens (van Dam et al., 2009) and soil organisms with myrosinase activity have been shown to mediate the conversion of GSLs to ITCs. Moreover, GSLs and ITCs can affect the composition of rhizosphere communities which may also suppress soilborne plant diseases and some common beneficial microbial species such as *Trichoderma* show high tolerances to ITCs (Galetti et al., 2008; Gimsing and Kirkegaard, 2009, Smith and Kirkegaard, 2002).

Incorporation of biofumigants

This is the most recognised use of biofumigant plants where a crop is grown specifically for incorporation with the aim of converting GSLs to ITCs. To achieve high levels of ITC release, comprehensive maceration of plant tissue is required followed by rapid incorporation into soil and addition of water if required to ensure complete hydrolysis (Matthiessen & Kirkegaard, 2006; Kirkegaard, 2009). As some ITCs are quite volatile, sealing/smearing the soil with a roller or covering the soil with plastic mulch may be beneficial (Kirkegaard and Matthiessen, 2004).

Seed meals and other processed biofumigants

Defatted seed meal produced after the processing of brassica seeds for oil (e.g. in mustard crops) also offer a convenient source of high GSL material for soil amendment as the myrosinase required for hydrolysis to ITCs remains intact (Brown and Mazzola, 1997). These materials have shown promise against a number of soilborne plant pathogens including *Rhizoctonia* spp. (Mazzola et al., 2007) and *Meloidogne* spp. (Lazzeri et al., 2009). A liquid formulation has also been developed from defatted *B. carinata* seed meal which had activity against *Meloidogyne incognita* (De Nicola et al., 2012). Other products based on pellets of dried-high GSL plants have also been developed and showed good

funded by European Commission

activity *in vitro* against *Pythium* and *Rhizoctonia* (Lazzeri et al., 2004). Simple drying of biofumigant plants can also be effective at conserving GSLs/myrosinase as reported by Michel (2014) where dried brown mustard plants (mustard hay) significantly reduced the number of *Verticillium dahliae* microsclerotia in a greenhouse soil. The main advantages of this approach are that these products can be used at times of year when growth of biofumigant plants is restricted (e.g. in the winter), can be more easily integrated in rotations, and are more amenable to intensive production systems where break crops are not used and there is only a short non-cropped period (e.g. protected horticulture).

Green manures and trap crops

As indicated earlier, use of biofumigant crops can have additional benefits in addition to ITC-based disease suppression such as potential (transient) increase in organic matter, better soil structure and nutrient release, all of which may increase plant vigour and growth, hence indirectly reducing the impact of soilborne plant pathogens. The use of green manures and cover crops to control soilborne diseases is the subject of another EIP-AGRI mini-paper and is not further addressed here. Some specific brassica green manures are also used as trap crops for the control of nematodes (Jaffee et al., 1998) but again this is outside the scope of this mini-paper.

Maximising ITC-mediated disease suppression

The reviews of Matthiessen & Kirkegaard (2006) and Kirkegaard (2009) outline very well the main ways in which biofumigation can be optimised. In summary these are:

1. Establish a relationship between GSL, ITC levels and pathogen suppression: effectively different biofumigant crops need to be screened for activity against the target pathogen. This can be done through *in vitro* studies particularly focussing on the effect on resting structures such chlamydospores, sclerotia and microsclerotia or ideally in soil-based assays under controlled conditions to establish the best biofumigant for a particular soilborne disease before extensive field experiments are performed. Recently an optical platform has been established that could be used as a real-time biological screen to assess effect on target pathogens post ITC application (Downie et al., 2012).
2. Select most appropriate biofumigant or product: in addition to considering activity against the target pathogen (1), brassica species giving rise to aliphatic short chained ITCs may be more efficient than those resulting in long chained aromatic ITCs due to increased volatility and reduced sorption of these compounds to organic matter. The biofumigant species may also need to be selected based on winter hardiness, growth rate and GSL production at different times of year depending on when it is intended to be incorporated. Seed meals and processed biofumigants may be more appropriate 1) for small, intensively cropped areas such as in greenhouses and polytunnels, and 2) for the control of more resistant resting structures such as microsclerotia of *Verticillium dahliae* (Neubauer et al., 2014).
3. Optimise agronomy: as high amounts of biomass are required for biofumigation, agronomic factors such as seed rate, time of sowing, fertiliser application and optimal incorporation time all need to be considered in order to maximise biofumigant crop yield and GSL level. For instance, GSL concentration in plant tissue has been reported to be modified by nitrogen and sulphur supply mediated by fertilization (Li et al., 2007).
4. Grow and incorporate high amounts of biofumigant biomass: unpublished data from J.A Kirkegaard suggest that up to 5% w/w fresh biomass is required to maximise pathogen suppression and typically 50 t ha^{-1} is required to achieve an efficacious result (Tozer Seeds, pers. comm.).
5. Maximise incorporation efficacy and ITC release: cell disruption is key to efficient conversion of GSLs to ITCs and equipment for pulverising and crushing plant material is superior to

funded by European Commission

chopping. Immediate incorporation is then required with addition of water to maximise GSL hydrolysis and sealing the soil or tarping will maximise ITC retention.

6. Allow 1-2 weeks before planting following crops: ITCs and other products of GSL hydrolysis can be phytotoxic.

Opinion: future directions and challenges

Since the term 'biofumigation' was first introduced just over 20 years ago there has been relatively little large scale research and commercial exploitation of the technique. However, the political and social landscape is now changing, with an increased desire from supermarkets and other end users for reduced pesticide inputs and driven also by EU legislation through the introduction of the Sustainable Use Directive and associated National IPM plans for each member state. Moreover, many of the traditional chemically-based approaches to soilborne disease control have been banned or restricted and the rate of development of new actives has declined. Hence the scene is potentially set for renewed interest and potential funding initiatives in this area.

Research

Although there has been some good progress in aspects of biofumigation research, there has largely been a failure to implement the approach for soilborne disease control on a large scale in Europe despite some adoption by certain areas of the USA. To a certain extent this has been due to a fragmented and underfunded research community and the lack of consistent approaches and results in the field. However, the approaches and framework for future work as advocated by Matthiessen & Kirkegaard (2006) and more recently Motisi et al., (2010) give some direction for researchers. This includes separating out the effects of the growing biofumigant crop and the incorporation phase, standard measurement of GSLs / ITCs, identifying the most appropriate biofumigant for a particular target pathogen, understanding the relative importance of ITCs compared to other potential mechanisms of control (e.g. benefits related to organic matter incorporation such as increased microbial community activity), and selection or breeding of new high GSL brassica lines adapted to the local environmental conditions. More robust experimental approaches and new technologies such as next generation sequencing based analysis of microbial communities will potentially help address some of these challenges and begin to unravel the complexity of the biological systems associated with biofumigation. The research base for biofumigation needs to expand and engage a multidisciplinary approach and this is only just beginning to be co-ordinated through meetings such as the International Biofumigation Symposium which first took place in 2004.

Commercial implementation

Historically, social and cultural barriers have impeded the uptake of biofumigation with the dual concerns that adoption would accelerate the removal of synthetic pesticides and the lack of trust regarding the equivalent efficacy of biofumigant crops. However, there now appears to be an increasing interest by farmers and growers in biofumigation but the variability in levels of disease control or the lack of any evidence for the benefits of this approach for particular crop-pathogen combinations are still major barriers to widespread adoption. This urgently needs to be addressed, ideally through collaborative approaches and projects between researchers and industry. There is also still a lack of consistent advice and information on some of the basic agronomy associated with growing biofumigants for maximum GSL production such as seed rate, fertiliser applications, sowing dates and biofumigant crop selection which could be further addressed by the biofumigant seed producers. In addition, appropriate machinery optimised for maceration and incorporation is not universally accessible to growers and farmers. However, despite these barriers to implementation, there are some innovative growers who have already adopted biofumigation and integrated this technique into their farming practice. This might be in response to specific problems and it's perhaps more often the case that plant parasitic nematodes are targeted more often than soilborne fungal diseases. This may be because there is more research evidence and experience in using biofumigation for nematode control. Hence, some early adopters of the technique include potato farmers where

funded by European Commission

potato cyst nematode (PCN) is a universal problem and biofumigants can be easily integrated into rotations in combination with the use of potato cultivars partially resistant to PCN.

In conclusion, biofumigation has good potential for management of a range of soilborne diseases but much more evidence-based research and development is needed to implement the technique more widely in order to address the main issue of variability. It is most likely that biofumigation will be promoted on the basis of its multiple benefits to farmers in addition to potential disease control and that it will form just one part of an integrated strategy for the more intractable soilborne diseases that could include other approaches such as biological control (see EIP-AGRI biological control mini-paper). To overcome social and cultural reticence in the use of biofumigants and to promote adherence to recent EU directives on pesticide usage, an incentivisation scheme perhaps as a component of CAP reform could also be a way forward.

Acknowledgements

The authors acknowledge the help and advice of John Kirkegaard (CSIRO, Australia) and Matthew Back (Harper Adams University, UK) in preparing this minipaper.

References

Brown PD, Morra MJ, 1997. Control of soilborne plant pests using glucosinolate-containing plants. In: Donald LS, ed. *Advances in Agronomy Volume 61*. Academic Press, 167-231.

Chew FS, 1987. Biologically active natural products - Potential use in agriculture. In: Comstock MJ, ed. *ACS Symposium Series.* USA: American Chemical Society.

De Nicola GR, D'avino L, Curto G, Malaguti L, Ugolini L, Cinti S, Patalano G, Lazzeri L, 2013. A new biobased liquid formulation with biofumigant and fertilising properties for drip irrigation distribution. *Industrial Crops and Products* **42**, 113-8.

Downie H, Holden N, Otten W, Spiers AJ, Velntine TA, Dupuy LX, 2012. Transparent soil for imaging the rhizosphere. *PLoS One* **7**, e44276.

Fahey JW, Zalcmann AT, Talalay P, 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **56**, 5-51.

Fenwick GR, Heaney RK, Mullin WJ, 1983. Glucosinolates and their breakdown products in food and food plants. *Critical Reviews in Food Science and Nutrition* **18**, 123-201.

Galletti S, Sala E, Leoni O, Burzi PL, Cerato C, 2008. *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. *Biological Control* **45**, 319-27.

Gimsing A, Kirkegaard J, 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. *Phytochemistry Reviews* **8**, 299-310.

Jaffee BA, Ferris H, Scow KM, 1998. Nematode-trapping fungi in organic and conventional cropping systems. *Phytopathology* **88**, 344-50.

Kirkegaard J, 2009. Biofumigation for plant disease control – from the fundamentals to the farming system. In: *Disease Control in Crops*. Wiley-Blackwell, 172-95.

Kirkegaard J, Matthiessen J, (2004). Developing and refining the biofumigation concept. *Agroindustria* **3**, 233-239.

Kirkegaard JA, Gardner PA, Desmarchelier JM, Angus JF, 1993. Biofumigation - using Brassica species to control pests and diseases in horticulture and agriculture. In: *Proceedings of the 9th Australian Research Assembly on Brassicas* pp 77-8. N. Wratten and RJ Mailer eds.

Lazzeri L, Curto G, Dallavalle E, D'avino L, Malaguti L, Santi R, Patalano G, 2009. Nematicidal efficacy of biofumigation by defatted *Brassicaceae* meal for control of *Meloidogyne incognita* (Kofoid et White) Chitw. on a full field zucchini crop. *Journal of Sustainable Agriculture* **33**, 349-58.

Lazzeri L, Leoni O, Manici LM, 2004. Biocidal plant dried pellets for biofumigation. *Industrial Crops and Products* **20**, 59-65.

Li S, Schonhof I, Krumbein A, Li L, Stützel H, Schreiner M, 2007. Glucosinolate concentration in turnip (*Brassica rapa* ssp. *rapifera* L.) roots as affected by nitrogen and sulfur supply. *Journal of Agricultural and Food Chemistry* **55**, 8452-8457.

Manici LM, Lazzeri L, Palmieri S, 1997. *In vitro* fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. *Journal of Agricultural and Food Chemistry* **45**, 2768-73.

Matthiessen JN, Kirkegaard JA, 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Critical Reviews in Plant Sciences* **25**, 235-65.

Mazzola M, Brown J, Izzo AD, Cohen MF 2007. Mechanism of action and efficacy of seed meal-induced pathogen suppression differ in a *Brassicaceae* species and time-dependent manner. *Phytopathology* **97**,454-460.

Michel VV, 2014. Ten years of biofumigation research in Switzerland. *Aspects of Applied Biology* **126**, 33-42.

Motisi N, Doré T, Lucas P, Montfort F, 2010. Dealing with the variability in biofumigation efficacy through an epidemiological framework. *Soil Biology and Biochemistry* **42**, 2044-57.

funded by European Commission

Neubauer C, Heitmann B, Müller C, 2014. Biofumigation potential of *Brassicaceae* cultivars to *Verticillium dahliae*. *European Journal of Plant Pathology* **140**, 341–352.
Potter M, Vanstone V, Davies K, Rathjen A, 2000. Breeding to increase the concentration of 2-phenylethyl glucosinolate in the roots of *Brassica napus*. *Journal of Chemical Ecology* **26**, 1811-20.
Smith BJ, Kirkegaard J, 2002. *In vitro* inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. *Plant Pathology* **51**, 585–593.
Van Dam N, Tytgat TG, Kirkegaard J, 2009. Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochemistry Reviews* **8**, 171-86.
Vig, AP, Rampal G, Thind, TS, Arora S, 2009. Bio-protective effects of glucosinolates – A review. *LWT - Food Science and Technology* **42**, 1561-72.

funded by European Commission