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Biofumigation: A control method for the soil-borne diseases

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KEY **W**ORDS : Biofumigation, Soil-borne diseases

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. Biofumigation is the suppression of soil born pests and diseases through the use of plants that produce inhibitory chemicals, also known as secondary metabolites. In most cases these biofumigant plants are chopped and incorporated into the soil so they can release their inhibitory chemicals.

So, "Biofumigation is the use of specialized cover crops, which are grown, mulched and incorporated into the soil prior to cropping. High biomass, especially roots, can provide the traditional benefits of green manure crops and if done right, naturally occurring compounds from the biofumigant crops can suppress soil-borne pests, diseases and weeds."

Common biofumigant crops :

Plants such as broccoli, cauliflower, mustard, rapeseed, and horseradish contain organic compounds called glucosinolates. When the tissues of these plants are damaged, biologically active chemicals are produced. One of the most important compounds released is isothiocyanate (ITC). The pungency of horseradish and the spicy taste of hot mustard are caused by ITCs released when the tissues are macerated. At low concentrations ITCs are considered beneficial to human health. At high concentrations ITCs are general biocides that behave much like commercial pesticides. In fact, several commercial pesticides including Dazomet, Vapam, and Vorlex depend on an ITC as the active ingredient for pest control.

Some commonly used biofumigant crops include:

– Mustard-oil seed crop
– Sorghum-cereal crop.

Why *Brassicas*?

Soil borne organisms are becoming more difficult to control due to pathogen resistance and restricted use of some chemicals. The cost of chemicals is also becoming a concern. Mustard is a well understood biofumigant. Its biofumigation properties have been studied for a number of years and scientists have developed a method to fully use these properties. Mustard and most other plants from the *Brassica* family produce chemicals called "glucosinolates". When glucosinolates come in contact with water and a family of enzyme myrosinase, contained in plant cells, they are transformed in another group of compounds called "isothiocyanate". It is these isothiocyanates that give mustard its biofumigation power. Isothiocyanates are also responsible for giving plants from the *Brassica* family their bitter/hot/spicy taste. The isothiocyanate that is produced by mustard is called "Allyl isothiocyanate" (AITC). AITC is a compound that is very similar to the compound that is contained in the commercial fumigant Vapam®.

Not all *Brassicas* are equal :

Mustard comes in many varieties but not all are equally as effective when it comes to biofumigation. Some mustard varieties produce more glucosinolates compared to others.

Brassica species produce a significant quantity of glucosinolates (GSLs) in their tissue. When these are hydrolyzed by the enzyme myrosinase which is also present in the *Brassica* tissues a range of products are produced that include the volatile, biocidal isothiocyanate (ITCs) which is similar to the active ingredient in the nematicide, metam-sodium (Vapam). For maximum biofumigant activity the plants need to be chopped and incorporated into moist soil when the tissue is lush and green. The glucosinolates are highest in the leaf tissue, but glucosinolate activity in leaves and roots too. So production practices need to promote lush leafy biomass. Plants should be flail chopped and incorporated no later than full bloom for best glucosinolate production. Brassica species can be utilized as late summer and early spring cover crops. Caliente Mustard Blends can tolerate temperatures to 19.4°F (-7°C), so they can be utilized as late summer and early spring cover crops.

Managing a biofumigant crop :

Growing a biofumigant crop requires good management and attention to detail similar to a vegetable crop. Unlike many of the low input, low management green manure crops, biofumigant crops may need some fertilizer and irrigation.

To get the most out of biofumigant crops you need to:

– Choose the right variety. There are several varieties available, each with specific requirements and benefits.

– Have the necessary equipment to manage the crop correctly.

– Plant at the best time within your rotation.

– Test soils to ensure appropriate nutrient management for the biofumigant crop as well as subsequent crops in your rotation. Make sure sulphur levels are adequate.

– Time biofumigant crop growth to maximize ITC production. GSL levels are highest at mid flowering.

– Seed at the rate recommended by the seed supplier

to get the most benefit.

– Macerate and incorporate. These should only be done when soil moisture levels are not too high, otherwise soil structure will be damaged.

– Incorporate the well-macerated biofumigant crop straight away to release the ITCs. Soil temperatures >12 degrees improve ITC formation.

– Benefits of biofumigants will not always occur after the first crop.

– Biofumigant crops cannot be grazed.

Soil incorporation :

As the growing season unfolds mustard should be monitored for flowering. Mustard has to be incorporated into the soil before full bloom and before it starts to produce seeds for the following reasons; 1) If mustard is left to produce viable seeds there is the potential for mustard to become a weed problem the following season; 2) Glucosinolate levels quickly decline once mustard plants begin to produce seeds. Ideally, mustard should be soil incorporated before full bloom (about 2 weeks after flowering has started).

In order to be successful with biofumigation the following procedure must be followed rigorously.

– Soil incorporation should be done before the mustard crop has reached full bloom.

– Soil incorporation should be done in the morning or evening. Avoiding hot sunny days.

– Soil incorporation should be done when soil has a good level of moisture. Do not incorporate mustard when the soil is dry.

– Prior to the actual incorporation, it is critical to chop and crushed as much plant material as possible to release the fumigant from plant cells. This can be done with a flail mower.

– Mustard must be incorporated immediately after mowing, 80 per cent of the fumigant gas will be released in the first 20 minutes after mowing.

– For ideal incorporation, choose a tool that will place as much plant material as possible into the top 15 to 20 centimeters. Do not use a plow.

– If possible, after incorporation the field should be rolled and packed to trap the fumigant gas in the soil. For small scale production systems and when possible, cover the area with a tarps to trap the gas in the soil. This will enhance the biofumigation effect.

– Finally, once the incorporation process is complete, leave the field undisturbed for 14 days to ensure that all the plant material can break down. Attempting to plant another crop before the 14 day period has passed will cause significant crop injury and hinder germination. If soil temperature is less than 10°C, a longer post incorporation period may be required for plant material to break down.

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 soil borne plant diseases and some common beneficial microbial species such as *Trichoderma* show high tolerances to ITCs (Galletti *et al.*, 2008; Gimsing and Kirkegaard, 2009 and Smith and Kirkegaard, 2002).

Incorporation of biofumigants :

This is the most recognized 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 and Kirkegaard, 2006 and 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. (Morra *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.,* 2013). Other products based on pellets of dried-high GSL plants have also been developed and showed good 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.

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) (Fig. 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) (Fig. 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).

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 and Kirkegaard, 2006) and may explain why some low GSL *Brassica* crops have been shown to have suppressive activity against soil borne diseases.

Other effects

As researchers have been trying to understand, demonstrate and optimize the biofumigation process, and as more studies have now employed quantification of

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

Common name	GSL	ITC
Brownmustard (*Brassica juncea*)	Sinigrin	2-propenyl-ITC (= allyl-ITC)
Blackmustard(*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

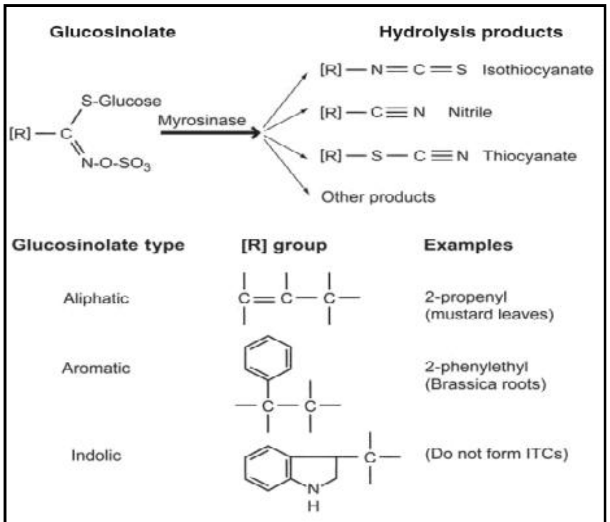

Fig. 1 : Glucosinolate structure and products of hydrolysis (Kirkegaard, 2009)

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 characterize microbial populations associated with the observed disease suppression may provide further insights for optimizing ITC and non-ITC benefits of biofumigants.

Biofumigant crops for control of soil borne diseases:

Growing a *Brassica* as a rotational crop is not ideal for all situations. It is important to know your major disease issues as *Brassicas* can increase levels of some pathogens. *Brassicas* are a host for *Sclerotinia sclerotiorum*, causal agent of white mould and therefore, if this is a significant problem in your potato production, growing *Brassicas* as a biofumigant may not be desirable. In addition, if crucifer cash crops such as broccoli are grown, the *Brassica* cover crop should be used several years before or after these cash crops due to potential issues with club root caused by *Plasmodiophora Brassicae*. This organism causes serious malformation of the roots which will reduce yield and marketability of the cash crop.

Brassicas have been shown to have a role in controlling *Rhizoctonia* (canker and black scurf), common scab (*Streptomyces scabies*), powdery scab (*Spongospora subterranean*), and *verticillium* wilt (*Verticillium dahliae*). Populations of other pathogen *viz.*, *Aphanomyces* spp., *Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Sclerotinia* spp., nematodes *viz.*, *Heterodera* (cyst), *Globodera*, *Pratylenchus* and *Tylenchus* and *Meloidogyne species* (*M. chitwoodi* and *M. hapla*) have be also reduced. The use of mustard as a biofumigant has also shown a decrease in damage caused by wireworm.

Growing a *Brassica* is not a silver bullet, and having one season/year of *Brassicas* in a 3- year rotation with potato may not result in dramatic changes in disease level.

The benefits are as :

Biofumigant crops in your farm's rotation can improve overall efficiency and productivity.

The benefits of correctly incorporating biofumigant crops include improvements in soil health and a reduction in farm inputs. In order to reap the full rewards of biofumigation,certain crop management and incorporation techniques must be used. Benefits are dependent on local climate and soil conditions, the type of biofumigant crop used and its management.

Soil biology :

Biofumigant crops act as break crops, disrupting the lifecycle of pests and diseases. Suppression may result from direct biocidal toxicity as well as indirectly through changes in the soil fauna and microbial community. Populations of beneficial micro-organisms, including mycorrhizal fungi, have been found to increase after biofumigant crops.

Weed suppression :

Early vigorous growth and improved plant vigour help to outcompete weeds. When incorporated correctly, the release of isothiocyanates (ITCs) from the biofumigant crop leads to the biocidal burning of weed seedlings.

Soil organic matter:

Organic matter is replenished in the soil after incorporation of the biofumigant crop. As micro-organisms break down organic matter they produce sticky substances that bind soil particles together into soil aggregates. This, in turn improves:

–Water infiltration, water and air holding capacity.

– Structural stability,reducing the risk of compaction.

– Soil friability, making the soil easier to work.

– The soil's resilience to wind and water erosion.

– Nutrient holding capacity.

– Overall biological activity.

– Root growth.

Organic matter also buffers against changes in pH, salinity or sodicity and it inactivates or filters toxic elements.

Nutrient cycling:

Deep-rooted break crops can access nutrients stored deeper within the soil profile that are unavailable to shallow-rooted crops. Better biological activity can lead to improved nutrient cycling and crop nutrient uptake. The nutrients become available to the next cash crop. Increased rates of nitrogen mineralisation following *Brassica* and other break crops have been recorded.

Maximizing ITC-mediated disease suppression:

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

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 soil borne 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).

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 micro-sclerotia of *Verticillium dahliae* (Neubauer *et al.*, 2014).

Optimise agronomy:

As high amounts of biomass are required for biofumigation, agronomic factors such as seed rate, time of sowing, fertilizer 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).

Grow and incorporate high amounts of biofumigant biomass:

J. A. Kirkegaard suggest that upto 5 per cent w/w fresh biomass is required to maximize pathogen suppression and typically 50 t ha^{-1} is required to achieve an efficacious result.

Maximize incorporation efficacy and ITC release:

Cell disruption is key to efficient conversion of GSLs to ITCs and equipment for pulverizing and crushing plant material is superior to chopping. Immediate incorporation is then required with addition of water to maximize GSL hydrolysis and sealing the soil or tarping will maximize ITC retention.

Allow 1-2 weeks before planting following crops:

ITCs and other products of GSL hydrolysis can be phytotoxic.

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.

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, fertilizer applications, sowing dates and biofumigant crop selection which could be further addressed by the biofumigant seed producers. In addition, appropriate machinery optimized 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 soil borne 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 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.

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 soil borne diseases that could include other approaches such as biological control.

Facts:

- Glucosinolates (GSLs) or cyanogenic gluco sides are responsible for the fumigation effect and are found *in Brassicas* or specialised sor ghums.
- When the biofumigant crop is macerated, GSLs are broken down by the enzyme myrosinase and isothiocyanates (ITCs) are produced immediately.
- ITCs are highly toxic compounds to many soil-borne pests, diseases and weed seedlings.
- To contain ITCs in the soil, the biofumigant crop must be finely macerated, incorporated directly and the soil surface sealed through irrigation, rain or rolling.

Conclusion :

When managed properly mustard offers another tool to help growers control soil born pests and diseases. The use of mustard as a biofumigant is particularly interesting for organic producers. It is important to strictly follow the outlined cultural practices if you want to have any chance of success using mustard as a biofumigant. Proper chopping of plant material and soil incorporation is of utmost importance. Although mustard is a remarkable biofumigant, it has similar benefits that is expected from any other cover crop such as; prevention of soil erosion, recycling of soil nutrients, improved soil structure and maintaining soil organic matter. Mustard can also acts as a deterrent to many insects (wireworm) and pests therefore it may prevent many problems from occurring in your field. Interestingly, there are other crops that show possible biofumigation effect such as but not limited to; buckwheat, pearl millet, sorghum-sudan grass, rape seed and oil seed radish.

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