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CHAPTER 2. EFFICIENCY OF OIL RADISH IN THE SYSTEM OF SOIL BIOFUMIGATION TECHNOLOGIES

2.1. Cruciferous plant species in the system of biofumigation as an innovative technology for the rehabilitation of degraded soils

Clarkson et al. (2020) noted that 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.

Biofumigation is the suppression of soil pests, nematodes and diseases by sowing plants that release certain chemicals into the soil that inhibit the development of pests (secondary metabolites). These plants include, among others, some types and varieties of mustard. To achieve a positive effect, these biofumigant plants are usually crushed and incorporated into the soil.

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The biofumigation market was estimated to be worth USD 100 million in 2023, and is expected to reach USD 140.71 million by 2030, growing at a CAGR of 5% from 2024 to 2030. The agricultural industry focused on controlling soil pests and diseases with natural substances and procedures is known as the biofumigation market. The process of biofumigation involves

the introduction of certain plants, mainly members of the Brassicaceae family (broccoli, radish and mustard), into the soil. These plants release glucosinolates when they are cut and added to the soil. Glucosinolates break down into biologically active chemicals, such as isothiocyanates, which are toxic to a variety of pests and soil-borne diseases. The growing popularity of this natural approach as a long-term replacement for chemical fumigants is in line with the growing need for environmentally responsible agricultural practices.

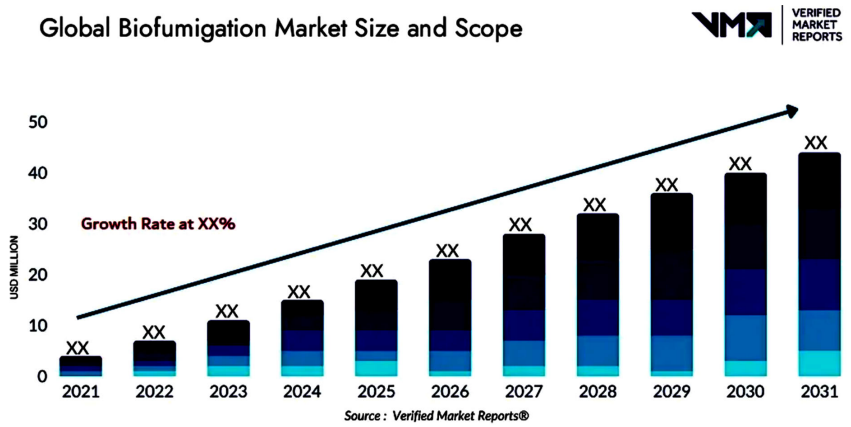

Figure 2.1 – Global biofumigation market by type (mustard seeds, cauliflower seeds), by application (fruits, vegetables), by geographic coverage and forecast. URL: https://www.verifiedmarketreports.com/download-sample/?rid=776826

The biofumigation market is fueled by several factors such as the growing trend towards organic farming and growing awareness about the harmful effects of synthetic chemical fumigants on the environment and human health. As part of Integrated Pest Management (IPM) practices, farmers and agribusinesses are increasingly using biofumigation to increase crop yields, improve soil health, and reduce dependence on chemicals. Moreover, biofumigation promotes biodiversity and reduces chemical residues in soil and crops and is in line with sustainable agriculture practices.

The biofumigation market can be segmented on the basis of crop type, end-user, and geographical region. Biofumigation methods are highly beneficial for crops such as ornamental plants, fruits, and vegetables. Research institutes, organic farmers, and commercial agricultural producers are some of the end users. Geographically, the market is spread across North America, Europe, Asia-Pacific, and other regions, with Europe driving adoption due to strict laws regulating chemical fumigants and active promotion of organic farming practices. The market is expected to expand as research and development in biofumigation continues to evolve, providing creative answers to the challenges associated with sustainable agriculture.

The biofumigation market is expanding significantly due to the growing interest in sustainable agricultural practices. Key changes include improved formulations of natural fumigators and increasing public awareness about the environmental benefits of biofumigation. In addition, changes in regulations supporting environmentally friendly pest management practices are fueling the market growth.Market research shows a growing preference for organic pest control methods among consumers and agricultural businesses. This is due to concerns about the health and environmental impact of synthetic fumigants. Biofumigants derived from natural materials such as essential oils and plant extracts offer a safer and sustainable alternative. This trend is expected to drive the biofumigation market, owing to increasing demand for organic food production and stringent regulations regarding synthetic fumigants.

Market research shows that advancements in biofumigation technology are increasing its efficiency and widening its application scope. New formulations, application methods, and combinations with other biocontrol methods are being developed to control a wider range of pests and improve efficacy. Research on biofumigants with specific target characteristics for different pests is also gaining momentum. These advancements are expected to make biofumigation a more competitive option in the overall pest control market.

Market studies show a shift towards regional production and distribution of biofumigants. This trend is driven by factors such as reducing transportation costs, minimizing environmental impact, and ensuring the quality and efficacy of biofumigants. Locally produced biofumigants can

be customized to address specific regional pest problems and can provide greater freshness and efficacy than those transported long distances. This trend is expected to drive the regional biofumigation markets along with the established global players.

Leading companies in the biofumigation market:BASF SE, UPL Group, Isagro USA, Inc, Marrone Bio Innovations, Inc, Eastman Chemical Company, PH Petersen, Mighty Mustard, Tozer Seeds, Harley Seeds. Taking into account the data synthesis of Clarkson et al. (2020), who noted that 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).

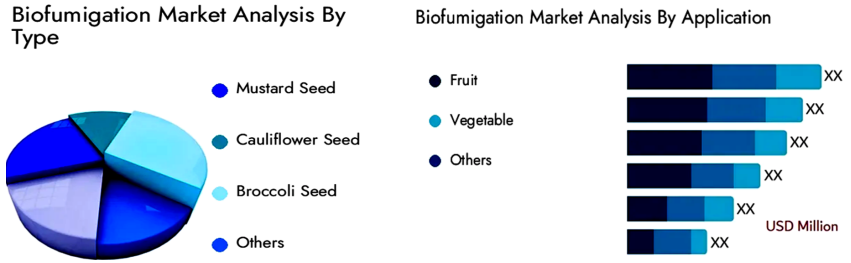

Figure 2.2 – Biofumigation market segmentation

The world experience of bofumigation is summarized in the review article by Kruger et al. (2013), the main points of which are presented below in the form of a literature review:

Principles of chemical soil fumigation (in accordance with the author's text of Kruger et al. (2013)). The primary aim of soil fumigation is to suppress soil-borne problems such as diseases, nematodes and weeds that might otherwise have a negative economic impact on the production of crops (Louvet, 1979). The first application of fumigation for the control of nematodes was recorded as early as in the 1870s (Van Berkum & Hoestra, 1979). In the years after the Second World War, several soil fumigants reached the market, including products such as chloropicrin, methyl bromide, 1,3-dichloropropene, ethylenedibromide, 1,2-dibromo-3-chloropropane and methyl isothiocyanate (ITC) (Lembright, 1990). However, for soil

fumigation to be effective in the control of soil-borne pest and diseases, intensive research on the application rate and a sound knowledge of the soil and of the environmental conditions involved are required. It is also necessary to bear in mind the secondary, negative impacts of the use of this method on the soil (Louvet, 1979). Soil fumigation should be used as part of a holistic programme that follows a long-term approach (Louvet, 1979). Products such as methyl bromide, chloropicrin and combinations of chloropicrin and 1,3-dichloropropene must be applied by trained pest control operators to lower the risks involved when using fumigation products.

Fumigation of the soil is done before the planting or transplanting of seedlings to prevent a negative impact of the product on the crops planted. To increase the efficacy of soil fumigation, factors such as a knowledge of the crop involved, its correct seeding or planting date, the presence of soil-borne pests and diseases that might pose a problem to the specific crop, the availability of cultivars with resistance to certain soil-borne pest and diseases, and soil preparation should be taken into consideration before applying the product. Furthermore, knowledge of the pest or disease and its survival in the soil is also imperative for successful fumigation (Louvet, 1979).

Principles of soil biofumigation (in accordance with the author's text of Kruger et al. (2013)). Biofumigation takes place when certain soil-borne pests and diseases are suppressed as a result of the biocidal activity of glucosinolate-containing plants when they are incorporated into the soil (Kirkegaard et al, 1993, 1998). The fumigant action of the volatile compounds that are released during the biodegradation of organic matter suppresses plant pathogens (Piedra Buena et al, 2007).

Glucosinolates (GSLs) (glucose- and sulphur-containing organic anions) and ITCs are the main active compounds involved in biofumigation. The first observations of the unique properties of GSLs and ITCs were recorded at the beginning of the 17th century during efforts that were made to understand the reason for the sharp taste of mustard seeds (Challenger, 1959). GSLs are sulphur-containing secondary metabolites produced by certain crops that are hydrolysed by the enzyme myrosinase (MYR) to form ITCs, in a process that is known as the GL-MYR system (Wathelet et al, 2004). ITCs have a toxic effect on many soil-borne pathogens (Sarwar et al., 1998). Breakdown products, including the active compound

ITC, are released when the plant cell walls are damaged or broken during maceration of the plant biomass (Sarwar et al, 1998; Wathelet et al, 2004).

The role played by biofumigation in integrated pest management (IPM) (in accordance with the author's text of Kruger et al. (2013)). The positive biological activity of the GSL degradation products used for the suppression of some pathogenic fungi (Manici et al, 1997) and nematodes (Lazzeri et al, 1993) serves to open up new perspectives on IPM (Lazzeri et al, 2004), because it has been proven to be effective against weeds, plant diseases and nematodes (Van Dam et al., 2009). Numerous studies in the literature confirmed the ability of certain plants to suppress nematodes through the nematicidal activity of the secondary metabolites (Chitwood, 2002; Zasada & Ferris, 2004). Research has furthermore proved that many Brassica spp. show nematicidal activity on plant-parasitic nematode species such as M. incognita, M. javanica, Heterodera schachtii and Pratylenchus neglectus (Thierfelder & Friedt, 1995; Potter et al, 1998; Monfort et al., 2007).

Plants containing GSL (in accordance with the author's text of Kruger et al. (2013)). The Family Brassicacea (brassicas) contains more than 350 genera with 3 000 species, of which many are known to contain GSL. However, GSLs are not confined to brassicas alone. At least 500 species of non-brassica dicotyledonous angiosperms have also been reported to contain one or more of the over 120 known GSLs (Fahey et al., 2001). Each of the GSLs has its own chemical properties and can be placed in one of three different classes, namely aliphatic, aromatic or indole forms (Zasada & Ferris, 2004; Padilla et al, 2007). Most GSL-containing genera, however, are clustered within the Brassicaceae, Capparaceae and Caricaceae families (Rodman, 1981). The GSL concentration in the cells of the various plants in the families differs substantially. Therefore, it is crucial to identify species that will be effective in supressing soil-borne pests and diseases, including nematodes. Rotation crops tested for the presence of GSLs are provided in Table 1, which shows that it is mostly the brassicas that contain GSLs and that different levels of GSL exist within different genera (Larkin & Griffin, 2007). Therefore the plant species that generally are considered for biofumigation are found mostly in the family Brassicaceae, and include Brassica oleracea (broccoli, cabbage, cauliflower, kale), Brassica rapa (turnip), Raphanus sativus (radish), Brassica napus (canola, rapeseed) and

various mustards, such as Sinapis alba (white mustard) and Brassica juncea (Indian mustard) (Sarwar et al., 1998; Ploeg, 2007).

Aspects that influence GSL release and ITC activity (in accordance with the author's text of Kruger et al. (2013)). Techniques that ensure the maximum rupturing/maceration of the cells involved, as well as effective incorporation, ensure the best release of ITC. This aspect, along with a variety that has a high GSL content and with enough water present for hydrolysis to take place, ensure optimum biofumigation (Brown *et al.,* 1991; Poulton & Moller, 1993; Morra & Kirkegaard, 2002; Matthiessen *et al.,* 2004). One way to ensure the effective release of ITC is to slash the leaves with a slasher and then to plough the slashed residues into the soil as soon as possible, using a rotavator or disc harrow (Fig. 2). A flail chopper ensures the best maceration results and, consequently, a good GL-MYS interaction for the release of ITC (D. Gies, personal communication). The latter technique remains applicable particularly for the *Brassica* spp. such as mustards, which have a high GSL concentration in the above-ground parts of the plant.

The growth stage of the crop (emergence, rosette, flowering, seed filling, ripening), the amount of biomass produced and the correct incorporation into the soil all contribute towards the success of biofumigation (Bellostas *et al.,* 2004) (Fig. 3). The flowering stage of the plant maintains a higher GSL content than the vegetative plant parts. The GL-MYS interaction can be expected to take place more effectively later in the growing season, prior to seed set. In the root tissue, the concentration of GSL is higher in the earlier root growth stage, with decreasing concentrations during the root growth cycle. Different types of GSLs are present in the roots and shoots of different plant species (Van Dam *et al,* 2009). Studies that were conducted by Van Dam *et al.* (2009), in which the root and shoot GSL of 29 plant species were evaluated for their GSL concentration and profiles, showed that the roots had a higher GSL concentration, as well as more diversity than the shoots. The root and shoot concentration of specific GSLs was found to differ from one another, with the most prominent indole GSL in the shoots being indol-3- yl GSL, and with the roots having higher concentrations of aromatic 2-phenylethyl GSL.

Low soil temperature slows down the enzymatic reaction during biofumigation, and therefore incorporation of green manure is not

recommended at soil temperatures close to 0°C. The presence of organic matter seems to have an immobilising effect on the degradation products, thus preventing them from reaching the target pests (L. Lazzeri, personal communication).

The inclusion of sulphur fertilisers may improve the nutritional value of *Brassica* spp. Sulphur forms part of the process that takes place in the formation of secondary metabolites, *inter alia* GSLs. The level of GSLs is dependent on the genetic factors of the plant, but can also vary according to environmental conditions and the availability of soil sulphur (De Pascale *et* al., 2007).

Nematode host status of different biofumigation crops (in accordance with the author's text of Kruger et al. (2013)). The ideal cover crop to be applied in vineyards for nematode suppression should either be resistant or have a poor host status, in addition to having a biofumigation suppressing effect on the target nematode when applied to the soil as a green manure (Vianene & Abawi, 1998). The possibility exists that *Brassica* spp., if used as cover crops in vineyards, could also be susceptible to specific nematodes species that require suppressing. If the target pest manages to reproduce on the cover crop species before it is ploughed in as a green manure, these *Brassica* spp. cannot be recommended as a cover crop (McLeod & Warren, 1993).

Root-knot nematode species can complete their life cycle on several *Brassica* spp., but there are major differences in their susceptibility (McLeod & Steel, 1999). In a glasshouse study, Curto *et al.* (2005) evaluated the host status of different brassicas for *M. incognita.* Although all of the brassicas act as hosts, the life cycle of the nematode was in general much slower in comparison to tomato. These authors rated certain brassicas as poor or non-hosts (resistant), maintenance hosts (tolerant) or good host (susceptible). *Eruca sativa* cv. Nemat was evaluated for its potential as a trap crop for root-knot nematode. No eggs were produced in 80% of the plants, indicating that it has the potential to act as trap crop for *M. hapla* (Melakeberhan *et al.,* 2006).

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 2.3). ITCs have a wide range of biocidal characteristics and are acutely toxic to a variety of pests

and pathogens (Chew, 1987). GSLs are в-thioglucoside N-hydroxysulfates, with a side group (R) and a sulphur-linked в-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 2.3). The R group is retained in the ITCs and influences its biological activity.

Biofumigation matter to the soil. This will increase soil aeration, water infiltration rates and soil water holding capacity. It also increases soil porosity if used as a green manure. It adds more organic carbon to the soil (Balesh et al., 2005) which is needed to increase the activity of soil fauna and flora. In particular, the activities of biological control agents like predaceous nematodes, protozoa, fungi and bacteria will be enhanced as the presence of organic carbon in the soil increases the saprophytic activities of microorganisms (Karavina and Mandumbu, 2012).

Biofumigation is the use of green manures crops which release biocidal molecules into the soil after their incorporation. This best practice was developed in several countries to cope with the withdrawal of methyl bromide, a most effective but controversial chemical soil fumigant. The effect of biofumigation is partly based on the release of natural toxic substances but also on their effect as a green manure plant.

For Brassicas, the transformation of glucosinolates into toxic and volatile isothiocyanates happens during the breakdown of the plant cells. The more cells which are broken and release glucosinolates, the higher the peak of isothiocyanates will be (Morra & Kirkegaard, 2002). This is critical for the efficacy of biofumigation. Therefore, the biofumigation crop should be shredded as finely as possible before soil incorporation (Figure 1), with the best method to use are mulching devices equipped with hammers rather than blades (Matthiessen et al., 2004).

The amount (concentration) of isothiocyanates needed for successful control depends on the targeted soilborne pathogens, nematodes and weed seeds (Klose et al., 2008). For the more resistant microsclerotia of the soilborne pathogen *Verticillium dahliae*, *Brassica* plants will not liberate sufficient isothiocyanates for a successful control in the field (Neubauer et al., 2014).

While biofumigation holds a lot of promise as a crop protection tool, its broad spectrum toxicity might harm non-target beneficial soil biota

such as biocontrol agents or other pest antagonists (Ramirez *et* al., 2009). This means the switch to brassica biofumigants might not eliminate all the harmful non-target effects associated with synthetic chemicals, thus potentially complicating the integration of cultural and biological control. Henderson *et al.* (2009) reported that biofumigation interfered with the biological control of *Meloidogyne chitwoodi* by the entomopathogenic nematodes *Steinernema feltiae* and *Steinernema riobrave.* Biofumigants are also non-persistent. Thus, it does not provide a long term control option for pests. Farmers might have to complement biofumigation with other crop protection tools.

The nature of the soil is also an important factor when biofumigation is used as a control method. Lighter-textured soils with low organic matter content are better suited to this approach (Kirkegaard, 2009). Isothiocyanates get fixed to organic matter (sorption) and are therefore less active against soilborne pathogens and nematodes. Therefore, the lower the organic matter content, the less sorption of the isothiocyanates occurs in a soil. Lighter soils i.e., soils with a higher part of sand, allow a better diffusion of the toxic gases in the soil.

An alternative to increasing the amount of isothiocyanates in the soil is the use of defatted seedmeals from *Brassica* cultivars with high content of glucosinolates (Patalano, 2004). Such products are commercially available, and in most cases sold as organic fertilizers (Figure 2.2.1). Therefore, their efficacy is not known as such products do not undergo efficacy evaluation, as is the case when products are registered as pesticides. However, the amount of seedmeal added to the soil is limited by its nutrient content, usually nitrogen in the first instan ce. The addition of too much seedmeal can result in an over-fertilization and potentially in the leaching of different nutrient elements (such as nitrate).

Seedmeal products are mostly applied by broadcast in form of pellets or powder (Figure 2.2.2) and incorporated into the soil before the planting of the crop. Once in contact with water in the soil, the transformation of the glucosinolates into isothiocyanates takes place. Irrigation after the incorporation of these products accelerates this transformation and also favors the diffusion and dispersal of the isothiocyanates in the soil.

- Pelletted organic fertilizer
- Produced from 100% GMO FREE Plant Material

OMRI Listed

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Guaranteed Analysis:

TOTAL NITROGEN (N):	6%
Water Soluble Organic Nitrogen	6%
AVAILABLE PHOSPHORIC ACID (P_2O_5):	2%

Derived From Mustard Seed Meal

DIRECTIONS FOR USE:

- Apply product to dry soil
- Mechanically incorporate to a depth of 4-6 inches
- Irrigate with 0.50 - 1.00 inches of water
- Allow 7 days after irrigation before planting treated areas

- Formulation d'azote organique à action d'amendement
- 100% d'origine végétale
- 100% non OGM

ENGRAIS AZOTÉ ORGANIQUE
TOURTEAU N + C 6 + 45

AZOTE (N) ORGANIQUE:	6%
CARBONE (C) ORGANIQUE D'ORIGINE BIOLOGIQUE:	45%

ADMIS EN AGRICULTURE BIOLOGIQUE

Origine: tourteau déshydraté issu de graines oléagineuses

CONSEILS D'UTILISATION:

- Epandre le produit en presemis.
- Enfouir à 10-15 cm de profondeur.
- Irriguer pour humidifier la couche superficielle du sol.
- Attendre 7 jours environ pour installer la culture.

Figure 2.2.1 – Example of an organic fertilizer based on defatted mustard seedmeal (Michel and de Cara García, 2020)

Figure 2.2.2 – Pellets of defatted mustard seedmeal before incorporation in the soil (Michel and de Cara García, 2020)

Another way to apply isothiocyanates to the soil is the use of liquid *Brassica* seedmeal products. In this case, the seedmeal is manipulated before application. Through this manipulation, the glucosinolates are transformed into isothiocyanates and then dissolved in liquid which is applied to the soil through a drip irrigation system.

It is noted (Karavina and Mandumbu, 2012) that the most studies on biofumigation have been done with brassicas (Kirkegaard et al., 1993; Gouws, 2004; Kumar, 2005; Ramirez et al., 2009; Szczyglowska et al., 2011), but as already noted, other families contain plants with biofumigant properties. The decomposition of biofumigant plant tissues mainly releases isothiocyanates (ITCs), in addition to thiocyanates, nitriles and oxazolidinethiones (Kirkegaard and Sawar, 1998; Fahey et al., 2001). The ITCs are related to the active ingredients of metham sodium and dazomet. They are released following tissue damage, when endogenous myrosinase enzymes hydrolyze glucosinolates (GCs). GCs are sulphur-containing chemicals that are produced by plants as secondary metabolites (Agrios, 2005). They are found in the vacuole. In the plant, GCs are relatively inactive against microbes. But when hydrolyzed, the ITCs in particular, are biocidal to nematodes, bacteria, fungi, insects and germinating seeds (Sarwar et a/., 1998). There are over 120 ITCs (Fahey et al., 2001) that have been identified mainly from plants in the Brassicaceae family (van Dam et al., 2009) (Table 1).

The same studies (Karavina and Mandumbu, 2012) indicate that differences in structure of individual ITCs depend on their organic side-chain, which in turn, influence their biocidal activity (Rosa et al., 1997; Clark, 2010). The side chains may be aliphatic, aromatic or indole. Aromatic ITCs are mainly produced in roots and are very toxic (at least 50 times more toxic than metham sodium's methyl ITC) to a range of soil borne fungal pathogens (Vaughn et al., 1993). They have low volatility and long persistence in the soil.

This long persistence benefits crops grown after the GC-producing plants. Roots may release ITCs both during growth and decomposition. Aliphatic ITCs are more common in shoots. They are of higher volatility than root ITCs. Thus, shoots tend to have a lower concentration of ITCs than roots. Exposure of dry shoot residues to the surface after crop harvest, burning and grazing of residues reduce the amount of ITCs in shoots. The indole ITCs are found in both roots and shoots. In shoots, the predominant ITC is derived from the GC indol-3-ylmethylglucosinolate, while roots have 1-methoxyindol-3-ylmethylglucosinolate and 4-metho-xyindol-3-ylmethylglucosinolate (van Dam et al., 2009).

The amount and profile of ITCs produced by brassicas vary with the species and with the soil conditions. Table 2 gives common sources of some ITCs. For example, the three major ITCs identified from Brassica juncea roots are 3-butenyl, 4-pentenyl and 2-phenyethyl, while from Brassica campestris and Brassica napus, the major ITCs are 3-butenyl, 4-pentenyl, 2-phenyethyl, 5-methylthiopentyl and benzyl.

Table 2.1

Plants with glucosinolates in their leaves and stems (Karavina and Mandumbu, 2012)

Plant Species	Common Name	Family
1	2	3
Alliaria petiolata	Garlic mustard	Brassicaceae
Arabidopsis thaliana	thale cress	Brassicaceae
Alima tetracantha	needle bush	Salvadoraceae
Brassica campestris rapa	turnip	Brassicaceae
Brassica carinata	Ethiopian mustard	Brassicaceae
Brassica fruticulosa	Mediterranean cabbage	Brassicaceae
Brassicajuncea	Indian mustard	Brassicaceae
Brassica napus	rape/canola	Brassicaceae
Brassica nigra	black mustard	Brassicaceae
Brassica oleraceae acephala	kale	Brassicaceae
Brassica oleraceae	cabbage	Brassicaceae
Cardamine cordifolia	Heartleaf bittercress	Brassicaceae
Cardamine diphylla	pepper root	Brassicaceae
Carica papaya	pawpaw	Caricaceae
Diplotaxis tenuifolia	Perennial wall-rocket	Brassicaceae
Eruca sativa	salad rocket	Brassicaceae
Lepidium sativa	garden cress	Brassicaceae
Moringa oleífera	Moringa	Moringaceae
Moringa stenopetala	cabbage tree	Moringaceae
Rhaphanus sativus	radish	Brassicaceae
Sinapis alba	white mustard	Brassicaceae
Thlaspi arvense	field pennycress	Brassicaceae
Tropaeolum majus	Indian cress	Tropaeolaceae
Alliaria petiolata	Garlic mustard	Brassicaceae
Arabidopsis thaliana	thale cress	Brassicaceae
A%ima tetracantha	needle bush	Salvadoraceae
Brassica campestris rapa	turnip	Brassicaceae
Brassica carinata	Ethiopian mustard	Brassicaceae
Brassica fruticulosa	Mediterranean cabbage	Brassicaceae

(End of Table 2.1)

1	2	3
Brassicajuncea	Indian mustard	Brassicaceae
Brassica napus	rape/canola	Brassicaceae
Brassica nigra	black mustard	Brassicaceae
Brassica oleraceae acephala	kale	Brassicaceae
Brassica oleraceae	cabbage	Brassicaceae
Cardamine cordifolia	Heartleaf bittercress	Brassicaceae
Cardamine diphylla	pepper root	Brassicaceae
Carica papaya	pawpaw	Caricaceae
Diplotaxis tenuifolia	Perennial wall-rocket	Brassicaceae
Eruca sativa	salad rocket	Brassicaceae
Lepidium sativa	garden cress	Brassicaceae
Moringa oleifera	Moringa	Moringaceae
Moringa stenopetala	cabbage tree	Moringaceae
Rhaphanus sativus	radish	Brassicaceae
Sinapis alba	white mustard	Brassicaceae
Thlaspi arvense	field pennycress	Brassicaceae
Tropaeolum maius	Indian cress	Tropaeolaceae

Table 2.1.1

Common sources of ITCs (Karavina and Mandumbu, 2012)

Isothiocyanates	Common Sources
Methyl	*Capparales,* metham sodium
2-Propenyl	B juncea, B carinata, B nigra
3-Butenyl	B napus, B campestris
4-Pentenyl	B napus, B campestris
Benzyl	*Sinapis* spp
2-Phenylethyl	*Brassica* roots

Table 2.1.2

Some commonly used biofumigant crops and their respective GSLs and ITCs (Clarkson et al., 2020)

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

Table 2.1.3

Glucosinolates associated with brassica plants (Musa, 2021)

Glucosinolate	Common names	Brassica species	Plant organ	Author(s)
Aliphatic				
Gluconapin	Wild mustard	*Brassica rapa*	Shoot	Park *et al.* (2017)
Sinigrin	Indian mustard	*Brassica juncea*	Shoot	Zasada and Ferris (2004); Ngala *et al.* (2015)
	Ethiopian mustard	*Brassica carinata*	Shoot	Potter *et al.* (1998)
Sinalbin	White mustard	*Sinapis alba*	Shoot	Hopkins *et al.* (1998)
	White mustard	*Brassica hirta*	Shoot	Zasada and Ferris (2004)
Glucoerucin	Rocket	*Eruca sativa*	Shoot	Di Gioia *et al.* (2018)
Glucobrassi-canapin	Wild mustard	*Brassica rapa*	Shoot	Kim *et al.* (2010)
Progoitrin	Canola	*Brassica napus*	Seed	Cartea *et al.* (2008)
Glucoraphanin	Radish	*Raphanus sativus*	Shoot	Ngala *et al.* (2015)
	Rocket	*Eruca sativa*	Shoot	Di Gioia *et al.* (2018)
Aromatic				
Gluconasturtiin	Indian mustard	*Brassica juncea*	Root	Park *et al.* (2017)
	Radish	*Raphanus sativus*	Root	Ngala *et al.* (2015)
Glucotropaeolin	White mustard	*Sinapis alba*	Root	Sarwar *et al.* (1998)
	White mustard	*Brassica hirta*	Root	Zasada and Ferris (2004)
Glucosinalbin	White mustard	*Sinapis alba*	Seed	Borek and Morra (2005)
Indolyl				
Neoglucobrassicin	Canola	*Brassica napus*	Shoot	Potter *et al.* (1998)
4-methoxyglucobrassicin	Canola	*Brassica napus*	Shoot, Seed	Potter *et al.* (1998)

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 2.1.2–2.1.3). 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 BB. 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 & Kirkegaard, 2006) and may explain why some low GSL brassica crops have been shown to have suppressive activity against soilborne diseases.

Clarkson et al. (2020) were noted that 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 disease suppression may provide further insights for optimising ITC and non-ITC benefits of biofumigants.

Edwards & Ploeg (2014) noted that biofumigation is a rotation-based approach to crop protection in which *Brassica* cover crops in the family Brassicaceae (also called Cruciferae) are macerated and incorporated into soil prior to planting vegetables. During the leaf maceration process, sulphur-containing compounds known as glucosinolates combine with an enzyme called myrosinase to produce isothiocyanates and other chemical compounds. These broad-spectrum biocides are detrimental to weed seeds

and a range of soilborne pests and pathogens, including nematodes. In this case, the flail mower on the rear tractor is mowing and macerating a Nemfix mustard (*Brassica juncea*) cover crop (shown in the background), while the rotary hoe is being used to immediately incorporate the leaves and shoots into the soil. The field was irrigated after cultivation to enhance the biofumigation effect, and cherry tomatoes were planted four weeks later.

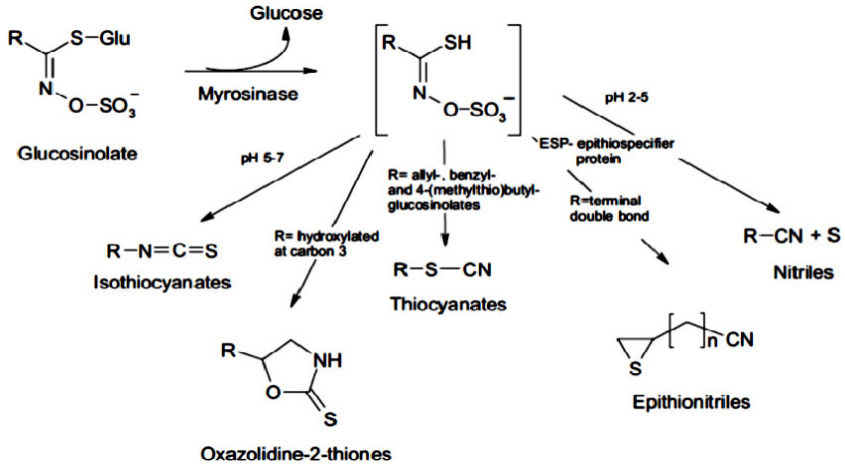


Figure 2.3.1 – Structure of possible glucosinolate degradation products (Musa, 2021)

The authors of the study (Edwards & Ploeg, 2014) also emphasize that although the term 'biofumigation' implies that the active ingredients are volatile, and act in much the same way as commercial fumigants, some of the most potent chemicals released from decomposing *Brassica* residues are not gaseous at ambient temperatures. Thus, it is important to recognise that these chemicals principally diffuse through soil in the water phase and do not act as true fumigants. Another point that must be recognised is that the term 'biofumigation' was initially used to describe the production of isothiocyanates from plant residues. However, the term is now commonly used when volatile substances are produced through microbial degradation of animal and plant organic amendments. Thus, plants such as neem,

marigold and castor contain compounds that are toxic to nematodes and when they are used as soil amendments and nematode populations are reduced, it is considered a biofumigant effect. Similarly, when microbial fermentation of nutrient rich amendments such as velvet bean, sunn hemp and elephant grass produces toxins that are detrimental to nematodes, the term biofumigation is also used.

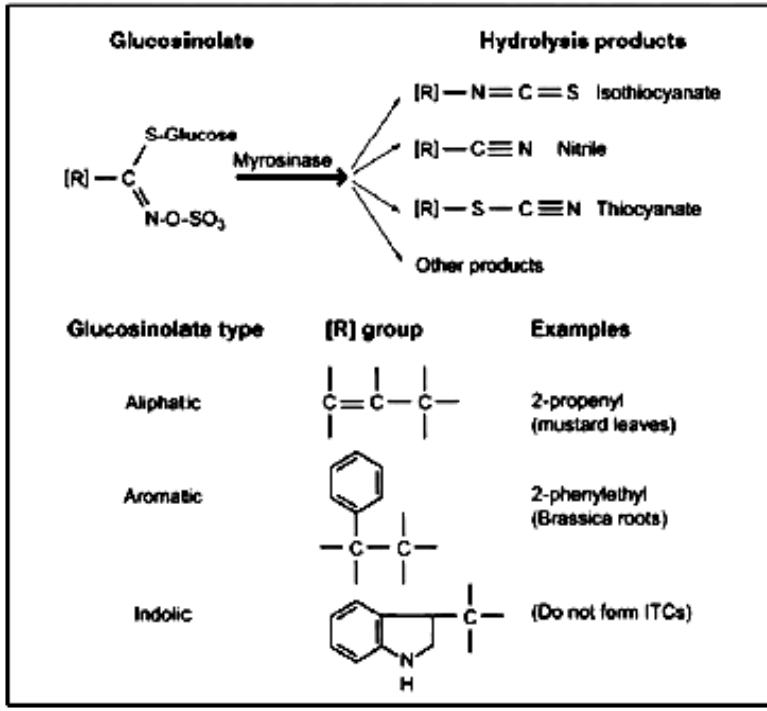


Figure 2.3.2 – Glucosinolate structure and products of hydrolysis (from Kirkegaard, 2009)

Based on the findings of these studies (Björkman & Shail, 2010; Edwards & Ploeg, 2014), the following generalizations were made:

– *Brassica* cultivars capable of producing large quantities of glucosinolates must be selected, and because sulphur is an important

component of glucosinolates, the brassicas must be grown with optimal nutrition, particularly with regards to sulphur.

– Glucosinolate release from *Brassica* tissue must be maximised by incorporating the cover crop into wet soil at the time of maximum leaf area (i.e. before flowering). During the incorporation process, the leaf tissue must be macerated and disrupted as much as possible.

– To ensure that the volatile chemicals are retained within the soil profile, the field should be irrigated immediately with sprinklers to seal the soil surface, or the soil should be covered with plastic. As this is logistically difficult to do on a large scale, it is a major limitation of biofumigation.

When *Brassica* cultivars with high leaf glucosinolate concentrations are incorporated into soil as described above, nematicidal activity is not always high enough to control the nematode pest being targeted. For example, the damage threshold for root-knot nematode on crops such as carrot, tomato, potato, and sweetpotato is very low, and so biofumigation will often fail to reduce nematode populations to the required level. Also, research has shown that some nematodes (e.g. *Pratylenchus*) are less sensitive to brassicaceous seed meals than others.

Another major drawback of brassicas is that some species and cultivars are good hosts of root-knot nematode, the most important nematode pest of vegetables. Thus, *Brassica* cultivars need to be chosen carefully as there is a danger that the biofumigant crop will increase rather than decrease nematode populations. A paper by Edwards and Ploeg (2014) is worth consulting, as 31 plants in the family Brassicaceae were assessed in the greenhouse for their capacity to host three species of root-knot nematode (*Meloidogyne javanica*, *M. incognita* and *M. hapla*). Indian mustard (*Brassica j*uncea) and turnip (*B. rapa*) were generally good hosts of the two warm-climate species whereas most oil radish cultivars (*Raphanus. sativus* ssp. *oleiferus*) were poor hosts. Some oil radish cultivars were among the best hosts for *M. hapla*, while arugula (*Eruca sativa*) cv. Nemat was a poor host of all three nematode species.

In situations where cyst nematodes cause problems on vegetables, biofumigant crops also need to be chosen carefully because many cruciferous plants are good hosts of species such as *Heterodera schachtii* and *H. cruciferae.*

It is noted (Edwards & Ploeg, 2014) that there were many situations where brassicas will prove to be a useful rotation or cover crop. However, one question vegetable growers need to answer was whether they gain additional benefits from macerating the tissue and pulverising wet soils to achieve a biofumigation effect. This process not only destroys soil structure and is detrimental to soil health but also kills or disrupts the larger beneficial soil organisms, many of which are predators. *Brassica* cover crops provide the traditional benefits of a green manure (i.e. a break in the vegetable rotation and inputs of organic matter), but the additional costs of macerating the biomass and incorporating it into soil may not be justified due to the negative effects on soil health.

The benefits obtained from using brassicas as a green manure rather than a biofumigant crop can be determined by establishing strip trials in which the *Brassica* biomass is incorporated aggressively into the soil in some parts of the field and treated as a cover crop in other areas. The costs of the incorporation process, together with data on disease incidence and severity in the following vegetable crop, will indicate whether it is economically worthwhile spending additional time and money trying to achieve a biofumigation effect.

In their publications, Duff et al. (2020) note that biofumigation is the practice of growing specialised cover crops for suppression of soilborne pathogens, pests and weeds. The cover crop produces naturally occurring compounds that are toxic to many soilborne pathogens that impact on Australian vegetable crops. Some soilborne diseases can survive for many years, even in the absence of a suitable host. The resting stages of some soilborne pathogens, can remain dormant until conditions are favourable, resulting in the development of symptoms on the plant. For some diseases like white rot (*Sclerotium cepivorum*) in onion, the pathogen can survive in the soil for 20 years or more.

It also indicates (Duff et al., 2020), що the challenge from a disease management perspective is reducing disease inoculum in the soil, whilst maintaining or enriching soil health so that crops are able to become more resilient to soilborne pathogens. An integrated approach utilising biofumigant cover crops can be an effective tool in the management of soilborne diseases in horticultural production systems. This offers growers a solution that does not involve the use of synthetic pest control. Brassica

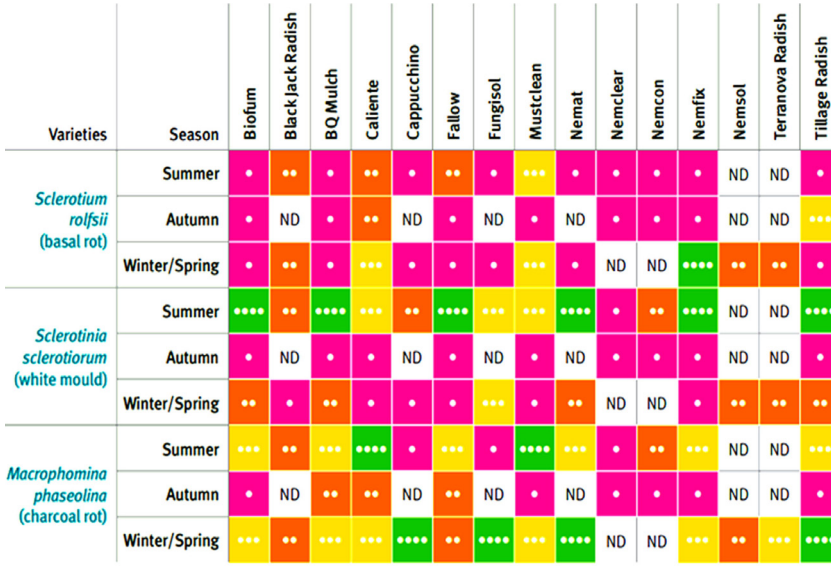
species, such as mustard, radish and rocket, have been shown to suppress soilborne diseases such as basal rot (*Sclerotium rolfsii*), Onion white rot (*Sclerotium cepivorum*), charcoal rot (*Macrophomina phaseolina*) and white mould (*Sclerotinia sclerotiorum*) (Figure 2.4). They achieve this through processes resulting in the release of naturally occurring chemicals contained in plant tissue.

Brassicas naturally produce a group of chemicals known as glucosinolates (GSLs). The highest concentration of glucosinolates tends to occur at approximately 25% flowering, which is the recommended timing for incorporating biofumigants. Through the process of mulching and incorporation, glucosinolates are released from the plant cells. Once released from plant cells and with the addition of irrigation water, glucosinolates are converted by the enzyme myrosinase into isothiocyanates (ITCs), gases that are toxic to various soilborne pathogens and pests. Irrigation and/or rolling helps to seal the gas in the soil so that they are most effective in suppressing pathogens (Duff et al., 2020).

There are over 137 glucosinolate compounds commonly found in brassica plants. The concentration and type of GSLs will vary between varieties, as does the type of ITCs produced from the GSLs. The ITCs determine the biofumigants' toxicity to various soilborne pathogens and pests. All biofumigant varieties have positive soil health benefits, but some may be better suited for a particular cropping program. This will depend on the soilborne disease being targeted as well as other considerations such as cropping window and agronomic management of the biofumigant (Figure 2.4) (Duff et al., 2020).

The soilborne disease that requires management, and time of year available to plant a cover crop, will impact which variety is most suitable. The matrix (Table 2.1) is a tool designed to assist decision making about variety selection for disease management. The table is based on key production breaks when a biofumigant cover crop is most likely to be included as part of a cropping system and the relative efficacy of biofumigant varieties against a range of soilborne diseases.

The authors of the study (Duff et al., 2020) also suggest the days to incorporation varied between different varieties and growing seasons. Generally, biofumigants reached incorporation (or 25% flowering) faster and produced less biomass in summer compared to winter (Figure 2.6).



Percentage mortality pink 0–20; orange 2–40; yellow 41–60; green 61–80; white 81–100.

Figure 2.4 – Suppress soilborne diseases due to biofumigation with cruciferous crops (Duff et al., 2020)

As brassicas are predominantly a winter-grown crop, biomass production is greater in the cooler months, compared to summer. However, having high biomass does not mean that these varieties will produce more GSLs.

Glucosinolates or GSLs are the precursors to the toxic compounds, isothiocyanates or ITCs, that have suppressive activity against soilborne pathogens. As the ITCs are volatile gases, GSLs within the plant are measured instead as they are less volatile. Biofumigant varieties can have a range of individual glucosinolates, with varying concentrations.

While some GSLs are known to be more toxic in the ITC they are converted to, glucosinolate data in this guide is presented as total glucosinolate concentration rather than individual GSLs. However, higher total GSLs does not necessarily equate to greater activity against soilborne diseases.

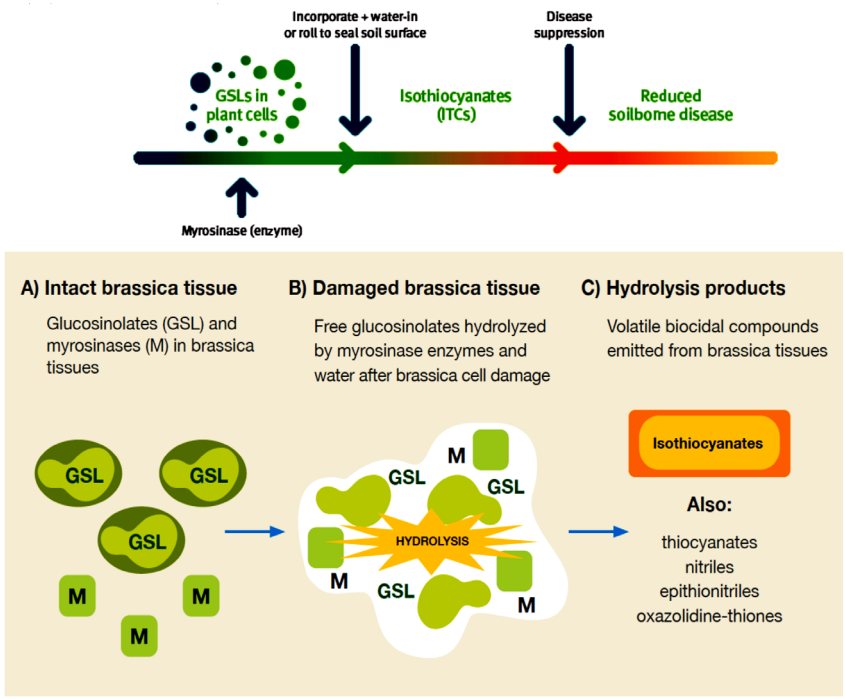


Figure 2.5 – The biofumigation process in brassica plants (Duff et al., 2020)

Brassica biofumigant cover crops are prone to a similar pest spectrum as commercially grown brassica crops, such as broccoli and cabbage. Generally speaking, biofumigant cover crops will not require the intensive pest management of commercial crops for various reasons:

The authors of the study (Duff et al., 2020) also draw conclusions:

– There is greater flexibility in thresholds and acceptable damage limits in crops not destined for human consumption.

– There are some benefits to the crop's performance as a biofumigant if it is allowed to experience a moderate level of stress, whether this is insect feeding or some other stress (such as water stress) – this results in some increase in potency in GSLs, but this is variety dependant.

– The biofumigant cover crop can also provide functions beyond its soilborne disease suppression properties. For example, they can act as a nursery for beneficial insects, which would colonise vegetable crops and attack the relevant pests.

– Management of insect pests in biofumigant crops is generally not required, and may be considered economically unfeasible. However, you should still monitor biofumigant crops if you wish to get the most benefit from growing them. Biofumigants are highly attractive to all beneficial insects particularly if flowers are present as many beneficial species are nectar feeders (e.g. parasitoids and hoverflies).

– Biofumigants have high requirements for nitrogen and potassium as well as sulphur, as the glucosinolates are sulphur containing compounds. Nutrient requirements for summer grown biofumigants were roughly half of that when grown in winter. This reflects the difference in biomass between growing seasons. Tillage radish was the exception with similar biomass and nutrient requirements whether grown in summer or winter. As biofumigants are fully mulched and incorporated, any applied nutrients will be available through nutrient recycling for future crops.

– Research looking at the effect of drought stress on GSLs and biofumigant efficacy has shown that moderate to high water stress increases the concentration of GSLs per kg of plant tissue, even though the amount of biomass is less. Comparison of biofumigants grown under high, medium and low irrigation frequencies showed biomass was reduced by 45–55% between high and low irrigation treatments for 3 out of the 4 varieties in summer. The impact of irrigation strategy on biomass was not as significant for winter grown biofumigants with at most a 24% lower biomass in Nemat with low irrigation frequency.

– Irrigation treatment. Winter growing season The low irrigation treatment was established and then grown on rainfall only receiving 0.7–0.75 ML/ha, medium irrigation received 1.57–2.27 ML/ha and the high irrigation received 2.07–3.17 ML/ha depending on harvest date. Summer growing season: The low irrigation treatment was established and then grown on rainfall only receiving 1.4–2.5 ML/ha, medium irrigation received 2.5–3.2 ML/ha and the high irrigation received 3.3–6.I ML/ha depending on harvest date.

– Biofumigants do not have to produce high levels of biomass to be effective in suppressing plant pathogens. Results from the irrigation trial

showed that low irrigation produced a lower biomass crop yet the highest production of GSLs per hectare in 3 out of the 4 varieties with a winter planting. A summer planting was the reverse with 3 out of the 4 varieties producing more GSLs with the higher irrigation. While total GSLs have been measured, these values are only indicative and do not reflect the toxicity of the resulting ITCs on plant pathogens.

– Weed management for Biofumigants: it is recommended that weeds are managed as per a commercial crop. Pre-emergent herbicides like Dacthal® 900 WG (Nufarm) (active ingredient: Chlorthal-dimethyl) is registered for use in mustard crops as is Dual Gold (active ingredient: s-metolachlor). Care needs to be taken when using herbicides as most will have plant back issues for following cash crops. Biofumigant crops that are left to go to seed can be a weed in their own right. To avoid this, incorporate plant material at 25% flowering stage to maximise the disease suppression properties of the crop and to avoid plants going to seed and becoming weeds themselves. Before using any herbicide always read and comply with label requirements.

– Rotary hoe has been the recommended incorporation method for biofumigant cover crops. However, as not all vegetable growers have access to a rotary hoe, DAF also looked at a range of incorporation methods for biofumigant cover crops. These include: Mulching followed by Disc Plough or Rotary Hoe, then Irrigation or Rolling, and Strip till followed by irrigation. Strip tillage showed the most variability in biofumigant activity. Mulch/rotary hoe/irrigation and mulch/disc plough/irrigation were similar in their biofumigant activity. There was also little difference between irrigation and rolling post incorporation (Figure 2.7–2.8, Table 2.3–2.4).

Spraying off the biofumigant cover crop was also evaluated to see if it still retained its suppressive characteristics once incorporated post spraying. The crop was sprayed off at 25% flowering and incorporated 4 weeks later. Total GSL levels were measured 2 weeks post spraying and 4 weeks post spraying (incorporation). Comparison of GSL data showed a progressive decline in total GSL levels by 50% at 2 weeks post spraying and by 90% at 4 weeks, incorporation.

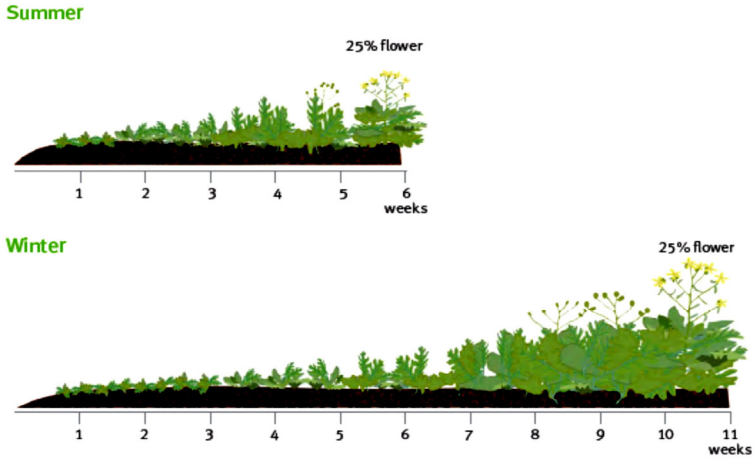


Figure 2.6 – Graphic depicting biofumigant cover crop days to incorporation across different growing seasons (Duff et al., 2020)

Figure 2.7 – Biofumigant varieties: Caliente (left), Nemat (right) and Oilseed Radish (bottom)

Table 2.2

Common commercially available biofumigant varieties compared in DAF trials (Duff et al., 2020)

Trade name	Variety	Species name
Biofum™	Doublet oilseed radish and Achilles white mustard mix	*Raphanus sativus* and *Sinapis alba mix*
Black Jack Radish™	Oil Radish	*Raphanus sativus*
Black Mustard	Black mustard	*Brassica nigra*
BQ Mulch®	Black mustard and Ethiopian mustard	*Brassica nigra* and *Brassica carinata*
Caliente™ including Caliente Rojo™	Indian mustard	*Brassica juncea*
Cappucchino™	Ethiopian mustard	*Brassica carinata*
FungiSol™	Ethiopian mustard and Terranova oilseed radish mix	*Brassica carinata* and *Raphanus sativus*
Mustclean™	Indian mustard	*Brassica juncea*
Nemat™	Rocket	*Eruca sativa*
Nemfix™	Indian mustard	*Brassica juncea*
Nemclear™	Fodder mustard	*Brassica napus*
Nemcon™	Fodder mustard	*Brassica napus*
NemSol™	Terranova oilseed radish and Nemat mix	*Raphanus sativus* and *Eruca sativa*
Terranova Radish™	Oilseed radish	*Raphanus sativus*
Tillage Radish®	Oilseed radish	*Raphanus sativus*
White Mustard	White mustard	Sinapis alba

Biofumigant activity against known pathogens varied greatly with the spray-off/incorporation methods. While some of these results reflect the significant decline in total GSL with time after spraying off, others do not. Further work on this as an option for biofumigant cover crop management is required.

Apply nitrogen at rates of 100–150 kg/ha and sulphate at 25-50 kg/ha. Biofumigant crops should also be succulent at early to mid-flowering to aid maceration implements in cutting and pulping biofumigant material. Nitrogen helps to produce a lush canopy which breaks down easily. Irrigation can also help to prevent early senescence.

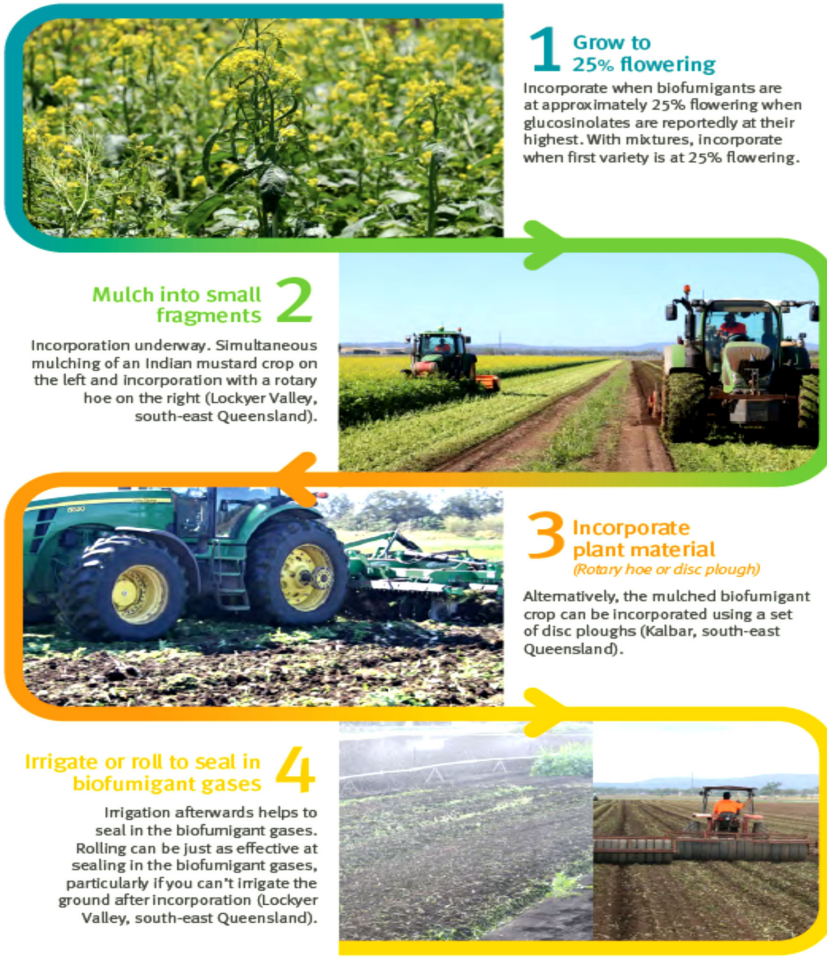


Figure 2.8 – Steps involved in mulching and incorporation of biofumigant cover crops (in accordance with Duff et al., 2020)

Table 2.3

Efficacy matrix showing the extent of pathogen mortality after different methods of incorporation (in accordance with Duff et al., 2020).

Pathogen	Fallow (Field control)	Mulch + Disc plough + Irrigation	Mulch + Disc plough + Roll	Mulch + Rotary hoe + Irrigation	Mulch + Rotary hoe + Roll	Mulch + Strip till implement + Irrigation
Sclerotium rolfsii (basal rot)			••••			
Sclerotinia sclerotiorum (white mould)	••••	•••	••••	••••	••••	•••
Rhizoctonia sp. (wire stem)			•••	••••		••••
Macrophomina phaseolina (charcoal rot)	••••	••••	•••	•••	••	••
Verticillium dahliea (verticillium wilt)		••••	••••			••••
Sclerotium cepivorum (onion white rot)	••••	••••	••••	••••	••••	•••

Percentage mortality pink 0–20; orange 2–40; yellow 41–60; green 61–80; white 81–100.

Leafy biofumigants such as Indian mustard and rocket are more easily macerated and so release isothiocyanates more readily. Large root crops, such as oil radish, do not break down easily. However, brassica roots release glucosinolates into soil during growth in what is known as partial biofumigation. Many brassica species, including Indian mustard, oilseed radish and rocket, have been shown to suppress PCN during the growing period.

Biofumigant maceration and incorporation techniques. Many options are available for biofumigant maceration and incorporation. Maceration techniques should pulp, twist or tear biofumigant material rather than just cut. This is to cause widespread rather than localised tissue damage.

Isothiocyanates disappear within minutes, so incorporate immediately after maceration. The time gap should be no more than 20–30 seconds. Mix biofumigant material evenly into soil, to a depth of 25–35 cm. If the crop has high biomass, incorporate to 35 cm. Some spaders can incorporate to 40 cm. Then seal the soil to retain toxic gases. A rotavator hood or spader smear roll can create an effective soil surface seal. The best systems use a front-mounted haulm topper, followed by a rear-mounted rotavator or spader, (Figure 2.8.1). If a rotavator has a shallow working depth, follow it with a plough to mix the material to a greater depth. Then use a plough-press, power-harrow with packer roll, or other rolls to reseal the surface (Figure 2.8.2). Minimise the number of passes because each extra operation moves soil and allows escape of toxic isothiocyanate gas. Figure 4 shows a single-pass system.

Table 2.4

Efficacy matrix showing the extent of pathogen mortality with different incorporation methods (in accordance with Duff et al., 2020).

Pathogen	Fallow (Field control)*	Spray-off + Disc plough + Irrigation	Spray-off + Disc plough + Roll	Spray-off + Rotary hoe + Irrigation	Spray-off + Rotary hoe + Roll
Sclerotium rolfsii (basal rot)					
Sclerotinia sclerotiorum (white mould)	••••	...	...		
Rhizoctonia sp. (wire stem)	••••	••			
Macrophomina phaseolina (charcoal rot)	...				
Verticillium dahliea (verticillium wilt)					
Sclerotium cepivorum (onion white rot)	••••	...			

Percentage mortality pink 0–20; orange 2–40; yellow 41–60; green 61–80; white 81–100.

*Fallow samples were taken at incorporation, 4 weeks after the biofumigant was sprayed.

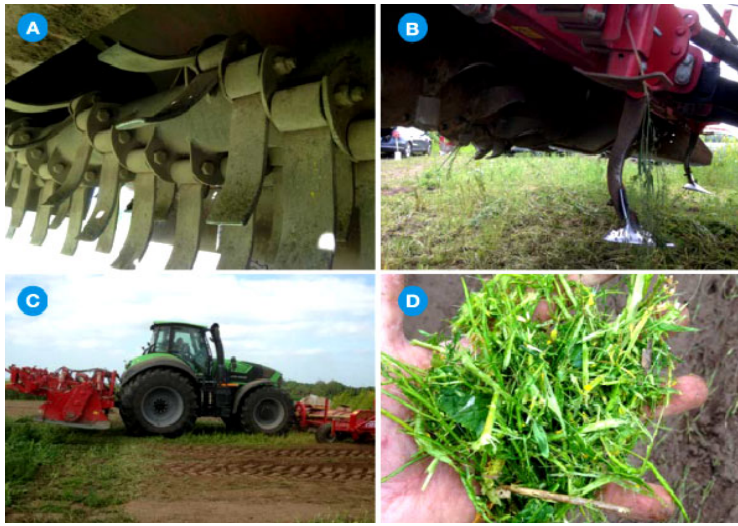


Figure 2.8.1 – Single-pass biofumigation system: A = tine arrangement on a haulm topper, B = rotavator with rigid tines and S-blades, C = a single-pass system using front-mounted haulm topper and rear-mounted rotavator, and D = good-quality brassica residues following maceration (AHDB PCN, 2020)

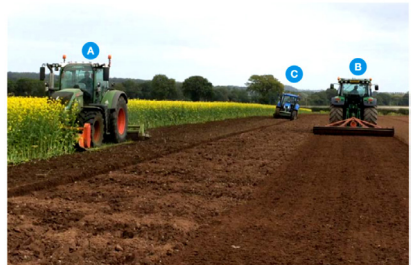


Figure 2.8.2 – Multiple-pass biofumigation system: tractor A flail topping and shallow rotavating, followed by tractor B ploughing to 30 cm, followed by tractor C power-harrowing with packer-roll. The two implements on tractor A achieve full maceration and partial incorporation, allowing a short gap before tractor B fully incorporates and tractor C seals (AHDB PCN, 2020)

Soil moisture at incorporation affects biofumigation success. In sandy loam soils, aim for 25–75% of field capacity. Soil at this moisture range feels damp and will hold its shape when moulded or pressed. Isothiocyanate gases escape too easily from drier soils and cannot spread as effectively through wet soils. Incorporate lower biomass crops in soil at 25–50% of field capacity. Incorporate higher biomass crops at 75% soil moisture.

In his research, Caldbeck (2022) notes:

– crop type-to maximise on glucosinolate, the choice of crop species and cultivar is important. Plants have been found to be the most consistent in causing reductions in pests and pathogens. Oilseed radish had a big potential. White mustard should not be considered because it does not produce the right type of glucosinolates. It is important to maximise the volume of material produced from the brassica plants (which need adequate water and nutrition). Aim at producing 50 tonnes of fresh weight, up to 10 tonnes of dry weight per hectare. In contrast to standard cover cropping, you are trying to maximise biomass and glucosinolate content (quantity and quality). Nitrogen (N) is important for biomass and sulphur (S) for glucosinolate content. Aim at 100 kg per hectare (ha) of N and 25 kg per ha of S. For organic systems, aim at utilising nutrients from previous crops such as peas or legumes to feed the biofumatory green manure (mustards can be very good at mopping up and recircu-lating surface nutrients). If incorporating a 2-year break crop into your rotation (i.e. with legumes or clover), consider reducing it to 18 months and utilising the nutrition to feed the biofumatory crop.

– sowing date should be as early as possible (in an arable rotation, sowing in June – July has been found to produce the highest biomass and highest glucosinolate content – due to higher UV, temperatures and longer day length). Plant seeds into clean moist ground, so they can race ahead of and suppress weeds.

– damaging the plant cells allows the glucosinolates and myrosinase to interact with one another – this can be done by flailing, cutting and bruising the tissue as much as possible. Early flowering is when there is a peak of glucosinolates in the plants so this is the best time to cut (this would typically be within 10–16 weeks into growing time). Flowers may be at different stages – cut when the majority are closest to early flower as possible (once seed pods are formed it is too late). Outside, applying a light

roll over can be beneficial. This should ideally be done coming up to early flowering.

– the volatile organic compounds are lost quickly (within the first 5 hours) after chopping and incorporating the plant material, therefore it needs to be buried quickly. Ideally, incorporate straight after chopping. It is recom-mended to use a front mounted flail followed by a rear mounted rotavator, and to compress in a one pass system if possible. If growing in protected cropping, it is possible to strim plant material into small pieces, then rotavate it in. Do this a bit at a time, and then apply water. A mower could alternatively be used – pull the whole plant up, roll over it lightly with the mower, then work it in.

– when about to incorporate the plant biomass, make sure the soil is moist enough. It may be best to wait for rain before incorporating (but avoid soaking wet soil). The aim is to enable the release of as much of the volatile gases in the soil as quickly as possible. 75% field capacity is best for optimising the hydrolysis and the biofumigation. The soil temperature needs to be above 8 ^{0}C for optimal movement of the volatile compounds.

– watering-In protected cropping, consider covering (i.e. with polythene) to lock in the volatile gases and leaving it on for a few days to a week (most of the biofumigation happens in the first 24–48 hours). Make sure the soil moisture is good beforehand, watering on top also creates a bit of a cap. On a field scale, irrigate after incorpo-rating – the equivalent of about half an inch. This will create the chemistry and seal it in.).

– Crop management-It is important to leave a gap between incorporating the biofumigant crop and planting a cash crop, in order to allow sufficient time to ventilate the soil. Allow a 2-week margin to be safe (even though isothiocyanates are lost within the first 5 hours, there are other volatile compounds that come later). Growing successive crops may lead to a greater suppression of certain soilborne pests and pathogens e.g. potato cyst nematodes.

According to a number of estimates (International Biofumigation Network, 2020; AHDB PCN, 2020; AHDB Biofumigation Report, 2022) biofumigant seed usually costs £5–9/kg. Seed costs are £60–100/ha for Indian mustard, rocket and oil radish. Establishment costs £15–30/ha. For spring and summer systems, fertiliser improves efficacy. Nitrogen at 100–150 kg/ha costs £80–160/ha, and sulphate at 25 kg/ha costs around

£25–50/ha. One 25 mm irrigation event would cost £85–155/ha. Machinery running costs are usually £100–150/ha. Total cost for a low-input overwintered system is about £270/ha for an expected efficacy of, at most, 10–30%. A spring or summer system is more likely to cost in the region of £400–450/ha but could extend to £675/ha. A maximum of 40–70% efficacy should be expected. Biofumigants also provide benefits from adding organic matter to the soil. A drawback of biofumigation is that it requires more time and attention from the grower than the use of synthetic nematicides.

Table 2.4.1

Summary of biofumigation recomendations for spring and summer windows (AHDB PCN, 2020)

Description of operation or crop management input	Comments
1	2
Biofumigant selection	Select a high-sinigrin-content Indian mustard biofumigant, and/or a rocket variety high in gluconasturtiin or glucotropaeolin glucosinolates
Preparation of soil and drilling	Drill biofumigants, between mid-July and mid-August, to a depth of 2-3 cm
Seed rate	Use a 8-10 kg/ha seed rate
Nutrient inputs	Apply nitrogen at 100-150 kg/ha, and sulphate at 25-50 kg/ha
Herbicides	Generally not required. If weed burden is high, seek advice from a qualified agronomist
Irrigation	May be required for establishment, to prevent early senescence or ahead of incorporation if soils are below 50% of field capacity (target 25-75% of field capacity)
Timing of maceration and incorporation	Macerate at early to mid-flowering when brassica foliage is still succulent. The best crops should be 50 t/ha of fresh biomass or greater, probably at 1.2-2.0 m in height
Foliage maceration	Use a flail or haulm topper for maceration, fitted with blunt hammer or solid V-tines. Front-mount maceration implements where possible. Keep tractor forward speed as slow as practicable to reduce the bite length of the macerator. The aim is to produce biofumigant pulp
Incorporation of residues	Ideally, rear-mount a rotavator or spader. Other incorporation implements can be used, provided material is well mixed into the top 30 cm of soil and incorporated within 20-30 seconds of maceration. Seal the soil either by smear-roll, heavy flat roll, or by the hood of a rotavator

(End of Table 2.4.1)

1	2
Planting the next crop in the rotation	Leave at least 2 weeks between incorporating a biofumigant and planting a new crop. This is to avoid phytotoxic effects in the new crop from biofumigant organic matter breakdown
Summary of biofumigation recomendations for winter windows	
Biofumigant selection	Select an oil radish rich in the glucosinolates gluconasturtiin or glucotropaeolin. Some Ethiopian mustards *(Brassica carinata)* may also be suitable. They are similar to Indian mustard, but hardier
Preparation of soil and drilling	Drill biofumigants to a depth of 2-3 cm between early and mid-September for best establishment
Seed rate	Use a 15-20 kg/ha seed rate (10 kg/ha-for Ethiopian mustard)
Nutrient inputs	Apply nitrogen at 30-40 kg/ha, and sulphate at 15-20 kg/ha
Herbicides	Generally not required. If weed burden is high, seek advice from a qualified agronomist
Irrigation	Typically not required
Timing of maceration and incorporation	Macerate ahead of planting a spring crop. Leave the biofumigant as long as possible to capitalise on partial biofumigation
Foliage maceration	Use best practice where possible. Efficacy is less than in summer and spring systems
Incorporation of residues	Use best practice where possible. Ensure residues are incorporated to at least 25 cm depth. Efficacy is less than in summer and spring systems
Planting the next crop in the rotation	Leave a minimum of 2 weeks between incorporating an overwintered biofumigant and planting a new crop. This is to avoid phytotoxic effects in the new crop from biofumigant organic matter breakdown

Biofumigation success is also subject to environmental conditions, more so than with granular nematicides. The combination of both management systems is advised for long-term PCN management.

Biofumigants are susceptible to pest damage and disease, the same as any cash crop. Crops can recover from pigeon feeding, but it delays biomass production, enables weeds to compete and causes early senescence. Biofumigant crops can increase the risk of introducing clubroot to oilseed rape or vegetable brassicas. Request information from seed suppliers on varietal resistance to clubroot. Some oilseed radish varieties are resistant, but most mustards and rocket are not.

The biofumigation efficiency of cover crops has been studied in terms of their glucosinolate content. Thus, Hansen (2011) noted that all forms

of fumigation, biofumigation can impact living organisms in the soil, such as beneficial microbes including earthworms. Biofumigant activity against beneficial microorganisms such as *Trichoderma* spp. and *Bacillus amyloliquefaciens* was assessed in the laboratory. The biofumigants tested were found to be more suppressive against *Trichoderma spp.* than *Bacillus amyloliquefaciens*. There was variability in the suppression of beneficial microorganisms between varieties with some varieties suppressing more than others. Caliente in particular, showed high levels of suppression of *Trichoderma* spp. There was a trend for greater activity against beneficial microorganisms from biofumigant material grown during summer, however, this was not always the case. Therefore, if using biocontrol products such as *Trichoderma* spp. or *Bacillus* spp., it is recommended to only use these products when planting your cash crop and not in conjunction with the biofumigation crop.

The suppression of soilborne pathogens has also been linked to factors other than biofumigation. These include competition by a range of copiotrophic soil microorganisms, which thrive under the addition of fresh organic matter, proliferation of *Streptomycetes* (filamentous bacteria that have a role in breaking down plant material), and elevated soil populations of ammonia-oxidising bacteria, or the formation of additional bioactive sulphur containing compounds.

Biofumigation activity is known to increase soil bacterial diversity, but also significantly reduces soil fungal diversity, possibly due to reduced pathogenic fungi. This will obviously depend on the biofumigant variety, as different varieties contain varying types of GSLs with varying levels of toxicity when converted into ITCs.

Brassica biofumigant cover cropping is an option for vegetable system rotations as part of an integrated disease management strategy. Key information from this guide highlights several key considerations for those wanting to incorporate brassica biofumigants into their rotation.

Back et al. (2019) studied the glucosinolate profile of a number of cruciferous crops from the point of view of their use as a biofumigant (Figure 2.9).

A full-fledged assessment of the glucosinolate structure of cruciferous crops was carried out in the studies of Kirkegaard & Sarwar (1998) (Table 2.5).

Aliphatic GSLs dominated the shoot profiles of all Brassica species while some weed species contained higher concentrations of aromatic

GSLs (Tables 2.6–2.7). Indolyl GSLs were ubiquitous, but present in relatively low concentrations (<1 ^mol g-1) except in some of the *B. oleracea* vegetables. The concentration of major individual GSLs generally varied from two to ten fold within species (Table 2.5) as did total glucosi-nolates (Table 2.6). These differences were significant based on the LSDs calculated for individual or total GSLs in the representative entry selection (Tables 2.5 and 2.6). *B. napus* and *B. campestris* entries had similar shoot GSL profiles dominated by 3-butenyl GSL, 4-pentenyl GSL and their hydroxy-substituted forms, and also contained significant levels of the aromatic 2-phenylethyl GSL.

		Glucosinolate content (μmol g^{-1} dry weight)					
Species	**Cultivar**	**Glucoraphanin**	**Sinigrin**	**Sinalbin**	**Glucotropaeolin**	**Glucobrascicin**	**Gluconasturtiin**
B. caratina	Cappuccino		11.70			0.20	
B. juncea	ISCI 99		26.73			0.32	
B. juncea	Pacific Gold		25.95				
B. juncea	Scala		7.57			0.09	
B. juncea	Vitasso		20.79			0.50	
B. juncea	Keva			46.76	0.72	0.24	
S. alba	Ida Gold			31.20	0.48	0.13	
S. alba	Smash			32.65	1.54	0.04	
S. alba	Loti			12.25	0.22	0.07	
E. sativa	Trio	19.18		0.04			0.16
E. sativa	Nemat	6.62				2.59	
R. sativus	Bento					0.20	
R. sativus	Doublet					1.24	0.21
R. sativus	Anaconda					0.60	0.11
R. sativus	Terranova					3.24	0.28

Figure 2.9 – Glucosinolate profile of the biofumigant lines used in the poly-tunnel experiment at Harper Adams University (Back et al., 2019).

The authors of the study (Kirkegaard & Sarwar, 1998) note that maximum concentrations of these glucosinolates were two to ten times higher in B. napus oleifera biennus than *B. napus* oleifera annua while *B. napus* rapifera was intermediate.The B. oleracea vegetables had lower concentrations of the three alkenyl aliphatic GSL dominant in other species, but higher concentrations of the methylthio and methylsulphinyl analogues as well as significant concentrations of the indolyl GSLs particularly 3-indolyl-methyl GSL and 1-methoxy-3-indolyl methyl GSL. The dominant GSL types varied within the subspecies (Table 2.5) although the total GSL concentrations were similar (Table 2.6). B. carinata and B. nigra entries had simple profiles dominatedby 2-propenyl GSL and a similar range in total GSL concentration was evident in the two species. In B. júncea, while 2-propenyl GSL was the major GSL detected, significant levels of 3-butenyl GSL and 4-pentenyl GSL were also present in some entries.

However, unlike B. napus and B. campestris, the OH-substituted forms of these alkenyl GSL were not present in appreciable quantities in B. júncea. B. carinata and B. nigra entries contained minimum 2-propenyl GSL concentrations of 10 μmol g^{-1}, while some *B. júncea* entries had concentrations as low as 0.1 μmol g^{-1}.

S. alba and weed species tended to have relatively simple profiles dominated by one or two GSLs in high concentrations. *S. alba, S. arvensis* and *D. tenuifolia* had high concentrations of the aromatic GSLs, benzyl GSL and p-hydroxybenzyl GSL, which were absent from the shoots of the Brassica species. *B. tornefortii, B. fruticulosa* and *S. orientale* were dominated by 3-butenyl GSL while the latter also contained 3-methylthiopropyl GSL, which was not found in appreciable quantities in any of the other entries. Despite the high concentrations of the individual aliphatic and aromatic GSLs in the weed species, the concentration of the indolyl forms was low. Entries containing total GSL concentrations >20 μmol g^{-1} were represented in *B. napus oleifera biennis*, *B. carinata, B. nigra, B. juncea, S alba* and all of the weed species, although the ranges within species were large (Table 2.6). Maximum total GSL concentrations in *B. napus oleifera annua*, *B. campestris* and *B. oleracea* were <15 μmol g^{-1}. In all *B. napus*, *B. campestris* and *B. oleracea* species only ~50% of the total GSLs were ITC liberating, while for *B. nigra, B. juncea, B. carinata,*

S. alba and the weed species almost all of the GSLs contained in the plant were ITC liberating (Table 2.6).

Table 2.5

Glucosinolates identified in Brassica samples, their HPLC retention times and major hydrolysis products (Kirkegaard & Sarwar (1998))

Number	Chemical name	Trivial name	Retention time (min)	Hydrolysis products
Aliphatic				
1	3-Methylthiopropyl	Glucoibervcrin	16.80	ITC, ш triles
2	3-Metbylsiilplimylpropyl	Ghicoberin	7.27	ITC. nitriles
3	2-Propeuyl	Sinigrin	12.53	ITC. nitriles
4	4-Metbylthiobutyl	Glucoerusin	19.79	ITC, nitriles
	4-MethyIsulphinyIbutyI	Glucoraphanin	13.51	ITC. nitriles
6	3-Butcuyl	Gtucoiiapin	16.52	ITC. nitriles
7	2-Hydroxy-3-butenyl	Progoitnn	9.51	Oxazolidinc-2-thioncs
8	5-Metkylsulpliinylpentyl	Glucoalyssiu	11.96	ITC. nitriles
9	4-Puiienyl	Glucobiassicanapin	18.83	ITC. nitriles
10	2-Hydroxy-4-pcntcnyl	Gluconapoleiferin	15.24	Oxazol idine-2-thiones
Aromatic				
II	2-Phenylethyl	Ghiconastiirtiin	21.42	ITC
12	2-Hydroxy-2-pheuylethyl	Glucobaibaiiii	17.82	ITC
13	Benzyl	Glucotropneokn	19.11	ITC
14	p-Hydioxybeiizyl	Ghicosiaalbin	15.58	ITC
Itidolil				
15	3-Itidolylinetbyl	Glucobiassicin	20.20	Iudolyl-3-caibiool
16	4-Hydroxy-3-uidolyhnethyl	4-Hydioxyglucobrassicin	17.56	Tliioc valíate
17	4-Methoxy-3-indolylmethyl	4-N lethoxyghicobrassic m	2104	Auxins?
18	1-Meiboxy-3-ilklolylmethyl	Neoglucobrassicui	23.09	Pbytoalexins?

Range in the concentration of individual glucosinolates (µmol g^{-1}) found in shoots of field grown Brassica and related species at flowering. Glucosinolates are numbered as in Table 2.4 (Kirkegaard & Sarwar, 1998)

Species	No. of entries	Aliphatic 2	3	5	6	7	9	10	Aromatic 11	Indolyl 15	16	17	18
B. napus oleifera													
biennis (oil)	6	–	–	0.2–0.3	0.5–3.3	1.6–9.2	0.8–7.8	0.6–1.9	0.6–2.3	0.2–0.4	–	0.1	0.1–0.3
biennis (fodder)	7	–	–	0.5–0.8	0.3–8.7	0.5–5.9	0.5–4.5	0.8–2.9	0.4–0.9	0.1–0.6	0.1	0.1	0.1–0.4
annua	7	–	–	0.1–0.5	0.1–0.8	0.2–3.8	0.1–1.8	0.1–1.5	0.1–1.3	0.1–0.6	0.1	0.1	0.1
B. napus rapifera	3	–	–	–	0.4–0.6	0.5–5.1	0.6	0.5–3.7	0–1.4	0.1–0.4	–	–	0.1–0.4
B. campestris oleifera													
biennis	1	–	–	–	1.6	1.7	3.8	1.8	1.6	0.2	0.1	–	–
annua	5	–	–	–	0.4–1.3	0.4–3.0	1.3–3.8	1.1–5.6	0.8–3.6	0.2–1.7	0.1–0.5	0.1–0.3	0.1–0.5
B. campestris rapifera	5	–	–	–	0.7–4.0	0.9–4.6	1.9–6.5	1.1–2.1	0.6–1.7	0.1–0.2	0.1	–	0.1–0.3
B. oleracea													
var. *gemmifera*	1	2.2	1.3	1.0	0.5	0.5	–	–	–	2.9	0.2	–	0.3
var. *capitata*	1	3.2	1.3	–	–	–	–	–	–	3.0	0.1	0.1	0.6
var. *botrytis*													
sv. *cauliflora*	1	1.4	–	–	–	–	–	–	–	1.6	0.1	0.7	3.2
sv. *cymosa*	1	–	–	2.4	–	–	–	–	–	1.3	–	0.1	4.1
var. acephala	2	1.4–1.5	2.2–3.2	0.5–1.1	0.5–1.6	0–1.4	–	–	–	3.1–4.0	0.2–0.3	–	1.0–1.1
B. carinata	6	–	10.0–20.2	–	–	–	–	–	–	0.4–0.9	0–0.1	–	0–0.1
B. nigra	5	–	10.7–26.4	–	–	–	–	–	0.3–0.4	0.1	–	–	–
B. juncea	14	–	0.1–18.7	–	0–7.5	–	0.1–2.0	0.1–0.3	0–1.3	0–0.2	0–0.1	–	0.1–0.2

Species	No. of entries	Aliphatic 1	5	6	7	8	9	10	Aromatic 11	13	14	Indolyl 15	16	17	18
S. alba	5	–	–	–	0.4–0.5	–	1.0–3.4	–	0.3–0.4	1.9–9.6	9.0–14.4	0–0.1	–	–	0–0.1
E. sativa	1	–	4.4	–	–	–	–	–	–	–	–	0.5	–	–	
B. fruticulosa	1	–	–	33.8	–	–	–	0.6	0.4	–	–	–	–	–	–
B. tournefortii	1	–	–	34.8	–	0.8	0.7	–	0.9	–	–	–	–	0.1	–
S. orientale	1	13.2	–	30.2	–	–	–	–	–	–	–	–	1.5	–	–
S. arvensis	1	–	–	–	0.6	–	–	–	1.4	–	15.3	–	–	0.7	1.6
D. tenuifolia	1	–	8.1	1.2	–	–	–	–	1.1	12.0	–	2.1	0.7	0.5	–

LSD's ($P < 0.05$) for individual glucosinolate concentrations: 3 – 1.0; 5 – 3.4; 6 – 2.1; 9 – 1.2; 10 – 0.4; 11 – 0.6; 15 – 0.2; 16 – 0.1; 17 – 0.5; 18 – 0.5.
(–) = not detected (<0.01 mol g^{-1}).

It was also found (Kirkegaard & Sarwar, 1998) that in contrast to the shoots, the roots of all Brassica species contained significant concentrations of aromatic GSLs, predominately 2-phenylethyl GSL (Tables 2.7 and 2.8). Significant quantities of benzyl GSL and fbhydroxybenzyl GSL were also present in the roots, while they were not detected in the shoots. In common with the shoots, indolyl GSLs were ubiquitous but in low concentrations (<2 μmol g^{-1}). Aliphatic GSLs were present in appreciable quantities in most species, and in general the profile in the shoots and roots was similar (Tables 2.5 and 2.7). As for the shoots, the concentrations of individual GSLs varied within species by two to ten fold (Table 2.7) while total GSLs in roots varied by two to five fold (Table 2.8). These differences within species were significant based on the LSDs constructed from the representative entry selection (Tables 2.7 and 2.8).

B. napus profiles were dominated by 2-phenylethyl GSL although significant quantities of the aliphatic GSLs found in the shoots (3-butenyl GSL, 4-pentenyl GSL and their OH-substituted forms) were also present. Although the maximum concentrations of some individual aliphatic GSLs were similar in the roots and shoots (e.g. 3-butenyl GSL, 4-pentenyl GSL) (Tables 2.5 and 2.7), total aliphatic GSL concentrations were generally higher in the shoots (see Tables 2.6 and 2.8). Consistent with the shoots, the maximum observed concentrations of individual and total root GSLs within *B. napus* sub-species followed the pattern biennis > rapifera > annua, although the range within sub-species was considerable.

In common with their shoot GSL profiles, *B. napus* oleifera annua and B. campestris oleifera had similar root profiles although 3-butenyl GSL and 2-hydroxy-3-butenyl GSL were absent from the latter. *B. campestris* spp. *rapifera* had significantly higher individual and total concentrations of aliphatic GSLs in the roots than ssp. *oleifera*.

B. oleracea vegetables had similar profiles of aliphatic GSLs in roots and shoots, lower concentrations of indolyl GSLs in the roots but higher levels of the aromatic 2-phenylethyl GSL.

As for *B. napus* and *B. campestris*, 2-phenylethyl GSL was the dominant GSL found in the roots of *B. carinata, B. nigra* and *B. juncea*. These species also had significant concentrations of 2-propenyl GSL in their roots although the maximum concentrations were approximately half of that found in the shoots (Tables 2.5 and 2.7). Consistent with shoots, 3-butenyl

GSL was also found in the roots of some *B. juncea* entries. The low levels of 2-propenyl GSL observed in the shoots of double low *B. juncea* lines were also observed in the roots, some of which had no detectable level present (Table 2.7, Figure 2.9).

Table 2.7

Range in concentration of glucosinolate classes found in the shoots of field grown Brassica and related species at flowering (Kirkegaard & Sarwar, 1998)

Species	No. of entries	Glucosinate concentration (μmol g^{-1})				Total ITC liberating
		Total aliphatic	Total aromatic	Total indolyl	Total	
B. napus oleifera						
biennis (oil)	6	3.5-22.2	0.6-2.3	0.2-0.6	4.6-23.8	1.9-12.3
biennis (fodder)	7	4.1-17.2	0.4-0.9	0.1-0.7	5.5-18.3	1.3-12.8
annua	7	0.1-8.4	0.0-1.3	0.0-0.7	0.2-10.4	0.1-4.4
ssp. *rapifera*	3	1.0-10.0	0.0-1.4	0.2-0.8	1.2-12.2	0.1-2.6
B. campestris oleifera						
biennis	1	8.4	1.6	0.3	10.3	7.0
annua	5	3.4-8.6	0.8-3.6	0.3-2.5	5.3-14.7	2.8-7.0
ssp. *rapifera*	5	6.4-13.4	0.6-1.7	0.2-0.6	7.8-15.5	3.6-9.7
B. oleracea						
var. *gemmifera*	1	5.5	-	3.4	8.9	5.0
var. *capitata*	1	4.5	-	3.8	8.3	4.5
var. *botrytis*						
svar. *cauliflora*	1	1.4	-	5.6	7.0	1.4
svar. *cymosa*	1	2.4	-	5.5	7.9	2.4
var. *acephala*	2	4.7-8.7	-	4.5-5.2	9.2-13.9	4.7-7.3
B. carinata	6	10.0-20.2	-	0.3-0.9	10.5-21.1	10.0-20.2
B. nigra	5	10.7-26.4	0.0-0.4	0.0-0.1	11.2-26.4	11.1-26.4
B. juncea	14	1.1-20.5	0.0-1.3	0.1-0.4	1.4-21.7	1.2-21.6
S. alba	5	1.4-3.8	12.6-24.0	0.0-0.1	15.8-25.6	15.7-25.6
E. sativa	1	4.4	-	0.5	4.9	4.4
B. fruticulosa	1	34.3	0.5	0.3	35.1	34.2
B. tournefortii	1	36.3	0.9	0.1	37.3	37.2
S. orientale	1	43.4	-	1.5	44.9	43.4
S. arvensis	1	0.6	16.8	2.3	19.7	17.4
D. tenuifolia	1	9.3	13.0	2.8	25.1	21.7
LSD ($P < 0.05$)		3.2	5.3	0.3	6.6	*4.3*

(-) = not detected (<0.01 fimol g^{-1}).

Table 2.8

Range in the concentration of individual glucosinolates (µmol g flowering. Glucosinolates are numbered as in Table 3) found in roots of field grown Brassica and related species (Kirkegaard & Sarwar, 1998)

Species	No. of entries	Aliphatic							Aromatic		Indolyl			
		2	3	5	6	7	9	10	11	13	15	16	17	18
B. napus oleifera														
biennis (oil)	6	–	–	0.4–0.7	0.4–1.0	0.3–3.7	0.4–4.4	0.7–2.6	7.9–19.3	1.0–2.7	0.3–0.5	–	0.2–0.7	0.1
biennis (fodder)	7	–	–	0.5–0.9	0.7–10.2	1.3–5.0	0.6–7.5	0.8–2.0	7.1–19.5	1.0–3.4	0.2–1.1	–	0.4–1.1	0.1–0.4
annua	7	–	–	0.1–0.8	–	0.3–0.7	0.4	0.5–1.5	2.3–11.6	0.2–1.3	0.2–0.4	0.1	0.2–0.4	0.1–0.4
B. napus rapifera	3	–	–	–	–	0.6–1.4	–	0.6–1.6	1.5–12.8	0.4–1.3	0.3–0.7	0.1–0.2	0.1–0.2	0.2–1.1
B. campestris oleifera														
biennis	1	–	–	–	–	–	0.3	0.6	9.4	–	0.3	–	0.4	0.3
annua	5	–	–	–	–	–	0.3	0.4–0.6	2.1–9.0	0.2	0.2–0.4	0.1–0.8	0.2–0.8	0.2–1.0
B. campestris rapifera	5	–	–	–	0.3–2.0	0.3–4.5	0.5–2.7	0.9–1.8	7.1–11.9	–	0.2–0.6	–	0.1–0.2	0.1–0.5
B. oleracea														
var. *gemmifera*	1	0.3	1.2	0.4	0.7	0.5	–	–	2.5	–	0.1	0.3	0.1	0.3
var. *capitata*	1	1.3	6.2	–	–	–	–	–	3.3	–	0.5	2.0	0.5	0.4
var. *botrytis*														
sv. *cauliflora*	1	0.7	–	–	–	–	–	–	1.7	–	0.7	0.8	0.7	1.1
sv. *cymosa*	1	–	–	1.5	0.3	0.3	–	–	3.1	–	0.2	0.2	0.2	0.4
var. *acephala*	2	0.3	1.1–2.3	0.6–0.8	0.4	0–0.9	–	–	4.7–5.2	2.2–3.0	0.2–0.4	0.6–0.7	0.2–0.4	0.3–0.4
B. carinata	6	–	2.2–10.4	–	0–0.4	0–1.0	–	–	3.9–12.2	–	0.1–0.4	0–0.2	0.1–0.4	0.1–0.6
B. nigra	5	–	0.5–5.1	–	–	–	–	–	0.9–8.8	–	0–0.1	0–0.2	0.1–0.4	0–0.1
B. juncea	14	–	0–4.8	–	0–1.9	–	0–0.5	–	2.5–12.5	0–0.6	0–0.9	0–0.2	0.1–1.1	0–0.7

Species	No. of entries	Aliphatic					Aromatic			Indolyl			
		2	5	6	7	8	11	13	14	15	16	17	18
S. alba	5	0–0.2	0.9–1.7	–	0–0.4	0–0.7	2.1–4.1	2.0–5.3	2.7–3.9	0–0.2	–	0.1–0.9	0.2–1.2
E. sativa	1	–	0.7	–	–	–	1.8	–	–	–	–	–	–
B. fruticulosa	1	–	–	12.9	–	1.9	18.5	–	–	0.5	–	0.3	0.4
B. tournefortii	1	–	–	21.7	–	7.1	26.4	–	–	–	–	0.3	–
S. orientale	1	–	–	13.7	–	–	25.7	–	5.3	0.7	–	0.8	1.3
S. arvensis	1	–	–	–	0.9	–	19.0	–	23.1	–	0.2	–	0.1
D. tenuifolia	1	–	14.7	–	–	–	1.7	50.0	2.0	0.5	–	–	–

LSD's for individual glucosinolate concentrations: 5 – 1.5; 6 – 1.1; 8 – 1.0; 11 – 2.3; 13 – 3.6; 14 – 0.4; 15 – 0.1;17 – 0.2; 18 – 0.2
(–) = not detected (<0.01 μmol g^{-1}).

In *S. alba* and the weed entries, the dominant GSLs found in shoots also dominated in roots, but as for all the other species, this was in association with high levels of 2-phenylethyl GSL. In some species the concentrations of individual GSLs were higher in the roots than the shoots (e.g. D. tenuifolia, S arvensis). The high concentration of 3-butenyl GSL in the shoots of B. fruiticulosa, S orientale and B. tournefortii were also present in roots, along with similarly high concen- trations of 2-phenylethyl GSL. The roots of the weed species contained the highest concentrations of both individual and total GSLs measured in the study. A greater portion of the total root GSLs were ITC liberating compared to the shoots due to the dominance of 2-phenylethyl GSL in the roots (Table 2.8).

Significant positive correlations between total root and total shoot GSL concentrations were only found within B. napus and B. carinata entries (bothr2 = 0.81) while there were neither significant nor consistent correlations within the other species. There was a significant ($P < 0.05$) positive correlation between total seed and total shoot GSL concentration for the 65 entries for which seed was collected, although the relationship accounted for only 22% of the variation. There were no significant or consistent relationships within species, with the exception of B. juncea where seed GSL and shoot GSL were positively correlated ($r^2 = 0.56$).

There was no relationship between seed GSL concentration and root GSL concentration generally, or within any of the individual species. GSL production on a ground area basis (mmol m^{-2}) is the product of the biomass and GSL concentration of a particular tissue and is shown for shoot and root tissues and whole plants for individual entries in Figure 2.10. Isolines of GSL production on a ground area basis are shown for combinations of GSL concentration and biomass production.

In shoot tissues (Figure 2.10 a) there was large variation in GSL production both within and between species. High shoot GSL production (>20 mmol m^{-2}) arose from high biomass (e.g. *B. oleracea*), from high GSL concentration (e.g. *B. napus oleifera biennis*) or from combinations of moderate to high levels of each (e.g. *B. nigra*).

Table 2.9

Range in concentration of glucosinolate classes and total glucosinolates found in the roots of field grown Brassica and related species at flowering (Kirkegaard & Sarwar, 1998)

Species	No. of entries	Glucosinate concentration (μmol g^{-1})				
		Total aliphatic	Total aromatic	Total indolyl	Total	Total ITC liberating
B. napus oleifera						
biennis (oil)	6	1.8-12.1	8.9-21.4	0.3-1.2	11.5-33.9	9.7-27.0
biennis (fodder)	7	4.6-21.7	9.0-21.3	0.7-2.2	20.7-34.9	13.8-29.7
annua	7	0.1-3.2	2.5-12.5	0.5-1.2	3.2-15.9	2.7-13.7
ssp. *rapifera*	3	1.2-3.0	1.9-14.1	0.6-2.2	3.7-19.3	1.9-14.1
B. campestris oleifera						
biennis	1	0.9	9.4	1.0	11.3	9.7
annua	5	0.0-0.9	2.1-9.4	0.7-1.9	3.8-11.3	2.1-9-.0
ssp. *rapifera*	5	2.8-8.5	7.1-11.9	0.6-0.9	13.2-21.2	8.3-14.8
B. oleracea						
var. *gemmifera*	1	3.1	2.5	0.8	6.4	5.1
var. *capitata*	1	7.5	3.3	3.4	14.2	10.8
var. *botrytis*						
svar. *cauliflora*	1	0.7	1.7	3.3	5.7	2.4
svar. *cymosa*	1	2.1	3.1	0.9	6.1	4.9
var. *acephala*	2	2.6-4.5	6.9-8.2	1.3-1.9	12.1-13.3	10.5-10.8
B. carinata	6	2.2-10.9	3.9-12.2	0.5-1.2	8.0-24.3	7.1-23.1
B. nigra	5	0.5-5.1	0.9-8.8	0.2-0.6	4.0-11.1	1.4-10.5
B. juncea	14	0.4-4.8	2.5-12.8	0.1-1.2	4.6-14.5	4.1-11.9
S. alba	5	0.9-2.4	7.1-12.3	0.3-1.9	8.8-16.5	8.0-14.7
E. sativa	1	0.7	2.5	nd	3.2	2.5
B. fruticulosa	1	14.8	18.6	1.0	34.4	33.5
B. tournefortii	1	28.8	26.4	0.3	55.5	55.2
S. orientale	1	13.7	31.0	2.8	47.5	44.7
S. arvensis	1	0.9	42.1	0.3	43.3	43.0
D. tenuifolia	1	14.7	53.6	0.6	68.9	68.2
LSD (P < 0.05)		2.7	3.3	0.2	4.9	3.7

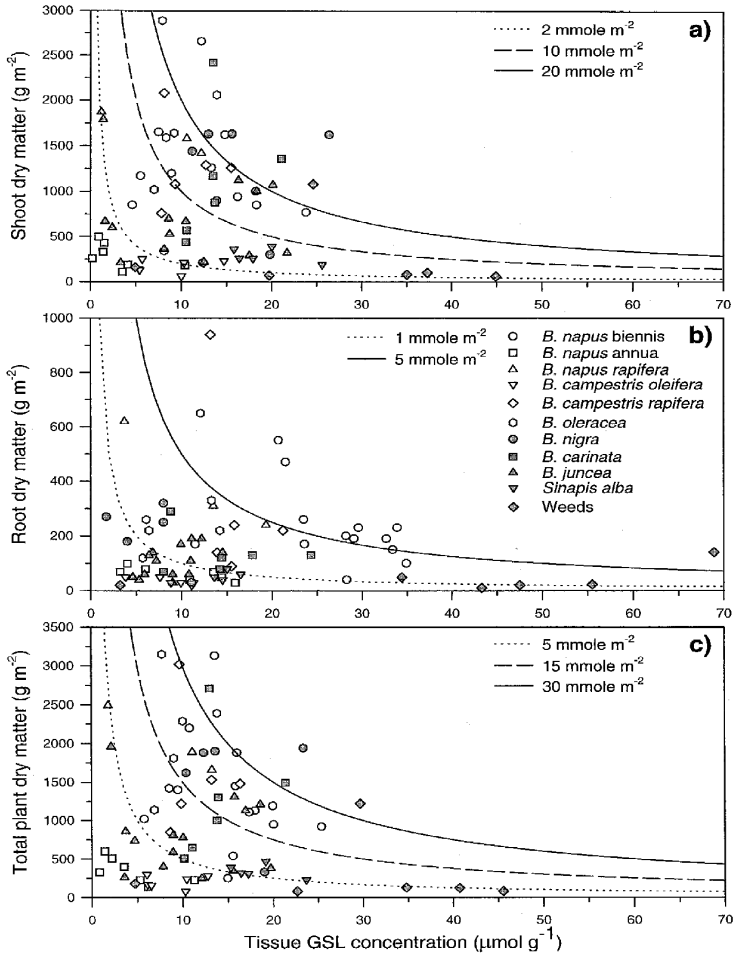


Figure 2.10 – Relationship between tissue glucosinolate concentration and biomass for (a) shoot tissue, (b) root tissue and (c) total plant for Brassica and related weed species.

(O) *B. napus oleifera biennis*, (□) *B. napus oleifera annua*, (Δ) *B. napus rapifera*, (v) *B. campestris oleifera* (>) *B. campestris rapifera*, (O) *B. oleracea*, (•) *B. nigra*, (■) *B. carinata*, (a) *B. juncea*, (▼) *S. alba*, (♦) weed species. (Kirkegaard & Sarwar, 1998)

Despite the large variation some general patterns emerged. All of the *B. napus oleifera annua* and *B. campestris oleifera* entries had shoot GSL production which fell on or below the 2 mmol m^{-2} isoline while no other species had all entries in this low category. All of the other species, with the exception of *S. alba*, had entries with >10 mmol m^{-2} and six species had entries with >20 mmol m^{-2}. There was no significant correlation found between shoot biomass and shoot GSL concentration in any of the species, indicating that high biomass production did not lead to a dilution of GSL concentration, although no entries had both high GSL concentration and high biomass

It is proved (Kirkegaard & Sarwar, 1998) that GSL production in roots was lower than that of shoots primarily due to the lower root biomass, while the range in GSL concentrations in roots and shoots was similar (Figure 2.10 a, b). High levels of root GSL production resulted from high biomass in some entries (e.g. *B. campestris rapifera*) and from high glucosinolate concentration in others (e.g. *D. tenuifolia*), while most of the *B. napus oleifera biennis* combined moderate to high levels of biomass and GSL concentration. GSL production of all *B. napus oleifera annua*, *B. campestris oleifera* and *S. alba* entries fell below the 1 mmol m^{-2} isoline while *B. napus oleifera biennus* entries were predominately above the 5 mmol m^{-2} isoline. The entries of the other species/subspecies fell into the low (<1 mmol m^{-2}) or moderate (<5 mmol m^{-2}) categories. Despite the high GSL concentrations found in weed species, low root biomass resulted in low levels of GSL production with the exception of *D. tenuifoli*a which fell into the > 5 mmol m^{-2} category. Interestingly, there were significant ($P < 0.05$) negative correlations between root biomass and root GSL concentrations in B. napus oleifera annua ($r^2 = 0.47$), and *B. campestris oleifera* ($r^2 = 0.75$), the two species with the lowest GSL concentrations, but no significant relationships within any of the other species.

It also indicates (Kirkegaard & Sarwar, 1998) that the pattern of total plant GSL production generally followed that of the shoots due to the relatively large contribution of the shoots to total plant biomass (Figure 1c). All entries of *B. napus oleifera annua* and *B. campestris oleifera* produced <5 mmol m^{-2} GSL, as did several of the *B. juncea* entries, and the *S. alba* entries fell just above the 5 mmol m-2 isoline. Entries from all other species were distributed within the 530 mmol m^{-2} range. Entries from

B. napus oleifera biennis, *B. campestris rapifera, B. oleracea, B. nigra, B. carinata* were represented in the > 30 mmol m^{-2} category along with the weed species *D. tenuifolia*. The ranking of entries for total plant GSL production changed when only the ITC liberating GSLs were considered (Figure 2.11). Only half of the total GSLs present in *B. napus, B. campestris* and *B. oleracea* species yield ITCs upon hydrolysis due primarily to significant concentrations of OH-substituted aliphatic GSLs (GSL 7, 10) or indolyl GSLs in their tissues, which do not yield ITCs upon hydrolysis (Table 2.3). Despite several entries within these species producing high total GSLs, only one entry produced >20 mmol m-2 of ITC liberating GSL (Figure 2.10). In contrast, *B. nigra, B. carinata, B. juncea, S. alba* and the weeds species contained predominately ITC liberating GSLs and consequently several entries within these species produced >20 mmol m^{-2} of ITC liberating GSLs.

The results of this study illustrate the need to consider the contribution of root GSLs to the sup-pressive potential of green manure crops and as the principal contributor in rotation or companion crops where shoot material is not incorporated. The universal presence of 2-phenylethyl GSL in significant concentrations in the roots of brassicas raises questions regarding its function. The ITCs produced by hydrolysis of aromatic glucosinolates such as 2-phenylethyl are generally less volatile than aliphatic types and may therefore persist for longer in the soil.

This persistence may have provided greater suppression of soil-borne pests, pathogens and weeds and led to selection pressure for higher levels of aromatic types in root tissues in the same way that selection for particular GSLs from pest pressure in the aerial environment has lead to selection for specific aliphatic types in the leaves (Mithen et al., 1995).

Relatively little is known about the activity of 2-phenylethyl ITC in the soil since many previous studies have concentrated on aliphatic types such as methyl ITC (a commercial soil fumigant) or 2-propenyl ITC (allyl) due to its early recognition as the active constituent of mustard oils. However 2-phenethyl ITC has been shown to be significantly more toxic than 2-propenyl ITC in vitro to fungi (Drobnica et al., 1967; Sarwar et al., 1998), insects (Borek et al., 1995) and germinating wheat seeds (Bialy et al., 1990). The predominance in roots of the aromatic GSLs, particularly 2-phenylethyl GSL revealed in this study suggests that their activity and persistence in soil warrant further investigation.

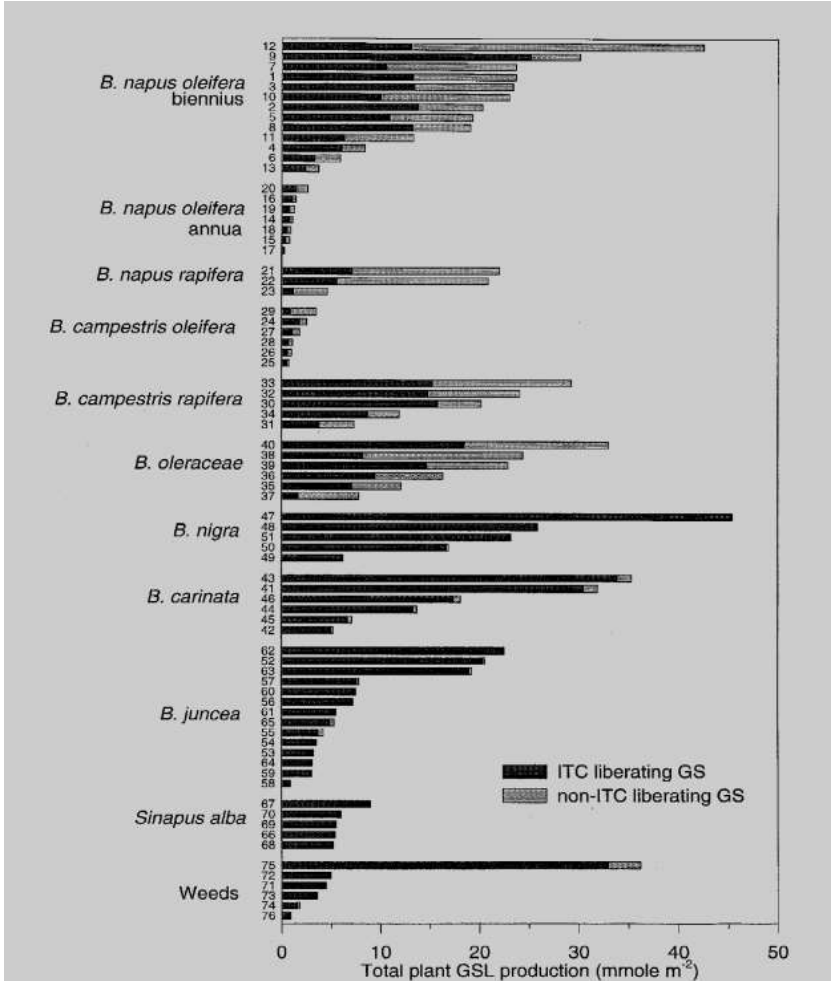


Figure 2.11 – Proportion of total plant glucosinolate production (root + shoot) comprised of ITC liberating (dark bar) and non-ITC liberating (light bar) glucosinolates for 76 Brassica and related species at mid-flowering. Individual entries are numbered as in Table 2.3 (Kirkegaard & Sarwar, 1998)

Brown et al. (1991) have estimated that the amount of methyl ITC used commercially for soil sterilization ranges from 517 to 1294 nmol g^{-1} of soil depending upon the specific crop and control required. The maximum total plant GSL at mid-flowering measured in this experiment was 45.3 mmol m^{-2} which is equivalent to 324 nmol g^{-1} of soil assuming a soil bulk density of 1.4 g cm^{-3} and incorporation to 10 cm. In a subsequent spring-sown experiment at the same site, GSL production was doubled in some entries (Part II, Sarwar and Kirkegaard, 1998). The potential production of ITCs by hydrolysis of GSLs in brassicas (up to 700 nmol g^{-1}) is therefore in a range likely to contribute to pathogen suppression, considering that commercial rates of methyl ITC are aimed at total control.

The effectiveness of brassicas for biofumigation will ultimately depend on many factors beside the BP of the particular Brassica used. The timing of incorporation or exudation of the GSL containing tissue must coincide with a susceptible stage in the life cycle of the pest organism and the suppression must persist to provide protection for the crop of interest. In addition, effectiveness of biofumigation will be influenced by efficiency of incorporation, activity of the hydrolysing myrosinase enzyme, and losses due to volatilisation, sorption onto clay and organic matter, leaching, and microbial degradation (reviewed by Brown and Morra, 1997).

The diversity of GSL profiles, concentrations and production within and between Brassica and related species demonstrated in this experiment suggest that where pest or disease suppression by Brassica rotation or green manure crops can be linked to GSL hydrolysis products, opportunities will exist to select or develop brassicas with enhanced biofumigation potential. In addition to the existing genetic variation in glucosino-late production demonstrated here, further genetic manipulation of glucosinolate concentration, profile and distribution is possible using wide crossing (e.g. Gia-moustris and Mithen, 1995), interspecific crosses (e.g. Chopra et al., 1996) and molecular genetic techniques (Mithen and Campos, 1996).

As for the biochemical composition of oil radish in its synonymous name, according to the research of Ricardo et al. (2018), which is cited in the author's version, the biochemical composition of the aboveground mass of oil radish is as follows:

– phytosterols в-sitosterol (1);

– stigmasterol (2) and />-sitosterol-3-/>-O-D-glucopyranoside;

– D-glucose based on 13C NMR spectra [102.0 (C-1'), 74.5 (C-2'), 77.5 (C-3'), 71.1 (C-4'), 77.1 (C-5'), 62.4 (C-6')];

– norisoprenoid dehydrovomifoliol;

– S-(+)-de-hydrovomifoliol;

– flavonoids kaempferol 3-O-a-L-rhamnopyranosyl-7-O-a-L-rhamnopyranoside and quercetin 3-O-a-L-rhamnopyranosyl-7-O-a-L-rhamnopyranoside, respectively;

– phenylpropanoid ferulic acidm;

– sitosterol (1) (1H NMR (500 MHz, CDCl3) 5: 3.53 (1H, m, H-3), 5.36 (1H, dl, J = 4.0 Hz, H-6), 0.68 (3H, sl, H-18), 1.01 (3H, sl, H-19), 0.92 (3H, d, J = 6.4 Hz, H-21), 0.83 (3H, d, J = 6.5 Hz, H-26), 0.80 (3H, sl, H-27). 13C NMR (75.45 MHz, CDCl3) 5: 37.4 (C-1), 32.1 (C-2), 72.0 (C-3), 42.4 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.3 (C-9), 36.3 (C-10), 21.2 (C-11), 39.9 (C-12), 42.2 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.6 (C-19), 36.3 (C-20), 18.9 (C-21), 34.1 (C-22), 26.2 (C-23), 46.0 (C-24), 29.3 (C-25), 19.6 (C-26), 19.2 (C-27), 23.1 (C-28), 12.2 (C-29));

– estigmasterol (2): 1H NMR (500 MHz, CDCl3) 5: 3.53 (1H, m, H-3), 5.36 (1H, dl, J = 4.0 Hz, H-6), 0.68 (3H, sl, H-18), 1.01 (3H, sl, H-19), 5.15 (1H, dd, J = 8.6/15.1 Hz, H-22), 5.01 (1H, dd, J = 8.6/15.1 Hz, H-23). 13C NMR (75.45 MHz, CDCl3) 5: 37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.4 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.3 (C-9), 36.3 (C-10), 21.2 (C-11), 39.9 (C-12), 42.4 (C-13), 57.0 (C-14), 24.5 (C-15), 29.1 (C-16), 56.1 (C-17), 12.2 (C-18), 19.6 (C-19), 40.7 (C-20), 21.2 (C-21), 138.5 (C-22), 129.4 (C-23), 51.4 (C-24), 32.1 (C-25), 21.3 (C-26), 19.1 (C-27), 25.6 (C-28), 12.2 (C-29);

– daucosterol (3): 13C NMR (75.45 MHz, CD3OD) 5: 38.1 (C-1), 30.3 (C-2), 79.6 (C-3), 39.3 (C-4), 141.2 (C-5), 122.6 (C-6), 32.7 (C-8), 51.1 (C-9), 37.4 (C-10), 21.8 (C-11), 40.6 (C-12), 43.0 (C-13), 57.6 (C-14), 24.9 (C-15), 28.9 (C-16), 56.9 (C-17), 12.2 (C-18), 19.7 (C-19), 36.9 (C-20), 19.2 (C-21), 34.7 (C-22), 26.7 (C-23), 46.7 (C-24), 29.9 (C-25), 19.3 (C-26), 20.1 (C-27), 23.7 (C-28), 12.2 (C-29), 102.0 (C-1'), 74.5 (C-2'), 77.5 (C-3'), 71.1 (C-4'), 77.1 (C-5'), 62.4 (C-6');

– S-(+)-Dehydrovomifoliol (4): [a]D = +98, 1H NMR (300 MHz, CDCl3) 5: 2.33 (1H, d, J = 15.0 Hz, H-2a), 2.50 (1H, d, J = 15.0 Hz, H-2b), 5.95 (1H, qt, J =1.5 Hz, H-4), 6.83 (1H, d, J = 15.0 Hz, H-7), 6.46 (1H, d, J = 15.0 Hz, H-8), 2.3 (3H, s, H-10), 1.88 (3H, d, J = 1.5 Hz, H- 11), 1.02 (3H, s, H-12), 1.10 (3H, s, H-13). 13C NMR (75.45 MHz, CDCl3) 5: 41.6 (C-1), 49.7 (C-2), 197.1 (C-3), 128.0 (C-4), 160.5 (C-5), 79.5 (C-6), 145.1 (C-7), 130.5 (C-8), 197.6 (C-9), 28.6 (C-10), 18.8 (C-11), 24.5 (C-12), 23.1 (C-13);

– Kaempferol 3,7-rhamnoside (5): 1H NMR (300 MHz, CD3OD) 5: 6.68 (1H, d, J = 2.4 Hz, H-6), 6.42 (1H, d, J = 2,4 Hz, H-8), 7.76 (2H, d, J = 8.7 Hz, H-2'/6'), 6.92 (2H, d, J = 8.7 Hz, H-3'/5'), 5.54 (1H, d, J = 1.2 Hz, H-1»), 4.22 (1H, m, H-2»), 3.28-3.90 (1H, H-3»), 3.32 (1H, m, H-4»), 4.02 (1H, m, H-5»), 1.25 (3H, d, J = 6.3 Hz, CH3), 5.38 (1H, d, J = 1.5 Hz, H-1»), 3.28-3.90 (1H, H-3»'), 3.47 (1H, m, H-4»'), 3.59 (1H, m, H-5»), 0.92 (3H, d, J = 5.7 Hz, CH3). 13C NMR (75.45 MHz, CD3OD) 5: 159.7 (C-2), 136.4 (C-3), 179.7 (C-4), 162.9 (C-5), 95.5 (C-6), 163.4 (C-7), 99.8 (C-8), 122.3 (C-1'), 131.9 (C-2'/6'), 116.5 (C-3'/ 5'), 161.7 (C-4'), 100.5 (C-1»), 71.8 (C-2»), 72.0 (C-3»), 73.5 (C-4»), 71.6 (C-5»), 18.0 (CH3), 103.4 (C-1»), 72.0 (C-2»), 72.0 (C-2»), 73.1 (C-4»), 71.2 (C-5»), 17.6 (CH3);

– Quercetin 3,7-rhamnoside (6): 1H NMR (300 MHz, CD3OD) 5: 6.68 (1H, d, J = 2.4 Hz, H-6), 6.42 (1H, d, J = 2,4 Hz, H-8), 7.35 (1H, d, J = 2.1 Hz, H-2'), 6.88 (1H, d, J = 8.4 Hz, H-5'), 7.30 (1H, dd, J = 8.4, 2.1Hz, H-6'), 5.54 (1H, d, J = 1.2 Hz, H-1»), 4.22 (1H, m, H-2»), 3.28-3.90 (1H, H-3»), 3.32 (1H, m, H-4»), 4.02 (1H, m, H-5»), 1.25 (3H, d, J = 6.3 Hz, CH3), 5.38 (1H, d, J = 1.5 Hz, H-1»), 3.28-3.90 (1H, H-3»), 3.47 (1H, m, H-4»), 3.59 (1H, m, H-5»), 0.92 (3H, d, J = 5.7 Hz, CH3). 13C NMR (75.45 MHz, CD3OD) 5: 159.7 (C-2), 136.4 (C-3), 179.7 (C-4), 162.9 (C-5), 95.5 (C-6), 163.4 (C-7), 99.8 (C-8), 122.3 (C-1'), 131.9 (C-2'/6'), 116.5 (C-3'/5'), 161.7 (C-4'), 100.5 (C-1»), 71.8 (C-2»), 72.0 (C-3»), 73.5 (C-4»), 71.6 (C-5»), 18.0 (CH3), 103.4 (C-1»), 72.0 (C-2™), 72.0 (C-2»), 73.1 (C-4»), 71.2 (C-5»), 17.6 (CH3);

– ferulic acid (7): 1H NMR (300 MHz, D2O) 5: 7.25 (1H, sl, H-2), 6.92 (1H, d, J = 16,0 Hz, H-5), 7.13 (1H, dd, J = 1.2/8.1 Hz, H-6), 7.44 (1H, d, J = 16.0 Hz, H-7), 6.37 (1H, d, J = 16.0 Hz, H-8), 3.89 (3H, s, CH3). 13C NMR (75.45 MHz, D2O) 5: 130.6 (C-1), 114.0 (C-2), 150.5 (C-3),

149.8 (C-4), 118.5 (C-5), 125.5 (C-6), 146.0 (C-7), 121.9 (C-8), 177.1 (C-9), 58.8 (CH3).

In conclusion (Ricardo et al., 2018), the results showed that straw of the aerial parts of *Raphanus sativus* var. *oleifer* Stokes possess a phytotoxic activity, and that the dichloromethane fraction was the most active against *E. heterophylla*, *B. pilosa* and *I. grandifolia*. So, it can be concluded that the aerial parts of *R. sativus* possess phytotoxic compounds able to reduce the emergence and growth of weed species, properties that further support the benefit of its use as a cover plant.

A detailed assessment of the biofumigation potential of cruciferous plant species was made in the study by Santos et al. (2021), the results of which are consistently presented in Tables 2.10–2.15.

Clarkson et al. (2020) were noted that 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 Vertcciiiium 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 vttro 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.

Table 2.10

Promising experimental results obtained for the control of fungi using biofumigation with plant species of the Brassicaceae family (Santos et al., 2021)

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
Brassica oleracea	*R. solani*	Tomato	Use of fresh residues	Bioassay, greenhouse, field	Reduced symptoms and increased yield.	Tsror et al. (2007)
Various species	Various species	N/A	Powder	In vitro	Reduced *Ceratobasidium fimbriata* by 68.6% and *V. dahliae* by 68.7%.	Fan et al. (2008)
Brassica napus, B.juncea, B. campestris	Sclerotinia sclerotiorum	N/A N/A	Macerated and dry tissues	In vitro	Growth reduction and sclerotia formation.	Ojaghian et al. (2012)
N/A	*Sclerotium rolfsii, S. sclerotiorum*		Mustard synthetic essential oil	*In vitro,* field	Delayed sclerotia germination.	Dhingra et al. (2013)
B. juncea	*R. solani*	Sugar beet	Planting and incorporation	Field	Consistent control of primary infection.	Motisi et al. (2013)
Various species	*V. dahliae*	N/A	Residue incorporation	*In vitro,* field	*B. juncea* significantly reduced the number of viable microsclerotia.	Neubauer et al. (2014)
Various species	Various species	N/A	Dry powdered plants	In vitro	Released volatiles showed inhibitory effects.	Prasad et al. (2016)
Various species *B. carinata*	*S. sclerotiorum V. dahliae*	N/A N/A	Dry and ground residues *Pellets* (DSM), liquid formulation	*In vitro* *In vitro,* field	Effect on mycelial growth and germination. 67% efficiency using the liquid formulation.	WarmingtoN & Clarkson (2016) Wei et al. (2016)
B. juncea	*Sclerotinia homoeocarpa*	Grass	(DSM)	*In vitro,* field	Reduced mycelial growth and incidence.	Pan et al. (2017)
B. nigra, B. oleracea	*R. solani*	Potato	Leaf extract	*In vitro,* field	*B. nigra* was the most effective at inhibiting growth.	Rubayet et al. (2018)

N/A – Not applicable. DSM – defatted seed meals.

Table 2.11

Promising experimental results obtained in the control of *Fusarium* spp. using biofumigation with plant species of the Brassicaceae family (Santos et al., 2021)

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
B. oleracea	*Fusarium oxysporum*	N/A	Crushed residues	*In vitro*	Reduced the population of *F. oxysporum.*	Iriarte et al. (2011)
B. juncea, S. alba	*F. graminearum*	N/A	Crushed residues	*In vitro*	Suppressed the growth of *F. graminearum.*	Perniola et al. (2012)
B. juncea	*Fusarium* sp.	Bitter melon, calabash	Macerated leaves	Bioassay	Reduced mycelial growth and incidence. Biofumigation combined with	RelevantE & Cumagun (2013)
B. juncea	*F. graminearum*	N/A	Crushed residues	*In vitro*	*Trichoderma* spp. had a synergistic effect against the pathogen. Inoculum control	Perniola et al. (2014) Morales-Rodríguez et al. (2018)
B. carinata	*F. circinatum*	Pine	Pellets (DSM)	Bioassay	and reduced seed mortality.	
B. juncea, Diplotaxis tenuifolia	*F. oxysporum* f. sp. *cucumerinum*	Cucumber	Planting and incorpo-ration	Green-house	Suppressed *Fusarium* wilt.	Jin et al. (2019)
B. carinata	*Fusarium* spp.	Wheat	Planting and incorpo-ration	Field	Reduced incidence and severity; increased yield.	Campanella et al. (2020)

N/A – Not applicable. DSM – defatted seed meals.

Table 2.12

Promising experimental results obtained in the control of oomycetes using biofumigation with plant species of the Brassicaceae family (Santos et al., 2021)

Biofumigaiit	Target	Host	Strategy	Test type	Comments	Reference
Brassica júncea, lberis amara	*Pythium* spp.	NA	Dehydrated plants (pellets)	*In vitro*	After adding water, the dried plants showed ftmgitoxic activity against *Pythiwn* spp. Bioftimigation. combined with solarization reduced the occurrence of the pathogen and increased yield.	Lazzeri et al. (2004)
Brassica carinara	*Phytophrhora cactorum*	Strawberry	Residue incorporation	Field		Barrau et al. (2009)
B. oleracea	Various species	Sweet pepper	Fresh residues – solanzalion	Field	Reduction of plants with symptoms and increased yield. Reduced incidence through changes in the structure of the soil microbial community.	VillaloboS el al. (2013)
B. napas	*Phytophrhora capsici*	Pepper	Ground seeds	Field		Wang et al. (2014a)
B. napas	*P. capsici*	Pepper	Ground seeds	Bioassay	Reduced disease and increased bacterial diversity in the soil. Inliibited mycelial growth and germination of clilamydospores and zoospores, and reduced inoculum potential. *B. carinara* and *B. júncea* reduced mycelial growth aud decreased *P. cinnamonii* viability in the soil.	Wang el al. (2014b)
B. carínata	*P. citntantonti*	*Quercus cerris*	Pellets (DSM)	*In vitro,* bioassay		Morales-Rodríguez et al. (2016)
B. ca/inata, B. júncea, B. napus	*P cinnamotni*	Yellow lupin	Ground seeds	*In xitro,* bioassay		Ríos et al. (2017)
B. carinara	*P. nicoriatiae*	N/A	Pellets (DSM)	*In vitro*	Reduced chlamydospore germination.	Serrano-Pérez et al. (2017)

N/A – Not applicable. DSM – defatted seed meals.

Table 2.13

Promising experimental results obtained in the control of nematodes using biofumigation with plant species of the Brassicaceae family (Santos et al., 2021)

Biofiunigant	Taiget	Host	Stiategy	Test type	Comments	Reference
Raphanns sativus. B. júncea, Sinapis alba	Various specie»	Onion. celery	Planting and incorporation	Field	Reduced parasitic nematodes m celery	Wang et al (2006)
B carinara	*A. chitwoodi*	Potato	Pellets (DSM)	Field	Rednced nematode damage in the root and increased yield.	Henderson et al. (2009)
Various species	*Globodera pallida*	N/A	Leaf extract	*In vitro*	Presented toxicity to nematodes and inhibited the activity of juveniles.	Lord ct al. (2011)
B juncea	*A. incognita*	Tomato	Various	*In vitro.* greenhouse	Reduccd number of galls, egg mass, and eggs ni tomato plants by over 90%.	Oliveira et al. (2011)
B. carinara	*Sí. incognita*	Tomato	Liquid formulation	Bioassay	Statistically significant dose-efl'ect correlations related to isothiocyanate release.	Nicola et al. (2013)
B. júncea	*Sí.incognita*	N/A	Leaf macerates	*In vitro*	Macerate and volatile organic compCHUids released have nematicidal effect.	Barros et al. (2014)
B. júncea. R satmis, Eruca sativa	*G. pallida*	Potato	Planting and mcoiporation	Field	*B. júncea* and *R. sativus* showed to be promising for integrated nematode management systems in potatoes.	Ngala et al. (2015)
Various species	Si. *incognita*	Tomato	DSM	Greenliouse	Better results with *Eruca sativa. Barbaiva venia,* and *Brassica nigra.*	Curto et al. (2016)
R sartvus, B. júncea, S. alba	*Si. incognita*	Sweet pepper	Planting, incorporation + solarizatiou	Greenhouse	Biosolarizatiou with brassicas reduccd the population of juveniles in the soil.	Ros ct al. (2016)
R sativus. E. sativa	*M arenaria*	Tomato	Planting aud incorporation	Greenliouse	Galls aud egg masses decreased significantly.	Aydinli & Mennan (2018)
E. sativa, R sativus. B. júncea	*Sí. incognita. Sí. javanica*	Tomato, potato	Planting and uicoipoiation	Greenliouse	Reduced inoculum density and increased yield.	Daneel et al. (2018)
Crambe abyssinica	*Sí. incognita*	Tomato	Leaf extract	Greenliouse	Crambe extract weekly incorporated into the soil was promising for *Si. incognita* management in tomato.	Roncato et al (2018)

N/A – Not applicable. DSM – defatted seed meals

Table 2.14

Promising experimental results obtained in the control of phytopathogenic bacteria and protozoa using biofumigation with plant species of the Brassicaceae family (Santos et al., 2021)

Biofumigant	Target	Host	Strategy	Test t>pc	Comments	Rcfcrencc
B. oleráceo	*S. scabies*	Potato	Iucorpoiatiou of dry aud ground residues	Field	Disease suppression by 90%	Gouws and Wehner (2004)
B júnceo, R sanvus, B. oleráceo	*R. solanacearum*	Potato	Residue incorporation	Field	Significant suppression of wilt by up to 50%.	Kirkegaard (2009)
B. oleráceo	*R solanacearum.*	Ginger	Residue incorporation Must aid	Field	Lowered incidcncc of wilt and higher crop yield. Cell moitality and íuhibilion of colony Reduced seventy in roots with density greater than plants in *B. rapa* reduccd severity aud increased mass in cauliflower plants	BandyopadhyaY & Khalko (2016)
B. jtntcea	*R. solaiiaceaum* Protozoa	Tomato	essential oil	In vitro		Pontes et al. (2019)
B. rapa. B napus	*P. brassicae*	Chinese cabbage	Planting and iticotporation	Greenhouse field		Chelh et al. (2001)
B. rapo, B.napus	*P. brassicae*	Various	Planting and iucoipoiatiou	Field		Chelh et al. (2006)

N/A – Not applicable. DSM – defatted seed meals

Table 2.15

Promising experimental results obtained in the control of weeds and insects using biofumigation with plant species of the Brassicaceae family (Santos et al., 2021)

Biofuniigant	Target	Host	Strategy	Test type	Comments	Reference
B. júncea. S. alba	Weed various species	N/A	Crushed residues	*In viiro*	At the evaluated doses. *B. jmicea.* significantly inhibited weed germination.	Perniola et al. (2016)
B. júncea	Various spccics	N/A	Powder	Bioassay	Biotunugarion showed potential to be included in weed management programs. Highly effective (mortality 85% against small seeds. |0% to 20%) ngamst hard aud large seed species. Propagule mortality – 90%	Lefebyre et al. (2018)
B. Júncea	Various species	N/A	Incorporation ofcrushed residues. Powdei	Bioassav, field		Cauwer ct al. (2019)
B. júncea	Various species Inscets	NA	Crushed residues	Greenhouse	Biofiimieation with 2.5 kg m was an alternative tool for integrated weed iiuinagemeut.	Perniola el al (2019)
E. sativa	Stored product pests	N/A	ITCs extracted from seeds	Bioassay	Effects of isothiocyaiiates on adults and larv ae of stored product pests.	Shaayal Kostyukovsky (2010)
B. carinóla; B Júncea	*Agrióles bres. A sordidus. usndatus*	Lettice	DSM. chopped residues	Bioassav. field	Insecticidal eftect with high larval moHality and crop protection.	Fitilan et al. (2010)

N/A – Not applicable. DSM – defatted seed meals

It should be noted that the biofumigation activity of different parts of cruciferous plants is different. Thus, in the studies of Bellostas et al. (2007) was noted that the four species followed different patterns during growth with respect to total glucosinolate concentration (Figure 2.12). Total glucosinolate content increased from the first (leaves) to the second (buds) growth stage in *B. carinata*, *B. nigra* and *B. juncea*, with the last species being the one that showed the greatest increase. *B. nigra* and *B. juncea* subsequently showed the same pattern with a decrease in glucosinolate content at the third stage (flower) followed by a new increase towards the last stage monitored (green seeds). Total glucosinolate concentration increased in B. carinata until the flower-stage, decreasing afterwards until the green seed stage, while in B. rapa a steady decrease from the first to the last growth stage was observed.

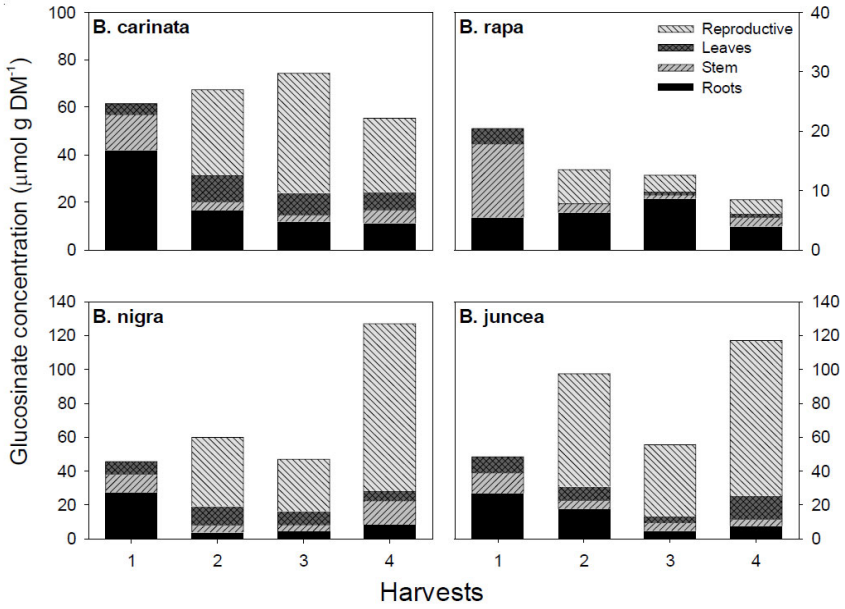


Figure 2.12 – Glucosinolate concentration (imol g DM^{-1}) in the different plant parts at the four growth stages monitored for the four species (Bellostas et al., 2007)

Glucosinolate distribution among plant parts varied with plant age and species (Figure 2.12). The roots generally showed a higher glucosinolate concentration in the first growth stage monitored (leaves) than later in the growth. Glucosinolate content of stem and leaves showed a slight decrease during the growth with total concentrations generally below 15 imol g^{-1} DM. Buds showed a large glucosinolate concentration in all species except for B. rapa, with amounts that varied from 40 to 65 imol g^{-1} DM in *B. nigra* and *B. juncea* respectively. The glucosinolate concentration in the reproductive organs decreased from this stage to the flowering stage, although values as high as 40 imol g^{-1} DM were reported in flowers of *B. juncea*. Total glucosinolate concentration increased in reproductive organs towards the end of the growth, and in the last growth stage values as high as 98 imol g^{-1} DM were found in pods of *B. nigra*.

The distribution of the different types of glucosinolates varied among plant parts during the growth cycle. Figure 2.13 shows the type of glucosinolates (aliphatic, aromatic, indol-3-ylmethyl) present in the different plant parts of the Brassica species at the last growth stage. Aliphatic glucosinolates were mainly present in the vegetative parts of the species, although they also accounted for approximately 50% of the glucosinolate content of the roots in all species, with the exception of *B. rapa* Phenethylglucosinolate was the only aromatic glucosinolate present in the species studied, being the dominant compound in the roots of B. rapa from the second growth.

The total glucosinolate production of the different plant tissues as well as of the whole plant was calculated on the basis of the glucosinolate concentration and the biomass production (Table 2.16). The maximum values were achieved at the last harvest, when both the total glucosinolate concentration in the plant and the biomass produced are at a maximum. The final glucosinolate concentration per g of soil was also estimated based on a plant density of 100 plants m^{-2}, a depth of 10 cm for incorporation of the plant material and a soil bulk density of 1.4 g cm^{-3}.

Differences in the biofumigation potential of the species studied can be expected on the basis of their glucosinolate profiles as well as of their total glucosinolate production. In general, the combination of a high glucosinolate production with the highest dry matter production towards the end of the growing period would make these stages optimal for biofumigation. However, these should not be the only factors to take

into account, since many other parameters might influence the outcome of biofumigation (Figure 2.14). Incorporation of plant material into the soil plays an important role in determining a maximum glucosinolate release and different techniques for an appropriate tissue breakdown have already been investigated (Matthiessen et al., 2004). Knowledge on the cycle of the pathogen in the soil and potential side-effects towards beneficial microorganisms is also necessary other bioactive compounds present in the plant tissue, which may act independently or synergistically with glucosinolate derived products has also been suggested.

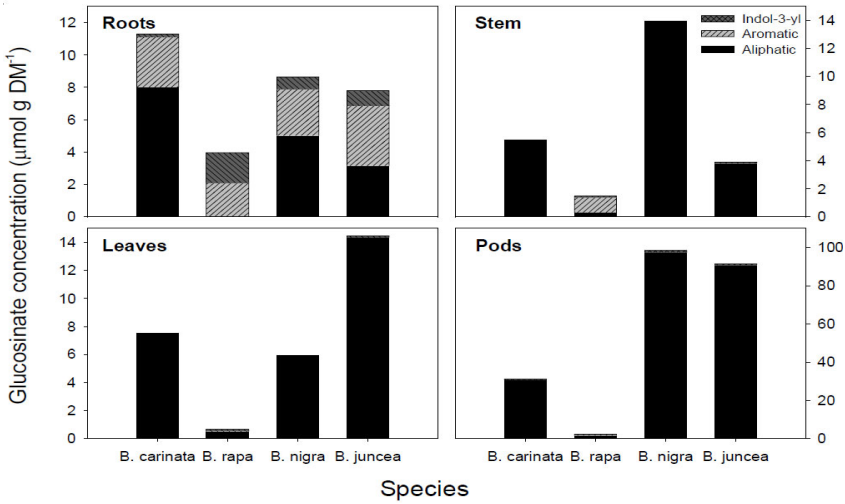


Figure 2.13 – Concentrations (imol g DM^{-1}) of different glucosinolate types (aliphatic, aromatic and indol-3-yl) present in plant parts of each species at the last growth stage monitored (green seeds in pods) (Bellostas et al., 2007)

Bellostas et al. (2007) was noted that determining the type of glucosinolates present in a certain species might be the first step for the assessment of its biofumigation potential, since transformation products resulting from glucosinolate hydrolysis have different toxicities due to their variation in structural types, physical and chemical properties. However,

environmental conditions, such as pH or the presence of certain ions, influence the outcome of the myrosinase hydrolysis of a given glucosinolate. Further investigation of the different types of products resulting from myrosinase catalyzed hydrolysis of glucosinolates at different reaction conditions is therefore important in order to better define the opportunities of biofumigation as a realistic control method of soil-borne pathogens.

Table 2.16

Whole plant and tissue contribution to total glucosinolate production (imol plant^{-1} and nmol g^{-1} soil) of the four species at the four growth stages monitored (Bellostas et al., 2007)

Total glucosinolate production (nmol plant^{-1})							
Species	**Growth stage**	**Root**	**Stem**	**Leaves**	**Reproductive**	**Total**	**Total *(nmol g^{-1} soil)**
B. carinata	1	0.29	0.16	0.70	/	1.16	0.83
	2	16.62	27.64	63.46	6.01	113.73	81.23
	3	27.44	25.31	52.03	22.39	127.17	90.83
	4	49.34	188.31	35.24	243.10	516.00	368.57
B. rapa	1	0.04	0.33	0.36	/	0.73	0.52
	2	6.26	12.89	0.48	1.38	21.01	15.01
	3	5.61	3.55	1.26	1.71	12.13	8.66
	4	12.37	23.46	2.05	16.33	54.22	38.73
B. nigra	1	0.14	0.13	0.67	/	0.94	0.67
	2	5.63	40.81	56.53	26.31	129.28	92.34
	3	9.52	54.73	41.01	82.94	188.21	134.44
	4	24.06	531.66	42.48	2709.86	3308.06	2362.90
B. juncea	1	0.22	0.19	1.35	/	1.76	1.26
	2	12.96	11.40	25.25	15.46	65.06	46.47
	3	7.53	27.03	11.72	29.89	76.17	54.41
	4	12.50	44.78	53.35	1379.37	1490.00	1064.29

* Assuming 100 plants m^{-2}, 10 cm depth incorporation & soil bulk density of 1.4 g m^{-2} (Kirkegaard and Sarwar, 1998).

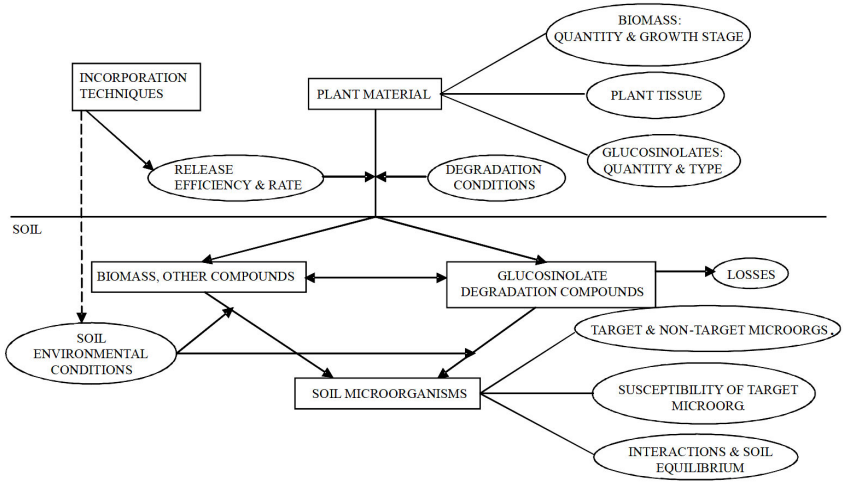


Figure 2.14 – Summary of some of the different factors influencing the outcome of biofumigation (Bellostas et al., 2007)

According to the generalizations of Clarkson et al. (2020) (in the author's original interpretation), the use of biofumigant cultures has the following aspects and expediency:

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 incogntta (De Nicola et al., 2012). 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.

According to the generalizations of Clarkson et al. (2020) with reference to Matthiessen & Kirkegaard (2006), Kirkegaard (2009) and others researchers outline very well the main ways in which biofumigation can be optimised in such ways:

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 dahiiae (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 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.

7. *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.

8. *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 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.

According to the generalizations and conclusions of Clarkson et al. (2020) 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.

Tagged (Biomass Production of Biofumigant Cover Crops, 2014) a new group of cover crops for winter and summer use include mustards, oilseed radishes, and turnips. These crops belong to the family Brassicaceae, which also includes the vegetable crops radish, turnip, collards, broccoli, cauliflower, and cabbage. When young, these plants resemble turnip greens, are very succulent, and have a low C:N ratio, resulting in rapid decomposition when incorporated into the soil. However, if allowed to mature, bolt, and flower, they produce a large amount of biomass in a short period of time and become woody, resulting in slower decomposition than when killed at an immature stage (Figure 2.14.1–2.14.2).

Biofumigant cover crops, including mustards and oilseed radish, produce a group of compounds known as glucosinolates. When these Brassica cover crops are chopped with a shredder or flail mower to destroy plant cells, chemical reactions convert these glucosinolates to isothiocyanates (ITCs). ITCs are used commercially in the control of numerous crop pests including nematodes, disease-causing pathogens, and weeds. Biofumigant cover crops may offer a natural way to fumigate soil with the use of these isothiocyanates.

These potential biofumigants become especially important with the loss of methyl bromide, a fumigant used in the production of high-value vegetable and fruit crops like tomato, pepper, and strawberry. The potential fumigant properties and the physical benefits of cover cropping make Brassica cover crops valuable in cropping systems. However, little is known about the biomass production of oilseed radish and 'Caliente' mustard. Therefore, field experiments were conducted in both spring and summer to determine the biomass produced by these two Brassica cover crops at three different seeding rates.

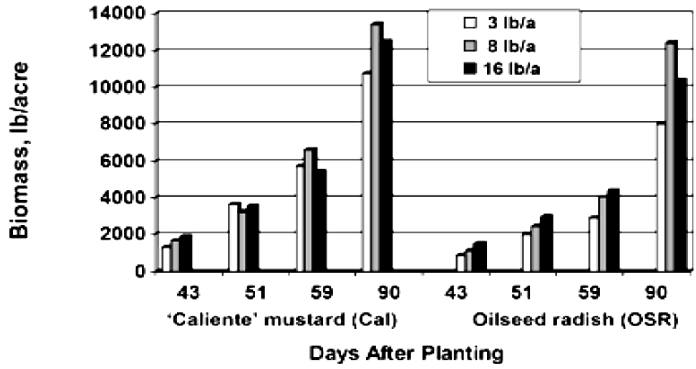


Figure 2.14.1 – 'Caliente' biomass production in spring 2007. 'Caliente' mustard and oilseed radish biomass production in spring 2007. Seeding date was March 31, seeding rate was 3, 8, 16 pounds per acre, and harvest dates correspond to 43, 51, 59, and 90 days after planting (DAP) (Biomass Production of Biofumigant Cover Crops, 2014)

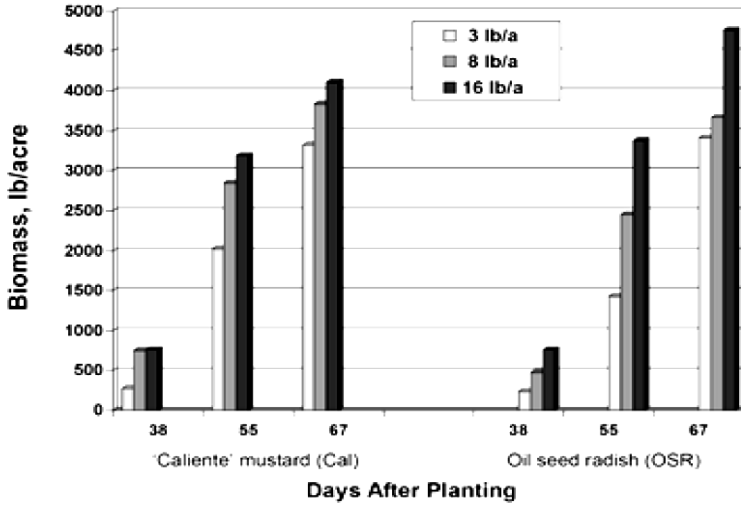


Figure 2.14.2 – 'Caliente' mustard and oilseed radish biomass production in spring 2008. Seeding date was April 1, seeding rate was 3, 8, 16 pounds per acre, and harvest dates correspond to 38, 55, and 67 days after planting (DAP) (Biomass Production of Biofumigant Cover Crops, 2014)

Like any cover crop, Brassica cover crops have limitations. Because these cover crops are closely related to important vegetable crops, (cabbage, collards, broccoli, and greens), crop rotation limitations may exist. A grower producing cabbage may not want to use a Brassica species as a cover crop for fear of disease and insect pests in the cover crop affecting the cabbage crop. However, growers producing vegetables not related to the Brassicas, such as tomato, pepper, squash, and melons, or row crops such as corn, cotton, soybeans, wheat, and tobacco, can include Brassicas in a rotation without fear of any pests moving from the cover crop to these marketable crops. Biomass production can be beneficial for the nutrients recycled, carbon added to the soil organic matter pool, and isothiocyanate produced for potential pest reductions.

2.2. Practical application of oilseed radish in soil biofumigation options

The strategy of using oilseed radish to limit the number of soil nematodes is known and widely used in production practice. Thus, in the scientific work of Mbiro (2016) it is indicated that due to a policy to increase biodiversity farmers are financially rewarded if they use mixtures of green manures. For farmers with *P. penetrans* and *M. chitwoodi* problems the options are limited and therefore research on green manure mixtures is needed. Based on this study the classical clover-ryegrass mixtures are not ideal but fodder radish (resistant cultivar) and arugula (cv. Nemat) can be successful (Table 2.17–2.18, Figure 2.15–2.18).

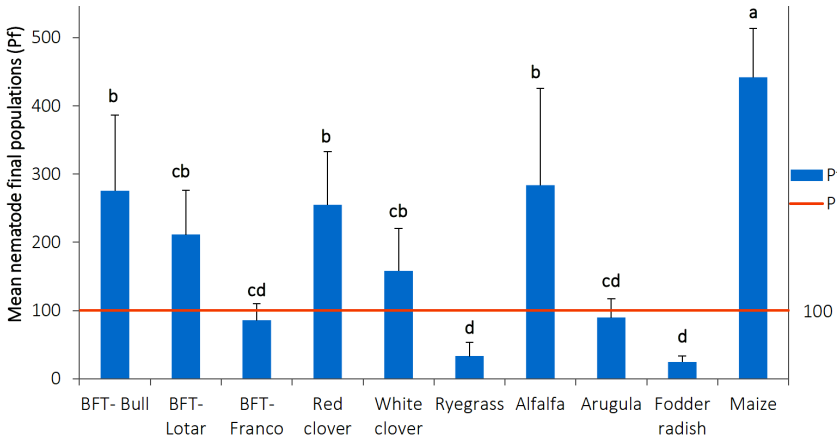


Figure 2.15 – Mean population of *P. penetrans* from a combination of organic and mineral soil fractions of different green manure plant cultivars extracted 8 weeks after inoculation with 100 (juveniles and adults) in yellow tubes. Error bars show standard error and letters indicate significantly similar and dissimilar groups (n= 6 and P < 0.05). Pf = Nematode final population, Pi = Nematode initial inoculation, BFT = Bird's-foot trefoil (Mbiro, 2016)

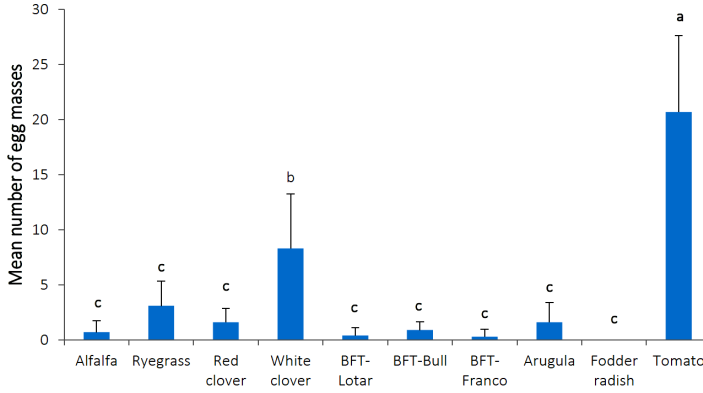


Figure 2.16 – Mean number of egg masses observed on roots of different green manure cultivars 8 weeks after inoculation with 100 second-stage juveniles of M. chitwoodi. Error bars show standard error and letters indicate significantly similar and dissimilar groups (n= 10 and *P < 0.05*), BFT = Bird's-foot trefoil (Mbiro, 2016)

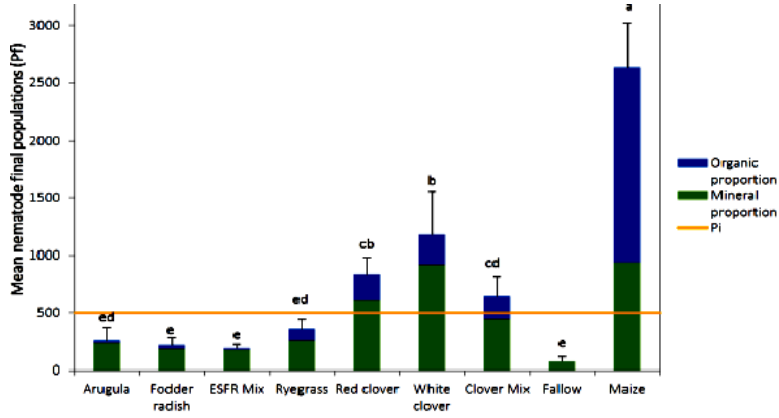


Figure 2.17 – Mean population of *P. penetrans* (organic and mineral soil fraction) of different green manure plant cultivars extracted 8 weeks after inoculation with 500 juveniles and adults. Error bars show standard error and letters indicate significantly similar and dissimilar groups (n = 5 and *P < 0.05*). Pf = Nematode final population, Pi = Nematode initial inoculation, ESFR = Arugula-fodder radish (oilseed radish) mix (Mbiro, 2016)

Putting into consideration that different geographical areas have differing soil properties and abiotic factors. It is essential first to select potential green manure plants that are adapted and best fit into the local climatical crop rotation. It is recommended to carry out greenhouse or field micro-plots for the various selected green manure plants to test for their host suitability to the target pathogens before attempting larger scale field experience through farmers. For a Belgian farmer willing to adopt organic farming, increase his or her crop yields and obtain monetary incentives from the use of green manure mixtures provided by the European Union, it should be a collaborative approach and advice from plant pathologists in different disciplines, plant breeders and geneticists as well.

Table 2.17

The number of eggs, juveniles and adults in organic and mineral fraction and their respective reproductive factor (Rf) on different green manure plant cultivars extracted 8 weeks after inoculation with 500 *P. penetrans* (mixture of juvenile and adult stages). A susceptible maize control and a fallow were subjected to the same inoculation (Mbiro, 2016)

Plants	Root weight (g)	Nematode population after 8 weeks			Rf	Host status
		Organic fraction g^{-1} fresh root	Mineral proportion 2000 g^{-1} soil	Final Populations (Pf)		
Arugula	3.65±0.27	6.0	236±116.10 ^{de}	258±110.97^{e}	0.52	Poor
Fodder radish (Oilseed radish)	3.54±0.78	9.3	184±60.66^{de}	217.2±65.52^{e}	0.43	Poor
ESFR Mix	2.30±0.20	5.9	176±43.36^{de}	189.6±35.65^{e}	0.38	Poor
Ryegrass	14.23±1.44	7.2	256±63.88^{de}	358±81.895^{e}	0.72	Poor
Red clover	4.32±1.27	52.6	612±114.54^{bc}	839.2±145.74^{bc}	1.68	Mainte-nance
White clover	3.70±0.85	77.9	920±367.97^{ab}	1177.2±375.88^{b}	2.35	Good
Clover Mix	13.0±2.46	15.6	444±84.14^{cd}	647.2±167.47^{cd}	1.29	Mainte-nance
Fallow			76±38.47^{e}	76.0±38.47^{e}	0.15	
Maize	29.47±3.66	57.47	940±167.33^{a}	2632.4±391.97^{a}	5.26	Excellent

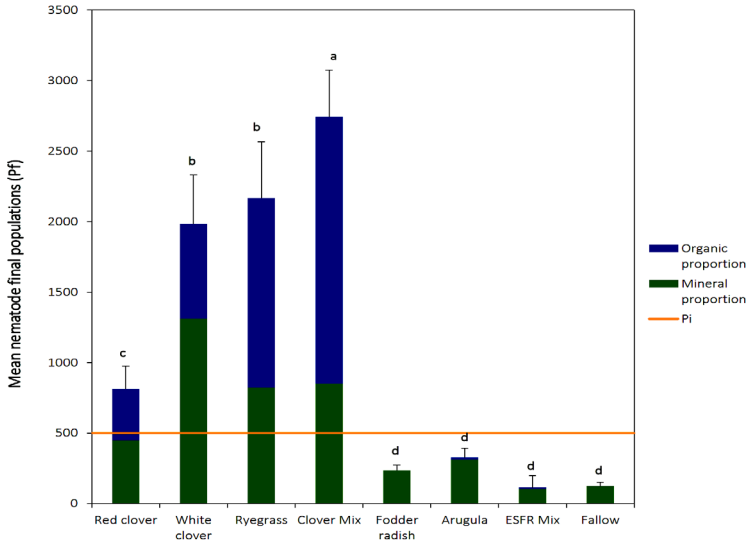


Figure 2.18 – Mean population of *M. chitwoodi* from a combination of organic and mineral soil fraction of different green manure plant cultivars extracted 8 weeks after inoculation with 500 second-stage juveniles. Error bars show standard error and letters indicate significantly similar and dissimilar groups (n = 5 and P < 0.05). Pi = initial nematode inoculation, ESFR = Arugula-fodder radish (oilseed radish) mix (Mbiro, 2016)

Table 2.18

The mean number of eggs, juveniles and adults in organic and mineral fraction and their respective reproductive factor (Rf) on different green manure plant cultivars extracted 8 weeks after inoculation with 500 second-stage juveniles of *M. chitwoodi.* A negative control (fallow) was subjected to the same inoculation (Mbiro, 2016)

Plant	Root weight (g)	Nematode population after 8 weeks			Rf	Host status
		Organic fraction g^{-1} fresh root	Mineral proportion 2000 g^{-1} soil	Final populations (Pf)		
1	2	3	4	5	6	7
Red clover	3.64±0.56	100.3	448±180.33^{c}	813.2±163.65^{c}	1.63	Main-tenance

(End of Table 2.18)

1	2	3	4	5	6	7
White clover	3.33±1.29	201.8	1312±263.29 [a]	1984±347.69[b]	3.97	Good
Ryegrass	11.09±1.76	121.1	824±12.81[b]	2167.6±396.91[b]	4.33	Good
Clover mix	11.81±1.06	160.1	852±136.09[b]	2743.2±332.22[a]	5.49	Good
Fodder radish (Oilseed radish)	3.02±0.22	0	236±38.47[cd]	236±38.47[d]	0.47	Non
Arugula	2.29±0.71	6.8	312±71.55[cd]	327.6±64.92[d]	0.65	Poor
ESFR mix	2.64±0.51	3.9	104±81.73[d]	114.4±83.51[d]	0.23	Poor
Fallow			124±26.08[d]	124±26.07[d]	0.25	

The research by Back et al. (2019) proved the effectiveness of using cruciferous plant species, including oil radish, against potato cyst nematode (PCN) (Table 2.19–2.21).

Table 2.19

Viable population densities of potato cyst nematodes (viable eggs g-1 soil) sampled from field plots with Brassica juncea, Sinapis alba, Raphanus sativus, Eruca sativa or left fallow in field experiment 2013–2014 (Samples collected pre-sowing, pre, incorporation and post-incorporation of the brassica cover crops) (Back et al., 2019)

Treatment	Viable eggs g^{-1} soil pre-sowing (August 2013)	Viable eggs g^{-1} soil pre-incorporation (November 2013)	Viable eggs g^{-1} soil post-incorporation (January 2014)	Viable eggs g^{-1} soil post-incorporation (April 2014)
Untreated	20	13	20	24
Caliente 99 *(Brassica juncea)*	11	16	18	32
Ida Gold *(Sinapis alba)*	14	16	26	28
Bento *(Raphanus sativus (oilseed radish))*	9	12	19	22
Nemat *(Eruca sativa)*	17	20	30	27
Significance (P-value)	NS	NS	NS	NS
SED	6.57	3.55	7.08	8.21
%CV	53.8	36.6	49.6	48.8

Table 2.20

The viability of potato cyst nematode eggs (%) sampled from field plots with Brassica juncea, Sinapis alba, Raphanus sativus, Eruca sativa or left fallow in field experiment 2013–2014 (Samples collected pre-sowing, pre, incorporation and post-incorporation of the brassica cover crops) (Back et al., 2019)

Treatment	% PCN eggs viable pre-sowing (August 2013)	% PCN eggs viable pre-incorporation (November 2013)	% PCN eggs viable post-incorporation (January 2014)	% PCN eggs viable post-incorporation (April 2014)
Untreated	75	67	43	43
Caliente 99 *(Brassica juncea)*	65	71	46	50
Ida Gold *(Sinapis alba)*	71	68	53	52
Bento *(Raphanus sativus)*	49	67	42	47
Nemat *(Eruca sativa)*	70	72	42	47
Significance (P-value)	NS	NS	NS	NS
SED	5.21	7.14	6.97	7.20
%CV	12.4	16.3	24.4	23.8

Table 2.21

Population densities of potato cyst nematodes (eggs g -1 soil) sampled from field plots with Brassica juncea, Sinapis alba, Raphanus sativus, Eruca sativa or left fallow in field experiment 2013–2014 (Samples collected pre-sowing, pre, incorporation and post-incorporation of the brassica cover crops) (Back et al., 2019)

Treatment	Eggs g^{-1} soil pre- sowing (August 2013)	Eggs g^{-1} soil pre-incorporation (November 2013)	Eggs g^{-1} soil post-incorporation (February 2014)	Eggs g^{-1} soil post-incorporation (April 2014)
1	2	3	4	5
Untreated	20.4	5.2	6.2	9.6
Caliente 99 *(Brassica juncea)*	13.8	4.0	7.6	11.2

(End of Table 2.21)

1	2	3	4	5
Ida Gold *(Sinapis alba)*	13.4	4.8	7.0	4.2
Bento *(Raphanus sativus)*	12.6	6.8	5.0	7.0
Nemat (Eruca sativa)	13.4	5.6	5.20	4.8
Significance (P-value)	NS	NS	NS	NS
SED	4.91	2.97	2.43	4.36
%CV	52.7	89.1	61.8	93.7

At the same time, Hansen (2011) proved the effectiveness of oil radish as a biofumigant in terms of transformation of glucosinolates in the soil (Table 2.22-2.23).

Table 2.22

Mean amount of isothiocyanate detected in soil samples after incorporation (Fall-planted brassicas) (Hansen, 2011)

Sampling Time[z] Isothiocyanate	**Pacific Gold Mustard ITCs fag g^{-1} dry soil) (data range)**	**Oilseed Radish ITCs fag g^{-1} dry soil) (data range)**	**Winter Rapeseed ITCs fag g^{-1} dry soil) (data range)**	**Weedy Fallow ITCs fag g^{-1} dry soil) (data range)**
4 hours				
Allyl	5.91 (4.36-8.58)	nd	nd	nd
Benzyl	nd*	nd	nd	0.14 (nd-0.43)
2-phenylethyl	nd	nd	nd	nd
Subtotal	5.91	nd	nd	0.14
2 Days				
Allyl	3.01 (0.65-5.89)	0.56 (0-1.67)	4.99 (0-14.96)	nd
Benzyl	nd	nd	nd	nd
2-phenylethyl	0.13 (nd-0.39)	nd	nd	nd
Subtotal	3.14	0.56	4.99	nd

[y]All values are the mean of three replicates. [z]Sampling times correspond to time elapsed after brassica incorporation. * nd = none detected above the GC-MS limit of detection (limit of detection = 0.005 picogram ITC mL^{-1}.

Table 2.23

Mean amount of isothiocyanate detected following incorporation (Spring-planted brassicas) (Hansen, 2011)

Sampling Time[z] Isothiocyanate	Pacific Gold Mustard ITCs fag g^{-1} dry soil) *(data range)*	Oilseed Radish ITCs fag g^{-1} dry soil) *(data range)*	Winter Rapeseed ITCs fag g^{-1} [dr]y soil) *(data range)*	Weedy Fallow ITCs fag g^{-1} dry soil) *(data range)*
4 hours				
Allyl	1.58 (nd-4.86)	nd	nd	0.13 (nd-0.53)
Benzyl	nd*	nd	0.16 (nd-0.63)	0.17 (nd-0.36)
2-phenylethyl	nd	nd	0.28 (nd-l.12)	nd
Subtotal	1.58	nd	0.44	0.3
2 Days				
Allyl	0.57 (0.5-0.62)	nd	nd	nd
Benzyl	0.06 (nd-0.23)	nd	nd	0.11 (nd-0.43)
2-phenylethyl	0.15 (nd-0.32)	nd	0.44 (nd-0.88)	nd
Subtotal	0.78	nd	0.44	0.11
4 Days				
Allyl	0.22 (nd-0.89)	nd	nd	nd
Benzyl	nd	nd	nd	0.07 (nd-0.27)
2-phenylethyl	nd	nd	0.14 (nd-0.54)	nd
Subtotal	0.22	nd	0.14	0.07

[y] All values are the mean of four replicates.

[z] Sampling times correspond to time elapsed after brassica incorporation. * nd = none detected above the GC-MS limit of detection (limit of detection = 0.005 picograms ITC mL^{-1}.

The effectiveness of using oilseed radish in the control of Fusarium pathogens in the soil through the process of biofumigation has been proved (Table 2.24)

In addition to the positive impact of biofumigation, the impact of this process on improving the microbiological portfolio of soil was also studied (Wieczorek et al., 2024). In the review by Wieczorek et al., 2024, with references to other literature sources, it is noted that plants used for biofumigation provide the soil with a large amount of organic matter.

Table 2.24

Type and concentration of glucosinolates in freeze-dried tissue of brassicas grown in a field experiment to assess the effect of biofumigation on *Fusarium grami* nearum in a wheat-maize rotation (Ashiq et al., 2022)

Glucosinolate (nmol g^{-1}	B.juncea	'Brons'	B. carinata 'Cappucchino'		R. sativus	'Bokito'	R. sativus (oilseed radish) 'Terranova'		E. sativa 'Trio'		B. oleracea var. caulorapa L. 'Kolibri'	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Glucoberin	2.25 (0.32) [a]	2.17 (0.38)	2.01 (0.63)	1.89 (0.39)	2.33 (0.74)	2.22 (0.53)	2.35 (0.41)	2.25 (0.75)	2.06 (0.58)	2.18 (0.49)	2.24 (0.21)	1.83 (0.50)
Progoitrin	0.42 (0.13)	0.37 (0.08)	0.34 (0.23)	0.25 (0.17)	0.42 (0.31)	0.54 (0.39)	0.33 (0.22)	0.34 (0.27)	2.08 (1.49)	1.65 (1.02)	0.39 (0.27)	0.18 (0.13)
Sinigrin	26.39 (12.42)	19.97 (12.33)	13.06 (6.21)	4.81 (3.24)	-	-	-	-	-	-	-	-
Gluconapin	0.20 (0.14)	0.14 (0.17)	-	-	-	-	-	-	-	-	-	-
Glucobrassicin	0.17 (0.07)	0.32 (0.04)	0.65 (0.30)	0.43 (0.31)	1.43 (0.79)	1.54 (1.48)	2.05 (1.29)	0.78 (0.67)	0.38 (0.38)	0.05 (0.07)	1.19 (0.76)	0.91 (0.19)
Gluconasturtiin	1.32 (0.70)	18.04 (3.76)	0.42 (0.29)	6.17 (3.50)	1.12 (0.49)	2.47 (1.74)	1.28 (0.61)	2.38 (2.22)	0.90 (0.42)	2.96 (2.18)	0.52 (0.37)	5.42 (1.75)
Neogluco-brassicin	0.07 (0.03)	0.90 (0.17)	0.11 (0.07)	0.61 (0.44)	-	0.42 (0.38)	0.02 (0.03)	0.12 (0.13)	0.03 (0.02)	0.00	0.53 (0.36)	4.35 (1.82)
Glucoraphanin	-	-	-	-	7.84 (4.79)	1.39 (0.95)	5.24 (4.43)	1.47 (1.11)	7.36 (3.96)	3.94 (2.07)	3.45 (2.72)	1.30 (0.67)
Glucoraphenin	-	-	-	-	0.45 (0.11)	-	0.35 (0.19)	-	-	-	-	-
4 hydroxy glucobrassicin	-	0.04 (0.05)	-	-	-	-	-	0.02 (0.03)	-	-	-	0.27 (0.12)
Glucoraphasatin	-	-	-	-	2.43 (1.69)	14.90 (9.77)	3.45 (2.60)	17.60 (10.92)	-	-	-	-
Glucoalyssin	-	-	-	-	-	-	-	-	0.50 (0.21)	0.24 (0.21)	-	-
Glucoerucin	-	-	-	-	-	-	-	-	7.03 (3.65)	12.28 (7.30)	0.14 (0.16)	3.80 (1.71)
4-mercaptobutyl	-	-	-	-	-	-	-	-	4.89 (2.04)	1.26 (1.94)	-	-
unknown	-	-	-	-	-	-	-	-	0.53 (0.37)	-	-	-
unknown	-	-	-	-	-	-	-	-	0.93 (0.70)	-	-	-
Total glucosinolates	30.83 (13.76)	41.91 (15.63)	16.59 (7.62)	14.19 (7.31)	16.02 (8.66)	23.47 (14.65)	15.06 (7.33)	24.96 (15.10)	26.69 (12.33)	24.57 (12.69)	8.46 (4.55)	18.06 (6.22)

[a] n = 4, numbers in parentheses represent the standard error of the mean.

In consequence, there are a sufficient amount of nutrients for microorganisms, which produce more enzymes. These are the reasons for the increased enzyme activity and respiratory activity of the replanted soil in the variants with biofumigation treatment. Other researchers indicate a positive correlation between the content of organic matter in the soil and its enzyme activity (Yuan and Yue, 2012; Zhan and Sun, 2014].

The activity of soil enzymes depends on the physicochemical properties of soil (pH, organic matter content, heavy metal contamination), climate, and cultivation system (Zhan and Sun, 2014). According to Weaver (2012), an insufficient amount of water in the soil may significantly limit the enzyme activity. In our experiment, the enzyme activity and respiratory activity of the of soil were analysed in spring, summer, and autumn. There were differences in the results depending on the vegetation period.

The activity of soil enzymes because increased moisture facilitates the solution of organic matter in the soil (Geisseler et al., 2011). Sardans et al. (2005) observed that a 10% decrease in the soil moisture caused the protease activity to drop by 15-66%. The high enzyme activity and respiratory activity of soil depends on the count and diversity of microbial communities. The analysis of the count of soil microorganisms conducted in our experiment confirmed the positive effect of biofumigation treatment on this parameter. The most effective plant was French marigold *(Tagetes patula)*. The total bacterial count in the *French marigold* variant was almost two times greater than in the variant without it (Figure 2.19 A).

Hanschen and Winkelmann (2020) also observed an increase in the count of plant growth- promoting bacteria in the replanted soil after the application of *Brassica juncea* and *Sinapis alba.* The analysis of the count of oomycetes and fungi gave similar results. The total count of oomycetes and fungi in the variants with French marigold and white mustard was more than two times greater than in the replanted soil without forecrops (Figure 2.19 B). There was a slightly different result in the count of actinobacteria. The total count of these microorganisms (17.92 CFU 10^5 g^{-1} d.m.) was the highest in the oilseed radish variant *(Raphanus sativus* var. oleifera) (Figure 2.19 C).

The increase in the soil enzyme activity increased the rate of mineralisation of organic matter, and consequently, the amount of macro- and micronutrients available to plants. In our experiment, significant

differences in the content of macro- and micronutrients in the soil, depending on its earlier use, were noted. The content of all macro- and micronutrients (except Mn) in the replanted soil was significantly lower than in the crop rotation soil (Table 2.25). Other researchers also observed the low content of nutrients in replanted soil (Cesarano et al., 2017; Zydlik et al., 2021). The increase in the microbial activity manifested by the higher enzyme activity and respiratory activity of the soil in the variants with forecrops of three species of biofumigants translated into an increase in the content of soil macro- and micronutrients. There was a significant increase in the content of N, P, K, Zn, Cu, and Fe in the replanted soil with the phytosanitary plants, especially in the variant with Tagetes patula. The content of minerals in the oilseed radish variant (Raphanus sativus var. oleifera) was from about 9% (Fe) to about 90% (Zn) greater than in the replanted soil (RS) without forecrops (Table 2.25).

Table 2.25

Content of macro- and microelements (mg dm ^{3}) in the soil in 2021 (CRS = crop rotation soil; RS = replanted soil) (Wieczorek et al., 2024)

Mineral Elements	CRS	RS	French Marigold	White Mustard	Oilseed Radish
			Forecrop	Forecrop	Forecrop
N-NO_3	98.1 ± 8.62c	83.2 ± 10.4a	91.3 ± 9.77b	132.6 ± 11.2e	124.5 ± 10.7d
P	310.3 ± 16.4c	249.2 ± 21.7a	275.0 ± 26.6b	318.5 ± 30.9d	440.0 ± 36.5e
K	247.0 ± 20.6b	190.2 ± 19.8a	256.4 ± 22.3d	251.4 ± 19.6c	290.8 ± 21.9e
Ca	117.5 ± 10.3c	101.1 ± 9.4a	105.0 ± 9.90b	128.6 ± 11.2d	127.4 ± 13.6d
Mg	15.0 ± 1.34a	16.0 ± 1.72a	19.0 ± 1.63b	16.0 ± 1.45a	19.0 ± 1.9b
Zn	7.7 ± 0.67d	4.37 ± 0.74a	6.17 ± 0.56b	7.3 ± 0.60c	8.3 ± 0.49e
Cu	2.2 ± 0,11c	1.56 ± 0.09a	1.9 ± 0.08b	2.3 ± 0.10d	2.3 ± 0.94d
Mn	50.8 ± 2.34e	28.2 ± 1.96b	20.4 ± 1.36a	41.1 ± 0.45d	40.1 ± 2.17c
Fe	106.1 ± 10.3d	96.9 ± 9.75b	79.7 ± 8.32a	111.6 ± 9.69e	107.3 ± 9.73d

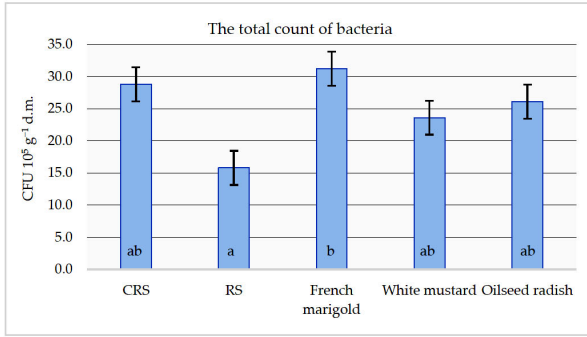
Means marked with the same letters do not differ significantly at a = 0.05.

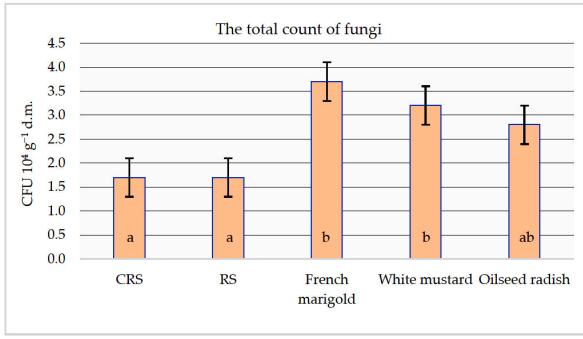
Wieczorek et al., 2024 The effect of oilseed radish in combination with other crops as a biofumigant agent on the abundance of certain species of

soil nematodes was also investigated. In their experiment, eleven species of nematodes were identified in the soil (Table 2.26). In the control variant (CRS), there were four species of nematodes, mostly Ecumenicus monohystera (about 34 individuals in 100 cm^3 of soil). The population of Mesorhabditis spiculigera was much smaller. The replanted soil (RS) had the most nematodes of the Mesorhabditis spiculigera and Tylenchorhynchus dubius species (79 and 60 individuals in 100 cm^3 of soil, respectively).

In this experiment, all the three species of biofumigants effectively reduced the number of nematodes, especially in the replanted soil. The French marigold (*Tagetes patula*) was highly effective, because in the variant with it, the number of nematodes of the Pratylenhus penetrans species was reduced from about 33 individuals in 100 cm^3 of soil to zero. This species belongs to the Pratylenchidae family, which is considered one of the most important pests in orchards as well as plantations of vegetables and ornamental plants. The *Pratylenhus penetrans* feed on roots, where they cause necrotic spots, which significantly reduce the active surface of the roots. In comparison with the variant without forecrops (RS), the forecrop of French marigold reduced the population of *Tylenchorhynchus dubius* several dozen times, the populations of Prismatolaimus sp. and Geocenamus nothus – about a dozen times, and the population of Mononhoides sp. – several times (Table 2.26). It is noteworthy that the population of *Tylenchorhynchus dubius* (another internal plant parasite after *Pratylenhus penetrans* that feeds on roots) in the replanted soil was significantly reduced. Tylenchorhynchus dubius is able to survive and develop in various environmental conditions. It occurs in the root zone of over one hundred plant species. The populations of species such as Ecumenicus monohystera and Mononhoides sp. in the soil with the white mustard forecrop (Sinapis alba) were completely reduced.

The populations of *Cephalobus persegnis* and *Geocenamus nothus* were also reduced several times. There were no significant variant-dependent differences in the population of Cuticularia oxycerca. Oilseed radish was relatively the least effective in reducing the number of nematodes in the replanted soil. In the variant with the forecrop of this plant, the number of nematodes of the *Geocenamus nothus* and *Terrtocephalus terrestris* species in the soil was reduced to zero. However, the populations of *Ecumenicus monohystera* and Mononhoides sp. did not change.





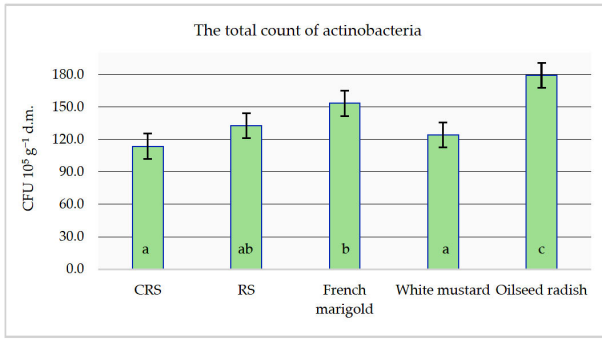


Figure 2.19 – Microbial abundance in the soil (CRS = crop rotation soil; RS = replanted soi)). Means marked with the same letters do not differ significantly at = 0.05. (Ah) The total count of bacteria; (B) The total count of fungi; (C) The total count of actinobacteria

Table 2.26

Average for years 2020–2021 nematode abundance in the soil (in 100 cm^3 of soil) (CRS = crop rotation soil; RS = replanted soil) (Wieczorek et al., 2024)

Species	CRS	RS	French Marigold Forecrop	Whight Mustard Forecrop	Oilseed Radish Forecrop
Cephalobus persegnis	4.1 ± 0.9 a	44.9 ± 9.2 b	0.0 ± 0.0 a	2.9 ± 1.0 a	1.0 ± 0.0 a
Cuticularia oxycerca	0.0 ± 0.0 a	3.3 ± 1.1 a	12.8 ± 3.4 b	3.3 ± 1.6 a	13.2 ± 3.1 a
Ecumenicus monohystera	33.9 ± 8.0 c	49.6 ± 5.6 c	44.2 ± 6.0 c	0.0 ± 0.0 a	42.7 ± 1.6 c
Geocenamus nothus	0.0 ± 0.0 a	16.5 ± 5.4 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Mesorhabditis spiculigera	19.2 ± 5.2 b	79.2 ± 8.3 c	39.5 ± 4.9 b	9.9 ± 2.2 a	75.9 ± 14.8 c
Mononhoides sp.	6.6 ± 2.0 a	13.2 ± 6.7 c	6.6 ± 1.1b	0.0 ± 0.0 a	9.9 ± 3.3 b
Panagralaimus rigidus	0.0 ± 0.0 a	56.2 ± 9.2 a	249.2 ± 24.5 b	240.9 ± 27.3 b	211.2 ± 28.1 b
Pratylenhus penetrans	0.0 ± 0.0 a	33.5 ± 5.8 c	0.2 ± 0.0 a	7.3 ± 2.0 b	7.2 ± 2.2 b
Prismatolaimus sp.	0.0 ± 0.0 a	49.5 ± 7.7 c	6.6 ± 1.0 a	19.5 ± 5.8 b	13.2 ± 3.5 a
Terrtocephalus terrestris	0.0 ± 0.0 a	21.3 ± 4.3 b	16.1 ± 3.7 b	36.0 ± 9.7 b	0.0 ± 0.0 b
Tylenchorhynchus dubius	0.0 ± 0.0 a	60.1 ± 8.8 c	3.7 ± 1.2 a	25.3 ± 4.4 b	25.1 ± 5.3 b

Means marked with the same letters do not differ significantly at a = 0.05.

The analysis of the populations (Wieczorek et al., 2024) of soil nematodes in individual years of the research showed that the nematocidal effect of the plants used for biofumigation was noticeable as early as one year after their application. The populations of eight of the eleven species of nematodes identified in 2020 decreased significantly in the following year of the research. These were mostly Geocenamus nothus, Mesorhabditis spiculigera, Mononhoides sp., Pratylenhus penetrans, and Prismatolaimus sp. In the second year of the experiment, no nematodes of these species were found in the soil more than double increase in the content of humus, as well as significant higher enzyme activity and respiratory activity in the replanted soil, in the variants with *French marigold*, white mustard, and oilseed radish was noted. Compared to the treatments without biofumi-gation, there was

also a significant increase in the number of bacteria in the soil, especially in the variant with the use of *Tagetes patula*. This significantly improved the growth strength of the apple trees. The leaves of the trees in the variants with the biofumigation treatment had a larger surface area and weight (increase by 50%) than those from the trees growing on the replanted soil without forecrops. Also, the trees were taller and had a greater total growth of side shoots. The experiment also confirmed the nematocidal effect of the three species of the biofumigants, especially French marigold. The biofumigation treatment with its use made it possible to reduce the population of nematode Pratylenhus penetrans species from 33 to 0 individuals in 100 cm^{-3} of soil.

The author of the study (Wieczorek et al., 2024) concluded that biofumigation treatments using *Tagetes patula*, *Sinapis alba* and *Raphanus sativus* on replanted soil should be considered a safer and futuristic alternative to thermal disinfection and chemical fumigation, because it improves the biological properties of replanted soil and reduces the number of parasitic nematodes feeding on plants. It restores the balance of soil microorganisms and improves the growth strength of fruit trees in nurseries.

In the results of a number of zonal studies on the effectiveness of bofumigation (International Biofumigation Network, 2020; AHDB PCN, 2020; AHDB Biofumigation Report, 2022; Biofumigation For management of potato cyst nematodes (PCN), 2022) were noted that biofumigation involves incorporating brassicaceous cover crops into the soil; they produce a range of secondary metabolites in-cluding glucosinolates which can control pests. Brassicas also contain an enzyme called myrosinase. Isothiocyanates inhibit cellular respiration and other PCN functions. They can cause juvenile PCN – the young wormlike stage which invades potato roots – to hatch from their protective eggs. This causes them to starve in the absence of a host. Isothiocyanates can also paralyse or kill juveniles outright. Longer isothiocyanate exposure times and higher doses kill more PCN. Some isothiocyanates are more volatile than others, that is, they easily evaporate at normal temperatures. These isothiocyanates have lower molecular weights and are generally more mobile in soil. However, the less mobile isothiocyanates tend to be more toxic due to having more binding sites within PCN tissues. This means that trade-offs between toxicity and mobility may sometimes have to be made. The most toxic isothiocyanates to PCN in laboratory studies are allyl, 2-phenethyl and benzyl.

The glucosinolate sinigrin (2-propenyl) produces allyl, gluconasturtiin produces 2-phenethyl and glucotropaeolin produces benzyl. Few field studies have investigated biofumigant varieties that produce these isothiocyanates. Allyl is similar in toxicity and volatility to methyl-isothiocyanate, which is encouraging for PCN management. Methyl-isothiocyanate is the gas given off from metam-sodium (METAM 510®, CERTIS) and dazomet (BASAMID®, BASF). Several seed companies supply biofumigant species. Most research has been on the Indian mustard variety 'ISCI 99'. It produces high concentrations of sinigrin glucosinolate in its tissues. ISCI 99 can also produce high fresh biomass during the summer window (c.40–70 t/ha). Indian mustard and other biofumigant species are shown in Figure 2.20. Rocket and oil radish glucosinolates depend greatly on variety, whereas Indian mustards mainly contain sinigrin.

Rocket and oilseed radish varieties can produce fresh biomass of 30–40 t/ha, with oil radish having the greater biomass potential. White mustard (*Sinapis alba*) often produces high biomass. However, its main glucosinolate is glucosinalbin, which is of limited use for PCN management. Figure 2.21 shows biofumigation efficacy against PCN for published field studies. Further information, including details of seed suppliers, can be obtained from the International Biofumigation Network.

It was noted (AHDB Biofumigation Report, 2022) that a biofumigant crop needs to meet its maximum potential before being macerated and incorporated into soil for the most effective biofumigation. Biofumigation potential depends on:

– The type of glucosinolate in the crop.
– The glucosinolate concentration in crop tissues.
– The biofumigant crop biomass.

As previously mentioned, a biofumigant needs to have appropriate glucosinolates in its tissues for the pest being targeted with biofumigation. Choose a variety with a potential glucosinolate concentration in dry tissue of at least 100 |jmol g (micromoles per gram). Look for seed suppliers that state the type of glucosinolate and its concentration. A biofumigant should produce lots of biomass. Crops of 1.2-2.0 m in height usually represent in excess of 50 t/ha fresh weight, which is the goal. The more biomass produced, the more glucosinolates and the greater the potential biofumigant dose at incorporation.

Figure 2.20 – Common biofumigant species investigated for PCN management: A = *Brassica juncea* (Indian mustard), B = *Eruca sativa* (rocket), C = *Raphanus sativus* (oilseed radish) and D = *Sinapis alba* (white mustard) (AHDB PCN, 2020)

According to the results of the 2016–2017 research of Duff et al. (2021) treatments comprised nine readily available brassica biofumigants, plus three grower standards; two commonly grown cover crop varieties, Fumigator sorghum and Lablab, and a Fallow (Table 2.27). Varieties were assessed for plant biomass, days to incorporation, glucosinolate content, and efficacy against three soilborne pathogens Sclerotium rolfsii, Sclerotinia sclerotiorum and Macrophomina phaseolina. The brassica varieties trialled were flowering between 5-10 weeks when grown in summer and 9-14 weeks when grown in winter as seen in Table 2.28. The fastest-growing variety of biofumigants was BQ Mulch, taking 36 days in summer and 59 days in winter from planting to incorporation. The greatest times taken to incorporation were the varieties Nemclear and Nemcon, which actually failed to flower, even after 100+ days. Earlier work in 2016 with these varieties never saw them flowering, even after 4 months. For this reason, they were excluded from further trial work and ongoing analysis. Brassica juncea types flowered

close together with Caliente maturing in 44 days when planted in summer and 98 days for it to reach flowering with a late autumn/winter planting. The two grower standard cover crops were incorporated close to 90 days after planting or when both Nemcon and Nemclear were incorporated.

Species (Common name)	Variety [supplier]	Dominant glucosinolates	Dominant isothiocayante	Recorded field efficacy (%)	Reference
Brassica juncea (Indian/brown mustard)	Caliente (ISCI) 99 [High Performance Seeds/Tozers]	sinigrin	2-propenyl/allyl	15–95	Ngala et al., 2014
		sinigrin	2-propenyl/allyl	23–37	Watts, 2018
	Variety 2 [Joordens Zaden/RAGT]	unknown	unknown	45	Watts et al., 2014
Eruca sativa (rocket/ arugula)	Nemat [High Performance Seeds/Tozers]	glucobrassicanapin	4-pentenyl	30–90	Ngala et al., 2014
	Trio [Joordens Zaden/ RAGT]	unknown	unknown	46	Watts et al., 2014
Raphanus sativus (oil radish)	Bento [P H Petersen]	glucoraphanin	(4-(methylsulfinyl) butyl/butyl	65–95	Ngala et al., 2014
		glucnsasturtiin	2-phenethyl		
Sinapis alba (white mustard)	Architect [Joordens Zaden/ RAGT]	unknown	unknown	41	Watts et al., 2014
	Zlata [Feldsaaten freudenberger]	unknown	unknown	0	Valdes et al., 2012
	Metex [P H Petersen]	unknown	unknown	16	Scholte & Vos, 2000

Figure 2.21 – Details of published biofumigation field studies focusing on potato cyst nematode management (AHDB PCN, 2020)

The research by Duff et al. (2021) also notes that up to 5 different GSLs were tested with 4 GSLs being consistently tested for over the 6 trials. Those tested for included Glucoberin, Sinigrin, Progoitrin, Gluconapin and Glucoraphanin, with the last GSL only being tested for in plants 5 and 6. Total glucosinolates, µmol/gram of dry matter, were greater during the hotter parts of the year with less during the cooler months of the year for the majority of the varieties evaluated. The levels of GSLs extracted from the different brassica biofumigants during the summer and winter growing periods or planting 1 and 4. They show that the majority of GSLs are

greater during the summer than the winter planting with large differences even between varieties of brassicas grown. Figures 2.22–2.23 show how the average total GSLs for all 6 plantings changed with the time of year with most varieties producing more GSLs per gram of dry material during the warmer months of the year. Caliente and Nemfix produced the greatest amount with 53.47 µmol g_{DM}^{-1} and 52.25 µmol g_{DM}^{-1} being measured from summer grown crops respectively. BQ Mulch was the next best.

Table 2.27

Biofumigant varieties trialled 2016–2017 at the Gatton research Facility, Queensland Australia (Duff et al., 2021)

Product Name	Company	Plant Species	Rate/ha (Kg)
B.Q. Mulch®	PPG Wrightson Seeds	*25% Brassica nigra & 75% Brassica napus* (Black mustard and Fodder mustard)	10
Biofum™ Mix	Australian Premium Seeds	*Raphanus sativus* & *Sinapis alba* (Doublet Oilseed radish, Achilles white mustard)	10
Caliente™	E. E. Muirs and Sons	*Brassica juncea* (Indian mustard)	10
Mustclean™	Graham's Seeds	*Brassica juncea* (Indian mustard)	10
Nemat™	E. E. Muirs and Sons	*Eruca sativa* (Rocket)	8
Nemfix™	Seedmark	*Brassica juncea* (Indian mustard)	15
Nemcon™	Pature Genetics	*Brassica napus* (Fodder mustard)	10
Nemclear™	Auswestseeds	*Brassica napus* (Fodder mustard)	10
Tillage Radish®	AFG Seeds	*Raphanus sativus* (Oilseed radish)	10
Fumig8tor™ sorghum	Pacific Seeds	*Sorghum bicolor*	25
Lablab		Lablab purpureus	30

Efficacy was variable between biofumigant varieties and planting dates. Summer (Planting 1) and late winter/spring (Planting 5) exhibited better control of the various pathogens tested, particularly *S. sclerotiorum*, while autumn (Planting 3) did not exhibit the same level of effectiveness.

It is emphasized (Duff et al., 2021) that Caliente, Mustclean and Nemfix, all B. juncea, were the better varieties for controlling *S. rolfsii* in the late winter/spring planting as shown in Figure 2.25, with between 67% and 93% mortality of the buried sclerotes. Tillage Radish was the best variety for managing S. rolfsii with autumn planting with 68% mortality, as opposed

to its poor performance during the summer and late winter/spring periods, where it managed only 6.7% mortality on both occasions. Caliente was the only other variety that had any significant effect on S. rolfsii when planted in autumn with 41% mortality. This was however not significantly better than the Fallow treatment which still exhibited some degree of control of S. rolfsii during summer and autumn.

Table 2.28

Days to incorporation (25% flowering) of brassica biofumigants trialled as well as grower standards (Duff et al., 2021)

	Planting date					
	15 Nov 2016	**23 Jan 2017**	**28 Mar 2017**	**16 May 2017**	**17 Jul 2017**	**20 Sept 2017**
Caliente	44	50	97	98	81	72
Mustclean	44	44	63	90	81	61
Nemfix	36	44	63	90	81	61
Nemat	70	87	69	79	67	78
Tillage Radish	70	94	97	98	67	81
Nemcon*	101	102	115	NP	NP	NP
Nemclear*	101	102	115	NP	NP	NP
Biofum	70	87	97	98	89	81
BQ Mulch	36	44	44	58	59	44
Fumig8tor sorghum	101	102	83	NP	96	88
Lablab	85	102	83	NP	NP	88

When biomass is incorporated into the equation, the amount of GSLs being produced per hectare reveals a mixed result. Some varieties producing more GSLs during winter with the greater biomass as with Caliente and Nemfix, and others still produce the least amount of GSLs during the cooler months as seen with BQ Mulch, Tillage Radish and Biofum as shown in Figure 2.24.

Sclerotinia sclerotiorum, Figure 2.25, was not managed to any great degree by any of the biofumigants with the Fallow treatment being just as effective if not better, at killing off the buried sclerotes. It wasn't until a winter/spring planting that Biofum outperformed all other treatments, including the Fallow treatment, with 50% mortality of the buried sclerotes. The autumn planting performed poorly with no significant differences between any of the treatments. This was regardless of the increased amount of plant material generated at this time of the year, Nemfix giving the greatest degree of mortality with an autumn planting at only 22% (Duff et al., 2021).

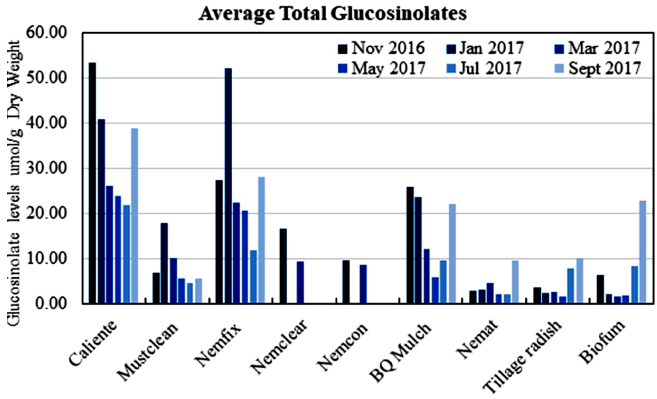


Figure 2.22 – Average total glucosinolates being produced per gram of dry material at different times of the year. performer with 25.96 µmol/g DM being produced with a summer planting also (Duff et al., 2021)

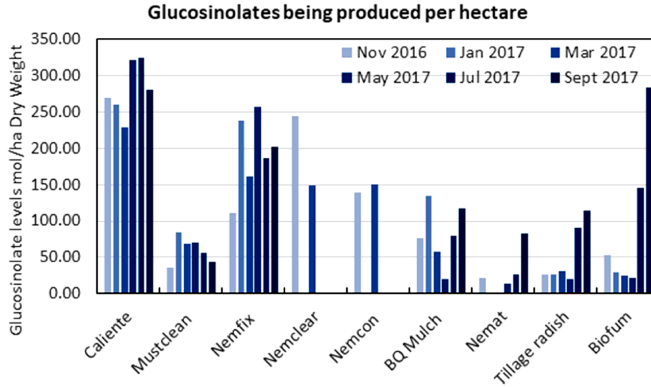


Figure 2.23 – Amount of glucosinolates being produced per hectare at different times of the year (Duff et al., 2021)

Macrophomina phaseolina (Figure 2.26) was effectively managed with Nemat and Tillage Radish during a late winter/spring planting with 93% and 100% mortality respectively of infested seed. Caliente, Mustclean and BQ Mulch were the best performers during a summer planting with

77.78%, 76.67% and 74.44% respectively. No treatment was better than the Fallow at controlling Macrophomina with an autumn planting. Bare ground Fallow treatment still managed to exert some level of control of this soilborne pathogen with 52%, 32% and 50% control being achieved during the three planting periods (Duff et al., 2021).

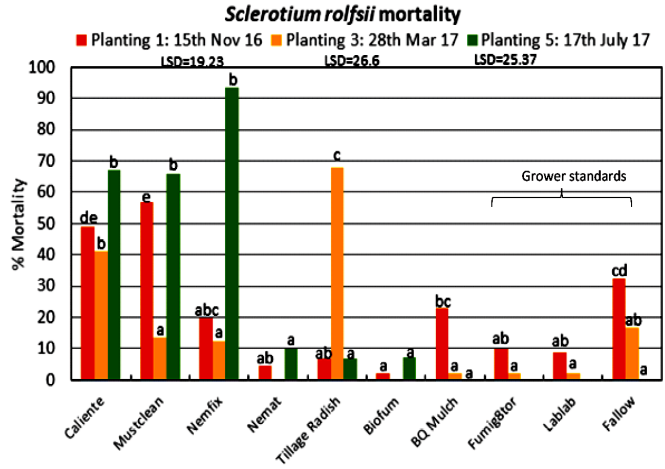


Figure 2.24 – Performance of various brassica biofumigants and grower standard practices against *Sclerotium rolfsii*. Bars with the same letter for the different plantings are not significantly different from one another (Duff et al., 2021)

The research of Wieczorek et al. (2023) proved the influence of cruciferous plant species, including oil radish, on the structure of the soil microbiological complex. The metapopulation analysis based on the analysis of the 16S rRNA sequence showed that the previous use of the soil in the nursery, the phytosanitary plants used in the experiment, and the years of research influenced the number of operational taxonomic units of bacteria in the soil (Table 2.29). Next-generation sequencing is an increasingly popular and extremely sensitive method of determining similarities and differences within the soil microbiome. This fact was confirmed by the results of our research (Table 2.30–2.32, Figures 2.27–2.29) Depending on the soil site and the year of the research,

there were 20–34 bacterial phyla and 378–554 genera identified. Due to the large number of operational taxonomic units (OTUs), only those with an average share of more than 1% were shown in Figures 2.27–2.29. Throughout the study period, the following bacterial phyla were dominant in the soil: Proteobacteria (the relative abundance ranged from 33.23% to 60.08%), *Firmicutes, Actinobacteriota, Acidobacteriota, Chloroflexi,* and *Verrucomicrobiota* (Figures 2.27–2.29). Depending on the year of the soil metagenomic analyses, the following phyla were also dominant: *Bacteroidetes, Planctomycetes, Tenericutes, Spirochaetes, Chlamydiae, Cyanobacteria, Gemmati- monadota, Bacteroidota,* and *Fatescibacteria* (Figures 2.27–2.29). Experiment (Wieczorek et al., 2023) showed that, regardless of the year of the study, the most intensive proliferation of Proteobacteria was observed in the soil in variant R3, slightly weaker – in the replanted soil with the white mustard forecrop *(Sinapis alba)* (R4), and then in variant R5 (Figures 2.27–2.29). The lowest percentage of the OTU content was found in the replanted soil (R2) in 2020. A year earlier, despite several attempts to isolate the bacterial DNA, it was impossible to obtain research material due to the degree of soil sterilisation.

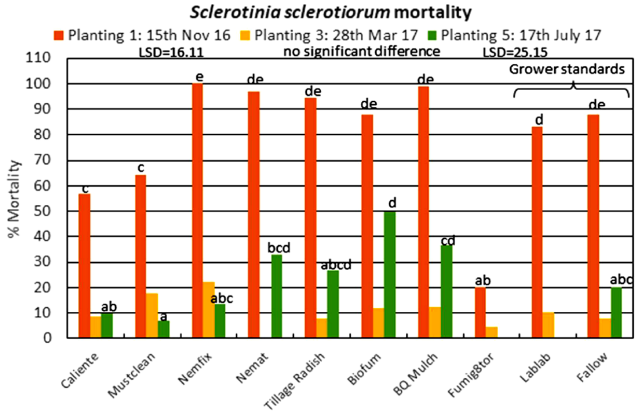


Figure 2.25 – Performance of various brassica bio fumigants and grower standard practices against *Sclerotinia sclerotiorum.* Bars with the same letter for the different plantings are not significantly different from one another. Planting 3 there was no significant difference between varieties (Duff et al., 2021)

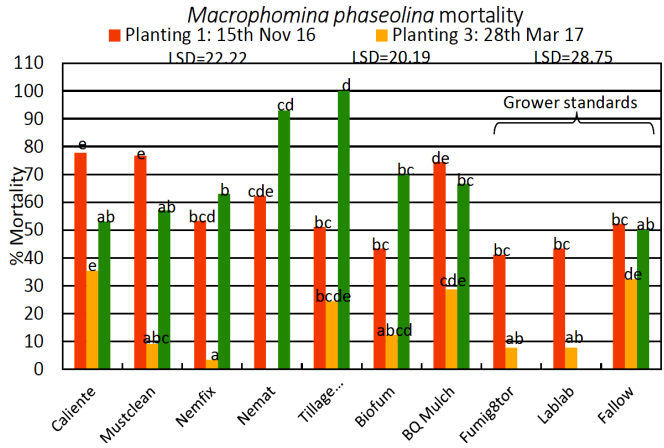


Figure 2.26 – Performance of various brassica biofumigants and grower standard practices against *Macrophomina phaseolina*. Bars with the same letter for the different plantings are not significantly different from one another (Duff et al., 2021)

Table 2.29

Number of bacterial taxonomic units according to experimental combinations (R1-agricultural soil; R2-replanted soil; R3-replanted soil with *Tagetes patula* L. foregut; R4-replanted soil with *Sinapis alba* foregut; R5-replanted soil with *Raphanus sativus* var. *oleifera* foregut) (Wieczorek et al., 2023)

Taxonomy units	R1	R2	R3	R4	Rn
1	2	3	4	5	6
2019 year					
Phylum	2o	-	22	23	23
Class	44	-	48	50	47
Order	87	-	96	93!	96
Family	180	-	201	I17	205
Genus	378	-	441	407	570
Species	456	-	591	532	704
2020 year					
Phylum	30	32	331	32	331
Class	84	89	89	90	85
Order	190	201	216	224	207
Family	280	294	320	338	317

(End of Table 2.29)

1	2	3	4	5	6
Genus	457	499	554	510	513
Species	832	906	1 00h	935	940
2021 year					
Phylum	32	30	30	33	30
Class	80	76	69	85	79
Order	180	170	105	200	189
Famüy	272	255	302	301	280
Genus	48e	458	552	553	505
Species	838	774	953	942	988

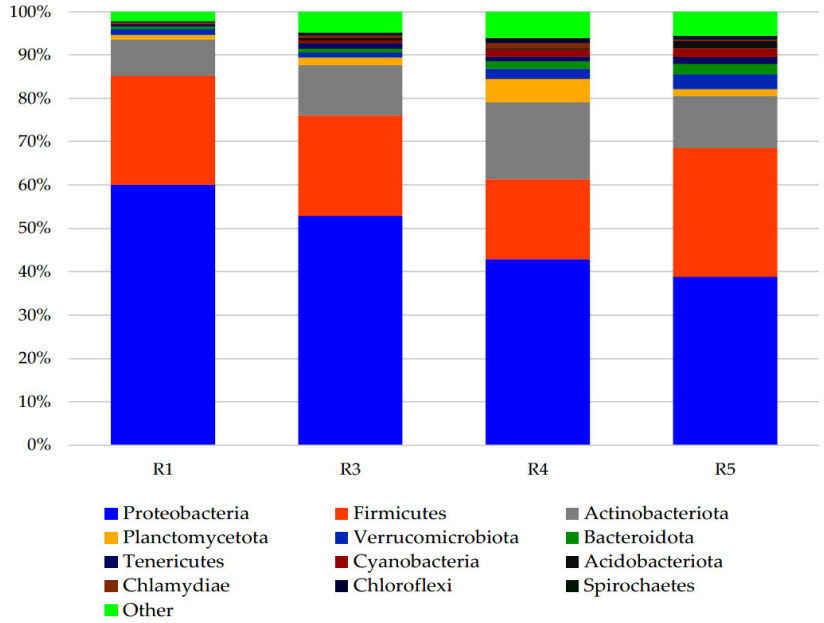


Figure 2.27 – Relative abundance o° dominant phyla o° bacteria in 2019. The classifications with less than 1% abundance are gathered into the category «Other» (R1–agricultural soil; R2–replanted soil; R3–replanted soil with *Tagetes patula* L. foregut; R4–replanted soil with *Sinapis alba* foregut; R5–replanted soil with *Raphanus sativus* var. *oleifera* foregut) (Wieczorek et al., 2023)

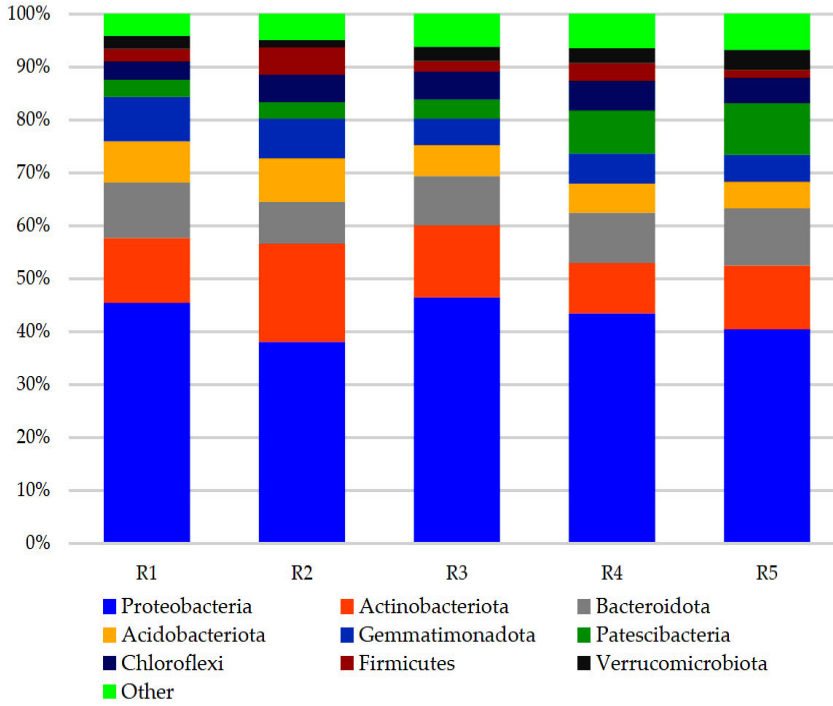


Figure 2.28 – Relative abundance of dominant phyla of bacteria in 22020. The classifications with less than 1% abundance are gathered into the category «other» (R1–agricultural soili R2–replanted soil; R3–replanted soil with *Tagetes patula* L. foregut; R4–replanted soil with *Sinapis alba* foregut; R5–replanted soil with *Raphanus sativus* -var. *oleifera f*oregut) (Wieczorek et al., 2023)

In 2019, regardless of the experimental variant, Firmicutes bacteria were the most dominant phylum (Figure 2.27). In the following years of the research, the count of Actinobacteriota increased and was greater than the counts of other bacterial phyla (Figures 2.28–2.29). The intensive growth and development of Actinobacteriota were particularly noticeable in variant R2. Actinobacteriota are a saprophytic group of actinobacteria that quickly adapt to unfavourable environmental conditions, such as desiccation.

Therefore, they actively decompose organic matter when the soil moisture is low (Wieczorek et al., 2023).

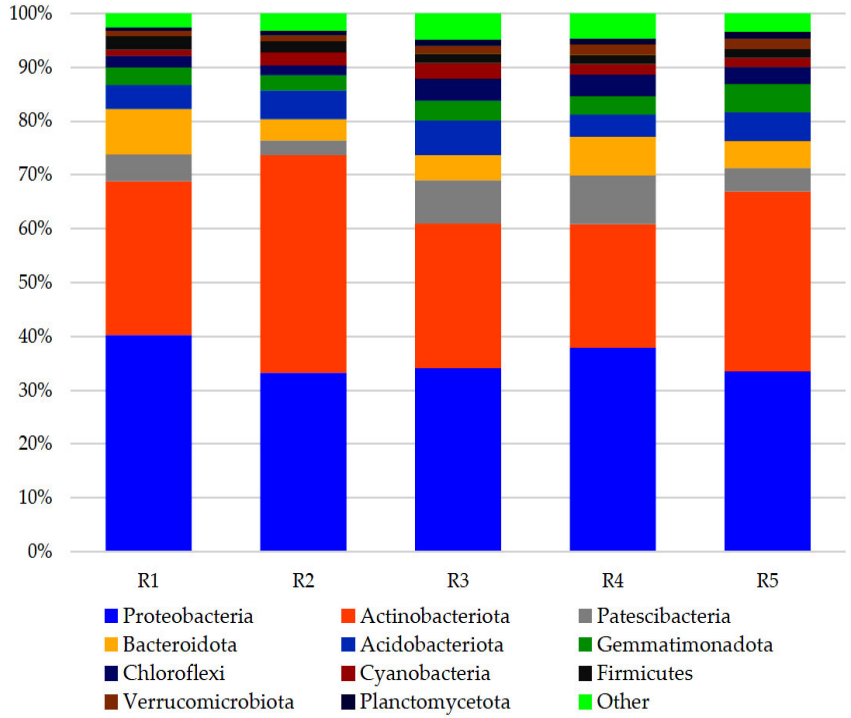


Figure 2.29 – Relative abundance of dominant phyla oo bacteria in 2021. The classifications wiih less than 1‰ abundance are gathered into the category «other» (R1-agricultural soili R2-replanted soii; R3-replanted soil with *Tagetes patula* L. foregut; R4-replanted soil with *Sinapis alba* foregut; R5–replanted soil with *Raphanus sativus* var *oleifera* foregut) (Wieczorek et al., 2023)

According to the same estimates by Wieczorek et al. (2023) Figures 2.27–2.29 show relative differences between the dominant types of bacteria in the control variant (R1) and the other experimental variants, expressed as a percentage of sequence. The analysis of the research results

showed that regardless of the year of the study, the OTU content of the Proteobacteria phylum in all experimental variants was lower than in the control variant (R1). However, it is necessary to stress the fact that the difference in the content of OTUs of the Proteobacteria phylum in the soils subjected to induced biofumigation with the phytosanitary plants – Tagetes patula L. (R3), Sinapis alba (R4), and Raphanus sativus var. oleífera (R5) – in relation to the agricultural soil was significantly lower than the difference observed in the soil with ARD (Figures 2.30–2.32). Apart from that, in 2020, the application of French marigold (R3–R1) caused a significant increase in the content in OTUs of the Proteobacteria phylum (Figure 2.31). Thus, it can be concluded that the application of phytosanitary plants to the soil with ARD causes an increase in the content of OTUs belonging to the Proteobacteria phylum.

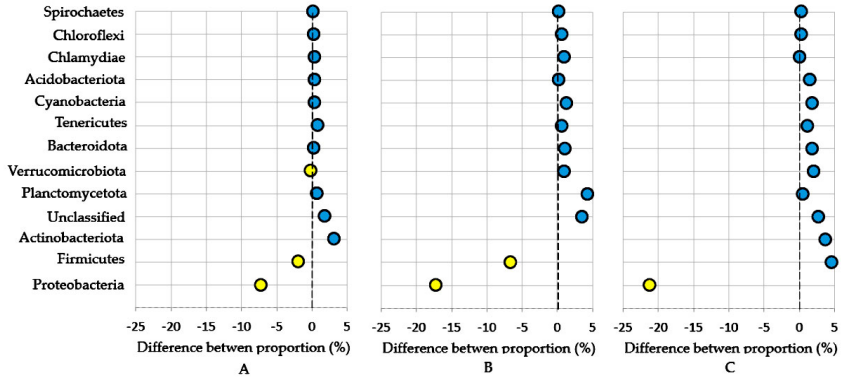


Figure 2.30 – Relative abundance of dominant classes of bacteria phyla in 2019 (A-R3 via R1; B-R4 via R1; C-R5 via R1). The classifications with less than 1% abundance are gathered into the category «other» (R1-agricultural so il; R3-replanted soil with *Tagetes patula* L. foregut; R4-replanted soil with *Sinapis alba* foragut; R5-replanted soil with *Raphanus sativus* var. *oleifera* foregut; «yellow» means negative difference; «blue» means positive difference) (Wieczorek et al., 2023)

A similar trend was also observed for the Firmicutes phylum. The metagenomic analysis of the soil conducted between 2019 and 2021 showed that the sequence content of seven bacterial phyla in the soil samples was lower than in the control variant. However, these differences were not observed in all experimental variants. They were ranked as follows according to the frequency of their occurrence in the soil: Bacteroidota > Acidobacteriota > Actinobacteriota = Gemmatimonadota > Patescibacteria.

The Verrucomicrobiota and Chloroflexi bacterial phyla, and especially Planctomycetota and Cyanobacteria, were isolated less often from the agricultural soil (R1), especially when compared with the replanted soil subjected to fumigation. The Chloroflexi phylum encompasses nitrifying bacteria developing in anaerobic or microaerophilic conditions. They can survive in intensively changing, extreme conditions. The incidence of the Chloroflexi phylum in soils with ARD after biofumigation was higher than in the control soil. This phenomenon can be explained by the fact that the development of the; community of these bacteria is based on the use of cellular compounds from dead microorganisms and their metabolites

In this study (Wieczorek et al., 2023), fumigation induced by the use of French marigold (R3) increased the content of OTUs in 2he Cyanobacteria phylum by 83% as compared with the soil with ARD and the agricultural soil. A similar trend was also observed for the Firmicutes phylum. The metagenomic analysis of the soil conducted between 2019 and 2021 showed that the sequence content of seven bacterial phyla in the soil samples was lower than in the control variant. However, these differences were not observed in all experimental variants. They were ranked as follows according to the frequency of their occurrence in the soil: Bacteroidota > Acidobacteriota > Actinobacteriota = Gemmatimonadota > Patescibacteria.

The Verrucomicrobiota and *Chloroflexi bacterial phyla*, and especially Planctomycetota and Cyanobacteria, were isolated less often from the agricultural soil (R1), especially when compared with the replanted soil subjected to fumigation. The *Chloroflexi phylum* encompasses nitrifying bacteria developing in anaerobic or microaerophilic conditions. They can survive in intensively changing, extreme conditions. The incidence of the *Chloroflexi phylum* in soils with ARD after biofumigation was higher than in the control soil. This phenomenon can be explained by the fact that

the development of the; community of these bacteria is based on the use of cellular compounds from dead microorganisms and their metabolites, which is typical of soils with ARD i60] (Wieczorek et al., 2023).

In this study (Wieczorek et al., 2023), fumigation induced by the use of *French marigold* (R3) increased the content of OTUs in 2he *Cyanobacteria phylum* by 83% as compared with the soil with ARD and the agricultural soil.

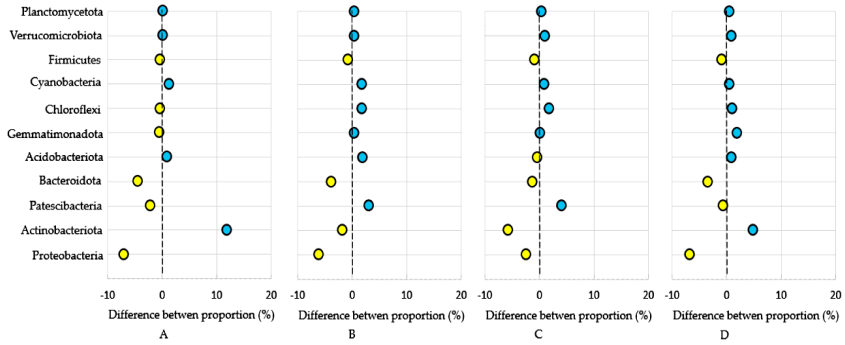


Figure 2.31 – Relative abundance of dominant classes of bacteria phyla in 2020 (A–R3 via R1; B–R4 via R1; C–R5 via R1). The classifications with less than 1% abundance are gathered into the category «other» (R1–agricultural so il; R3–replanted soil with *Tagetes patula* L. foregut; R4–re planted soil with *Sinapis alba* foragut; R5–replanted soil with *Raphanus sativus* var. *oleifera* foregut; «yellow» means negative difference; «blue» means positive difference) (Wieczorek et al., 2023)

The principal component analysis (PCA) revealed the relationships between the different types of soil bacteria in the experimental variants during the three years of the research (Figure 8). It showed that soil biodiversity ranged from 71.19% to 94.28%. It also showed that the relationships between the different types of bacteria were related to the year of the study (Wieczorek et al., 2023). Regardless of the! experimental variant, in the first year of the study, the analysis revealed a clear correlation between the percentage of taxonomic sequences oe the Verrucomicrobiota, Bacteroidota,

Cyanoeactetia, and Ttnericutes phyla and between Actinobacteriota and Planctomycetota.

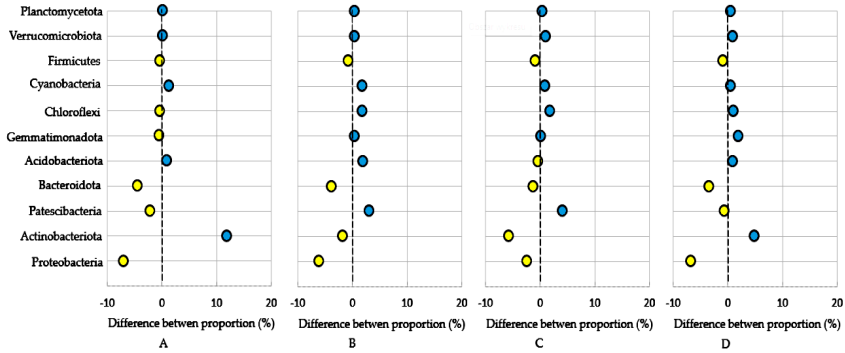


Figure 2.32 – Relative abundance of dominant classes of bacteria phyla in 2021 (A–R3 via R1; B–R4 via R1; C–R5 via R1). The classifications with less than 1% abundance are gathered into the category «other» (R1–agricultural so il; R3–replanted soil with *Tagetes patula* L. foregut; R4–re planted soil with *Sinapis alba* foragut; R5–replanted soil with *Raphanus sativus* var. *oleifera* foregut; «yellow» means negative difference; «blue» means positive difference) (Wieczorek et al., 2023)

A similar relationship between Verrucomicrobiota and Batterosdota was also observed in the second year of the study (Wieczorek et al., 2023) (Figure 2.33). However, in 2020 and 20221, there was a correlation between the percentage of OTUs in the Proteobacteria phylum and Bacteroidota.

As a result, the author of the study (Wieczorek et al., 2023) concluded that the use of metagenomics (functional analysis of genetic material isolated from the soil) as a tool for assessing soil biodiversity in the nursery after replantation proved to be a sensitive and precise method of assessment of the soil microbiome in the nursery. The analyses of the microbiome composition showed that biofumigation with phytosanitary plants – French marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), and oilseed radish (*Raphanus sativus* var. *oleifera*) – changed the structure and count of bacteria in the replanted soil in the fruit tree nursery. The phytosanitary

plants increased the abundance of operational taxonomic units (OTU) of the Proteobacteria, Bacteroidota, Patescibacteria, Chloroflexi, Fatescibacteria, and Verrucomicrobiota phyla, but decreased the abundance of the Firmicutes, Acidobacteriota, and Actinobacteriota phyla. The biofumigation also increased the content of some dominant bacterial genera in the replanted soil, such as Flavobacterium, Massila, Sphingomonas, Arenimonas, and Devosia. These genera are considered crucial in promoting plant growth and inducing plant systemic immunity, which may indicate the regeneration of replanted soil.

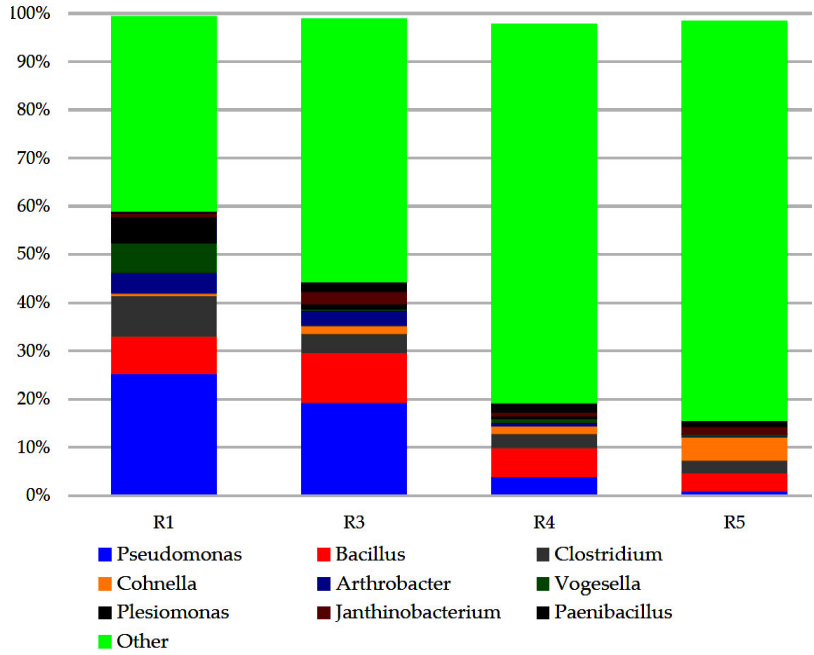


Figure 2.33 – Relative abundance of the dominant (rodzaj) genus of bacteria in 2019. The classifications with less than 1 % abundance are gathered into the category «other» (R1–agricultural soil; R2– replanted soil; R3–replanted soil with *Tagetes patula* L. foregut; R4–replanted soil with *Sinapis alba* foregut; R5–replanted soil with *Raphanus sativus* var. *oleifera* foregut) (Wieczorek et al., 2023)

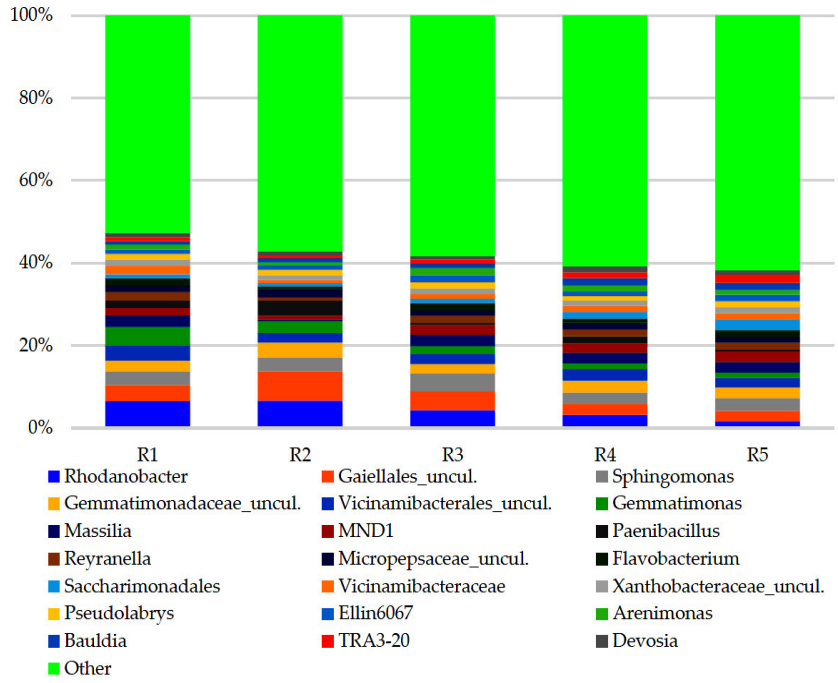


Figure 2.34 – Relative abundance of the dominant (rodzaj) genus of bacteria in 2020. The classifications with less than 1 % abundance are gathered into the category «other» (R1–agricultural soil; R2– replanted soil; R3–replanted soil with *Tagetes patula* L. foregut; R4–replanted soil with *Sinapis alba* foregut; R5–replanted soil with *Raphanus sativus* var. *oleifera* foregut) (Wieczorek et al., 2023)

Studies (Wieczorek et al., 2023) have shown that regardless of the species of phytosanitary plants used, there was an increase in the abundance of beneficial microbiomes. In practice, when planning production plantings, it is recommended to use a one-year break during which phytosanitary plants will be cultivated.

In the similar study (Nallanchakravarthula et al., 2021), strawberry yield was shown to be negatively affected by the application of oilseed radish

18 days before planting. In the experimental plots were sown on the day of incorporation, resulting in negative effects of the treatments on both stand establishment and yield in most crops.

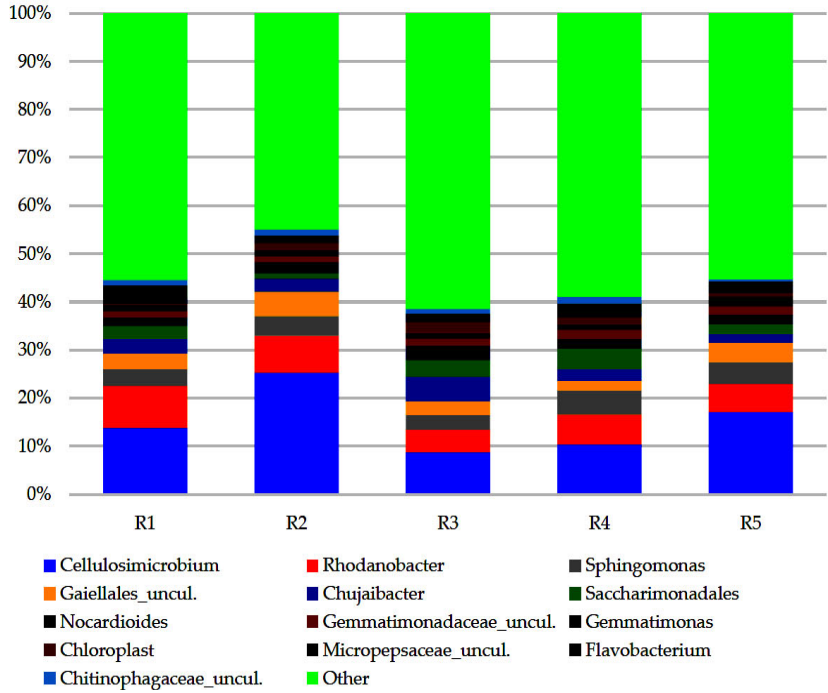


Figure 2.35 – Relative abundance of the dominant (rodzaj) genus of bacteria in 2021. The classifications with less than 1 % abundance are gathered into the category «other» (R1–agricultural soil; R2– replanted soil; R3–replanted soil with *Tagetes patula* L. foregut; R4–replanted soil with *Sinapis alba* foregut; R5–replanted soil with *Raphanus sativus* var. *oleifera* foregut) (Wieczorek et al., 2023)

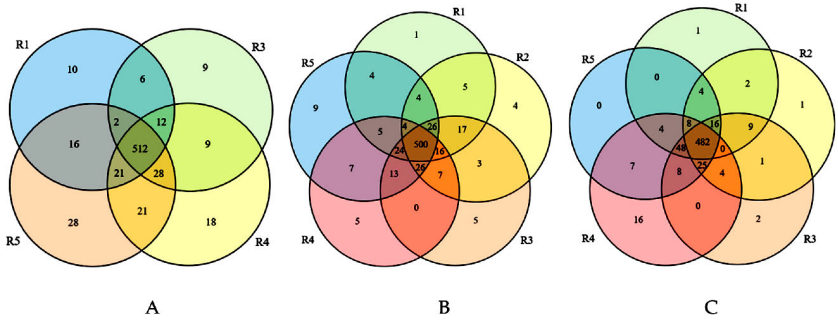


Figure 2.36 – Venn diagram of overlapping bacterial communities (phyla) (A–2019; B–2020; C–2021). (R1-agricultural soil; R2-replanted soil; 153-replanted soil with *Tagetes patula* L. foregut; R4-replanted soil with *Sinapis alba* foregut; R5-replanted soil with *Raphanus sativus* var. *oleifera* foregut.); the numbers indicate the number of unique bacterial sequences (Wieczorek et al., 2023)

However, if addition of biofumigant residues had any green manuring or nutritional effect then there should have been a significant increase in the abundance of the fungal 'saprotrophic guild' in rhizosphere soil (Figures 2.37–2.38) but we did not observe any major changes in abundance of dominant saprotrophs, suggesting that there was little or no green manuring effect. We therefore believe the observed effects of oilseed radish residues were more likely to be due to the ITCs and phytotoxic chemicals released after mulching of the biofumigant, although these chemicals were not measured directly in this study. In roots, the saprotrophic guild increased significantly in the +B+V treatment, with concurrent decrease in endophyte and arbuscular mycorrhizal guilds, possibly due to chemicals released from the biofumigant.

Oilseed radish incorporation was shown to change the composition of the glomeromycotan community, causing a significant decrease in the relative abundance of a taxon in the Glomeraceae. *V. dahliae*, there was a significant increase in relative enrichment of *Periconia macrospinosa* and *Microdochium bolleyi* ('dark septate endophytes'/DSE) and an unclassified taxon belonging to Pleosporales (presumably a root endophyte) compared to corresponding control treatment without *V. dahliae* (B–V).

This response of the root endophytes could have been a result of the presence of the pathogen, and this re-structuring of the root microbiome might enable the plant to tolerate deleterious effects of the pathogen.

In conclusion (Nallanchakravarthula et al., 2021), this study provides new data suggesting that biofumigation of strawberry plants by incorporation of *Raphanus sativus* var. *oleifera* has significant effects on the composition of root fungal communities and may decrease the relative abundance of arbuscular mycorrhizal fungi and some potentially beneficial root endophytic taxa.

Table 2.30

Unique bacterial taxa in individual experimental variants in 2019. (R1-agricultural soil; R2-replanted soil; R3-replanted soil with *Tagetss patula* L. foregut; R4-replanted loil with *Sinapis alba* foregut; R5-replanted soil with *Raphanus sativus* var. *oleífera* foregut) (Wieczorek et al., 2023)

R1	R2	R3	R4	R5
Number of Unique Taxa				
10	0	9	18	28
Amicrococcus *Dorea* *Eggerthella* *Enhydrobacter* *Fructobacillus* *Mitsuokella* *Paraprevotella* *Sarcina* *Thiocystis* *Vermineplirobacter*	–	*Chlamydia* *Desulfonatro-novibrio* *Desulfotalea* *Flectobacillus* *Helcococcus* *Muricatida* *Roseateles* *Sinomonas* *Spirochaeta*	*Citricoccus* *Cryobacterium* *Dehalobacterium* *Desulfomicrobium* *Entomoplasma* *Fusobacterium* *Jeotgalicoccus* *Kibdelosporangium* *Kytococcus* *Octadecabacter* *Odoribacter* *Peptostreptococcuss* *Roseococcus* *Saccharomonospora* *Salinivibrio* *Sporanaerobacter* *Thermococcus* *Xenococcus*	*Antarctobacter* *Aureispira Bulleidia* *Butyricimonas* *Coprococcus* *Haliscome-nobacter* *Lachnobacterium* *Limnothrix* *Luteococcus Nevskia* *Parabacteroides* *Porphyromonas* *Propionigenium* *Pseudanabaena* *Psychroflexus* *Rhodothalassium* *Roseburia Roseiflexus* *Roseivivax* *Salinimicrobium* *Snowella* *St reptomonospora* *Sulfuricurvum* *Sulfuritalea* *Teredinibacter* *Terriglolms* *Thermacetogenium* *Zobellia*

Table 2.31

Unique bacterial taxa in individual experimental variants in 2020. (R1-agricultural soil; R2-replanted soil; R3-replanted soil with *Tagetss patula* L. foregut; R4-replanted loil with *Sinapis alba* foregut; R5-replanted soil with *Raphanus sativus* var. *oleifera* foregut) (Wieczorek et al., 2023)

R1	R2	R3	R4	R5
Number of Unique Taxa				
1	4	5	4	3
Amycolatopsis	*Haliscomenobacter* *Novibacillus* *f_Enterobacte-riaceae;* Other *Hafnia-Obesumbac-terium*	*f_Nostocaceae;* Other *Archangium* *Aetherobacter* f_Polyangiaceae; g_uncultured *Leptospira*	*Vicingus* *Blastopirellula* *Chitinimona* *f_Parachlamy-diaceae;* g_uncultured	o_Babeliales; Other; Lactococcus 0M60(N0R5)_ clade

Table 2.32

Unique bacterial taxa in individual experimental variants in 2020. (R1-agricultural soil; R2-replanted soil; R3-replanted soil with *Tagetss patula* L. foregut; R4–replanted loil with *Sinapis alba* foregut; R5-replanted soil with *Raphanus sativus* var. *oleifera* foregut) (Wieczorek et al., 2023)

R1	R2	R3	R4	R5
Number of Unique Taxa				
1	1	2	16	0
Amycolatopsis	Rahnella1	f_Myxococcaceae; Other Candidatus_ Falkowbacteria	Paludibacter WCHB1-32 Vitellibacter Bacteriovorax Pseudarcobacter Candidatus_ Megaira Tolumonas Idiomarina Shewanella Noviherbaspirillum Candidatus_Accumulibacter f_Methylococcaceae;g_uncultured Methylophaga Halomonas Alkanindiges f_ Criblamydiaceae;g_uncultured	–

Further investigations should reveal whether negative effects of biofumigation on strawberry yield and root fungal microbiome are host genotype- or soil-dependent.

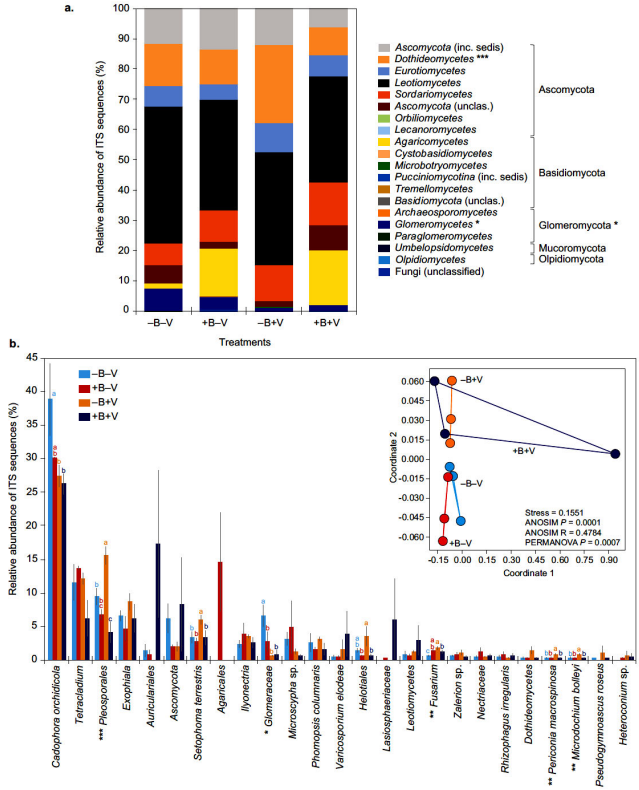


Figure 2.37 – Relative abundance of ITS sequences of different fungal classes in the roots of strawberry plants treated with factorial combinations of oilseed radish (*Raphanus sativus oleifera*) residues as biofumigant, (+/- B) and inoculation with the fungal pathogen *Verticillium dahliae* (+/- V) (a). Names of fungal phyla are given to the right of brackets grouping classes. The histogram depicts the twenty-five most abundant fungal taxa (that comprise around 95% of the ITS sequences from roots) found in all treatments, whereas the nonmetric multidimensional scaling (NMDS) ordination plot (inset) is based on all taxa (b). Analysis of variance (ANOVA) significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$ (Nallanchakravarthula et al., 2021)

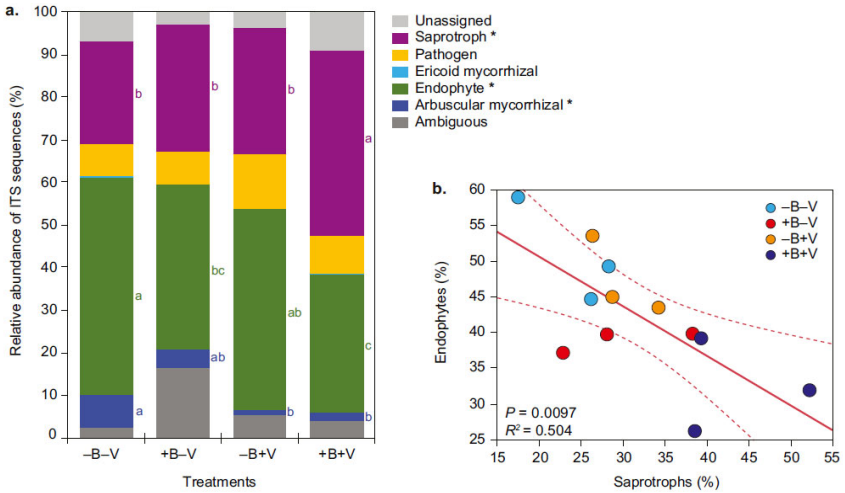


Figure 2.38 – Relative abundance of ITS sequences representing different functional guilds of fungi in roots of strawberry plants treated with factorial combinations of oilseed radish (*Raphanus sativus oleifera*) residues as biofumigant, (+/□ B) and inoculation with the fungal pathogen *Verticillium dahliae* (+/□ V) (a). Analysis of variance (ANOVA) significance level * = $P < 0.05$. Regression analysis of the proportion of ITS sequences belonging to endophyte and saprotroph functional guilds in the roots (b) show a statistically significant negative relationship between the two functional guilds. The dotted lines represent 95% confidence intervals (Nallanchakravarthula et al., 2021).

The antinematode effectiveness of oil radish has also been proven in terms of antinematode activity when growing vegetables in greenhouses. Thus, in the studies of Haroutunian (2013) was found that root-knot nematodes remain one of the most serious problems faced by greenhouse farmers of Lebanon and the Middle East region. The presence of these nematodes in the soil even in relatively small numbers causes serious damage to vegetable crops, specifically those lacking any resistance or tolerance to these pests, such as the cucurbits. In the past, soil fumigation with Methyl Bromide has been considered as the best control measure against root-knot nematodes.

However, Methyl Bromide was completely banned worldwide for Ozone preservation concerns. Biofumigation with the use of green manure crops has been one of the potential alternatives suggested by the Methyl Bromide Alternatives Project Lebanon. Under these circumstances, scientific evaluation of the efficiency and cost effectiveness of this technique in Lebanon is an important area of research.

As to the results achieved by the biofumigation crops with respect to Methyl Bromide, in experiment A yield produced by Methyl Bromide was significantly higher than oil radish with plastic cover. However, no significant difference was found between reduction of nematode population resulting from Methyl Bromide and oil radish with plastic cover. Difference was significant between Methyl Bromide and oil radish without plastic cover in both yield and reduction of nematodes (Figure 2.39).

In experiment B no significant difference was observed in neither yield nor reduction of nematode population between any of the two biofumigation crops used with plastic cover and Methyl Bromide. These differences were significantly in favor of Methyl Bromide only when oil radish was used without plastic cover.

In experiment C where Vydate (Oxamyl) was incorporated at the rate of 1 liter per 1,000 m^2 to all treatments, there was no significant difference in neither yield nor reduction of nematode population between any of the two biofumigation crops used with or without plastic cover and Methyl Bromide.

Cost-benefit analysis made on all treatments applied in the 3 experiments showed that in all cases, all these treatments whether chemical, non-chemical (with or without plastic cover) or in combination have produced higher net profits than Methyl Bromide, even when yield produced by Methyl Bromide was significantly higher.

Use of plastic for covering oilseed radish has generally produced better results in terms of production of higher yields, better reduction of soil populations of root-knot nematodes and leading to reasonable increases of net profits.

Based on these findings and in the light of global phase out of Methyl Bromide, it can be concluded that the use of oil radish and arugula as biofumigation crops with plastic cover can be considered as an alternative management tool for the root-knot nematode in greenhouse cucumber production under Lebanese conditions.

Besides adequate control of a wide range of nematodes and fungal diseases in the soil, biofumigation with the use of green manure crops presents several other major advantages. It improves soil texture and structure and increases organic matter and water holding capacity, doesn't harm most of the beneficial microorganisms of the soil and above all, it is one of the least expensive alternatives to Methyl Bromide. However, biofumigation has the major disadvantage of requesting a relatively a longer period of application time (45 days) as compared to Methyl Bromide (10 days). For most farmers this fact alone is a major handicap for the wide scale adoption of this technique as a viable alternative to Methyl Bromide.

Therefore, future research must focus on appropriate methods for the reduction of the application period of biofumigation crops. For this purpose, new fast growing varieties of oil radish (*Raphanus sativus oleífera*) and arugula (*Eruca vesicaria sativa*) producing more abundant vegetative parts within a shorter period of time, as well as other varieties of the Brassicaceae family must be developed and tested for their satisfactory performance under local conditions. The arugula cultivar tested in our research was a common variety purchased at the local market. It was till then used for rocket (salad) production purposes and its capacity as a biofumigant crop were unknown and not assessed. The results of our study indicate that arugula is worth of further development and additional testing is needed for the determination of its properties and eficacy as a biofumigant crop.

Finally, the combination of biofumigant crops with low doses of some chemical nematicides with limited impact on the environment remains a potential area of further investigation, having as purpose to evaluate new chemistry or to determine the minimal rates of the chemicals used in combination with the biofumigant crops and/or the maximum reduction of the application period of the biofumigation crops tested.

The second experiment (B) initiated immediately after the end of the following summer season in a greenhouse having the same area and adjacent to the one used in experiment A. The previous tomato crop was removed and the soil was plowed after the incorporation of preparatory organic and chemical fertilizers at usual rates. The total area of the greenhouse was divided into twenty (20) plots of 3.80 x 4.00 (15.20 m^2) for the application of the following five treatments with four replicates each:

– Control (untreated fallow).

– Methyl Bromide.

– Oilseed radish *(Raphanus sativus* spp. *oleifera)* variety "Boss" without plastic cover.

– Oilseed radish *(Raphanus sativus* spp. *oleifera)* variety "Boss" with plastic cover Arugula *(Eruca vesicaria* spp. *sativa)* with plastic cover.

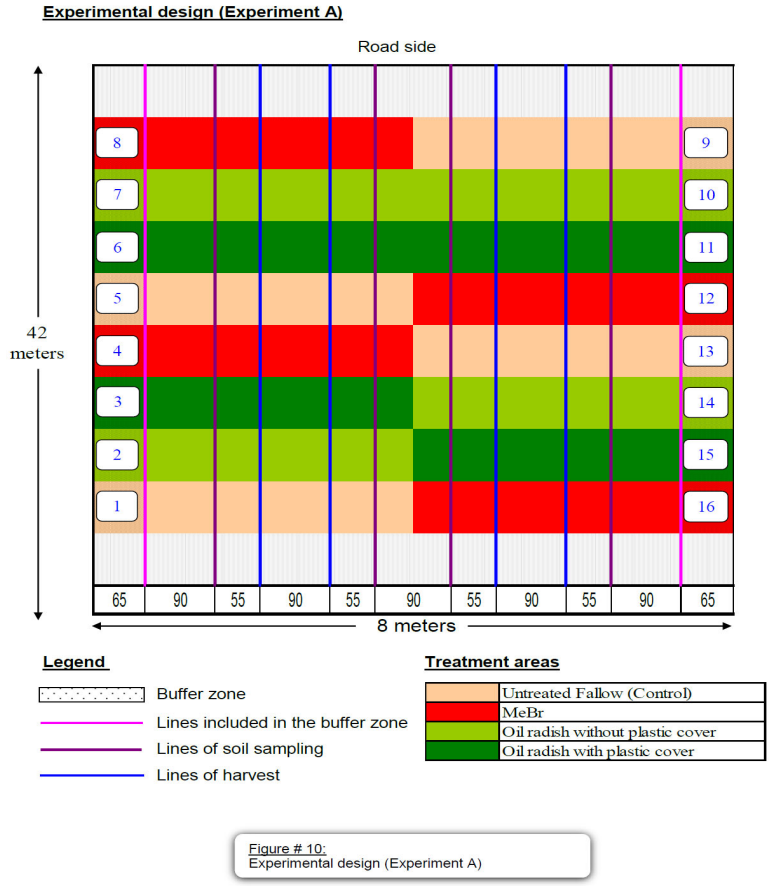


Figure 2.39 – Scheme of the experiment A (Haroutunian, 2013)

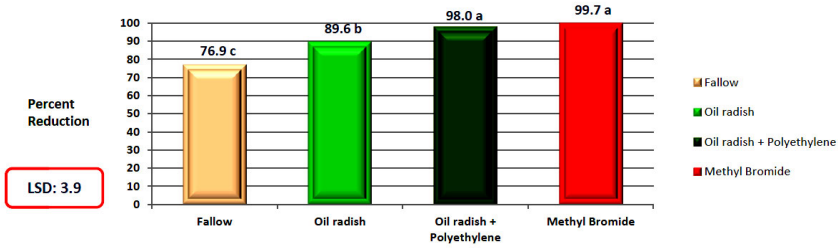


Figure 2.40 – Percent reduction of the soil population of root-knot nematodes (Experiment A) (Haroutunian, 2013)

In experiment B, the highest reduction was once again achieved with Methyl Bromide (99.7%). In plastic covered arugula or oil radish plots, M. incognita population was decreased by 97.7% and 94.2%, respectively, whereas in uncovered oil radish plots, population reduction was only 86.3%. In the untreated fallow, nematode population reduction was as low as 74.7% (Figure 2.41).

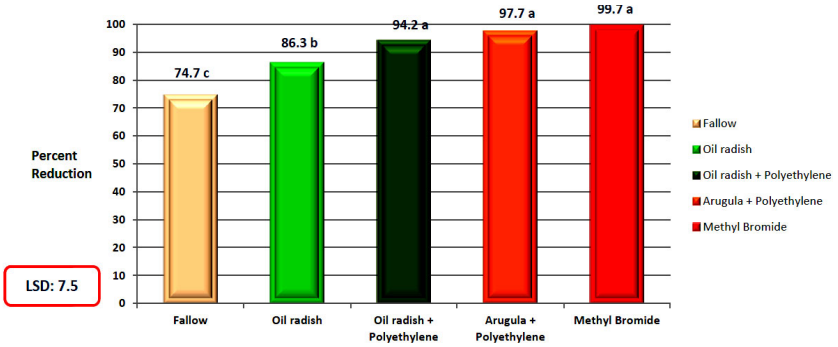


Figure 2.41 – Percent reduction of the soil population of root-knot nematodes (Experiment B) (Haroutunian, 2013)

In both experiments A and B, all treatments significantly reduced nematode population in comparison with the untreated fallow. Furthermore, there was no significant difference between the percent reduction of

nematodes achieved by any of the two green manure crops (oil radish and arugula) covered with plastic and Methyl Bromide. When covered with 50 microns transparent polyethylene film immediately following their incorporation to the soil, both oil radish and arugula have been as effective as Methyl Bromide in reducing nematode population in the soil (Figure 2.40–2.41).

Generally, in both experiments A and B both biofumigation crops used significantly reduced nematode population as compared to untreated fallow (control). This result is conform to findings of other studies describing oil radish and arugula as non-hosts (or least poor hosts among a variety of biofumigant crops tested) to root-knot nematodes, causing decline of soil populations by starvation at the first place. Results of our experiments also match other studies having found that these biofumigation crops are able to control nematodes, and more specifically those having associated the nematostatic effect of biofumigation crops to the release of glucosinolates which soon convert into isothiocyanates in the soil and thus suppress nematode populations.

In both experiments A and B, plastic cover has significantly increased the efficiency of oil radish and lead to better nematode control. These results indicate that plastic cover enhances the effectiveness of the biofumigation crops used in terms of nematode control. It is to note that there was remarkable difference in the initial soil populations of nematodes in plots treated with covered and uncovered oil radish in each of these two experiments. In fact, the average initial infestation with root-knot nematodes in all eight plots treated with oil radish (covered and uncovered) at the site selected for experiment A was considerably more severe than in plots treated similarly at the site where experiment B was conducted, as shown in table # 1.1 below. Furthermore, whereas in experiment B the average initial infestation in plots treated with oil radish and covered with plastic was lower (865) than in plots treated with oil radish and kept without cover (3,475), in experiment A plots treated with oil radish and covered with plastic had considerably higher average initial infestation (12,430) than those of with oil radish without cover (7,310).

Our results indicate that use of plastic cover with oil radish significantly enhances the effectiveness of the isothiocyanates, leading to a better control of the root-knot nematode. The beneficial effect of the plastic is not related

to the initial infestation levels. Furthermore, decreasing the covering period from 15 to 10 days does not negatively affect the efficiency of the plastic in the control of the root-knot nematode.

In experiment A root galling was significantly reduced by application of all treatments compared to fallow. The lowest root gall index was observed in the plots treated with Methyl Bromide (0.9). There was a significant reduction in the oil radish planted plots either covered with plastic or left uncovered compared to fallow. The gall reduction was less in plots planted with oil radish with or without plastic cover when compared to the untreated fallow plots (1.7 and 2.0 respectively). The highest galling was found in the roots of untreated fallow plots (7.1).

Figure 2.42 – Experiment A Galls on roots taken from the untreated fallow (A) & oil radish with plastic cover (B) (Haroutunian, 2013)

In experiment B root galling results were also quite similar to those of experiment A. On a scale of 1–10 the least root galling has occurred on plant roots taken from plots treated with Methyl Bromide (0.7). Galling on plant roots resulting from plastic covered arugula and oil radish plots was 1.7 and 2.4, respectively. Non-covered oil radish plots resulted into a root galling of 4.7, while roots taken from untreated fallow plots showed a gall index of 9.5.

Galls on roots taken from the different treatments: Methyl Bromide (A); arugula with plastic cover (B); oil radish with plastic cover (C); oil radish without plastic cover (D); untreated fallow (E) (Figure 2.44–2.45).

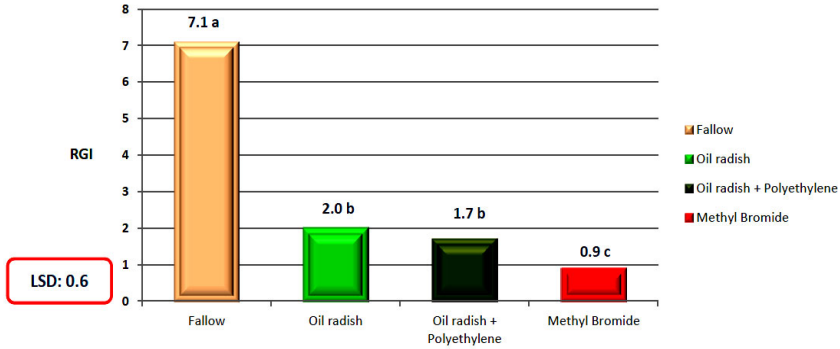


Figure 2.43 – Effect of different treatments on the cucumber root gall index at harvest (Experiment A) (Haroutunian, 2013)

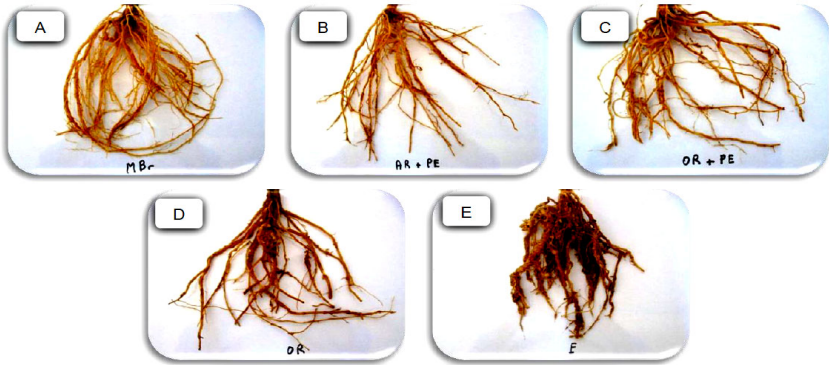


Figure 2.44 – (A, B, C, D, E): Experiment B (Haroutunian, 2013)

Both experiments A & B revealed similar results. Both biofumigation crops used significantly decreased root galls as compared to the untreated control. These results confirm the findings of several earlier studies classifying arugula and oil radish as non-host or poor host species to the root-knot nematode. The significant decline of root galls under the influence of these biofumigation crops justifies also the reduction of the soil population of the root-knot nematodes discussed earlier. Obviously, when there are

no juveniles (J2) or active juveniles proliferating in the soil, the root gall formation on the roots of the crop will definitely reduce.

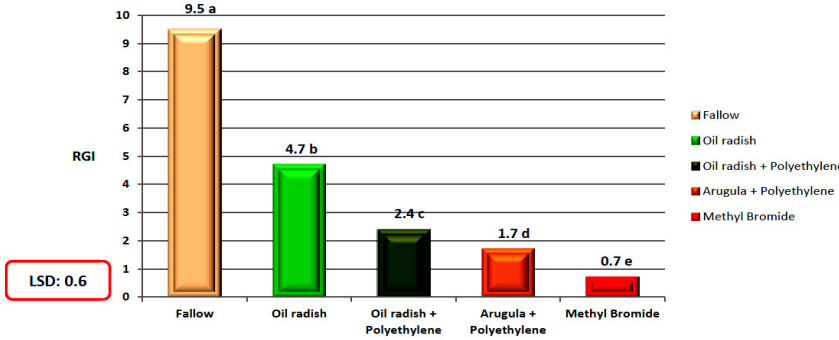


Figure 2.45 – Effect of different treatments on the cucumber root gall index at harvest (Experiment B) (Haroutunian, 2013)

These results further confirm that oil radish variety "Boss" and arugula can be considered as good cover crops and cause decline of the root-knot nematode population in the soil and on the roots of greenhouse cucumbers under Lebanese conditions. The efficacy of using sweet radish to reduce the number of beet cyst nematodes (BCN) and soybean cyst nematode (SCN) (Warner et al., 2017) has been demonstrated (Table 2.33–2.35).

Table 2.33

Average numbers of beet cyst nematode (BCN) females recovered per root system (number of trials in parentheses) (Warner et al., 2017)

Species	Common Name	Cultivar	BCN Females
1	2	3	4
Sinapsis alba	yellow mustard	Idagold (1)	267.75
Raphanus sativus	radish, tillage (1)		262.00
R. sativus	radish	Soilbuster (1)	220.25
R. sativus	radish	Driller (1)	211.25
R. sativus	radish	Daikon (1)	197.25
Brassica napus	rapeseed	Dwarf Essex (3)	190.00

(End of Table 2.33)

1	2	3	4
B. rapa	turnip, forage	Appin (3)	185.00
B. vulgaris	sugar beet	Prompt (1)	176.50
R. sativus	radish	Groundhog (3)	174.00
R. sativus	radish	Pile Driver (3)	173.58
B. juncea	brown mustard	Kodiak (3)	165.33
B. vulgaris	sugar beet	C-RR059 (1)	147.25
B. rapa	turnip, forage	Vivant (1)	137.75
R. sativus	radish	Graza (1)	135.25
S. alba	yellow mustard	Pacific Gold (2)	121.00
R. sativus	radish	GO-DRK (1)	87.00
B. oleracea	cabbage	Early Jersey Wakefield (1)	70.25
B. vulgaris	sugar beet	SX-1211NRR (1)	65.75
B. vulgaris	sugar beet	HM-50RR (1)	56.00
Phaseolus vulgaris	dry bean	Zorro (2)	26.58
R. sativus	radish	Carwoodi (3)	20.67
Raphanus sativus	radish	Cardinal (2)	7.00
Phaseolus vulgaris	dry bean	Puebla 152 (2)	5.88
R. sativus	radish	Toro(2)	5.75
Sinapsis alba	white mustard	Accent(3)	3.83
R. sativus	radish	Intermezzo (3)	2.40
S. alba	white mustard	Ludique (1)	1.75
R. sativus	radish	FumaRad (3)	1.33
Raphanus sativus	radish	Mercator (1)	0.75
R. sativus	radish	Tajuna (3)	0.58
R. sativus	radish	Image (2)	0.37
R. sativus	radish	Cannavaro (1)	0.25
R. sativus	radish	Defender: home grown (2)	0.25
R. sativus	radish	Maximus (1)	0.25
R. sativus	radish	Respect(2)	0.25
R. sativus	radish	Defender: certified (3)	0.13
Medicago sativa	alfalfa	Foregrazer (1)	0.00
Trifolium incarnatum	crimson clover (1)		0.00
T. pratense	red clover	Dynamite (1)	0.00
T. repens	white clover	Domino (1)	0.00

Table 2.34

Mean numbers of sugarbeet cyst nematode cysts, eggs and second-stage juveniles, eggs/cyst and males recovered from 100 cm³ soil in a greenhouse study after 8 weeks (Warner et al., 2017)

		sucarbeet cyst nematodes/100 cm³ soil						
		cysts		eggs + J_{2s}		eggs/cyst	males	
species	Cultivar	X	SE	X	SE	X	X	SE
cabbage	Early Jersey Wakefield	70.00	10.37	16860.00	3305.89	222.14	47.00	11.73
white mustard	Accent	1.50	0.75	220.00	110.00	146.17	1.00	0.50
mustard	Caliente 199 Blend	28.50	5.14	6572.50	1249.04	208.33	25.00	6.08
brown mustard	Kodiak	38.50	0.85	8210.00	517.84	181.04	107.00	18.17
yellow mustard	Pacific Gold	49.00	3.68	11730.00	691.73	208.98	57.00	3.30
oilseed radish	Cannava ro	0.50	0.14	133.50	44.63	265.00	1.00	0.50
oilseed radish	Carwoodi	3.75	0.94	497.50	100.36	109.33	0.50	0.25
oilseed radish	Defender[1]	0.00	0.00	0.00	0.00		3.00	0.96
oilseed radish	Defender[2]	0.00	0.00	0.00	0.00		5.00	1.50
oilseed radish	FumaRad	0.25	0.13	25.00	12.50	90.00	0.00	0.00
oilseed radish	Ground Hog	24.00	2.09	3950.00	749.31	137.92	78.00	13.33
oilseed radish	Intermezzo	1.00	0.20	234.00	43.57	227.50	0.00	0.00
oilseed radish	Pile Driver	42.25	3.03	7280.00	805.15	146.04	98.00	4.65
oilseed radish	Respect	0.25	0.13	50.00	25.00	200.00	4.00	0.71
oilseed radish	Taj una	1.00	0.29	132.50	40.49	122.50	7.00	2.50
oilseed radish	Toro	5.25	1.28	620.00	117.26	99.05	3.5	0.85
fodder turnip	Appin	31.25	6.66	5880.00	1475.81	165.44	81.00	13.91
turnip	Shogoin	14.00	1.21	2610.00	290.47	177.14	31.00	3.10

Table 2.35

Average numbers of soybean cyst nematode (SCN) cysts, eggs + second-stage juveniles per 100 cc soil on soybean (Pioneer 92Y91) following growth of the plants provided in the table (PI = 13050 SCN eggs + J2s) (Warner et al., 2017)

Plant	Cultivar	Tarp (Y/N)	cysts	Eggs + J2s	Pf/Pi
African marigold	Crackerjack	Y	112.50	13090	1.003
African marigold	Crackerjack	N	86.00	11840	0.907
annual ryegrass	Gulf	Y	104.25	13080	1.002
annual ryegrass	Gulf	N	82.00	12090	0.926
crimson clover		Y	102.50	13600	1.042
crimson clover		N	73.75	8640	0.662
yellow mustard	Pacific Gold	Y	59.00	7430	0.569
yellow mustard	Pacific Gold	N	35.50	4665	0.357
oilseed radish	FumaRad	Y	108.00	15040	1.152
oilseed radish	FumaRad	N	50.75	6820	0.523
rapeseed	Dwarf Essex	Y	80.25	10360	0.794
rapeseed	Dwarf Essex	N	102.00	13160	1.008
soybean	Pioneer 92Y91	Y	91.00	12270	0.940
soybean	Pioneer 92Y91	N	92.75	15220	1.166
fallow			157.25	15335	1.175
red clover	Medium red	Y	79.25	11295	0.866
red clover	Medium red	N	37.25	5085	0.390

Studies by Melakeberhan et al. (2008) and Edwards and Ploeg (2014) determined the effectiveness of oil radish against other nematode species (Table 2.36-2.37).

Table 2.36

Root galling and Meloidogyne incognita second-stage root-knot nematode juvenile numbers 8 wk after inoculating brassicaceous cultivars and tomato with 20,000 eggs (N = 5) per 3.8-liter pot. Ranking from 1 (highest) to 32 (lowest). (Melakeberhan et al., 2008; Edwards and Ploeg, 2014)

Species	Cultivar	Galling		*p*	Ranking	J2/100 g		*p*	Ranking
Experiment 1									
Brassica carinata	Bc007	3.4	(±0.2)[a]	hijk	13	502	(±57)[a]	fghij	18
Brassica juncea	ISCI99	5.0	±0.3)	de	5	2,422	± 540)	abc	4
	Nemfix	6.2	±0.4)	bc	3	2,134	±221)	abcd	5
	Pacific Gold	6.4	±0.5)	b	2	3,226	±1,174)	ab	2
Brassica napus	Greenland	3.8	±0.4)	fghi	10	566	± 149)	fghij	12
	Humus	3.0	±0.5)	ijkl	14	1,079	± 207)	cdefg	9
	Winfred	2.6	±0.2)		22	554	± 130)	fghij	15
Brassica oleracea	Liberty	1.0	±0.0)	op	31	9	± 2)	o	31
Brassica rapa	Br02205	4.6	±0.2)	def	6	995	± 205)	defg	10
	Br02206	5.4	±0.2)	cd	4	2,423	± 745)	abcd	3
	Rondo	4.4	±0.4)	efg	7	1,084	±210)	cdef	8
	Samson	4.2	±0.4)	efgh	8	1,369	± 355)	bcde	6
Eruca sativa	Nemat	3.6	±0.2)	ghij	11	267	±79)	jklm	25
Oilseed radish (Raphanus sativus)	Adagio	2.6	±0.2)	klm	23	141	±18)	lm	29
	Adios	4.2	±0.4)	efgh	9	277	±65)	ijkl	24
	Boss	0.4	±0.2)	p	32	7	± 5)	o	32
	Colonel	3.0	±0.5)	ijkl	15	201	± 64)	klm	27
	Comet	3.6	±0.2)	ghij	12	190	± 81)	mn	28
	Defender	2.8	±0.4)	jkl	17	257	± 70)	jklm	26
	Doublet	2.2	±0.2)	lmn	28	556	±107)	efghi	13
	Final	3.0	±0.3)	ijkl	16	468	±156)	hijk	21
	Rs05415	2.8	±0.4)	jkl	18	284	± 70)	ijkl	23
	TerraNova	2.6	±0.2)	klm	24	63	±19)	n	30
Sinapis alba	Abraham	1.6	±0.2)	no	30	351	± 59)	hijkl	22
	Absolut	2.8	±0.2)	jkl	19	546	±46)	efghi	16
	Accent	1.8	±0.2)	mno	29	648	±105)	efgh	11
	Achilles	2.6	±0.2)	klm	25	487	±106)	fghij	19
	Condor	2.8	±0.4)	jkl	20	1,234	±163)	bcde	7
	IdaGold	2.8	±0.2)	jkl	21	480	± 98)	ghij	20
	Maxi	2.4	±0.2)	lmn	26	554	± 140)	fghij	14
	Santa Fe	2.4	±0.2)	lmn	27	505	± 180)	hijk	17
Solanum lycopersicum	UC82	7.6	±0.2)	a	1	4,271	± 1,092)	a	1

(End of Table 2.36)

Experiment 2									
Brassica carinata	Bc007	3.4	±0.2)	ghi	12	316	±76)	defgh	17
Brassica juncea	ISCI99	5.6	±0.2)	cd	4	662	±68)	bcde	7
	Nemfix	6.6	±0.7)	b	2	1,630	± 849)	bc	3
	Pacific Gold	6.4	±0.5)	bc	3	1,273	± 813)	bcd	4
Brassica napus	Greenland	3.4	±0.2)	ghi	13	180	±21)	fghi	22
	Humus	1.8	±0.4)	ijkl	27	240	±73)	fghi	21
	Winfred	2.6	±0.2)	ijkl	20	172	±41)	fghij	23
Brassica oleracea	Liberty	0.6	±0.2)	op	32	0	± 0)	k	32
Brassica rapa	Br02205	5.0	±0.3)	de	6	360	± 115)	defgh	14
	Br02206	5.4	±0.4)	d	5	2,624	± 1,448)	ab	2
	Rondo	4.4	±0.2)	ef	7	458	± 298)	defgh	9
	Samson	4.4	±0.2)	ef	8	1,088	± 483)	bcde	5
Eruca sativa	Nemat	3.4	±0.2)	ghi	14	164	± 59)	hij	25
Oilseed radish (Raphanus sativus)	Adagio	3.4	±0.2)	ghi	15	116	± 29)	hij	27
	Adios	4.2	±0.2)	efg	9	308	±78)	defgh	18
	Boss	0.8	(±0.4)	p	31	6	±4)	k	31
	Colonel	3.0	(±0.0)	hij	17	164	±50)	fghij	24
	Comet	4.2	(±0.4)	efg	10	142	(±36)	ghij	26
	Defender	2.6	(±0.2)	ijkl	21	74	±42)	j	30
	Doublet	2.6	(±0.2)	ijkl	22	408	±128)	cdefgh	11
	Final	3.8	(±0.4)	fgh	11	422	(±111)	cdefg	10
	Rs05415	3.2	(±0.4)	hij	16	390	±152)	efgh	12
	TerraNova	2.8	(±0.2)	ijk	18	80	(±46)	j	29
Sinapis alba	Abraham	1.4	(±0.2)	no	30	88	(± 34)	ij	28
	Absolut	2.8	(±0.4)	ijk	19	332	(±230)	fghi	15
	Accent	2.0	(±0.5)	mno	25	324	(±63)	defgh	16
	Achilles	1.8	(±0.2)	klm	28	912	(±495)	cdef	6
	Condor	2.0	(±0.3)	jkl	26	304	(± 64)	defgh	19
	IdaGold	2.6	(±0.2)	ijkl	23	518	(±353)	fghi	8
	Maxi	1.8	(±0.4)	lmn	29	286	(±95)	defgh	20
	Santa Fe	2.4	(±0.2)	jkl	24	372	±190)	defgh	13
Solanum lycoper-sicum	UC82	7.8	(±0.2)	a	1	5,460	(±1,816)	a	1

a Values shown are the mean of five replicates (n = 5) ± SE. Root galling index on a scale from 0 to 10 with 0 = no galls, 10 = 100% of roots galled. Values in a column followed by different letters are significantly different (P # 0.05) according to Fisher's LSD-test within one experiment. Raw nematode data (egg counts) were log10(x+1)-transformed before analysis; nontransformed data are presented.

Table 2.37

Root galling and *Meloidogyne javanica* second-stage root-knot nematode juvenile numbers 8 wk after inoculating brassicaceous cultivars and tomato with 20,000 eggs (N = 5) per 3.8-liter pot. Ranking from 1 (highest) to 32 (lowest) (Melakeberhan et al., 2008; Edwards and Ploeg, 2014)

Species	Cultivar	Galling		*p*	Ranking	J2/100 g		*p*	Ranking
Experiment 1									
Brassica carinata	Bc007	4.0	(±0.3)[a]	gh	11	552	(±95)[a]	jk	25
Brassica juncea	ISCI99	6.0	±0.3)	cde	5	3,816	±1,070)	abcde	6
	Nemfix	7.0	±0.3)	b	2	3,980	±966)	abcd	4
	Pacific Gold	6.0	±0.5)	cde	6	2,812	±806)	bcdefg	11
Brassica napus	Greenland	4.8	±0.4)	fg	9	1,972	± 445)	defgh	13
	Humus	3.4	±0.2)	hij	14	1,620	±303)	efgh	17
	Winfred	5.8	±0.2)	de	7	3.096	±809)	abcdef	10
Brassica oleracea	Liberty	1.6	±0.2)	no	30	1,584	±413)	fghi	18
Brassica rapa	Br02205	6.8	±0.4)	bc	3	3,868	±819)	abcd	5
	Br02206	6.6	±0.2)	bcd	4	4,684	± 874)	abc	3
	Rondo	4.8	±0.4)	fg	10	6,524	±1,723)	a	1
	Samson	5.2	±0.4)	ef	8	3,584	±1,082)	abcdef	8
Eruca sativa	Nemat	3.0	±0.3)	ijkl	18	144	±76)	l	29
Oilseed radish (Raphanus sativus)	Adagio	3.2	±0.4)	hijk	17	450	±106)	k	28
	Adios	3.6	±0.4)	hi	12	1,280	±302)	ghi	19
	Boss	0.4	±0.2)	p	32	92	±48)	l	32
	Colonel	2.6	±0.4)	jklm	20	646	±73)	ijk	24
	Comet	3.4	±0.2)	hij	13	1,816	± 384)	defgh	15
	Defender	2.6	±0.2)	jklm	21	122	±52)	l	30
	Doublet	1.6	±0.2)	no	29	508	±166)	k	27
	Final	2.2	±0.4)	lmno	24	1,008	± 248)	ijk	21
	Rs05415	2.2	±0.2)	jkl	25	552	±121)	jk	26
	TerraNova	3.0	±0.3)	lmno	19	96	±26)	l	31
Sinapis alba	Abraham	2.2	±0.4)	lmno	23	3,544	±575)	abcde	9
	Absolut	3.2	±0.6)	hijk	15	1,620	±323)	efgh	16
	Accent	1.8	±0.2)	mno	27	732	±193)	ijk	23
	Achilles	3.2	±0.4)	hijk	16	3,708	±1,423)	abcdef	7
	Condor	2.4	±0.2)	klmn	22	2,526	±556)	cdefg	12
	IdaGold	2.0	±0.5)	mno	26	1,896	±477)	defgh	14
	Maxi	1.4	±0.2)	o	31	1,256	±329)	ghij	20
	Santa Fe	1.8	±0.2)	mno	28	908	± 84)	hij	22
Solanum lycopersicum	UC82	8.0	±0.3)	a	1	6,120	±1,405)	ab	2

(End of Table 2.37)

Experiment 2									
Brassica carinata	Bc007	2.8	±0.2)	de	7	74	±24)	mn	26
Brassica juncea	ISCI99	2.4	±0.2)	efg	11	1,364	± 449)	abc	3
	Nemfix	4.4	±0.3)	b	2	1,628	±575)	ab	2
	Pacific Gold	3.4	±0.8)	cd	5	670	±291)	bcdefg	11
Brassica napus	Greenland	2.0	±0.0)	efghi	17	602	±104)	bcdefg	13
	Humus	2.2	±0.5)	efgh	14	848	±119)	abcde	7
	Winfred	2.8	±0.4)	de	9	728	±126)	bcdef	9
Brassica oleracea	Liberty	1.0	±0.3)	jkl	29	8	±6)	pq	30
Brassica rapa	Br02205	3.4	±0.4)	cd	4	74	±12)	klm	27
	Br02206	2.8	±0.5)	de	8	954	± 444)	bcdefg	5
	Rondo	3.4	±0.2)	cd	6	972	±153)	abcd	4
	Samson	3.8	±0.2)	bc	3	754	±79)	bcdef	8
Eruca sativa	Nemat	1.0	±0.5)	jkl	30	44	±30)	op	28
Oilseed radish (Raphanus sativus)	Adagio	1.2	±0.4)	ijk	25	222	±95)	ijkl	20
	Adios	2.2	±0.2)	efgh	13	858	±106)	abcde	6
	Boss	0.2	(±0.2)	l	32	6(	±3)	pq	31
	Colonel	1.8	(±0.6)	$^{f}g^{hi}j$	19	150 (	± 104)	lmn	23
	Comet	1.4	(±0.2)	hij	24	722 (	±153)	bcdefg	10
	Defender	0.4	(±0.2)	kl	31	4(	±2)	^{q}	32
	Doublet	5.6	(±0.2)	bcd	20	486 (	± 184)	cdefgh	14
	Final	1.2	(±0.2)	ijk	26	330 (	± 97)	defghi	17
	Rs05415	1.2	(±0.4)	ijk	27	86 (	± 15)	jklm	25
	TerraNova	1.2	(±0.4)	ijk	28	40 (	± 15)	no	29
Sinapis alba	Abraham	1.8	(±0.4)	fghij	18	400 (	± 149)	defghi	15
	Absolut	2.6	(±0.4)	def	10	254 (	± 69)	fghijk	19
	Accent	1.6	(±0.2)	ghij	22	108 (	±27)	ijklm	24
	Achilles	1.4	(±0.2)	hij	23	274 (	± 24)	efghij	18
	Condor	2.0	(±0.3)	efghi	16	220 (	± 64)	ghijk	21
	IdaGold	1.8	(±0.4)	fghij	21	640 (	± 153)	bcdefgh	12
	Maxi	2.2	(±0.4)	efgh	15	208 (	±110)	hijkl	22
	Santa Fe	2.4	(±0.5)	efg	12	384 (	± 96)	cdefgh	16
Solanum lycopersicum	UC82	7.2	(±0.2)	a	1	2,840(	± 443)	a	1

a Values shown are the mean of five replicates (n = 5) ± SE. Root galling index on a scale from 0 to 10 with 0 = no galls, 10 = 100% of roots galled. Values in a column followed by different letters are significantly different (P # 0.05) according to Fisher's LSD-test within one experiment. Raw nematode data (egg counts) were log10(x+1)-transformed before analysis; nontransformed data are presented.

This results (Melakeberhan et al., 2008; Edwards and Ploeg, 2014) showed that there are significant differences within and between

brassicaceous species with regard to host status for RKNs, and furthermore that the host suitability of a particular brassicaceous cultivar for RKNs can differ depending on the nematode species. Therefore, identifcation of the target RKN to species level is important to optimize this management strategy. Results for *M. incognita* and *M. javanica* were similar, with *B. juncea* and *B. rapa* cultivars generally being good hosts, and *E. sativa* Nemat and the *R. sativus* (oilseed radish) cultivars Boss and TerraNova consistently ranking among the poorest hosts. Whether inoculated with M. incognita or *M. javanica*, reproduction on the latter two cultivars was reduced by at least 98% compared with reproduction on tomato. These results are in agree¬ment with findings by Curto et al. (2005) who also reported that *B. juncea* cultivars were among the better hosts for *M. incognita*, and that R. sativus (oilseed radish) Boss and E. sativa Nemat were poor or nonhost. Broccoli, in our study a poor host for *M. incognita* and to a lesser degree for *M. javanica* as a crop that showed potential to reduce Meloidogyne populations, although they used a different cultivar. *Brassica juncea* Nemfix, in our study among the best hosts for *M. incognita* and *M. javanica*.

Also was concluded that information on the level of susceptibility of Brassica species to RKN is needed for biofumigation to become a succesful nematode management strategy. The *R. sativus* (oilseed radish) cultivar Adagio, in our study a poor host for both *M. incognita* and *M. javanica*/

The response of M. hapla to the different brassica cultivars was sometimes very different from results with *M. incognita* or *M. javanica*. For example, *R. sativus* (oilseed radish) Colonel and TerraNova were among the best hosts for *M. hapla*, but poor hosts for *M. incognita* and *M. javanica*. Also, the variability in host status for *M. hapla* within the same brassica species was greater. For example, within *R. sativus* (oilseed radish) there were good hosts (Colonel, TerraNova) as well as poor hosts (Adagio, Condor). The fact that plants resistant to *M. incognita* and *M. javanica* are often susceptible to *M. hapla* is thought to be the result of basic differences in the inheritance of resistance.

Within *R. sativus* (oilseed radish) lines, it is also thought that differences in reponse to *M. incognita* or *M. javanica*, and *M. hapla* may be because of a single gene resistance mechanism for the former two species, and a polygenic resistance mechanism for *M. hapla*. Ongoing breeding efforts

may lead to more cultivars with resistance to the range of economically inportant Meloidogyne species. In this study (Melakeberhan et al., 2008; Edwards and Ploeg, 2014) poor hosts for all three Meloidogyne species include *R. sativus* (oilseed radish) Adagio and E. sativa Nemat.

Although this study did not include the biofumigant effect that occurs during cover crop decomposition after soil incorporation of the crop, it would be unproductive to grow a host crop as a biofumigant. This study clearly demonstrated that large differences occur between different brassica cultivars with respect to their host status for three Meloidogyne species. Based on this study, the *R. sativus* (oilseed radish) cultivars Boss, Terranova, or *E. sativa* Nemat would be good choices for cover crops on *M. incognita* – or *M. javanica*-infested sites. Conversely, *B. rapa* or *B. juncea* cover crops would carry a risk of substantial nematode multiplication. On *M. hapla*-infested sites, *E. sativa* Nemat would carry little risk of nematode multplication, whereas *B. juncea* cultivars should be avoided.

The use of oilseed radish as a biofumigant against soil pathogens in potato cultivation has been found to be effective. Thus, in studies (Laznik et al., 2014) it was found that Brassica crops and their secondary metabolites on the wireworm population dynamics in soil. The analysis was based on sampling seven different glucosinolates. By the quantity detected, three glucosinolates stood out: glucoiberin, glucotropin and gluconasturtiin. It was found out that among different Brassica crops there are differences in the amount of glucoiberin. Significantly, the highest amount of glucoiberin was confirmed in samples of white mustard (5.54 ± 0.01 μmol/g ds), whereas the lowest values were confirmed in rape-seed (0.82 ± 0.01 μmol/g ds; Figure 2.46). Based on our results, we conclude that the amount of gluconas-turtiin is not influenced by a specific Brassica plant species (P = 0.8258). In the beginning, the content of gluconasturtiin in the samples of white mustard was 62 ± 0.22 μmol/g, while in the samples of rapeseed was 1.13 ± 0.55 μmol/g ds. Also the concentration of the glucobrassicin differed between different Brassica species. The values ranged between 0.25 ± 0.01 ^mol/g ds (white mustard) and 0.45 ± 0.00 μmol/g ds (kale; Figures 2.46–2.48).

Figure 2.46 – Average values of glucosinolates in tested cruciferous species (μmol/g ds). Different small letters indicate significant differences (*P < 0.05*, Student-Newman-Keuls' multiple range test) of different

glucosinolates content within the same cruciferous species. Different capital letters indicate significant differences between tested cruciferous species in the amount of glucosinolates (Laznik et al., 2014)

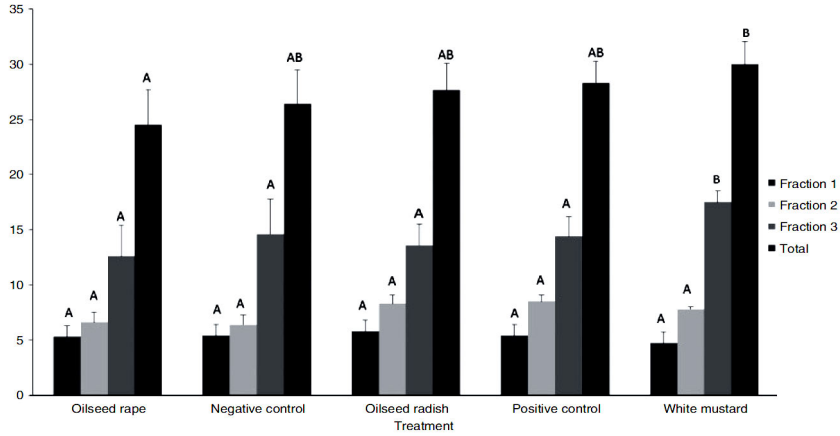


Figure 2.47 – The effect of different treatments on potato yield in 2011 in t ha-1. Different capital letters indicate significant differences (*P < 0.05*, Student-Newman-Keuls' multiple range test) within different treatments at the same fraction. Fraction 1 (tubers <4 cm); fraction 2 (tubers between 4 cm and 5 cm) and fraction 3 (tubers >5 cm)

ANOVA of pooled results in 2011 indicated a statistically significant effect of treatment (F = 3.61; df = 4, 149; P = 0.0080) and edge effect (F = 59.57; df = 2, 149; P < 0.0001) on the number of wireworm holes per potato tuber. In 2012, there were no significant differences among different treatments (F = 0.95; df = 6, 83; P = 0.4728) and edge effect (F =1.11; df = 3, 83; P = 0.3572) on the number of wireworm holes per potato tuber.

In 2011, there were significantly low number of wireworm holes per potato tuber in positive control (1.20 ± 0.3) and highest at white mustard (4.1 ± 1.1). Among other treatments, there were no significant differences and the number of holes per potato tuber ranged from 2.6 ± 0.7 (negative control) to 3.4 ± 0.9 (rapeseed; Figure 2.49). The edge effect influenced the

injuries rate of potato tubers in 2011. There was significantly low number of wireworm holes in potato in the right edge of the field (0.38 ± 0.1). In the middle part of the field, on average, 1.46 ± 0.2 holes per potato were found. The highest number of wireworm holes per potato was found in the left edge of the field (6.8 ± 0.9; Figure 2.50).

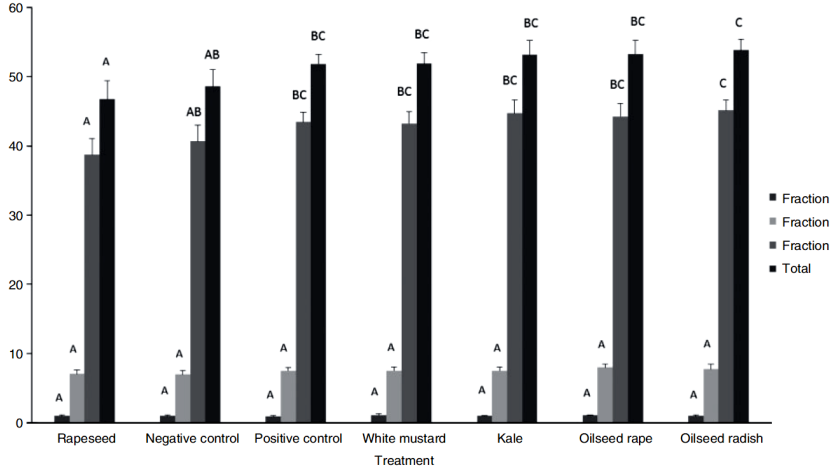


Figure 2.48 – The effect of different treatments on potato yield in 2012 in t ha^{-1}. Different capital letters indicate significant differences (*P < 0.05*, Student-Newman-Keuls' multiple range test) within different treatments at the same fraction. Fraction 1 (tubers <4 cm); fraction 2 (tubers between 4 cm and 5 cm); fraction 3 (tubers >5 cm)

In 2012, there were significantly low number of wireworm holes per potato tuber in rapeseed (0.22 ± 0.07) and kale (0.25 ± 0.04). Among other treatments, there were no significant differences, and the number of holes per potato tuber ranged from 0.39 ± 0.03 (negative control) to 0.47 ± 0.06 (positive control; Figure 2.51).The edge effect also did not have any influence on the number of wireworm holes per potato tuber in 2012, and the values ranged from 0.27 ± 0.03 (left edge of the field) to 0.45 ± 0.05 in the middle part of the field (Figure 2.52).

In both the years, big tubers (fraction 3) were significantly more damaged (2011: 1.69 ± 0.12 holes per potato tuber; 2012: 0.19 ± 0.04 holes per potato tuber) than smaller tubers [fraction 2 (2011: 0.93 ± 0.16 holes per potato tuber; 2012: 0.11 ± 0.02 holes per potato tuber) and Fraction 1 (2011: 0.24 ± 0.06 holes per potato tuber; 2012: 0.06 ± 0.02 holes per potato tuber)].

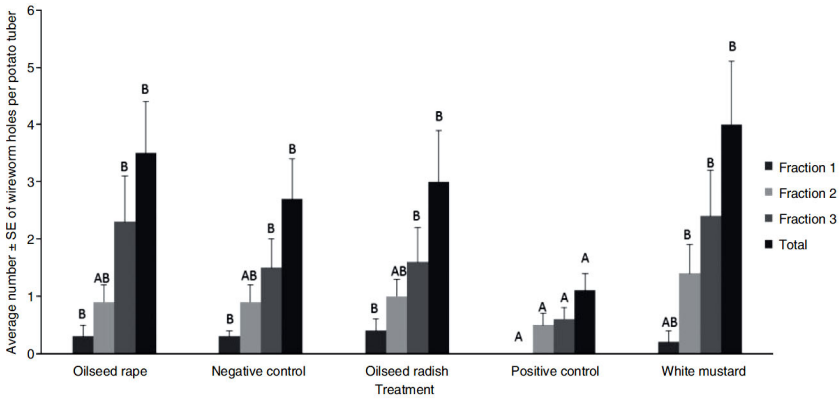


Figure 2.49 – The effect of different treatments on the average number of wireworm holes per potato tuber at different fractions in 2011. Different capital letters indicate significant differences (*P < 0.05*, Student-Newman-Keuls' multiple range test) within different treatments at the same fraction. Fraction 1 (tubers <4 cm); fraction 2 (tubers between 4 cm and 5 cm) and fraction 3 (tubers >5 cm)

The antinematode efficacy of oilseed radish has also been confirmed by other researchers (Menna and Melakeberhan, 2006; Hemayati et al., 2017; Hamidi and Hajihassani, 2020) (Figure 2.53-2.54).

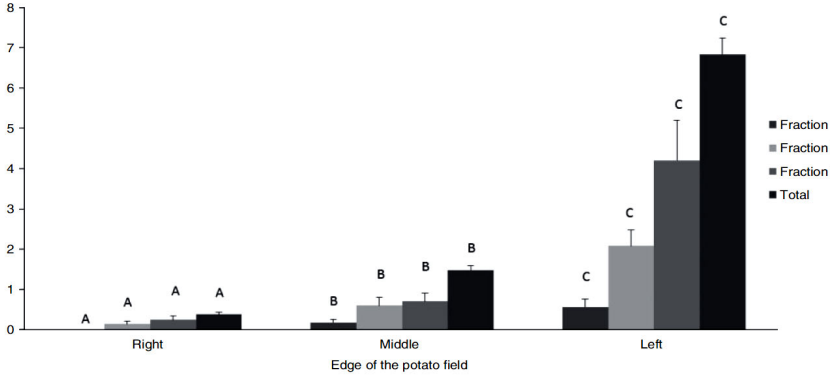


Figure 2.50 – The effect of the edge of the potato field on the average number of wireworm holes per potato tuber in 2011. Different capital letters indicate significant differences (*P < 0.05*, Student-Newman-Keuls' multiple range test) within the edge of the potato field at the same fraction. Fraction 1 (tubers <4 cm); fraction 2 (tubers between 4 cm and 5 cm) and fraction 3 (tubers >5 cm)

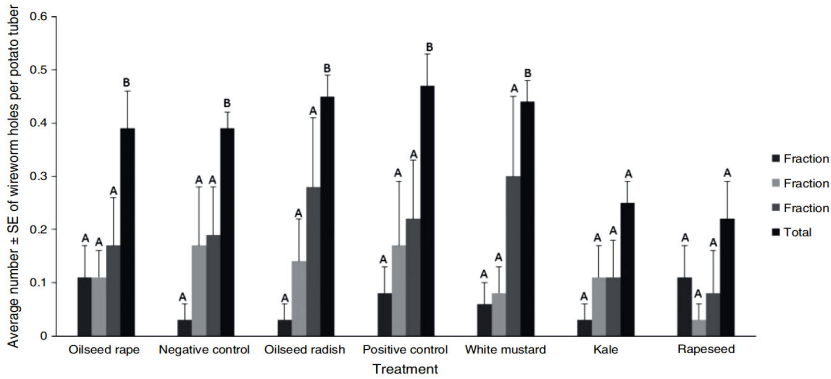


Figure 2.51 – The effect of different treatments on the average number of wireworm holes per potato tuber at different fractions in 2012. Different capital letters indicate significant differences (*P < 0.05*, Student-Newman-Keuls' multiple range test) within different treatments at the same fraction. Fraction 1 (tubers <4 cm); fraction 2 (tubers between 4 cm and 5 cm) and fraction 3 (tubers >5 cm)

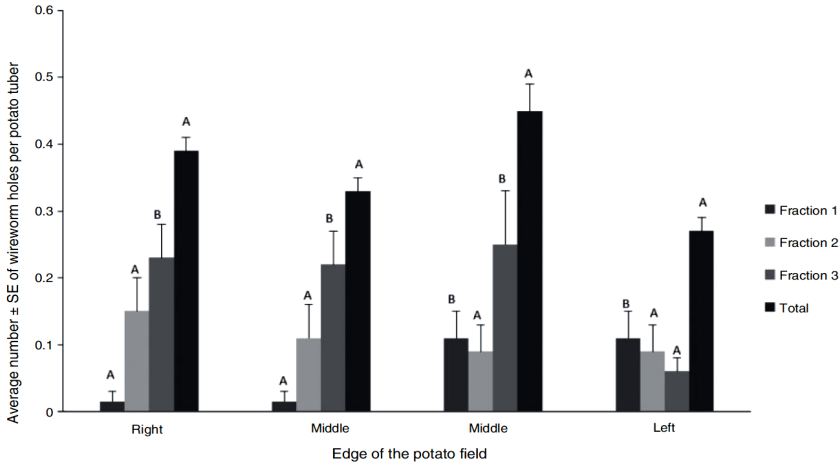


Figure 2.52 – The effect of the edge of the potato field on the average number of wireworm holes per potato tuber in 2012. Different capital letters indicate significant differences (P < 0.05, Student-Newman-Keuls' multiple range test) within the edge of the potato field at the same fraction. Fraction 1 (tubers <4 cm); fraction 2 (tubers between 4 cm and 5 cm) and fraction 3 (tubers >5 cm)

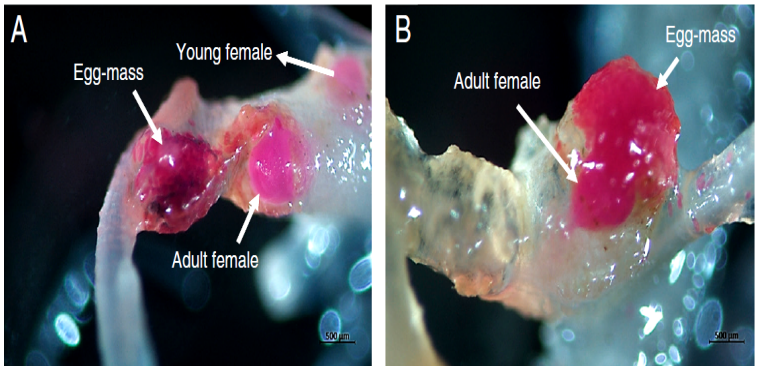


Figure 2.53 – Representative images of roots of oilseed radish cv. Eco-Till (A) and oat cv. Pratex (B) infected with Meloidogyne arenaria at 28 days after inoculation (Hamidi and Hajihassani, 2020)

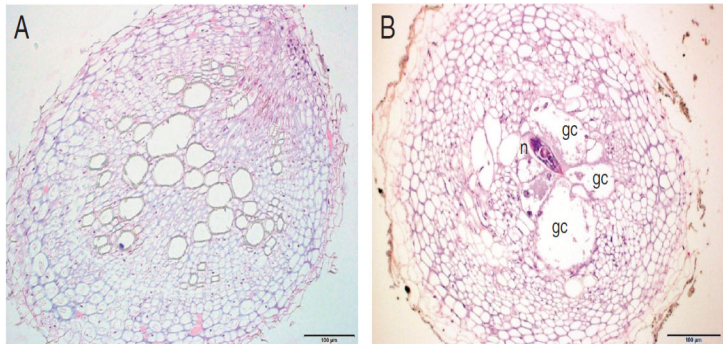


Figure 2.54 – Hematoxylin-Eosin stained cross sections of root tissues of oilseed radish cv. Carwoodi (A) and Eco-Till (B) infected with Meloidogyne incognita at 28 days after inoculation. N, female nematode; GC, giant cell (Hamidi and Hajihassani, 2020)

Oilseed radish stands out positively among other crops, which significantly reduces the number of nematodes when growing nematode-resistant varieties (Table 2.38) (Babych et al., 2011).

Table 2.38

Effect of nematode-resistant oil radish varieties on nematode abundance when grown as a main crop (Lykhochvor, 2008)

Variety	Originator	Impact on beet nematode population (+ increase, - decrease)	Flowering rate: 9 – high 1 – small	Mass growth rate: 9 – high 1 – small	Plant height: 9 – large 1 – small
Adagio	Saaten Union	- 70 – 90 %	2	5	2
Pegletta	Saaten Union	- 70 – 90 %	7	6	5
Colonel	Saaten Union	- 90 % і більше	5	5	4
Roma	St. Ramenda	- 90 %	5	5	5

The effective nematode-regulating role of oil radish has been proven, and the greatest inhibitory effect was provided by the use of oil radish as a green manure in combination with the resistance of the host plant. The lowest cyst viability was found in the variant with the use of oil radish green manure and a nematode-resistant potato variety (Table 2.39).

Table 2.39

Population density and viability of cystic fibrosis under different methods of substrate treatment (Babich et al., 2011)

Variant	Number of new generation cysts, pcs./100 g of soil	Reproduction rate	Cyst viability, %
1. Nevsky variety (K)	3.7	0.55	89
2. Sante variety	1.3	0.20	85
3. Oilseed radish (green manure)	2.0	0.30	75
4. Oilseed radish + Nevsky variety	1.0	0.15	92
5. Oilseed radish + Sante variety	0.3	0.05	83
LSD_{05}	1.1		

* – deviations from the control are significant $F_{fact} > F_{theor}$ (2.61).

The influence of green manure and biofumigation use of oilseed radish leaf mass in variants of influence on the microbiological activity of the soil profile was also proved. Thus, the studies Aiyer et al. (2022) have established that ITS1 sequencing produced a total of 69 005 920 reads from 360 samples. After trimming and chimera screening, a total of 28407 646 clean reads were clustered into 23 576 OTUs, of which 1356 OTUs remained after filtering out unclassified and low-abundance OTUs. Of the 1356 OTUs, 752 were predicted based on the reference database and 604 were de novo annotated. The most abundant fungal taxa identified at the family level were Plectosphaerellaceae (Fig. 2.55 A). Differences in abundance of fungal OTUs at different sample collection time points were observed at the family level (Fig. 2.55 B). An increase in abundance of Nectriaceae spp. in the cash crop phase of the second trial was also observed (Fig. 2.55 B).

Bacterial communities were characterized by sequencing the 16S rRNA gene, resulting in a total of 1 284 705 reads and 1 029 658 filtered and nonchimeric reads, with an average read length of 1451 bp, which clustered into 62 204 OTUs. After filtering based on low abundance and taxonomy, a total of 10 799 OTUs remained, of which 9967 were predicted based on the reference database, whereas 832 were de novo annotated. Bacillaceae was the most abundant bacterial family in the soil (Figure 2.56 A). Unlike with fungi, bacterial community relative abundance between sample collection time points was highly conserved at the family level (Fig. 2.56 B).

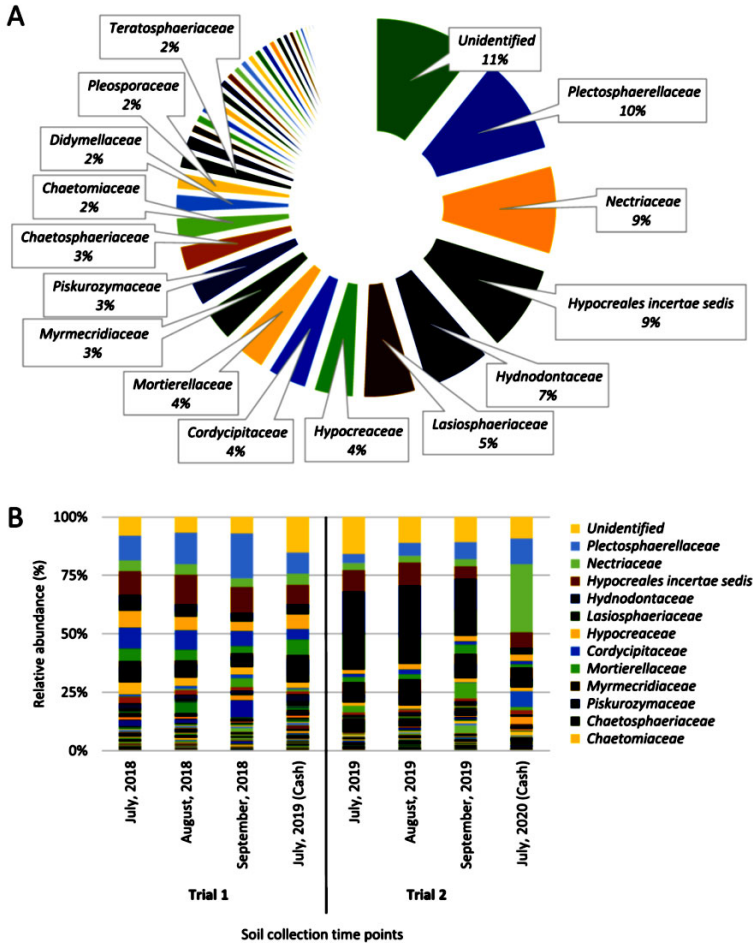


Figure 2.55 – Relative abundance of fungal families in soil samples. (A) Pie chart presenting the average relative abundance of fungal families in all soil samples. (B) Stacked bar chart presenting the changes in the relative abundance of fungal families over time in both trials. Soil samples were collected at three time points during the cover crop phase (July, August, and September), after which soil samples were collected in July of the cash crop phase (Aiyer et al., 2022)

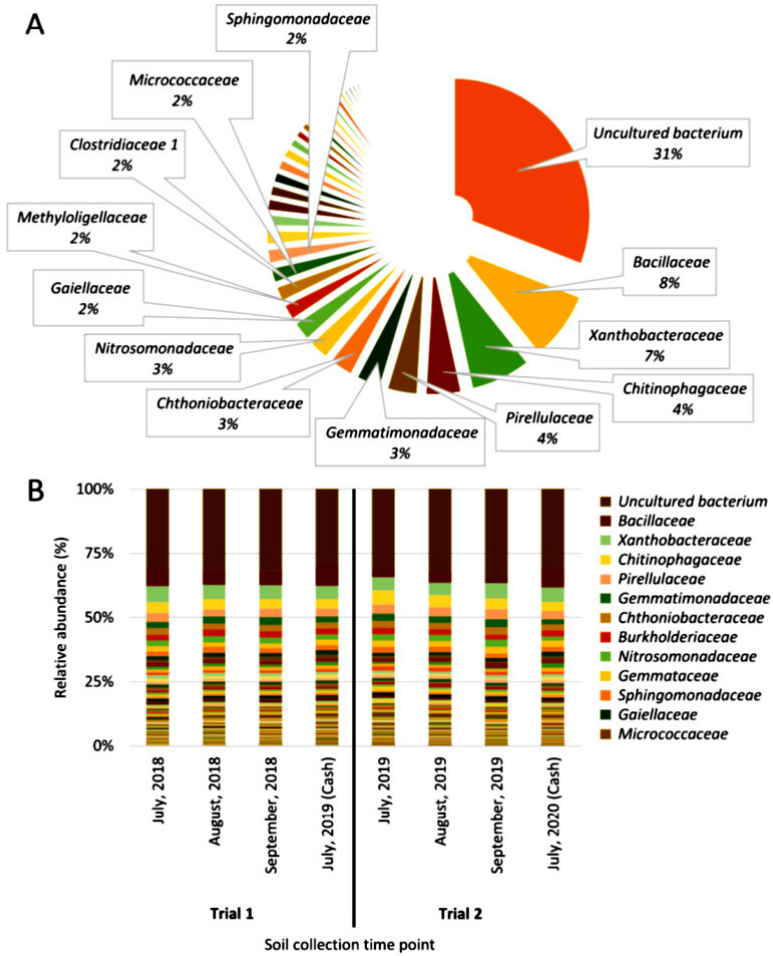


Figure 2.56 – Relative abundance of bacterial families in soil samples. (A) Pie chart presenting the average relative abundance of bacterial families in all the soil samples. (B) Stacked bar chart presenting the changes in the relative abundance of bacterial families over time in both trials. Soil samples were collected at three time points during the cover crop phase (July, August, and September), after which soil samples were collected in July of the cash crop phase (Aiyer et al., 2022)

Fungal alpha diversity was calculated, with a data set rari-fied to 19 729 reads per sample (Fig. 2.57), using Faith's phyloge-netic diversity index. Soil collected from the cash crop phase of the second trial had significantly higher fungal alpha diversity compared with that from the first trial (p value < 0.001). The fungal alpha diversity slowly increased throughout the growing season and the soil collected in the cash crop phase had significantly higher diversity than the soil from the start of the trial. Statistically significant differences in soil fungal alpha diversity between cover crops were observed in the cash crop phase (Table 2). The soil fungal alpha diversity after growing sorghum-sudangrass and Mix-1 was significantly higher than the alpha diversity after Mix-2 (Fig. 2.57A).

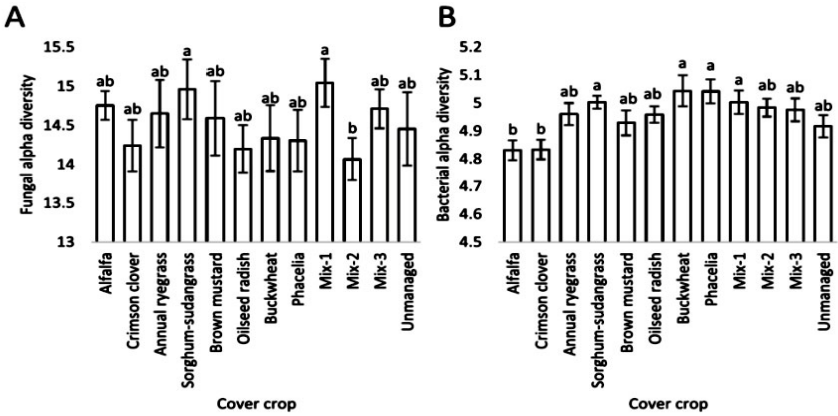


Figure 2.57 – Bar graphs representing average fungal alpha diversity rarified at 19 729 reads (A) and average bacterial alpha diversity rarified at 824 reads (B) for the cash crop phase; n = 12. Different letters indicate statistical significance. Mix-1: buckwheat + crimson clover; Mix-2: phacelia + brown mustard; and Mix-3: buckwheat + crimson clover + brown mustard. Connecting letters for fungal alpha diversity were determined using the SHASH-transformed data set. Error bars indicate standard error (Aiyer et al., 2022)

Bacterial alpha diversity was calculated with the data set rarified to 824 reads per sample (Fig. 2.57A). Similarly to fungal alpha diversity, bacterial alpha diversity also gradually increased over time, with the soil collected from the cash crop phase having significantly higher alpha diversity compared with the previous year. The choice of cover crops significantly influenced the soil bacterial alpha diversity in the cash crop phase. The soil after growing alfalfa and crimson clover had the lowest overall bacterial alpha diversity, while the soil after growing buckwheat, phacelia, sorghum-sudangrass and Mix-1 had significantly higher bacterial alpha diversity (Fig. 2.57 B).

Beta diversity analysis, using weighted UniFrac distance matrices, indicated that soil fungal communities became increasingly dissimilar between cover crops as the growing season progressed in both trials. By September, the soil fungal community composition was significantly affected by the cover crop and stayed different by cover crop in the subsequent year. Fungal community compositions between Mix-1 and Mix-3 soils were conserved in both trials. There were no significant differences in the soil fungal community composition between oilseed radish and phacelia soils in either trial. However, the soil fungal community composition after growing oilseed radish and phacelia was significantly dissimilar from that for every other crop in the first trial. In the second trial, the fungal community compositions associated with oilseed radish and phacelia soils were only dissimilar from those of alfalfa and sorghum-sudangrass soils. The fungal community composition associated with oilseed radish soil was also dissimilar from that of Mix-2 soil in the second trial. In the first trial, the fungal community in Mix-2 soil, which was composed of phacelia and brown mustard, was more similar in composition to that of brown mustard soil compared with that of phacelia soil in both the cover crop and cash crop phases. This trend was the same in the cover crop phase of the second trial, but it was the opposite in the cash crop phase.

The bacterial community composition was significantly affected by the cover crop choice in the cash crop phase the following growing season in both trials. In the first trial, the bacterial community composition associated with annual ryegrass soil was significantly different from that of every

other cover crop soil, and the bacterial community associated with alfalfa soil was significantly dissimilar to that of the soils of all the other cover crops except crimson clover. However, in the second trial, the bacterial community composition associated with annual ryegrass soil was only significantly different from that of unmanaged soil, while the community composition associated with alfalfa soil was significantly dissimilar from those of phacelia, oilseed radish, and sorghum-sudangrass soils. Like the fungal community composition, the bacterial community compositions associated with buckwheat, Mix-1, and Mix-3 soils were highly conserved in both trials. In both trials, the bacterial community composition associated with unmanaged soil was significantly dissimilar to those associated with alfalfa, crimson clover, annual ryegrass, sorghum-sudangrass, phacelia, and Mix-3 soils.

FUNGuild was used to taxonomically parse fungal OTUs into groups corresponding to their predicted ecosystem functions. A total of 950 fungal OTUs were classified into one or more of the three functional trophic groups in FUNGuild: 517 OTUs were classified as pathotrophs, 378 OTUs were classified as saprotrophs, and 55 OTUs were classified as symbiotrophs. Fungal OTUs were also subdivided into guilds All three fungal trophic groups were significantly affected by the choice of cover crop in the cash crop season of both trials). Of the 599 OTUs that were found to be differentially abundant by cover crop, 204 were identified as pathotrophs using genus-level classification in FUN-Guild (Figure 2.58 A). These OTUs included several well-known plant pathogens such as Fusarium spp. (Link), Septoria spp. (Desm.), Bipolaris spp. (Shoemaker), Diaporthe spp. (Nitschke), Rhizoc-tonia spp. (Kühn), and Colletotrichum spp. (Corda). The two most abundant Fusarium OTUs were differentially affected by cover crops. However, the two OTUs were both higher in alfalfa soil and lower in crimson clover, brown mustard, oilseed radish, and Mix-2 soils, compared with the un-managed soil (Figure 2.58A). Except for oilseed radish soil, where one OTU was higher in abundance compared with the un-managed soil, both Septoria OTUs were lower in all the cover crop soils than in the unmanaged soil.

Insect pathogenic fungi and fungal biocontrol agents Is-aria spp. ((Berk.) Lloyd), Metarhizium spp. (Sorokin), Beau-varia spp. (Vuil.), Clonostachys spp. (Corda), and Trichoderma spp. (Pers.) were also classified as

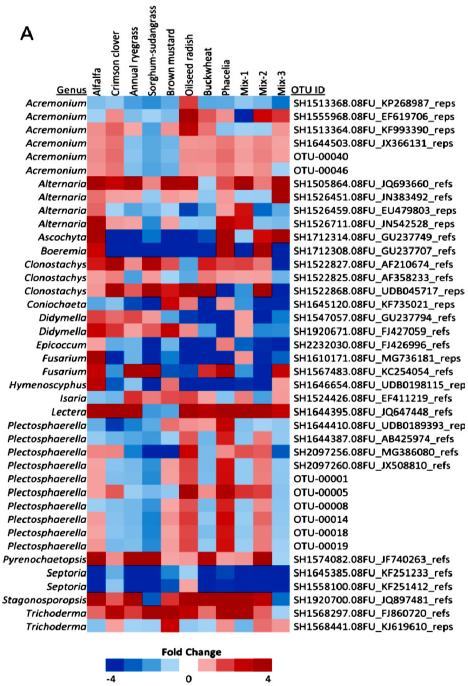


Figure 2.58 A – Differences in abundance of fungal OTUs classified as pathotrophs (A), symbiotrophs (B), and saprotrophs (C) present in the cash crop phase; n = 12. The heatmaps present fold change comparisons of the top 40 most abundant fungal pathotrophs and saprotrophs and the most abundant symbiotrophs found to be significantly different by cover crop. The relative abundance of fungal OTUs in soil from each cover crop was normalized against that of unmanaged soil (Aiyer et al., 2022)

pathotrophs (File S1a). Three Clonostachys OTUs, two Trichoderma OTUs, and one Is-aria OTU were among the top 40 most abundant pathotrophs (Fig. 2.58 A). Clonostachys OTUs were more abundant in alfalfa, crimson clover, sorghum-sudangrass, buckwheat, and Mix-2 soils compared with unmanaged soil, whereas they were less abundant or variable in all

the other cover crop soils. Trichoderma OTUs were consistently more abundant in brown mustard, phacelia, and Mix-2 soils compared with unmanaged soil. Isaria spp. were less abundant in alfalfa, crimson clover, phacelia, and Mix-2 soils, and more abundant in all the other cover crop soils, compared with unmanaged soil (Fig. 2.58A). A total of 33 OTUs, which were differentially abundant by cover crop, were classified as symbiotrophs, including several known arbuscular mycorrhizal fungi such as Funneliformis spp. (Walker and SchüEler), Glomus spp. (Tul. and Tul.), and Paraglomus spp. (Blaszk.) (Figure 2.58 B–C). Except for buckwheat soil, most Funneliformis OTUs were more abundant in all the cover crop soils compared with unmanaged soil.

Of 10 799 bacterial OTUs, only 188 OTUs were differentially abundant by cover crop ($a < 0.05$), with fold change comparisons between cover crop soils and unmanaged soils presented in File S1b. These included large bacterial genera such as Bacillus and Pseudomonas, as well as several unidentified taxa. Bradyrhizobium spp., which are known for their role in nitrogen fixation and commonly associated with legume crops, were not significantly different by cover crop. Meanwhile, three OTUs in the genus Pantoea, which includes several plant pathogenic species, were differentially abundant by cover crop, positively associated with alfalfa and Mix-1 soils. To better understand the bacterial community response to the choice of cover crop, bacterial OTUs were parsed into functional groups using FAPROTAX.

A total of 3195 bacterial OTUs were classified into 59 functional groups using the FAPROTAX package on Python. Cover crop effects on most bacterial functional groups in the cash crop phase significantly differed between the two trials, apart from methanol oxidation, methylotrophy, manganese oxidation, and hydrocarbon degradation. The choice of cover crop significantly affected the abundance of bacteria involved in methanol oxidation, methylotrophy, ammonia oxidation, nitrification, sulfate respiration, cellulolysis, manganese oxidation, pho-totrophy, and chemoheterotrophy in the cash crop phase. Bacteria involved in methylotrophy and methanol oxidation were more abundant after growing alfalfa and lowest after growing annual ryegrass. Cellulolytic bacteria were also most abundant after alfalfa, but least abundant after oilseed radish. Chemoheterotrophic bacteria were found in significantly higher

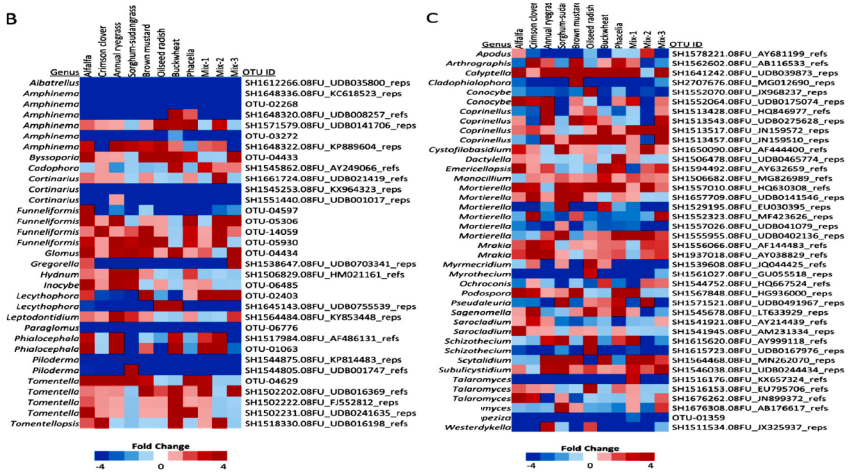


Figure 2.58 B–C – Differences in abundance of fungal OTUs classified as pathotrophs (A), symbiotrophs (B), and saprotrophs (C) present in the cash crop phase; n = 12. The heatmaps present fold change comparisons of the top 40 most abundant fungal pathotrophs and saprotrophs and the most abundant symbiotrophs found to be significantly different by cover crop. The relative abundance of fungal OTUs in soil from each cover crop was normalized against that of unmanaged soil (Aiyer et al., 2022)

abundance in soil collected after alfalfa compared with unmanaged soil. Bacteria involved in ammonia oxidation and nitrification were found in the highest abundance after buckwheat and lowest after Mix-3. Bacteria with the capacity to respire sulfate were more abundant in soil collected after brown mustard, and less abundant after phacelia. Bacteria with the capacity to oxidize manganese were more abundant after Mix-2 and less abundant after annual ryegrass. Genus-level taxonomic identification is listed on the left, with the corresponding OTU reference ID listed on the right. Mix-1: buckwheat + crimson clover; Mix-2: phacelia + brown mustard; and Mix-3: buckwheat + crimson clover + brown mustard.

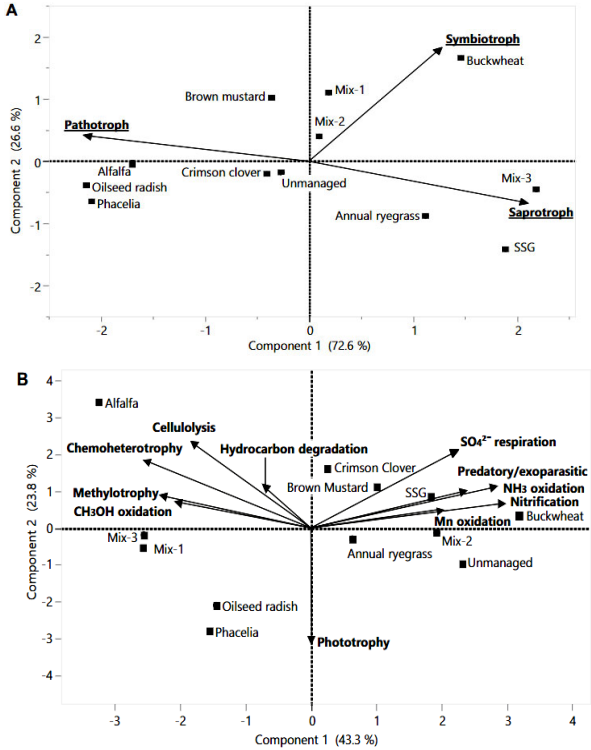


Figure 2.59 A–C – Principal component analysis (PCA) biplots. Principal components 1 and 2 were produced from the least-squares means table and represent the bacterial functional (bolded) and fungal trophic (bold underlined) group relationship with cover crop groups. The trophic group relationship is based on the data collected in the cash crop phase; n = 12. SSG: sorghum-sudangrass; Mix-1: buckwheat + crimson clover; Mix-2: phacelia + brown mustard; and Mix-3: buckwheat + crimson clover + brown mustard. (A) PCA biplot with fungal trophic groups (bolded and underlined) compared with different cover crops. (B) PCA biplot with bacterial functional groups (bolded) compared with different cover crops. (C) Combined PCA biplot with both bacterial and fungal groups in comparison with different cover crops (Aiyer et al., 2022)

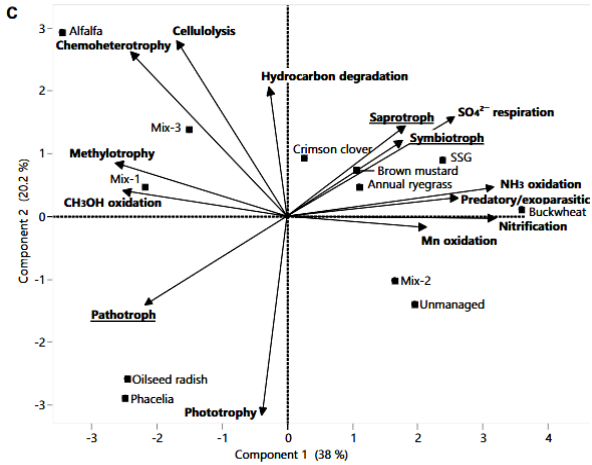


Figure 2.59 A–C (end)

The results from study of Aiyer et al. (2022) indicated that choice of cover crop has a significant influence on soil microbial community composition. The cover crop's influence on the soil microbial communities was found to carry over to the soil in the subsequent year, where it could potentially influence plant health. Choice of cover crop is important as it can affect soil health by manipulating the fungal community composition. The high proportion of plant pathotrophic fungi observed after growing oilseed radish, phacelia, and alfalfa may be an indication of a higher pathogen load, which could lead to increased root disease pressure in subsequently planted crops. Alternatively, the choice of sorghum-sudangrass as the cover crop may lead to disease-suppressive soils and buckwheat to pest-suppressive soils. These results verified the hypothesis regarding beta diversity, as well as functional and trophic group analysis. However, we hypothesized that cover crops would not affect the soil fungal and bacterial alpha diversity in a single growing season, but the results did not support this. This shows that the choice of cover crop has a profound influence on the soil microbiome in a single growing season, thereby influencing soil health.

List of references to the Chapter 2

Addison, P., Fourie, J.C. (2007). Cover crop management in vineyards of the Lower Orange River region, South Africa: 2. Effect on plant-parasitic 847–853.

Agrios, G.N. (2005). Plant Pathology. Fifth Edition. Elsevier ElsevierAcademic Press, China. 922 pp.

Agrofit. Sistema de Agrotóxicos Fitossanitários do Ministério da Agricultura (2020). URL: <http://agrofit.agricultura.gov. br/agrofit_cons/principal_agrofit_cons> (date of application 03.09.2024).

AHDB Biofumigation (2022) URL: potatoes.ahdb.org.uk/sites/default/files/pubNcation_upload/R476%20Final%20Report_180419%20to%20publish.pdf (date of application 03.09.2024).

AHDB PCN Grower Guide. (2022). URL: projectblue.blob.core.windows.net/media/ Default/ Imported%20Publication%20Docs/PCN-Guide659_181102_WEB.pdf (date of application 03.09.2024).

Akhtar, M., Mahmood, I., (1996). Control of plant-parasitic nematodes with organic and inorganic amendments in agricultural soil. Applied Soil Ecology, 4, 243–247.

Arnault, I. et al. (2013). Use of Alliaceae residues to control soil-borne pathogens. Industrial crops and products, 49, 265–272.

Ashiq, S., Edwards, S., Watson, A., Back, M. (2022). Biofumigation for the Management of Fusarium graminearum in a Wheat-Maize Rotation. Pathogens, 11, 1427.

Aydinli, G., Mennan, S. (2018). Biofumigation studies by using Raphanus sativus and Eruca sativa as a winter cycle crops to control root-knot nematodes. Brazilian Archives of Biology and Technology, 61.

Aiyer, H., Fofana, B., Fraser, T., Caldwell, C., McKenzie-Gopsill, A., Mills, A., Foster, A. (2022). Choice of cover crop influences soil fungal and bacterial communities in Prince Edward Island, Canada. Canadian Journal of Microbiology, 68(7), 465-482.

Babych, A.G., Sukhareva, R.D., Babych, O.A., Matvienko, O.P. (2011). Optimization of traditional and adaptive fertilization systems in the centers of cytoplasmic nematodes. In: Fundamentals of biological crop production in modern agriculture. K.: NULES of Ukraine, UNI of Biological Resources Quality and Life Safety,11 pp.

Back, M., Barker, A., Evans, A. (2019). Effectiveness of biofumigant crops for the management of PCN in GB. Ref: R476. Final Report. AH PB potatoes. 137 pp.

Baker, L.W. et al. (1996). Ambient air concentrations of pesticides in California. Environmental Science & Technology, 30, 1365–1368.

Balesh, T., Zapata, F., Aune, J.B. (2005). Evaluation of mustard meal as organic fertilizer on tef (Eragrostis tef (Zucc) Trotter) under field and greenhouse conditions Nutrient Cycling in Agroecosystems, 73, 49–57.

Bandyopadhyay, S., Khalko, S. (2016). Biofumigation – An eco-friendly approach for managing bacterial wilt and soft rot disease of ginger. Indian Phytopathology, 69(1), 53–56.

Barrau, C. et al. (2009). Brassica carinata for control of Phytophthora spp. in strawberry field crops. Revista de Ciências Agrárias, 32(2), 135–138.

Barros, A. F. et al. (2014). Exposure time of second stage juveniles to volatiles emitted by neem and mustard macerates and biofumigation against Meloidogyne incognita. Nematropica, 44(2), 190–199.

Bates, G.D., Rothrock, C.S. (2020).. Efficacy of a high-glucosinolate Indian mustard cover crop for biofumigation of soils to control reniform nematode on cotton. AAES Research Series 543, 135–139.

Bello, A., López-Pérez, J.A., Arias, M. (2001). Biofumigation and grafting in pepper as alternatives to methyl bromide. Centro Ciencias Edioambirntales. 119 pp.

Bellostas, N., Sørensen, J.C., Sørensen, H. (2007). Profiling glucosinolates in vegetative and reproductive tissues of four Brassica species of the U-triangle for their biofumigation potential. Journal of the Science of Food and Agriculture, 87, 1586–1594.

Bellostas, N., Serensen, J.C., Serensen, H. (2004). Qualitative and quantitative evaluation of glucosinolates in cruciferous plants during their life cycles. Agroindustria, 4, 267–272.

Bhering, A. S. et al. (2020). Soil management in a mountain agroecosystem and clubroot disease. Plant Pathology, 69(2), 302–309.

Bialy, Z., Oleszek, J., Lewis, J., Fenwick, G.R. (1990). Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. Plant Soil, 129, 277–281.

Bianco, V., Nicholls, J., Mattner, S., Allen, D., Porter, I. (2020). Biofumigation in Australian Horticulture: An Integrated Approach to MB Replacement. Agriculture Victoria, Institute for Horticultural Development, Scoresby Business Centre Vic, Australia, 3176, 4 pp.

Biofumigation For management of potato cyst nematodes (PCN) (2022). URL: https://archive.ahdb.org.uk/knowledge-library/biofumigation-for-management-of-potato-cyst-nematodes-pcn. (date of application 03.09.2024).

Biofumigation for Soil-Borne Diseases Management in Tropical Vegetable Production (2022). Biological Control, 48, 147–154.

Biomass Production of Biofumigant Cover Crops –'Caliente' Mustard and Oilseed Radish (2014). URL: https://content.ces.ncsu.edu/pdf/biomass-production-of-biofumigan/2014-09-29/biomass-production-of-biofumigant-cover-crops-caliente-mustard-and-oilseed-radish.pdf (date of application 03.09.2024).

Björkman, T., Shail, J.W. (2010). Cornell cover crop guide for forage radish. Cornell University, Ver. 1.100716, 2 pp.

Borek, V., Elberson, LR, McCaffrey, J.P., Morra, M.J. (1995). Toxicity of aliphatic and aromatic isothiocyanates to the eggs of the black vine weevil (Coleoptera, Curculionidae). Journal of Economic Entomology, 88, 1192–1196.

Borek, V., Morra, M.J. (2005). Ionic thiocyanate (SCN-) production from 4-hydroxybenzyl glucosinolate contained in Sinapis alba seed meal. Journal of Agricultural and Food Chemistry, 53 (22), 8650–8654.

Brown, P.D., Morra, M.J. (1997). Control of soil-borne plant pests using glucosinolate-containing plants. Advances in Agronomy. 61, 167–231.

Brown, P.D., Morra, M.J., McCaffrey, J.P., Auld, D.L., Williams, L. (1991). Allelochemicals produced during glucosinolate degradation in soil. Journal of Chemical Ecology, 17, 2021-2034.

Bruggen, A.H.C. et al. (2016). Plant disease management in organic farming systems. Pest Management Science, 72(1)1, 30–44.

Bruggen, A.H.C., Finckh, M.R. (2016). Plant diseases and management approaches in organic farming systems. Annual Review of Phytopathology, 54, 25–54.

Buckerfield, J.C., Webster, K.A. (1996). Earthworms, mulching, soil moisture and grape yields. Wine Industry Journal, 11, 47–53.

Burki, F. et al. (2010). Evolution of Rhizaria: new insights from phylogenomic analysis of uncultivated protists. BMC Evolutionary Biology, 10, 377.

Caldbeck, J. (2022). The potential of biofumigation to control soil borne pests, pathogens and weeds. Organic Research Centre, Trent Lodge, Stroud Rd, Cirencester GL7. 6JN, UK, 105 pp.

Campanella, V. et al. (2020). Management of common root rot and Fusarium foot rot of wheat using Brassica carinata break crop green manure. Crop Protection, 130.

Cartea, M.E., Rodríguez, V.M., de Haro, A., Velasco, P., Ordás, A. (2008). Variation of glucosinolates and nutritional value in nabicol (Brassica napus pabularia group). Euphytica, 159(1), 111–122.

Cauwer, B. et al. (2019). Impact of Brassica juncea biofumigation on viability of propagules of pernicious weed species. Weed Research, 59(3), 209–221..

Cesarano, G., Zotti, M., Antignani, V., Marra, R., Scala, F., Bonanomi, G. (2017). Soil sickness and negative plant-soil feedback: A reappraisal of Hypotheses. Journal of Plant Pathology. 99, 545–570.

Challenger, F. (1959). Aspects of the organic chemistry of sulphur. Butterworths, London, 457 pp.

Cheah, L.H. et al. (2001). Brassica crops and a Streptomyces sp. as potential biocontrol for clubroot of brassicas. New Zealand Plant Protection, 54, 80–83.

Cheah, L.H. et al. (2006). Clubroot control using Brassica break crops. Acta Horticulturae, 706, 329–332.

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. Annual Review of Phytopathology, 40, 221–249.

Clark, D. (2010). Glucosinolates, structure and analysis in food. Analytical Methods, 4, 301–416.

Clarkson, J. et al. (2015). Biofumigation for the control of soil-borne diseases. EPI-AGRI. Soil-borne diseases. URL: https://ec.europa.eu/eip/agriculture/sites/agri-eip/files/9_eip_ sbd_mp_biofumigation_final_0.pdf> (date of application 03.09.2024).

Clarkson, J., Michel, V., Neilson, R. (2020). Mini-paper - Biofumigation for the control of soil-borne diseases. Focus Group SOIL-BORNE DISEASES. URL: https://ec.europa.eu/eip/agriculture/sites/default/files/9_eip_sbd_mp_biofumigation_final_0.pdf (date of application 03.09.2024).

Curto, G. et al. (2016). Biofumigant effect of new defatted seed meals against the southern root-knot nematode, Meloidogyne incognita. Annals of applied biology, 169(1), 17–26.

Curto, G., Dallavalle, E., Lazzeri, L. (2005). Life cycle duration of Meloidogyne incognita and host status of Brassicaceae and Capparaceae selected for glucosinolate content. Nematology, 7, 203–212.

Daneel, M. et al. (2018). The host status of Brassicaceae to Meloidogyne and their effects as cover and biofumigant crops on root-knot nematode populations associated with potato and tomato under South African field conditions. Crop protection, 110, 198–206.

De Pascale, S., Maggio, A., Pernice, R., Fogliano, V., Barbieri, G. (2007). Sulphur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*. European Journal of Agronomy, 26, 418–424.

Deadman, M., Al Hasani, H., Al Sa'di, A. (2006). Solarization and biofumigation reduce Pythium aphanidermtum induced damping-off and enhance vegetative growth of greenhouse. Acta Horticulturae, 707, 258–272.

Dhingra, O.D. et al. (2013). Potential of soil fumigation with mustard essential oil to substitute biofumigation by cruciferous plant species. Tropical plant pathology, 38(4), 337–342.

Dixon, G.R.; Tilston, E.L. (2010). Soil-borne pathogens and their interactions with the soil environment. In: Dixon, G. R., Tilston, E.L. Soil Microbiology and Sustainable Crop Production. Dordrecht, Netherlands: Springer, Cap. 6, 197–271.

Di Gioia, F., Avato, P., Serio, F., Argentieri, M.P. (2018). Glucosinolate profile of Eruca sativa, Diplotaxis tenuifolia and Diplotaxis erucoides grown in soil and soilless systems. Journal of Food Composition and Analysis, 69, 197–204.

Donald, D. et al. (2019). Managing Soilborne Diseases in Vegetables: Rotation with green manure and biofumigant crops shows disease control & yield benefits. Victoria: Department of Primary Industries, URL: https://ausveg.com.au/app/data/technical-insights/docs/V G07125_Soilborne_Diseases_brochure. Pdf. (date of application 03.09.2024).

Downie, H., Holden, N., Otten, W., Spiers, A.J., Velntine, T.A., Dupuy, L.X. (2012). Transparent soil for imaging the rhizosphere. PLoSOne7, e44276.

Drobnica, L., Zemanova, M., Nemec, P., Antos, K., Kristian, P., Stullerova, A., Kuoppova, V., Nemec, P. (1967). Antifungal activity of isoth- iocyanates and their analogues. Applied Microbiology, 15, 701-703.

Duff, J.D., Firrell, M.C. (2021). Biofumigation: A Cover Crop Option 12 Months of the Year to Manage Three Soilborne Pathogens Ailing the Australian Vegetable Industry. Global Journal of Agricultural Innovation, Research & Development, 8, 104–116.

Duff, J., van Sprang, C., O'Halloran, J., Hall, Z. (2020). Guide to Brassica Biofumigant Cover Crops Managing soilborne diseases in vegetable production systems. Horticulture Innovation through VG16068 Optimising cover cropping for the Australian vegetable industry. State of Queensland. Department of Agriculture and Fisheries, 40 pp.

Dutta, T.K. et al. (2019). Plant-parasitic nematode management via biofumigation using brassica and non-brassica plants: Current status and future prospects. Current Plant Biology, 17, 17–32.

Edwards, S, Ploeg, A (2014). Evaluation of 31 potential biofumigant brassicaceous plants as hosts for three Meloidogyne species. Journal of Nematology, 46, 287–295.

Epstein, L. (2014). Fifty Years Since Silent Spring. Annual Review of Phytopathology, 52, 377–402.

Fahey, J.W., Zalcmann, A.T., Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, 56, 5–51.

Fenwick, G.R., Heaney, R.K., Mullin, W.J. (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, P.L., Cerato, C. (2008). Trichoderma spp. tolerance to Brassica carinata seed meal for a combined use in biofumigation. Biological Control, 45, 319–327.

Fahey, J.W., Zalcmann, A.T., Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemestry, 56, 5–51.

Fan, C. M. et al. (2008). Potential biofumigation effects of Brassica oleracea var. caulorapa on growth of fungi. Journal of Phytopathology, 156(6), 321–325.

Ferris, H., McKenry, M.V. (1976). Nematode community structure in a vineyard soil. Journal of. Nematology, 8, 131–137.

Fourie, J.C., Freitag, K. (2010). Soil management in the Breede River Valley wine grape region, South Africa. 2. Soil temperature. South African Journal of Enology and Viticulture, 31, 165–168.

Fourie, J.C. (2012). Soil management in the Breede River Valley wine grape region, South Africa. 4. Organic matter and macro-nutrient content of a medium textured soil. South African Journal of Enology and Viticulture, 33, 105–114.

Fourie, J.C., Agenbach, G.A., Louw, P.J.E., (2007). Cover crop management in a Chardonnay/99 Richter vineyard in the Coastal Region, South Africa. 3. Effect of different cover crops and cover crop management practices on organic matter and macro-nutrient content of a medium textured soil. South African Journal of Enology and Viticulture, 28, 61–68.

Fourie, J.C., Louw, P.J.E. & Agenbag, G.A., (2001). Effect of seeding date on the performance of grasses and broadleaf species evaluated for cover crop management in two wine grape regions of South Africa. South African Journal of Plant and Soil, 18, 118–127.

Fourie, J.C., Louw, P.J.E., Agenbag, G.A. (2005). Cover crop management in a Sauvignon blanc/Ramsey vineyard in the semi-arid Olifants River Valley, South Africa. 1. Effect of management practices on selected grass and broadleaf species. South African Journal of Enology and Viticulture, 26, 131–139.

Fourie, J.C., Louw, P.J.E., Agenbag, G.A., (2006). Cover crop management in a Chardonnay/99 Richter vineyard in the Coastal Region, South Africa. 1. Effect of

two management practices on selected grass and broadleaf species. South African Journal of Enology and Viticulture, 27, 167–177.

Furlan, L. et al. (2010). The efficacy of biofumigant meals and plants to control wireworm populations. Industrial crops and products, 31(2), 245–254.

Gamliel, A., Austerweil, M., Kritzman, G. (2000). Non-chemical approach to soilborne pest management – organic amendments. Crop Protection, 19.

Gamliel, A., Bruggen, A.H.C. (2016). Maintaining soil health for crop production in organic greenhouses. Scientia Horticulturae, 208, 120–130.

Gamliel, A., Katan, J. (2009). Control of plant disease through soil solarization. In: WALTERS, D. Disease Control in Crops: Biological and Environmentally Friendly Approaches. Oxford: Wiley-Blackwell, Cap. 10, 196–220.

Geisseler, D., Horwath, W., Scow, K. (2011). Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia, 54, 71–78.

Gies, D. (2004). Commercial use of mustards for green manure and biofumigation in the United States. Agroindustria, 3, 403–405.

Gimsing, A.L., Kirkegaard, J.A. (2009). Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochemistry Reviews, 8, 1, 299–310.

Global Biofumigation Market by type (mustard seed, cauliflower seed), by application (fruits, vegetables), by geographic coverage and forecast. URL: https://www.verifiedmarketreports.com/download-sample/?rid=776826 (date of application 03.09.2024).

Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. (2020). Phytochemistry Reviews 8, 299–310.

Godfery-Sam-Aggrey, W., Tekie, Y. (1990). Principles of Horticultural Crop Production: Vegetable Crops. Horticultural Training Manual. Agritex Training Branch, Harare, 327 pp.

Gouws, R., Mienie, N. (2000). Biofumigation of Common Scab of Potatoes in the Republic of South Africa. Agricultural Research Council-Roodeplaat, Private Bag X293, Pretoria, 0001, South Africa, 109 pp.

Gouws, R., Wehner, F.C. (2004). Biofumigation as alternative control measure for common scab on seed potatoes in South Africa. Agroindustria, 3, 309–312.

Gouws, R. (2004). Biofumigation: What is it, and what benefits can it add to existing farming systems? An international overview. CHIPS, 43–44.

GPC/RAS/191/AUL. Food and Agricultural Organization of the United Nations, 34 pp.

Gurel, F.B. et al. (2018). Biofumigation: Opportunities and challenges for control of soilborne diseases in nursery production. Plant Health Progress, 19, 4, 332–337.

Hamidi, N., Hajihassani, A. (2020). Differences in parasitism of root-knot nematodes (Meloidogyne spp.) on oilseed radish and oat. Journal of Nematology, 52, 1–10.

Hanschen, F.S., Winkelmann, T. (2020). Biofumigation for fighting replant disease – A Review. Agronomy, 10, 3, 425.

Hansen, Z. (2011) Potential of three brassica cover crops for biofumigation in the field and the relationship between soil myrosinase and biofumigation efficacy. All Theses. 1248. https://tigerprints.clemson.edu/all_theses/1248

Haroutunian, G. (2013). The use of biofumigation crops as an alternative to Methyl Bromide for the management of the root-knot nematode in greenhouse cucumber production. Agronomy. AgroParis-Tech, English. NNT: 2013AGPT0025.

Hemayati, S.S., Akbar, M.R.J.E., Ghaemi, A.R., Fasahat, P. (2017). Efficiency of white mustard and oilseed radish trap plants against sugar beet cyst nematode. Applied Soil Ecology, 119, 192–196.

Henderson, D.R., Riga, E., Ramirez, R.A., Wilson, J., Snyder, W.E.: (2009). Mustard biofumigation disrupts biological control by Steinernema spp. Nematodes in the soil. Biological Control, 48(3), 316–322.

Hopkins, R.J., Ekbom, B., Henkow, L. (1998). Glucosinolate content and susceptibility for insect attack of three populations of sinapis alba. Journal of Chemical Ecology, 24(7), 1203–1216.

Ibekwe, A.M. (2004). Effects of fumigants on non-target organisms in soils. Advances in Agronomy, 83, 1–35.

Iriarte, L.E. (2011). Efecto de la biofumigación con repollo sobre el control de Fusarium oxysporum en suelo. RIA. Revista de Investigaciones Agropecuarias, 37, 3, 231–237.

Jaffee, B.A., Ferris, H., Scow, K.M. (1998). Nematode-trapping fungi in organic and conventional cropping systems. Phytopathology, 88, 344–350.

Jin, X. et al. (2019). Rotations with Indian mustard and wild rocket suppressed cucumber Fusarium wilt disease and changed rhizosphere bacterial communities. Microorganisms, 7(2), 57.

Karavina, C., Mandumbu, R. (2012). Biofumigation for crop protection: potential for adoption in Zimbabwe. Journal of Animal & Plant Sciences, 14(3), 1996–2005.

Khan, M.J., Monke, E.J., Foster, G.R. (1986). Mulch cover and canopy effects on soil loss. Pap. ASAE no 86-253. Department of Agricultural Engineering, Purdue University, West Lafayette, IN, 97 pp.

Kim, J.K., Chu, S.M., Kim, S.J., Lee, D.J., Lee, S.Y., Lim, S.H., Ha, S., Kweon, S.J., Cho, H.S. (2010). Variation of glucosinolates in vegetable crops of Brassica rapa L. ssp. pekinensis. Food Chemistry, 119(1), 423–428.

Kirkegaard, J.A., Gardner, P.A., Desmarchelier, J.M., Angus, J.F. (1993). Biofumigation - using Brassica species to control pests and diseases in horticulture and agriculture. In 9th Australian Research Assembly on Brassicas Eds. N Wratten and R J Mailer. pp. 77–82. Agricultural Research Institute, Wagga Wagga.

Kirkegaard, J.A., Sarwar, M. (1998). Biofumigation potential of brassicas I. Variation in glucosinolate profiles of diverse field-grown brassicas. Plant and Soil, 201, 71–89.

Kirkegaard, J A, Wong, P.T.W., Desmarchelier, J.M. (1996). In vitro suppression of fungal root pathogens of cereals by Brassica tissues. Plant Pathology, 45, 593–603.

Kirkegaard, J. (2009). Biofumigation for plant disease control – from the fundamentals to the farming system. In: Disease Control in Crops. Wiley-Blackwell, 172–195.

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

Kirkegaard, J. (2009). Biofumigation for plant disease control – from the fundamentals to the farming system. IN: Walters D. (ed.) Disease control in crops: Biological and environmentally friendly approaches. John Wiley & Sons Ltd, Chichester, UK. pp 172–195.

Kirkegaard, J.A., Gardner, PA, Desmarchelier, J.M., Angus, J.F. (1993). Biofumigation- using Brassica species to control pests and diseases in horticulture and agriculture. In: Wratten, N., Mailer, R. (Eds.), Proceedings of the 9th Australian Research Assembly on Brassicas. NSW Agriculture, pp. 77-82.

Kirkegaard, J. (2009). Biofumigation for plant disease control – from the fundamentals to the farming system. In: Walters, D. Disease Control in Crops: Biological and Environmentally Friendly Approaches. Oxford: Wiley-Blackwell, Cap. 9, 172–195.

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

Kirkegaard, J.A., Sarwar, M., Matthiessen, J.N. (1998). Assessing the biofumigation potential of crucifers. Acta Horticulturae, 459, 105–111.

Kleynhans, K.P.N., Van den Berg, E., Swart, A., Marais, M., Bucley, N.H. (1996). Plant nematodes in South Africa. Handbook no. 8. Pretoria, ARC-Plant Protection Research Institute, 154 pp.

Klose, S., Ajwa, H.A., Brwone, G.T., Subbarao, K.V., Martin, F.N., Fennimore, S.A., Westerdahl, B.N. (2008). Dose response of weed seeds, plant-parasitic nematodes, and pathogens to twelve rates of metam sodium in a California soil. Plant Disease, 92, 1537–1546.

Krasnow, C.S.; Hausbeck, M.K. (2015). Pathogenicity of Phytophthora capsici to Brassica vegetable crops and biofumigation cover crops (Brassica spp.). Plant disease, 99(12), 1721–1726.

Kruger, D.H.M., Fourie, J.C., Malan, A.P. (2013). Cover Crops with Biofumigation Properties for the Suppression of Plant-Parasitic Nematodes: A Review. South African Journal of Enology and Viticulture, 34(2), 287–295.

Kumar, P. (2005). Biofumigation. Concept Note and Compilation of Session Guides on Biofumigation. Inter-country, 107 pp.

Kumar, G. N. K. et al. (2018). Disease management by Biofumigation in organic farming system. Journal of Pharmacognosy and Phytochemistry, 7(4), 676–679.

Ladhalakshmi, D. et al. (2015). Biofumigation in crop disease management. In: Ganesan, S. et al. Sustainable Crop Disease Management using Natural Products. Boston: CAB International, Cap.19, 389–402.

Lamberti, F. (1988). Nematode parasites of grapevine and their control. In: Proc. Int. Symp. on Plant-Protection Problems and Prospects of Integrated Control in Viticulture. Report EUR 111548 Commission of the European Communities,

International Organisation for Biological and Integrated Control, Zurich. | P. 163–172.

Larkin, R., Griffin, T. (2007). Control of soilborne potato diseases using Brassica green manure. Crop Protection, 26, 1067–1077.

Larregla, S. et al. (2015). Biodisinfestation with organic amendments for soil fatigue and soil-Borne pathogens control in protected pepper crops. In: Meghvansi M.; Varma A. Organic Amendments and Soil Suppressiveness in Plant Disease Management. Cham: Springer, Cap.21, 437–456.

Laznik, Ž., Trdan, S., Vučajnk, F., Bohinc, T., Vidrih, M. (2014). Cruciferous plants' use as biofumigants in potato against wireworms. Acta Agriculturae Scandinavica, Section B – Soil & Plant Science, 64(7), 606–614.

Lazzeri, L, Leoni, O, Manici, LM. (2004). Biocidal plant dried pellets for biofumigation. Industrial Crops and Products, 20, 5965.

Lazzeri, L. et al. (2011). Bio-based products from Brassica carinata A. Braun oils and defatted meals by a second generation biorefinery approach. Proc. 19th European Biomass Conference. Berlin, Germany, 6-10, 1080–1092.

Lazzeri, L. et al. (2013). The Brassicaceae biofumigation system for plant cultivation and defence. An Italian twenty-year experience of study and application. Acta Horticulturae, 1005, 375–382.

Lazzeri, L., Leoni, O., Manici, L.M. (2004). Biocidal plant dried pellets for biofumigation. Industrial Crops and Products. 20, 59–65.

Lazzeri, L., Tacconi, R., Palmieri, S. (1993). In vitro activity of some glucosinolates and their reaction products toward a population of nematode *Heterodera schachtii*. Journal of Agricultural and Food Chemistry, 41, 825–829.

Le Roux, F. (2012). Kanola juis puik wisselbougewas. Landbouweekblad, 1764, 27 Julie, 4.

Lefebvre, M. (2018). Seed dormancy and seed morphology related to weed susceptibility to biofumigation. Weed Science, 66, 2, 199–214.

Leite, I.C.H.; Lopes, U.P. (2018). Controle químico de patógenos radiculares. In: LOPES, U. P.; Michereff, S. J. (eds). Desafios do Manejo de Doenças Radiculares Causadas por Fungos. Recife: Edufrpe, Cap. 11, 179–192.

Lembright, H.W. (1990). Soil fumigation: Principles and application technology. Journal of Nematology, 22, 632–644.

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.

Likhochvor, V.V. Fertilizer alternative. Zerno, 3, 5–10.

Lobo Júnior, M.L. et al. (2018). Panorama da pesquisa com patógenos radiculares no Brasil. In: LOPES, U. P.; Michereff, S. J. (eds). Desafios do Manejo de Doenças Radiculares Causadas por Fungos. Recife: Edufrpe, Cap.2, 17–34.

Lord, J., Lazzeri, L., Atkinson, H., Urwin, P. (2011). Biofumigation for control of potato cyst nematodes: Activity of brassica leaf extracts and green manures on Globodera pallida in vitro and in soil. Journal of Agricultural and Food Chemistry, 59: 7882–7890.

Lord, J. S., et al. (2011). Biofumigation for control of pale potato cyst nematodes: activity of brassica leaf extracts and green manures on Globodera pallida in vitro and in soil. Journal of Agricultural and Food Chemistry, 59, 7882–7890.

Loubser, J.T., Meyer, A.J. (1986). Strategies for chemical control of root-knot nematodes (Meloidogyne sp.) in established vineyard. South African Journal of Enology and Viticulture, 7, 84–89.

Loubser, J.T., Meyer, A.J. (1987). Population dynamics of the root-knot nematodes Meloidogyne incognita Chitwood and Meloidogyne javanica Chitwood on grapevines in two different regions of South Africa. South African Journal of Enology and Viticulture, 8, 36–40.

Louvet, J. (1979). Aims of soil disinfestation. In: Mulder, D. (ed). Soil disinfestation. Elsevier Scientific, Amsterdam, 1-15.

Louw, P.J.E., Bennie, A.T.P. (1992). Water runoff and soil erosion in vineyard soils. Austr. Grapegrower & Winemaker Ann. Tech. Iss., 100–113.

Lu, P. et al. (2010). Biofumigation with Brassica plants and its effect on the inoculum potential of Fusarium yellows of Brassica crops. European Journal of Plant Pathology, 126, 3, 387–402.

Malan, A.P., Hugo, H.J. (2003). Present status of grapevine nepoviruses and their nematode vectors in South Africa. Wineland, 14, 120-123.

Malan, A.P., Meyer, A.J. (1992). Transmission of grapevine fanleaf virus by a South African population of Xiphinema index. Phytophylactica 24, 217–219.

Malan, A.P., Meyer, A.J. (1993). Interaction between a South African population of Xiphinema index and different grapevine rootstocks. South African Journal of Enology and Viticulture, 14, 11–15.

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, 2768–2773.

Manici, L.M., 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, 2768–2773.

Marschner, P., Rengel, Z. (2010). The effects of plant breeding on soil microbes. In: Dixon, G. R.; Tilston, E. L. Soil Microbiology and Sustainable Crop Production. Dordrecht, Netherlands: Springer, Cap. 8, 297–315.

Matthiessen, J.N., Warton, B., Shackleton, M.A. (2004). The importance of plant maceration and water addition in achieving high Brassica-derived isothiocyanate levels in soil. Agroindustria, 3, 277–280.

Matthiessen, J.N., Kirkegaard, J.A. (1998). Biofumigation for managing soil-borne pests- progress, pitfalls and prospects. Proceedings of the 6th Australasian Applied Entomology Research Conference (Eds Zalucki M, Drew R and White G). Volume 1, 364–372.

Matthiessen, J.N., Kirkegaard, J.A. (2006). Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Critical Reviews in Plant Sciences 25, 235–265.

Matthiessen, J.N., Warton, B., Schackleton, M.A. (2004). The importance of plant maceration and water addition in achieving high brassica-derived isothiocyanate levels in soil. Agroindustria, 3, 277–280.

Mawar, R., Lodha, S. (2015). Suppression of soilborne plant pathogens by cruciferous residues. In: Meghvansi M.; Varma A. (eds). Organic Amendments and Soil Suppressiveness in Plant Disease Management. Cham: Springer, Cap. 20, 413–433.

Mazzola, M., Brown, J., Izzo, A.D., Cohen, M.F. (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.

Mazzola, M. et al. (2017). Incorporation of Brassica seed meal soil amendment and wheat cultivation for control of Macrophomina phaseolina in strawberry. European journal of plant pathology, 149, 1, 57–71.

Mbiro, A. (2016). Host plant status of different green manure plants for Pratylenchus penetrans and Meloidogyne chitwoodi. Ghent university. Faculty of science. department of biology, 34 pp.

McGuire, A.M. (2003). Mustard green manures replace fumigant and improve infiltration in potato cropping system. Crop Management, 22.

McLeod, R.W., Steel, C.C. (1999). Effects of brassica-leaf green manures and crops on the activity and reproduction of Meloidogyne javanica. Nematology. 1, 613–624.

McLeod, R.W., Warren, M. (1993). Effects of cover crops of inter-row nematode infestation in vineyards. 1. Relative increase of root-knot nematode Meloidogyne incognita and M. javanica on legume, cereal and brassica crops. Aust. Grapegrow. Winemak, 357, 28–30.

McLeod, R.W., Gendy, M.Y., Steel, C.C. (1998). Observations on brassicas as cover crops and testing of biofumigation in vineyards. Aust. Grapegrow. Winemak, 414a, 83–86.

McLeod, R.W., Somers, M.Y., Gendy, M. (1995). Cover crops and nematodes – some field observations. Aust. Grapegrow. Winemak, 381, 53–57.

Meghvansi, M. K., Varma, A. (2015). Organic amendments and soil suppressiveness in plant disease management. Dordrecht: Springer, 347 pp.

Melakeberhan, H., Xu, A., Kravchenko, A., Mennan, S., Riga, E. (2006). Potential use of aragula (Eruca sativa) as a trap crop for Meloidogyne hapla. Nematology. 8, 793–799.

Melakeberhan, H, Mennan, S, Ngouajio, M, Dudek, T. (2008). Efect of Meloidogyne hapla on multi-purpose use of oilseed radish (Raphanus sativus). Nematology, 10, 375–379.

Menna, S., Melakeberhan, I.I. (2006). Comparative efficacy of oil seed radish and tomato root exudates on hatching of Meloidogyne hapla. Pakistan Journal of Biological Sciences, 6,485–489.

Michel, V.V. (2014). Ten years of biofumigation research in Switzerland. Aspects of Applied Biology, 126, 33–42.

Michel, V., de Cara García, M. (2020). Biofumigation: practical information, advantages and disadvantages. Best4Soil has received funding from the European

Union's Horizon 2020 Programme as Coordination and Support Action, under GA n 817696. URL: https://ec.europa.eu/eip/agriculture/sites/agri-eip/files/9_eip_sbd_mp_biofumigation_final_0.pdf (date of application 03.09.2024).

Michel, V.V. (2014). Ten years of biofumigation research in Switzerland. Aspects of Applied Biology, 126, 33–42.

Mithen, R (1992). Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica, 63, 71–83.

Mithen, R., Campos, H. (1996). Genetic variation of aliphatic glu- cosinolates in Arabidopsis thaliana and prospects for map based gene cloning. Entomologia Experimentalis et Applicata, 80, 202–205.

Mithen, R, Raybould, A.F., Giamoustris, A. (1995). Divergent selection for secondary metabolites between wild populations of Brassica oleracea and its implications for plant herbivore interactions. Heredity, 75, 472–484.

Mojtahedi, H., Santo, G.S., Hang, A.N., Wilson, J.H. (1991). Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. Journal of Nematology, 23, 170–174.

Mojtahedi, H., Santo, G.S., Wilson, J.H., Hang, A.N. (1993). Managing Meloidogyne chitwoodi on potato with rapeseed as green manure. Plant Disease, 77, 42–46.

Monfort, W.S., Csinos, A.S., Desaeger, J., Seebold, K., Webster, T.M. & Diaz-Perez, J.C. (2007). Evaluating brassica species as an alternative control measure for root-knot nematode (M. incognita) in Georgia vegetable plasticulture. Crop Protection, 26, 1359–1368.

Morales-Rodríguez, C. et al. (2018). Application of Trichoderma spp. complex and biofumigation to control damping-off of Pinus radiata D. Don caused by Fusarium circinatum Nirenberg and O'Donnell. Forests, 9, 7, 421.

Morales-Rodríguez, C. et al. (2016). Efficacy of biofumigation with Brassica carinata commercial pellets (BioFence) to control vegetative and reproductive structures of Phytophthora cinnamomi. Plant Disease, 100, 2, 324–330.

Morra, M.J., Kirkegaard, J.A. (2002). Isothiocyanate release from soil-in¬corporated Brassica tissues. Soil Biology and Biochemistry. 34, 1683–1690.

Morris, E.K. et al. (2020). Effective methods of biofumigation: a meta analysis. Plant and Soil, 446, 379–392.

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, 2044–2057.

Motisi, N. et al. (2013). Epidemiological analysis of the effects of biofumigation for biological control of root rot in sugar beet. Plant Pathology, 62, 1, 69–78.

Myburgh, P.A. (1998). Water consumption in South African vineyards: A modeling approach based on the quantified combined effects of selected viticultural, soil and meteorological parameters. Dissertation, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa, 117 pp.

Nallanchakravarthula, S., Marupakula, S., Alström, S., Finlay, R.D., Mahmood, S. (2021). Changes in the root fungal microbiome of strawberry

following application of residues of the biofumigant oilseed radish. Applied Soil Ecology, 168.

Neubauer, C., Heitmann, B., Müller, C. (2014). Biofumigation potential of Brassicaceae cultivars to Verticiilium dahliae. European Journal of Plant Pathology, 140, 341–352.

Neves, W.S. et al. (2008). The use of papaya-seed and solarization for the control of Meloidogyne javanica and Meloidogyne incognita. Nematologia Brasileira, 32, 4, 253–259.

Ngala, B. M. et al.(2015). Biofumigation with Brassica juncea, Raphanus sativus and Eruca sativa for the management of field populations of the potato cyst nematode Globodera pallida. Pest Management Science, 71, 5, 759–769.

Nicol, J.M., Stirling, J.R., Rose, B.J., May, P., Van Heeswijck, R. (1999). Impact of nematodes on grapevine growth and productivity: Current knowledge and future directions, with special reference to Australian viticulture. Australian Journal of Grape and Wine Research, 5, 109–127.

Nicola, G.R. et al. (2013). A new biobased liquid formulation with biofumigant and fertilizing properties for drip irrigation distribution. Industrial Crops and Products, 42, 113–118.

Noble, R.R.P., Sams, C.E. (1999). Biofumigation as an alternative to methyl bromide for control of white grub larvae. Department of Plant and Soil Science, The University of Tennessee, P.O. Box 1071, Knoxville, TN 37901.

Ntalli, N., Caboni, P. (2017). A review ofisothiocyanates biofumigation activity on plant parasitic nematodes. Phytochemistry Reviews, 6, 5, 827–834.

Ojaghian, M. R. et al. (2012). In vitro biofumigation of Brassica tissues against potato stem rot caused by Sclerotinia sclerotiorum. The Plant Pathology Journal, 28, 2, 185-190.

Oliveira, R. D. L. et al. (2011). Glucosinolate content and nematicidal activity of Brazilian wild mustard tissues against Meloidogyne incognita in tomato. Plant and Soil, 341, 1-2, 155–164.

Padilla, G., Cartea, M.E., Velasco, P., De Haro, A., Ordas, A. (2007). Variation of glucosinolates in vegetable crops of Brassica rapa. Phytochemestry, 68, 536–545.

Page, S.L.J. (1997). Natural Pest Management in Zimbabwe. LEISA Magazine, 13(4), 12¬13.

Pan, X. et al. (2017). Effect of oriental mustard (Brassica juncea) seed meal for control of dollar spot on creeping bentgrass (Agrostis stolonifera) Turf. International Turfgrass Society Research Journal, 13, 1, 166–174.

Patalano, G. (2004). New practical perspectives for vegetable biocidal molecules in Italian agriculture: Bluformula brand for commercialization of biocidal green manure and meal formulations. Agroindustria, 3, 409–412.

Perniola, O. S et al. (2012). Biofumigación con Brassicáceas: actividad supresora sobre Fusarium graminearum. Revista de la Facultad de Agronomía, 111, 1, 48–53.

Perniola, O. S. et al. (2019). Biofumigación con Brassicajuncea: efecto sobre la flora arvense. Revista de la Facultad de Agronomía, 118, 1, 25–35.

Perniola, O. S. et al. (2016). Biofumigación in vitro con Brassica juncea y Sinapis alba. Inhibición de la germinación y del crecimiento de plántulas de malezas. Revista de la Facultad de Agronomía, 115, 1, 91–98.

Perniola, O. S. et al. Control biológico de Fusarium graminearum: utilización de Trichoderma spp. y biofumigación con parte aérea de Brassica juncea. Revista de la Facultad de Ciencias Agrarias, v. 46, n. 2, pp. 45–56, 2014.

Piedra Buena, A., Garcia-Alvarez, A., Diez-Rojo, M.A., Bello, A. (2006). Use of cover crop residues for the control of Meloidogyne incognita under laboratory conditions. Pest Managment Science, 62, 919–926.

Piedra Buena, A., Garcia-Alvarez, A., Diez-Rojo, M.A., Ros, C., Fernadez, P., Lacasa, A., Bello, A. (2007). Use of pepper crop residues for the control of root-knot nematodes. Bioresource Technology, 98, 2846–2851.

Ploeg, A.T. Stapleton, J.J. (2001). Glasshouse studies on the effect of time, temperature and amendment of soil with broccoli plant residues on the infestation of melon plants by Meloidogyne incognita and M. javanica. Nematology, 3, 855–861.

Ploeg, A.T. (2007). Biofumigation to manage plant-parasitic nematodes. In: Ciancio, A. & Mukerji, K.G. (eds). Integrated management and biocontrol of vegetable and grain crops nematodes. Springer-Verlag, Berlin, 239–248.

Pontes, N. C. et al. (2019). Soil fumigation with mustard essential oil to control bacterial wilt in tomato. European Journal of Plant Pathology, 155, 435–444.

Potter, M., Vanstone, V., Davie, K., Rathjen, A. (2000). Breeding to increase the concentration of 2-phenylethyl glucosinolate in the roots of Brassica napus. Journal of Chemical Ecology, 26, 1811–1820.

Potter, M.J., Davies, K., Rathjen, A.J. (1998). Supressive impact of glucosinolates in Brassica vegetative tissues on root lesion nematode, *Pratylenchus neglectus*. Journal of Chemical Ecology, 24, 67–80.

Poulton, J.E., Moller, B.L. (1993). Glucosinolates. In: Lea, P.J. (ed). Methods in plant biochemistry, vol. 9. PJ Academic Press, London., 209–237.

Prasad, P. et al. (2015). Biofumigation: Success and Prospects in Soilborne Plant Disease Management. International Journal of Applied And Pure Science and Agriculture, 1, 6, 47–59.

Prasad, P. et al. (2016). Investigating disease controlling ability of Brassica volatiles and their compatibility with Trichoderma harzianum. The National Academy of Sciences, 97 pp.

Rahman, L., Somers, T. (2005). Suppression of root knot nematode (Meloidogyne javanica) after incorporation of Indian mustard cv. Nemfix as green manure and seed meal in vineyards. Australasian Plant Pathology, 34, 77–83.

Rahman, L., Somers, T., Creecy, H. (2000). Distribution of nematodes in vineyards and relationship of root knot nematode (Meloidogyne spp.) to vine growth and yield. Aust. Grapegrow. Winemak, 438a, 53–57.

Rahman, L., Weckert, M.,Orchard, B. (2009). Effect of three consecutive annual applications of brassica green manures on root-knot nematode suppression in soil. Austr. N.Z. Grapegrow. Winemak, 37, 10–12.

Rahman, L., Whitlaw-Weckert, M.A., Orchards, B. (2011). Consecutive applications of brassica green manures and seed meal enhances suppression. Journal of Chemical Ecology, 20, 27–40.

Rahman, M. et al. (2018). Brassicaceae mustards: Traditional and agronomic uses in Australia and New Zealand. Molecules, 23, 1.

Raja, N., Albert, S., Ignacimuthu, S., Dorn, S. (2001). Effect of plant volatile oils in protecting stored cowpea Vigna unguiculata (L.) Walpers against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) infestation. Journal of Stored Products Research, 37, 127–132.

Ramirez, R.A., Henderson, D.R., Riga, E., Lacey, L.A., Snyder, W.E. (2009). Harmful effects of mustard green manure on entomopathogenic nematodes. Biological Control, 48(2), 147–154.

Raski, D.J. (1986). Grapes. In: Union Carbide Agricultural Products Company Inc. (ed). Plant parasitic nematodes of bananas, citrus, coffee, grapes and tobacco. Union Carbide Agricultural Products Company Inc., Research Triangle Park, NC, 70 pp.

Relevante, C.A.; Cumagun, C.J.R. (2013). Control of Fusarium wilt in bittergourd and bottlegourd by biofumigation using mustard var. Monteverde. Archives of Phytopathology and Plant Protection, 46, 6, 747–753.

Republic of South Africa, Department of Agriculture, Forestry and Fisheries (2010). Canola: Production guideline. URL: http://www.nda.agric.za/docs/Brochures/prodGuideCanola.pdf (date of application 03.09.2024).

Ricardo, L.L., Bernardi, D.I., Mantovanelli, G.C., Moreno, B.P., Mito, M.S., Silva, A.A., Oliveira, J.R.R.S., Ishii-Iwamoto, E.L., Sarragiotto, M.H., Baldoqui, D.C. (2018). Hytochemical investigation and phytotoxic activity of aerial parts of oilseed radish (Raphanus sativus var. oleifer Stokes). Biochemical Systematics and Ecology, 78, 52–58.

Riga, E., Collins, H. (2004). Green manure effects on Meloidogyne chitwoodi and Paratrichodorus allius, economically important nematodes in the Pacific Northwest of the USA. Agroindustria, 3, 321–322.

Riga, E., Mojtahedi, H., Ingram, R., McGuire, A.M. (2004). (2nd ed). Green manure amendments and management of root knot nematodes on potato in the Pacific Northwest of USA. In: Cook, R.C. & Hunt, D.J. (eds). Nematology monographs and perspectives. Proc. 4th Cong. of Nematol,. 151–158.

Rodman, J.E. (1981). Divergence, convergence and parallelism in phytochemical characters: the glucosinolate-myrosinase system. In: Young, D.A. & Seigler, D.S. (eds). Phytochemistry and angiosperm phylogeny. Praeger, New York, 43–79.

Roncato, S. C. et al. (2018). Control of Meloidogyne incognita in tomato by crambe extract using different application forms. Summa Phytopathologica, 44, 3, 261–266.

Ros, C. et al. (2016). El cultivo de brásicas para biosolarización reduce las poblaciones de Meloidogyne incognita en los invernaderos de pimiento del Sudeste de España. Revista de la Asociación Interprofesional para el Desarrollo Agrario (AIDA), 112, 2, 109–126.

Ros, M. et al. (2008). Effects of biosolarization as methyl bromide alternative for Meloidogyne incognita control on quality of soil under pepper. Biology and Fertility of Soils, 45, 37–44.

Rosa, E.A.S., Heaney, R.K., Fenwick, G.R. (1997). Glucosinolates in crop plants. Horticultural Review, 19, 99–215.

Rosa, E.A.S. et al. (1997). GSLs in crop plants. Horticultural Reviews, 19, 99–215.

Rosskopf, E. N. et al. (2015). Anaerobic Soil Disinfestation and Soilborne Pest Management In: Meghvansi M.; Varma A. (eds). Organic Amendments and Soil Suppressiveness in Plant Disease Management. Cham: Springer, Cap. 13, 277–306.

Roth, C.H., Meyer, B., Frede, G., Derpsch, R. (1988). Effect of mulch rates and tillage systems on infiltrability and other soil physical properties of an oxisol in Parana, Brazil. Soil Tillage Research, 11, 81–91.

Rubayet, M.T. et al. (2018). Effect of biofumigation and soil solarization on stem canker and black scurf diseases of potato (Solanum tuberosum L.) caused by Rhizoctonia solani isolate PR2. Advances in Agricultural Science, 6, 3, 33–48.

Ríos, P. et al. (2017). Brassica-based seedmeal biofumigation to control Phytophthora cinnamomi in the Spanish «dehesa» oak trees. Phytopathologia Mediterranea, 56, 3, 392–399.

Santos, C.A., Abboud, A.C.S., Carmo, M.G.F. (2021). Biofumigation with species of the Brassicaceae family: a review. Ciencia Rural, 51(1), 1–17.

Sardans, J., Peñuelas, J., Ogaya, R. (2005). Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. Forest Soil Biology and Biochemistry, 37, 455–461.

Sarwar, M., Kirkegaard, J.A. (1998). Biofumigation potential of brassicas. II. Effect of environment and ontogeny on glucosino- late production and implications for screening. Plant Soil 201, 91–101.

Sarwar, M, Kirkegaard, J.A., Wong, P.T.W., Desmarchelier, J.M. (1998). Biofumigation potential of brassicas. III. ln vitro toxicity of isothiocyanates to soil-borne fungal pathogens. Plant Soil, 201, 103–112.

Schiavon, M., Malagoli, M. (2008). Role of sulphate and S-rich compounds in heavy metal tolerance and accumulation. In: Sulfur Assimilation and Abiotic Stress in Plants; Khan NA, Singh S, Umar S, Eds; Springer-verlag: Berlin, Germany, 253–269.

Schwelm, A. et al. (2015). The Plasmodiophora brassicae genome reveals insights in its life cycle and ancestry of chitin synthases. Scientific Reports, 5, 1–12.

Series. USA: American Chemical Society. De Nicola, G.R., 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, 113–118.

Serrano-Pérez, P. et al. (2017). Efficacy of Brassica carinata pellets to inhibit mycelial growth and chlamydospores germination of Phytophthora nicotianae at different temperature regimes. Scientia horticulturae, 216, 126–133.

Shaaya, E., Kostyukovsky, M. (2009). The Potential of Biofumigants as Alternatives to Methyl Bromide for the Control of Pest Infestation in Grain and

Dry Food Products. In: A Kirakosyan, P.B Kaufman. Recent Advances in Plant Biotechnology. P 389–403. ©Springer Science + Business Media, LLC.

Shaaya, E., Kostyukovsky, M. (2010). The potential of biofumigants as alternatives to methyl bromide for the control of pest infestation in grain and dry food products. In: 10th International Working Conference on Stored Product Protection. Lisbon: Portugal, 433–437.

Silva, M.F. et al. (2020). Medicinal plant volatiles applied against the root-knot nematode Meloidogyne incognita. Crop Protection, 130.

Smith, B.J., Kirkegaard, J. (2002). In vitro inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. Plant Pathology, 51, 585–593.

Smoliñska, U., Kowalczyk, W. (2014). The impact of the Brassicaceae plant materials added to the soil on the population of Fusarium solani (Mart.) Sacc. and Fusarium oxysporum Schlecht. Journal of Horticultural Research, 22, 1, 123–129.

Stirling, G.R., Stanton, J.M., Marchall, J. (1992). The importance of plant-parasitic nematodes to Australian and New Zealand agriculture. Australasian Plant Pathology, 24, 104–115.

Storey, S. (2007). Nematodes in vines: Practical guidelines for the short- and long-term control of nematodes. Wineland, 60–63.

Szczyglowska, M., Piekarska, A., Konieczka, P., Namiesnik, J. (2011). Use of Brassica Plants in the Phytoremediation and Biofumigation Processes. International Journal of Molecular Sciences, 12, 7760¬7771.

Thierfelder, A., Friedt, W. (1995). Development of novel rapeseed varieties (Brassica napus) resistant against beet cyst nematodes (Heterodera schachtii). In: Organising Committee of the Ninth International Rapeseed Conference Cambridge (ed). Ninth Int. Rapeseed Cong. (GCIRC), Cambridge, UK, 1208–1210.

Tsror, L. et al. (2007). Biofumigation for the control of soilborne diseases. Acta horticulturae, 747, 389–394.

Ugrenovic, V. et al. (2019). Effect of Brassicaceae as cover crops. Selekcija I Semenarstvo, 25, 2, 1–8.

Van Berkum, J.A., Hoestra, H. (1979). Practical aspects of chemical control of nematodes in soil. In: Mulder, D. (ed). Soil disinfestation. Elsevier Scientific, Amsterdam, 53–120.

Van Dam, N., Tytgat, T.G., Kirkegaard, J. (2009). Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. Phytochemistry Reviews, 8, 171–186.

Van Zyl, S., Vivier, M.A., Walker, M.A. (2012). Xiphinema index and its relationship to grapevine: A review. South African Journal of Enology and Viticulture, 33, 21–32.

Vaughn, S.F., Spencer, G.F., Loria, R. (1993). Inhibition of Helminthosporium so/ani by natural isothiocyanates. American Potato Journal, 70, 852–853.

Viaene, N.M., Abawi, G.S. (1998). Management of Meloidogyne hapla on lettuce in organic soil. Plant Disease, 82, 945–952.

Vig, AP, Rampal, G, Thind, T.S., Arora, S. (2009). Bio-protective effects of glucosinolates – A review. LWT – Food Science and Technology, 42, 1561–1572.

Villalobos, J.A.M. et al. (2019). Producción de chile (Capsicum annuum L.) a campo abierto con biofumigación del suelo. Durango: INIFAP, 2013. URL: http://biblioteca. inifap.gob.mx:8080/jspui/handle/123456789/4097. (date of application 03.09.2024).

Wang, K. H et al. (2006). Effects of cover cropping, solarization, and soil fumigation on nematode communities. Plant and Soil, 286, 1–2, .229–243.

Wang, Q. et al. (2014a). Effect of biofumigation and chemical fumigation on soil microbial community structure and control of pepper Phytophthora blight. World Journal of Microbiology and Biotechnology, 30, 2, 507–518.

Wang, Q. et al. (2014b). Integration of biofumigation with antagonistic microorganism can control Phytophthora blight of pepper plants by regulating soil bacterial community structure. European Journal of Soil Biology, 61, 58–67.

Warmington, R., Clarkson, J.P. (2016). Volatiles from biofumigant plants have a direct effect on carpogenic germination of sclerotia and mycelial growth of Sclerotinia sclerotiorum. Plant and Soil, 401, 1–2, 213–229.

Wathelet, J., Lori, R., Leoni, O., Rollin, P., Quinsac, A., Palmieri, S. (2004). Guidelines for glucosinolate analysis in green tissues used for biofumigation. Agroindustria, 3, 257–266.

Weaver, M., Zablotowicz, R., Krutz, L., Bryson, C., Locke, M. (2012). Microbial and Vegetative Changes Associated with Development of a Constructed Wetland. Ecological Indicators, 13, 37–45.

Wei, F. et al. (2016). Effects of individual and combined use of bio- fumigation-derived products on the viability of Verticillium dahliae microsclerotia in soil. Crop Protection, 79, 170–176.

Wieczorek, R., Zydlik, Z., Wolna-Maruwka, A., Niewiadomska, A., Kayzer, D. (2023). The Effect of Biofumigation on the Microbiome Composition in Replanted Soil in a Fruit Tree Nursery. Agronomy, 13(10), 2507.

Wieczorek, R., Zydlik, Z., Zydlik, P. (2024). Biofumigation Treatment Using Tagetes patula, Sinapis alba and Raphanus sativus Changes the Biological Properties of Replanted Soil in a Fruit Tree Nursery. Agriculture, 14, 1023.

Wratten, N., Mailer, R.J., 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, 349–358.

Yuan, B., Yue, D. (2012). Soil Microbial and Enzymatic Activities Across a Chronosequence of Chinese Pine Plantation Development On The Loess Plateau of China. Pedosphere, 22, 1–12.

Zasada, I.A., Ferris, H. (2004). Nematode suppression with brassicaceous amendments. Soil Biol. Biochem. 36, 1017–1024.

Zeist, A.R. et al. (2019). Combination of solarization, biofumigation and grafting techniques for the management of bacterial wilt in tomato. Horticultura Brasileira, 37, 260-265.

Zhan, J., Sun, Q. (2014). Development of microbial properties and enzyme activities in copper mine wasteland during natural restoration. Catena, 116, 86–94.

Zydlik, Z.; Zydlik, P., Wieczorek, R. (2021). The Effects of Bioinoculants Based on Mycorrhizal and Trichoderma spp. Fungi in an Apple Tree Nursery under Replantation Conditions. Agronomy, 11, 2355.

Musa, N.B. (2021). Management of the stem and bulb nematode (Ditylenchus spp.) in winter beans (*Vicia faba* L) using biofumigant Brassica spp. and other allelopathic cover crops. Harper Adams University, 204 pp.

Park, W., Kwang-Soo, K., Young-Seok, J., Lee, K., Sun-Ju, K., Sung-Ju, A., Hong, S.W. and Yong-Hwa, L. 2017. Variation in glucosinolate contents of cruciferous plants. Records of Natural Products, 11 (2), pp. 185.

Ngala, B.M., Woods, S.R., Back, M.A. (2015). In vitro assessment of the effects of Brassica juncea and Raphanus sativus leaf and root extracts on the viability of Globodera pallida encysted eggs. Nematology, 17(5), 543–556.

Zasada, I.A., Ferris, H. (2004). Nematode suppression with brassicaceous amendments: Application based upon glucosinolate profiles. Soil Biology and Biochemistry, 36(7), 1017-1024.