Published by Avanti Publishers

Global Journal of Agricultural Innovation, Research & Development

ISSN (online): 2409-9813

Biofumigation: A Cover Crop Option 12 Months of the Year to Manage Three Soilborne Pathogens Ailing the Australian Vegetable Industry

J.D. Duff* and M.C. Firrell

Gatton Research Facility, Department of Agriculture and Fisheries, Queensland, Australia

ARTICLE INFO

Article Type: Research Article

Keywords:
Biofumigation
Cover cropping
Sclerotium rolfsii
Sclerotinia sclerotiorum
Macrophomina phaseolina

Timeline:
Received: September 10, 2021
Accepted: October 13, 2021
Published: November 15, 2021

Citation: Duff JD, Firrell MC. Biofumigation: A Cover Crop Option 12 Months of the Year to Manage Three Soilborne Pathogens Ailing the Australian Vegetable Industry. Glob J Agric Innov Res Dev. 2021; 8: 104-116.

DOI: https://doi.org/10.15377/2409-9813.2021.08.8

ABSTRACT

Brassica biofumigant cover crops are being increasingly considered in vegetable crop rotations as part of an integrated disease management strategy and simply as a cover cropping choice. Nine biofumigant varieties were assessed to see if they could be grown year-round in the Lockyer Valley South East Queensland region, for yield, days to incorporation and glucosinolate concentrations, as well as efficacy against 3 soilborne pathogens; Sclerotinia sclerotiorum, Sclerotium rolfsii and Macrophomina phaseolina. The fastest growing brassica biofumigant was BQ Mulch which reached 25% flowering in 36 and 59 days from planting to incorporation with a summer and winter planting respectively. Nemcon and Nemclear took the longest to incorporation when planted in summer, 101 days and failed to flower, while Caliente, Tillage Radish and Biofum reached 25% flowering and incorporation in 98 days when planted in winter. BQ Mulch produced the least amount of biomass, 30.93 t/ha fresh weight and 2.92 t/ha dry weight with a summer planting. Biofum producing the greatest amount of biomass, 185.76 t/ha fresh weight and 17.34 t/ha dry weight with a summer and winter planting respectively. Most varieties produced more total glucosinolates during summer compared to winter. Caliente produced the highest levels of Total GSLs with 53.47 µmol/g DW in summer compared to 23.78 µmol/g DW in winter. This was reflected in their efficacy against the soilborne pathogens. Caliente and Mustclean were more efficacious at controlling Macrophomina and Sclerotinia in summer compared to winter while all varieties were more efficacious at controlling Sclerotinia with a summer planting compared to a winter planting.

*Corresponding Author
Email: john.duff@daf.qld.gov.au
Tel: +61418726597

1. Introduction

Cover crops or green manure crops are best described as crops planted as a break between commercial cropping. They are generally grown to fit into a crop rotation program and may only be for a small period of time. They are grown and incorporated back into the soil rather than for products that are removed off-farm, as is with a commercial crop. Cover crops provide numerous farming system benefits including: increasing soil organic matter, increased stability of nitrogen supply, fixing extra nitrogen in the soil (legumes), increasing soil microbial activity, improving soil structure such as improved water infiltration and soil porosity, scavenging nutrients that have leached beyond the root zone of commercial crops, preventing soil loss via water and wind erosion, and of course as biofumigants to combat soilborne pathogens. There are however some disadvantages when growing a cover crop, such as increased weeds, pest and disease pressures, and loss of income from not growing a cash crop [1].

Some soilborne diseases can survive for many years, even in the absence of a suitable host. The resting stages of certain soilborne pathogens can remain dormant until conditions are favourable, resulting in the development of symptoms on the plant. The soilborne pathogen *Sclerotium rolfsii* can survive for 3-4 years in the soil [2], with variable sclerotial survival from region to region dependent on both environmental and biological factors [3]. Sclerotes of *Sclerotinia sclerotiorum* generally survive in the soil for 3-8 years with the biological component of soils having a major impact on the survival of the sclerotia in the soil [4]. *Macrophomina phaseolina* has been shown to survive in the soil for between 2 and 15 years [5, 6]. 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, which offers growers a solution that does not involve the use of synthetic pesticides for pest and disease control.

With fewer and fewer fungicides available to vegetable growers for the control of soilborne pathogens, the need to find alternative control options is even more important. Improving soil health is one such control option and is based on supporting microbial communities to help fight micro-organisms that are deleterious to plant health [7], introducing composts and beneficial micro-organisms to the soil to protect and defend against soilborne pathogens [8], and since the 1980's the use of natural fumigation of the soil with plant-based volatile compounds targeting known plant pathogens (fungi, bacterial and nematodes), a term referred to as biofumigation [9].

Brassica biofumigants are a unique type of cover crop that produce compounds, glucosinolates (GSLs), which help in the suppression of certain soilborne pathogens, pests and weeds [10] that impact on Australian vegetable crops. In addition to the normal benefits of cover cropping, brassica biofumigants offer an alternative to synthetic fumigants for soilborne disease management if they can be successfully incorporated into a vegetable crop rotation program.

While brassicas biofumigants are considered to be a winter cropping option, this work covered multiple growing seasons to see how different growing conditions affected the performance of these biofumigant cover crops (Duff *et al*., in preparation), and whether they could be recommended when growers had a suitable window of opportunity to grow such a crop. This research will broaden the options for when biofumigants cover crops can be successfully incorporated into a crop rotation program. Growers generally only have a limited period when they can grow such crops and so as part of this research, 3 months was chosen as the cut off period in relation to the flowering of brassica biofumigants. Anything longer and most growers would not consider them if they were meant for managing soilborne pathogens, as flowering is when the peak concentration of GSLs are available.

The concentration and type of GSLs varies between varieties [11-13], as does the type of ITCs produced when GSLs and hydrolysed, which determine the biofumigants' toxicity to various soilborne pathogens and pests [14-16]. This research will looked at the types and concentrations of GSLs between varieties and between seasons and correlate this with the efficacy against the 3 soilborne pathogens.

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.

In undertaking this work, the Queensland Department of Agriculture and Fisheries aims to provide more detailed information on how effective biofumigant varieties are against the 3 soilborne pathogens mentioned above, so that growers may select particular varieties based on their disease spectrum and against the time of year that the grower is able to grow them.

2. Materials and Methods

2.1. Variety Selection

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 **1**). Varieties were assessed for plant biomass, days to incorporation, glucosinolate content, and efficacy against three soilborne pathogens *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Macrophomina phaseolina*.

Table 1: Biofumigant varieties trialled from November 2016 to September 2017 at the Gatton research Facility, Queensland Australia.

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

2.2. Field Trials

Six trials were planted every two months at the Gatton Research Facility, Queensland, Australia, from 2016 to 2017:

15th Nov 2016	16th May 2017
23rd Jan 2017	17th July 2017
28th March 2017	20th Sept 2017.

The trial design had plots that were 5x3m for plantings 1-3 and then 5x4.5m for plantings 4-6, each with 3 replications. Basal fertiliser CK 77 (S) 13.3N: 2.2P: 13.5K: 19.6S was applied at 400kg/ha prior to planting and

Ammonium Sulphate 21%N: 24%S at 4 weeks after planting at 120kg/ha. No other fertilisers were applied to the crop. Irrigation was applied when needed, particularly during the early stages of germination and growth and when the ammonium sulphate was applied through a fertigator.

2.3. Pests and Diseases

Each variety was monitored for pests and diseases during the year to determine how susceptible they were to insect pests and plant pathogens commonly found in other brassica crops grown in the area. If required, a pesticide spray was applied to reduce the impact on the biofumigant cover crops enabling the collection of other data such as days to incorporation, biomass, glucosinolates and efficacy at incorporation.

2.4. Biomass Measurements

Two 50cm square quadrats of plant material, tops and roots, were collected from each plot, averaged and converted to biomass per hectare. The plant material was then placed in large paper bags, marked accordingly with plant variety, replication and date, with fresh weight taken which was also written on the bag. Care was taken to minimise the cutting of the stems and roots to prevent any glucosinolates (GSLs) from being converted to isothiocyanates (ITCs). The roots were washed to remove soil, especially the dark clay soils of the research station. The bags of plant material were placed into a large air forced drier at 70°C for up to 7 days, depending on the amount and thickness of the material being dried. Some of the thick radish roots needed 7 days to dry fully. Once dry, the material was again weighed before grinding. The plant material was ground using a large plant grinding mill with a 2mm sieve attachment. Both tops and roots were ground together and mixed well before being placed in a plastic re-sealable bag, labelled with the variety, planting number, replicate and collection date, along with any other relevant information to the trial. These samples were double bagged to reduce the chance of tears, breakages, leaks and moisture getting to the ground material. They were then placed into a freezer at -18ºC.

2.5. Glucosinolate Extractions

Sample preparation and analysis of the individual (GSLs) were based on the published methods of [17, 18]. Briefly, to about 0.5 g of dried material, 15 mL of distilled deionised boiling water was added and the mixture boiled for 5 minutes. The mixture was cooled and transferred to a 50ml centrifuge tube, made up to 20 mL volume with distilled deionised water prior to using a vortex mixer for 30 seconds. The solution was centrifuged (≈ 5,000rpm, 5 min) before the supernatant was filtered through a PTFE 0.45 µm syringe filter. The filtered solutions were stored at –18°C ready for HPLC analysis.

2.6. Inoculum Preparation

Preparation of the soilborne pathogens used the following methods:

Macrophomina phaseolina, sourced from strawberry crown rot, was cultured on ½PDA+S (Potato Dextrose Agar + Streptomycin). Millet seed (50 grams) was placed into 250mL flasks, covered with distilled water, soaked overnight and drained. After autoclaving twice at 121°C for 30 minutes each, cooling between cycles, the seed was inoculated with four 5mm agar plugs of a 4 day old *M. phaseolina* culture. It was shaken daily for a week then maintained at 20°C for 4-6 weeks or until micro-sclerotia were produced and the seed was covered with the pathogen. The seed was then air-dried and stored in plastic containers at 4°C.

Sclerotium rolfsii was cultured on carrots to produce sclerotia . Moist soil used in this technique was sourced from the Gatton Research Facility and was autoclaves twice for 30 minutes at 121°C. Once autoclaved, the soil was transferred to a 2L plastic container with a lid. A 4 day old culture of *S. rolfsii* was used to inoculate the soil with 6 plugs of the advancing margin of the culture. The agar plugs were equally spaced on the moist soil surface. Once mycelial growth was observed on the soil surface, washed carrots were placed on top of the soil close together and the lid replaced. The pathogen was allowed to mature in a dark place for 4-6 weeks at which time the sclerotia were washed from the soil and carrot residue with suitably sized sieves. The harvested sclerotia were then air-dried and stored in plastic containers at 4°C.

Sclerotinia sclerotiorum sclerotia were produced on autoclaved wheat grains (50 grams) which were placed into 250mL flasks, covered with distilled water, soaked overnight and drained. After autoclaving twice at 121°C for 30 minutes each, cooling between cycles, the seed was inoculated with four 5mm agar plugs of a 4 day old *S. sclerotiorum* culture. It was shaken daily for a week then maintained at 20°C for 4-6 weeks or until sclerotia were produced on the seed. The sclerotia were removed from the seed, air-dried and stored in plastic containers at 4°C. These inoculum preparation techniques were taken and adapted from [3].

Inoculum for field trial assessment was bagged up using small pieces of polyester gauze material, which were folded into small squares held together with a paperclip. Sufficient inoculum was bagged up so that 10 pieces were plated out once recovered 7 days after the incorporation process.

2.7. Efficacy against Soilborne Pathogens

Brassica biofumigants were incorporated at approximately 25% flowering. All varieties were mulched when ready with a flail mulcher and then rotary hoed for maximum incorporation. Individual bags of inoculum of *S. rolfsii, S. sclerotiorum* and *M. phaseolina*, were buried at 10cm depth and retrieved at intervals 7 days after incorporation. Incorporated crops were watered in once the inoculum was buried. These inoculum studies were undertaken with plantings 1, 3 and 5 only.

2.8. Surface Sterilisation of Sclerotes and Infected Seed

A fresh solution of 5% sodium hypochlorite and 95% ethanol, 1:1 solution, was used for each batch of inoculum being cleaned. The inoculum was placed in metal tea strainers, which were placed individually into beakers of the made-up sterilant for 3 minutes, and then rinsed in distilled water for one minute and then rinsed in the second container of distilled water for a further one minute. The inoculum was then removed and dried on filter paper in a petri dish before being plated onto ½PDA+S. These plates were then placed into a 25°C incubator for up to a week, counting the number of inoculum propagules that were viable.

2.9. Statistical Analysis

The results were analysed by a general analysis of variance (ANOVA) using Genstat for Windows 16th edition (Copyright 2013, VSN International Ltd). Data was transformed, when required, before analysis to normalise and stabilise the variance. When significant treatment and interaction effects were determined the means of treatments and interactions were compared using LSD tests (5%).

3. Results

3.1. Variety Selection

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**. 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.

Knowing just how long it takes the varieties to flower, helps growers select varieties that can be grown as part of their crop rotation program, fitting into a time period when a cash crop is less likely to be grown.

Table 2: Days to incorporation (25% flowering) of brassica biofumigants trialled as well as grower standards.

	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

* These varieties did not flower and were incorporated regardless
NP Not planted due to wrong time of the year (Too cold) or no longer required.

3.2. Pests and Diseases

Brassica biofumigants attracted the same types of insect pests and diseases that crops such as broccoli, cabbage and cauliflowers could be expected to get during a typical growing season. A detailed description of all pests and diseases found in these crops can be found in the publication produced by Duff *et al*. [19].

3.3. Biomass Measurements

As might be expected, brassica biofumigants produced the most biomass during the cooler months, whereas the Lablab and Fumig8tor sorghum are warm-season crops producing their greatest biomass during summer. The three *B. juncea* varieties, Caliente, Mustclean and Nemfix, produced similar amounts of both fresh and dry weights as shown in Tables **3** and **4**. The highest biomass produced by the brassica biofumigants was achieved with Biofum, producing over 185 tonnes of fresh weight per hectare during summer and early autumn, while the greatest amount of dry matter per hectare produced was from Nemcon, producing 17.66 tonnes of dry matter per hectare with an autumn planting. BQ Mulch produced the least with just over 30 tonnes and 2.92 tonnes of fresh and dry weight per hectare respectively, when planted in late spring. The sorghum crop produced the greatest overall dry weight biomass with over 24 tonnes of dry weight per hectare during summer.

Table 3: Fresh weights of brassica biofumigants and grower standard cover crops, Gatton Research Facility, Queensland, Australia

	Caliente	**Mustclean**	**Nemfix**	**Nemat**	**BQ Mulch**	**Biofum**	**Tillage Radish**	**Nemclear**	**Nemcon**	**Lablab**	**Fumigator**
Nov 2016	48.85 d	47.50 b	33.66 d	63.14 b	30.93 d	90.24 b	82.46 b	128.85	118.96	53.62 a	103.02 b
Jan 2017	62.76 cd	42.79 b	46.74 cd	nd	67.15 b	185.76 a	157.69 a	nd	nd	48.028 a	nd
Mar 2017	72.5 c	65.07 b	66.47 c	nd	64.28 b	153.62 ab	172.10 a	183.39	173.76	21.54 b	59.78 c
May 2017	97.81 b	110.39 a	97.63 b	58.04 b	44.38 cd	91.53 b	160.46 a	nd	nd	nd	nd
July 2017	143.07 a	136.90 a	150.47 a	117.65 a	94.60 a	150.60 ab	145.57 a	nd	nd	nd	82.92 bc
Sept 2017	49.37 cd	71.21 b	56.28 cd	71.98 b	53.87 bc	133.30 ab	150.30 a	nd	nd	51.63 a	175.08 a
LSD (5%)	23.52	31.59	29.27	20.62	19.58	76.5	30.36			8.72	24.64

Means with same subscript for each variety are not significantly different at the P = 0.050 level.

Table 4: Dry weights of brassica biofumigants and grower standard cover crops, Gatton Research Facility, Queensland, Australia

	Caliente	Mustclean	Nemfix	Nemat	BQ Mulch	Biofum	Tillage Radish	Nemclear	Nemcon	Lablab	Fumigator
Nov 2016	5.04 d	5.25 c	4.03 b	7.60 b	2.92 d	8.29 b	7.19 b	14.58	14.65	10.65 a	17.58 b
Jan 2017	6.36 cd	3.90 c	4.57 b	nd	5.66 b	13.81 ab	11.25 a	nd	nd	9.37 a	24.17 a
Mar 2017	8.74 b	6.83 bc	7.21 b	nd	4.79 bc	15.05 a	11.84 a	16.16	17.66	4.07 b	10.88 c
May 2017	13.49 a	13.04 a	12.55 a	6.23 b	3.42 cd	11.57 ab	11.66 a	nd	nd	nd	nd
Jul 2017	14.82 a	12.68 a	15.67 a	12.03 a	8.18 a	17.34 a	11.32 a	nd	nd	nd	12.45 c
Sept 2017	7.25 bc	8.42 b	7.23 b	8.56 b	5.30 b	12.45 ab	11.42 a	nd	nd	9.06 a	23.54 a
LSD (5%)	2.157	3.129	3.345	2.667	1.853	6.443	2.05			2.192	4.02

Means with same subscript for each variety are not significantly different at the P = 0.050 level.

3.4. Glucosinolate Analysis

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. Table **5** highlights 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. Figure **1** shows 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 and 52.25 µmol/g DM being measured from summer grown crops respectively. BQ Mulch was the next best

Table 5: Range in concentration of individual glucosinolate (µmol/g DW) found in brassica biofumigants at 25% flowering after being planted late spring and autumn and grown over summer and winter.

Biofumigant	Planting date	Glucoiberin	Sinigrin	Progoitrin	Gluconapin	Total GSLs
Biofum	15-Nov-16	1.10	0.64	2.36	2.19	6.30
	16-May-17	0.92	0.11	0.34	0.49	1.86
BQ Mulch	15-Nov-16	0.54	21.46	1.59	2.37	25.96
	16-May-17	0.79	3.21	0.43	1.43	5.85
Caliente	15-Nov-16	nd	53.07	0.27	0.13	53.47
	16-May-17	0.23	22.50	0.20	0.85	23.78
Mustclean	15-Nov-16	nd	5.03	0.67	1.23	6.93
	16-May-17	0.91	2.07	0.15	2.53	5.65
Nemat	15-Nov-16	0.18	0.70	0.33	1.55	2.76
	16-May-17	0.54	0.76	0.21	0.70	2.20
Nemfix	15-Nov-16	1.41	17.26	0.76	1.93	21.36
	16-May-17	0.63	19.45	0.05	0.38	20.51
Tillage radish	15-Nov-16	1.29	0.14	1.13	1.09	3.65
	16-May-17	0.33	0.22	0.51	0.59	1.64

nd = no data.

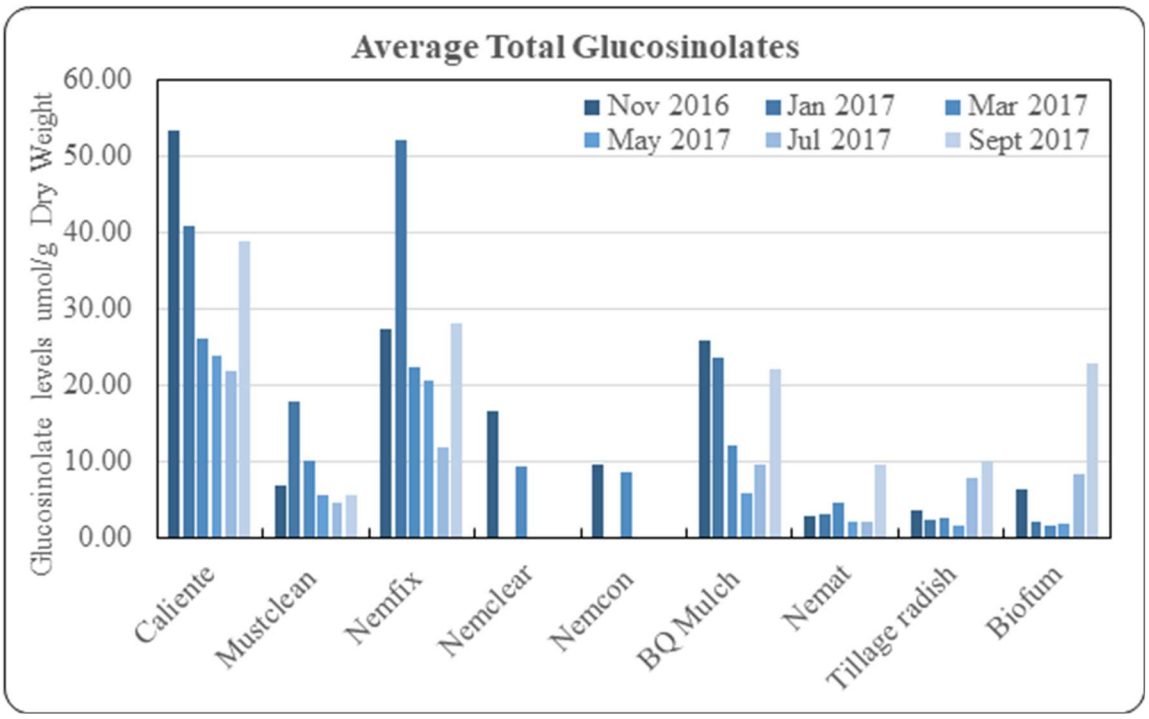

Figure 1: Average total glucosinolates being produced per gram of dry material at different times of the year.

performer with 25.96 µmol/g DM being produced with a summer planting also. 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**.

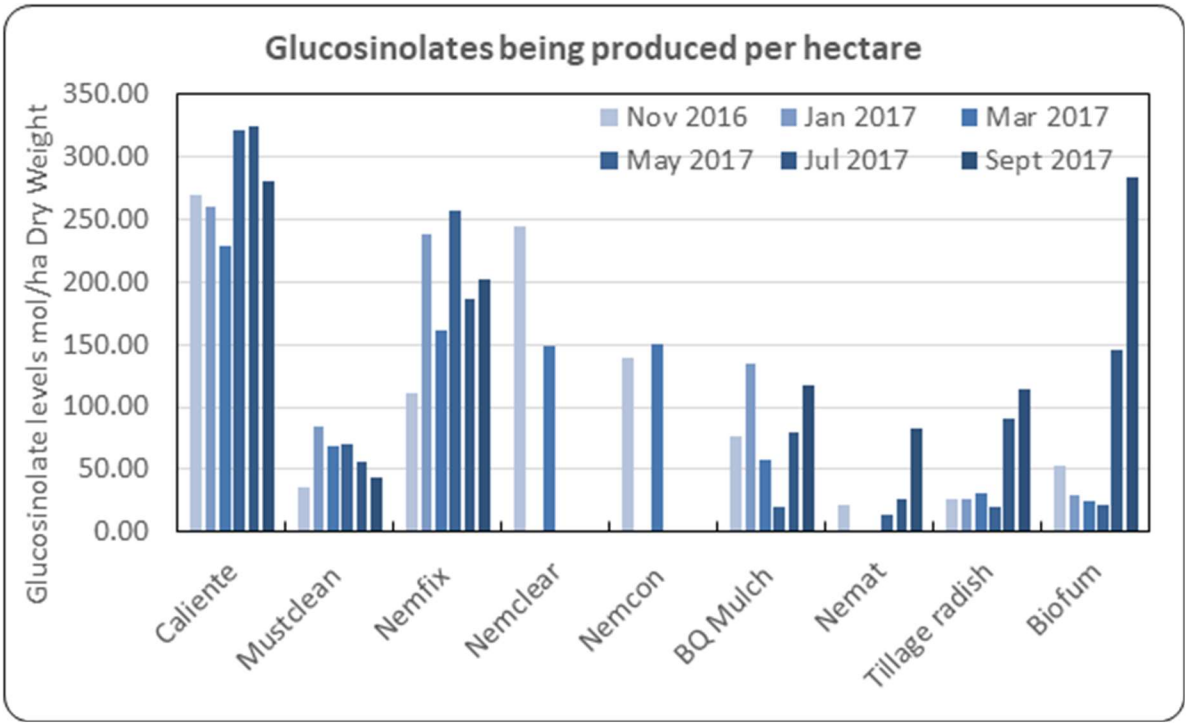

Figure 2: Amount of glucosinolates being produced per hectare at different times of the year.

3.5. Efficacy against Soilborne Pathogens

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.

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 **3**, 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.

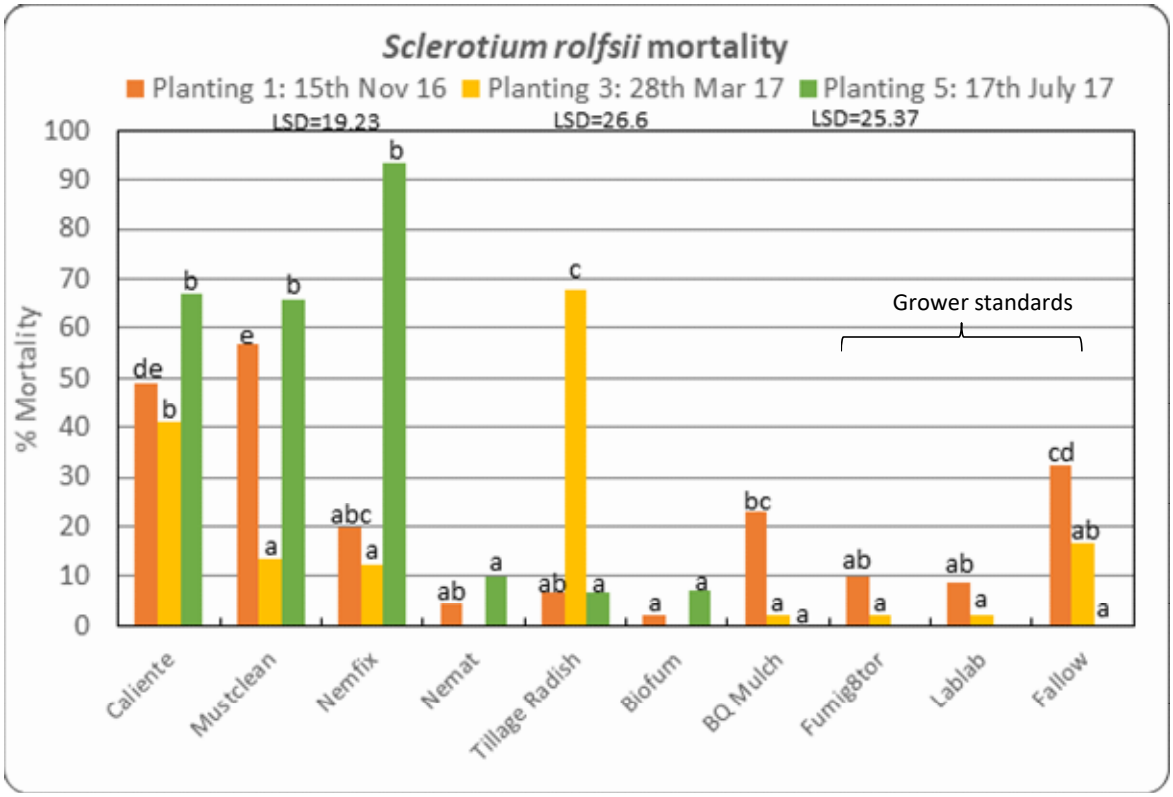

Figure 3: Performance of various brassica biofumigants and grower standard practices against Sclerotium rolfsii. Bars with the same letter for the different plantings are not significantly different from one another.

Sclerotinia sclerotiorum, Figure **4**, 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%.

Macrophomina phaseolina (Figure **5**) 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.

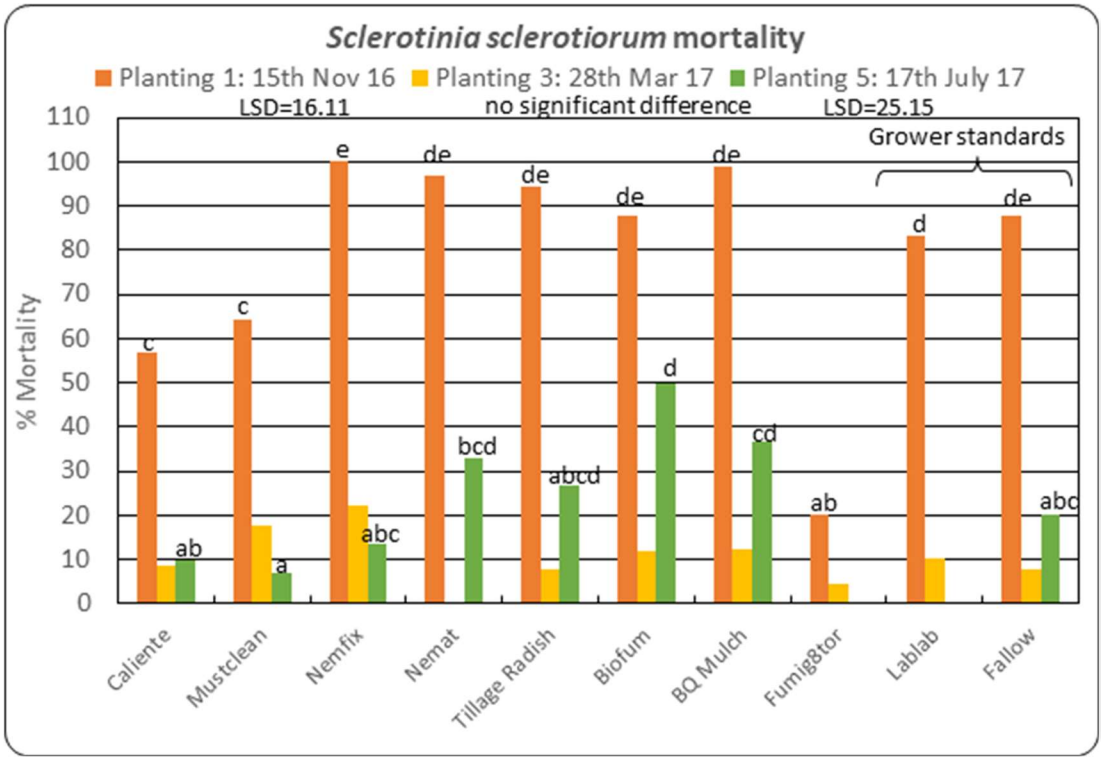

Figure 4: Performance of various brassica biofumigants and grower standard practices against *Sclerotinia sclerotiorum*. Bars with the same letter for the different plantings are not significantly different from one another. Planting 3 there was no significant difference between varieties.

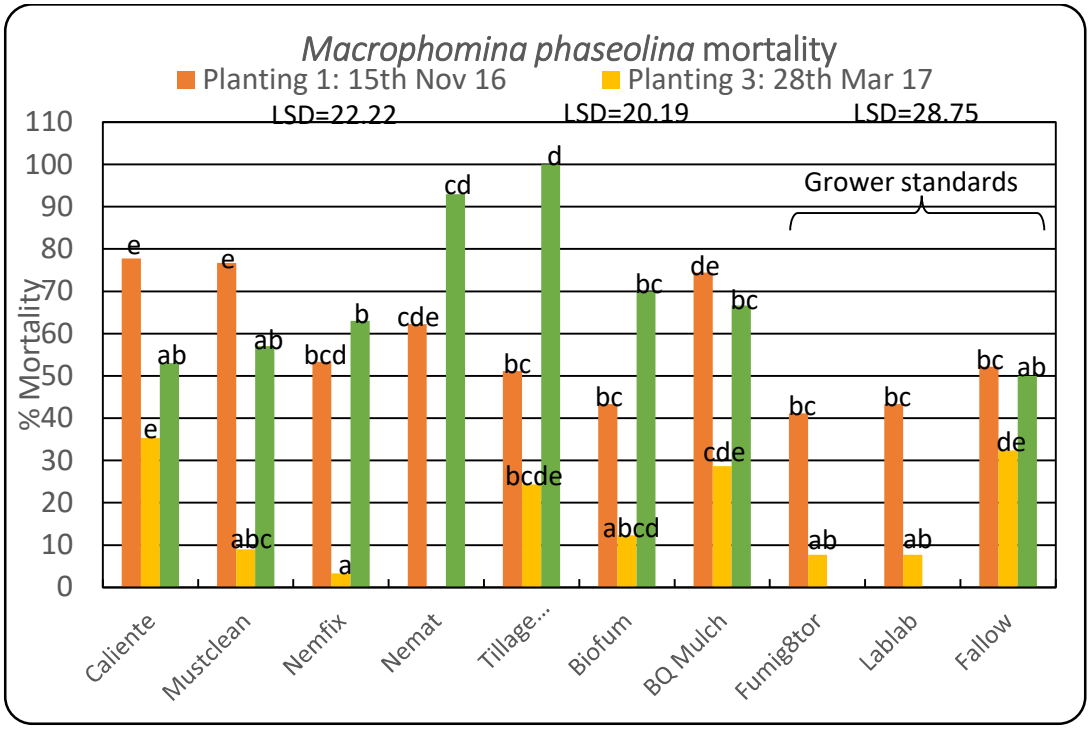

Figure 5: Performance of various brassica biofumigants and grower standard practices against *Macrophomina phaseolina*. Bars with the same letter for the different plantings are not significantly different from one another.

Nemclear and Nemcon are no longer being assessed, as their delayed flowering (greater than 3 months) would limit their consideration for cover cropping windows in vegetable production systems and the large number of insecticides that would be required to control whitefly and caterpillar pests.

4. Discussion

Although a traditionally grown cool-season crop, this work has shown that brassica biofumigants can be grown 12 months of the year with water being the greatest challenge to growing these crops during the hot summer months in south-east Queensland. They flower faster, allowing growers with only a small window of opportunity to grow a cover crop. They germinate quickly covering the ground, helping to reduce the chance of soil erosion and nutrient loss from high rainfall events, with some varieties still able to produce significant amounts of dry matter during summer. This was the case with both Nemcon and Nemclear, producing in excess of 14 t/ha of dry material. Even though these varieties were later abandoned, due to the length of time for them to flower, they would still make ideal cover crops if the grower was only interested in adding organic matter to the soil. It is possible that the temperature in our region when they were grown, was not cold enough to satisfy the requirements of these 2 varieties allowing them to flower. Perhaps if we had persisted with them, a winter planting may have seen them flower in spring. All other varieties flowered within 100 days, regardless of the time of year, which is just over the 3 months time limit we imposed on these cover crop options, fitting in with what growers were after. The cooler time of the year saw the greatest times to flowering or 98 days for Caliente and Tillage Radish. Growers are currently thinking of multispecies plantings and these 2 biofumigants may be a mix that could work, particularly if there were a range of soilborne pathogens needing to be managed. Tillage radish worked well against both *Macrophomina phaseolina* and *Sclerotium rolfsii* in autumn and winter while Caliente also performed well against these soilborne pathogens with more of an effect on the *S. rolfsii* with the winter planting. So they could complement each other well during this time period. Clearly, the different types of GSLs complement each other in the range of soilborne pathogens that they control.

The GSL sinigrin was the most dominant type found in Calinete and the other *B. juncea* varieties, whereas glucoiberin, progoitin and gluconapin are more common than sinigrin in most other varieties as shown in Table **6**. Unpublished data has also found glucoraphanin to be a commonly produced GSL of Tillage Radish, which was not consistently tested for during these field trial experiments.

The types and levels of GSLs varied considerably between varieties and throughout the year. Concentrating on a summer and winter grown crop, Table **6**, some of the varieties had more GSLs being produced with the summer grown crops compared to a winter grown crop. The review by Rosa *et al.* [20] mentions that winter and autumn seasons seem to induce lower GSL levels due to short days, cool temperatures and less radiation, and that periods of drought greatly increased GSL concentrations in oilseed rape and Portuguese cabbage in spring and summer growing seasons. Glucosinolate content has also been reported to vary in response to temperature and light quality with seasonal variation of GSL content in different *Brassica* spp such as oilseed rape [21, 22], turnip [23], and cabbage [13, 24]. Conditions in south-east Queensland can be extreme during spring and summer with average temperatures at 30ºC. When these brassica biofumigant trials started, monthly maximum temperatures

Table 6: Climatic data for Gatton Research Facility over the 12 months that biofumigants were planted

	2016		2017											
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Monthly average	33.1	33.8	33.7	35.1	30.7	25.8	24.3	23.0	23.1	25.5	30.0	28.1	28.6	32.4
Monthly Max	37.9	39.6	39.6	45.7	35.3	28	27.5	25.9	27.3	33.3	39.5	35.2	32.9	36.6
Mean	30.3	31.4	31.7	30.8	29.6	27.2	23.8	21.1	20.8	22.5	25.6	28.2	30.3	31.4
Rainfall	58.6	72.6	106.8	50.8	198	12.8	26.6	21.6	23.2	0.2	11	169.8	42.4	134
Mean rainfall	77.3	98.2	109.5	99.1	79.2	48	45.3	41.3	36	26.5	34.6	64.7	77.3	98.2

were well into the high 30ºC from November 2016 through until March 2017 and again the following spring, with a monthly maximum of 39.6ºC and 35.2ºC in December 2016 and October 2017 respectively, well above the monthly means of 31.4ºC and 28.2ºC. With below average rainfall during most of these periods, these brassica biofumigant varieties could have been further stressed to produce even more GSLs [25, 26].

Insects have also been shown to increase certain GSLs, with *Plutella xylostella* causing an increase in aliphatic glucosinolates in *B. juncea* [27] and *Myzus persicae* causing an increase in aliphatic GSLs in *Arabidopsis thaliana* [28]. Although insects were managed in this group of experiments, certain insect pests were more problematic during the warmer months of the growing period. Rutherglen bugs (*Nysius vinitor*) were particularly troublesome during plantings 1 and 2 (spring/summer), which also happened to coincide with the higher GSL levels measured in a number of varieties, particularly the *B. juncea* types as well as the mixture, BQ Mulch. If left unchecked, insect pests can quickly decimate a planting. Aphids have been shown to kill off large areas of *B. juncea* as observed in our own plantings and those of growers taking up this form of cover cropping option in the Lockyer Valley.

The elevated levels of GSLs in the warmer months appeared to contribute to improved control of one or more of the soilborne pathogens tested. The Fallow control exhibited significant levels of control of both *Sclerotinia* and *Macrophomina* at different times of the year, compared to the different cover cropping options, some of which were actually worse than the Fallow treatment. It is possible that these soils have some natural suppressive qualities inhibiting the growth and activity of soilborne pathogens to some extent, owing to the collective competitive and antagonistic activity of the total soil microbiome competing with the pathogens [29]. The soil at this set of trials is heavy clay with an organic matter content, between 3-4% OM, according to recent soil test results. Schlatter *et al* [29] mentioned that both soil organic matter and limited soil nutrient diversity are hypothesized to increase general suppression of soils to soilborne pathogens due to their direct impacts on total microbial densities and the intensity of competition within the soil microbiome. When recovering the soilborne pathogen propagules from the soil after 7 days, we did find a great deal of contamination, even after thoroughly surface sterilising them in an alcohol and bleach solution.

Selecting an appropriate brassica biofumigant is clearly an option for growers with specific soilborne pathogen issues. Although only 3 soilborne pathogens were compared, clear differences were evident between biofumigant varieties, but what was an important finding was the ability to be able to grow these biofumigants during our summer months with some of them performing better during this time period compared to the cooler months of the year. This will allow growers the ability to pick and choose a biofumigant variety depending on their needs [19].

The results from these plantings demonstrate the diversity of the GSL profiles, concentrations and production within and between Brassica biofumigants and related species when grown in the same environment and how they perform against certain soilborne pathogens.

Acknowledgement

This work was funded by the Queensland Government through the Department of Environment and Science as part of the Investing in the Environment for the Future program 2016 – 2019 and Horticulture Innovation through VG16068 Optimising Cover crops for Australian vegetable production. We wish to thank the following for their assistance in completing field trials and laboratory analyses: Fiona Giblin, Celia van Sprang, Julie O'Halloran, Zara Hall, David Williams, Sharon Punn and the Gatton Research Facility operational staff.

References

[1] Thorup-Kristensen K, Magid J, Jensen LS. Catch crops and green manures as biological tools in nitrogen management in temperate zones. Advances in agronomy. 2003; 79(79): 227-302. https://doi.org/10.1016/S0065-2113(02)79005-6

[2] Kator L, Hosea ZY, Oche OD. Sclerotium rolfsii: causative organism of southern blight, stem rot, white mold and sclerotia rot disease. Annals Biol Res. 2015; 6(11): 78-89.

[3] Alexander BJR, Stewart A. Survival of sclerotia of Sclerotinia and Sclerotium spp in New Zealand horticultural soil. Soil Biology and Biochemistry. 1994; 26(10): 1323-9. https://doi.org/10.1016/0038-0717(94)90213-5

[4] Adams P, Ayers W. Ecology of Sclerotinia species. Phytopathology. 1979; 69(8): 896-9. https://doi.org/10.1094/Phyto-69-896

[5] Watanabe T. Survivability of Macrophomina phaseoli (Maubl.) Ashby in Naturally-infested Soils and Longevity of the Sclerotia Formed in vitro. Annals of the Phytopathological Society of Japan. 1973; 39(4): 333-7. https://doi.org/10.3186/jjphytopath.39.333

[6] Gupta GK, Sharma SK, Ramteke R. Biology, epidemiology and management of the pathogenic fungus Macrophomina phaseolina (Tassi) Goid with special reference to charcoal rot of soybean (Glycine max (L.) Merrill). Journal of Phytopathology. 2012; 160(4): 167-80. https://doi.org/10.1111/j.1439-0434.2012.01884.x

[7] Larkin RP, Griffin TS, Honeycutt CW. Rotation and cover crop effects on soilborne potato diseases, tuber yield, and soil microbial communities. Plant Disease. 2010; 94(12): 1491-502. https://doi.org/10.1094/PDIS-03-10-0172

[8] Sharma A, Saha TN, Arora A, Shah R, Nain L. Efficient Microorganism Compost Benefits Plant Growth and Improves Soil Health in Calendula and Marigold. Horticultural Plant Journal. 2017; 3(2): 67-72. https://doi.org/10.1016/j.hpj.2017.07.003

[9] Kirkegaard J, Gardner P, Desmarchelier J, Angus J, editors. Biofumigation: using Brassica species to control pests and diseases in horticulture and agriculture. Proceedings 9th Australian Research Assembly on Brassicas; 1993; Agricultural Research Institute, Wagga.

[10] Kirkegaard J. Biofumigation for Plant Disease Control - from the Fundamentals to the Farming System. Disease Control in Crops: Biological and Environmentally Friendly Approaches: Wiley-Blackwell; 2009. p. 172-95. https://doi.org/10.1002/9781444312157.ch9

[11] Milford G, Evans E. Factors causing variation in glucosinolates in oilseed rape. Outlook on Agriculture. 1991; 20(1): 31-7. https://doi.org/10.1177/003072709102000107

[12] Kirkegaard JA, Sarwar M. Biofumigation potential of brassicas: I. Variation in glucosinolate profiles of diverse field-grown brassicas. Plant and Soil. 1998; 201(1): 71-89. https://doi.org/10.1023/A: 1004364713152

[13] Charron CS, Saxton AM, Sams CE. Relationship of climate and genotype to seasonal variation in the glucosinolate-myrosinase system. I. Glucosinolate content in ten cultivars of Brassica oleracea grown in fall and spring seasons. Journal of the Science of Food and Agriculture. 2005; 85(4): 671-81. https://doi.org/10.1002/jsfa.1880

[14] Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier JM. Biofumigation potential of brassicas III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. Plant and Soil. 1998; 201(1): 103-12. https://doi.org/10.1023/A: 1004381129991

[15] Warmington R, Clarkson JP. Volatiles from biofumigant plants have a direct effect on carpogenic germination of sclerotia and mycelial growth of Sclerotinia sclerotiorum. Plant and Soil. 2016; 401(1): 213-29. https://doi.org/10.1007/s11104-015-2742-8

[16] Smith BJ, Kirkegaard JA. In vitro inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. Plant Pathology. 2002; 51(5): 585-93. https://doi.org/10.1046/j.1365-3059.2002.00744.x

[17] West L, Tsui I, Haas G. Single column approach for the liquid chromatographic separation of polar and non-polar glucosinolates from broccoli sprouts and seeds. Journal of Chromatography A. 2002; 966(1-2): 227-32. https://doi.org/10.1016/S0021-9673(02)00734-3

[18] Williams DJ, Critchley C, Pun S, Nottingham S, O'Hare TJ. Epithiospecifier protein activity in broccoli: The link between terminal alkenyl glucosinolates and sulphoraphane nitrile. Phytochemistry. 2008; 69(16): 2765-73. https://doi.org/10.1016/j.phytochem.2008.09.018

[19] Duff JD, van Sprang Celia, O'Halloran Julie, Hall Zara. Guide to Brassica Biofumigant Cover Crops Managing soilborne diseases in vegetable production systems. State of Queensland: Department of Agriculture and Fisheries; 2020. https://www.publications.qld.gov.au/dataset/brassica-biofumigant-cover-crops/resource/a3694e67-0b1c-4972-a290-b8606a7c7360

[20] Rosa E, Heaney R, Fenwick G, Portas C. Glucosinolates in crop plants. 1997.

[21] Sarwar M, Kirkegaard JA. Biofumigation potential of brassicas II. Effect of environment and ontogeny on glucosinolate production and implications for screening. Plant and Soil. 1998; 201(1): 91-101. https://doi.org/10.1023/A: 1004333230899

[22] Justen VL, Fritz VA. Temperature-induced glucosinolate accumulation is associated with expression of BrMYB transcription factors. HortScience. 2013; 48(1): 47-52. https://doi.org/10.21273/HORTSCI.48.1.47

[23] Zhang H, Schonhof I, Krumbein A, Gutezeit B, Li L, Stützel H, *et al*. Water supply and growing season influence glucosinolate concentration and composition in turnip root (Brassica rapa ssp. rapifera L.). J Plant Nutr Soil Sci. 2008; 171(2): 255-65. https://doi.org/10.1002/jpln.200700079

[24] Charron CS, Sams CE. Glucosinolate content and myrosinase activity in rapid-cycling Brassica oleracea grown in a controlled environment. Journal of the American Society for Horticultural Science. 2004; 129(3): 321-30. https://doi.org/10.21273/JASHS.129.3.0321

[25] del Carmen Martínez-Ballesta M, Moreno D, Carvajal M. The physiological importance of glucosinolates on plant response to abiotic stress in Brassica. International journal of molecular sciences. 2013; 14(6): 11607-25. https://doi.org/10.3390/ijms140611607

[26] Björkman M, Klingen I, Birch AN, Bones AM, Bruce TJ, Johansen TJ, *et al*. Phytochemicals of Brassicaceae in plant protection and human health-Influences of climate, environment and agronomic practice. Phytochemistry. 2011; 72(7): 538-56. https://doi.org/10.1016/j.phytochem.2011.01.014

[27] Mathur V, Tytgat TO, de Graaf RM, Kalia V, Reddy AS, Vet LE, *et al*. Dealing with double trouble: consequences of single and double herbivory in Brassica juncea. Chemoecology. 2013; 23(2): 71-82. https://doi.org/10.1007/s00049-012-0120-z

[28] Mewis I, Appel HM, Hom A, Raina R, Schultz JC. Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiology. 2005; 138(2): 1149-62. https://doi.org/10.1104/pp.104.053389

[29] Schlatter D, Kinkel L, Thomashow L, Weller D, Paulitz T. Disease Suppressive Soils: New Insights from the Soil Microbiome. Phytopathology. 2017; 107(11): 1284-97. https://doi.org/10.1094/PHYTO-03-17-0111-RVW