Final Report

Effectiveness of biofumigant crops for the management of PCN in GB

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Report Authors: Matthew Back (Harper Adams University), Andy Barker (Barworth Agriculture) and Andy Evans (SRUC)

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1. SUMMARY

1.1. Aim

The aim of this project was to assess the use of biofumigation for the management of potato cyst nematodes in GB potatoes and develop recommendations for its optimal use.

1.2. Methodology

Polytunnel experiment

A polytunnel experiment was conducted at Harper Adams University to assess the performance of 15 commercially available biofumigant lines on the viability of the encysted eggs of *Globodera pallida*. Biofumigant species were grown for c.10 weeks until the majority of plants had reached flowering. The viability of the encysted eggs was assessed using Meldola's blue stain. Brassica biomass was assessed and tissue samples were taken for glucosinolate analysis.

Field experiments

Field experiments were conducted in Staffordshire (Harper Adams University), Lincolnshire (Barworth Agriculture) and Aberdeenshire (SRUC) in 2013-2016 to evaluate the use of biofumigation for the management of potato cyst nematodes. In 2013 and 2014, we evaluated the performance of brassica biofumigants including *Brassica juncea* (Indian mustard), *Raphanus sativus* (oilseed radish), *Sinapis alba* (white mustard) and *Eruca sativa* (rocket). In 2015, various incorporation techniques (rotovating, ploughing and discing) associated with biofumigation using *B. juncea* (Indian mustard) cv. Caliente 99 were compared. In 2016, we investigated the use of nitrogen and sulphur inputs for enhancing the biofumigant performance of *B. juncea* against PCN.

Each experiment monitored PCN populations during the development of the biofumigant crops and post maceration/incorporation of their biomass. In the 2013-2014 (Lincolnshire site only), 2014-15 and 2015-16 experiments, further monitoring took place in a successive potato crop to account for changes in PCN multiplication rates. Potato yield, tuber number and blemishing diseases were also assessed. The performance of the brassica species was monitored by recording root and foliar biomass and by taking tissue samples for glucosinolate analysis. The samples were analysed by staff at the Univeristy of York who were members of a consortium studying biofumigation as part of a separate BBSRC/AHDB-funded study "Establishing biofumigation as a sustainable replacement to pesticides for control of soil-borne pests and pathogens of potato and horticultural crops". Soil samples were collected in experimental plots, before and after growing biofumigants, to determine soil nutrients (N-P-K) in a number of the experiments.

1.3. Key findings

Results from the polytunnel study showed that all of the biofumigant species and varieties tested were able to cause a significant reduction of PCN viability (reductions

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between 49-65%). However, no significant differences could be found between the species or varieties tested.

The performance of biofumigants under field conditions was variable across sites and seasons. In 2013-14, the biomass produced at sites in Aberdeenshire and Shropshire was minimal and this is attributed to the later drilling dates (9th and 6th September, respectively). The Lincolnshire trial was drilled earlier (26th August) but there was no evidence of PCN suppression seen at any of the sites.

There was evidence of PCN suppression at the site in Shropshire in 2014-15 when all of the data post-biofumigant sowing was analysed. The trial consisted of five different biofumigant lines: *Brassica juncea* (brown mustard) cv. Caliente 99, *B. juncea* cv. Scala, *Sinapis alba* (white mustard) cv. Ida Gold, *Eruca sativa* (rocket) cv. Nemat and *Raphanus sativus* (oilseed radish) cv. Bento and an untreated (fallow) control. The crops were drilled on 11th August 2014. *Brassica juncea* cv. Scala and *R. sativus* cv. Bento were found to cause the greatest reductions in viable PCN eggs g^{-1} soil. However, comparisons made with the data generated at each sampling event only revealed a significant difference in viable PCN eggs g^{-1} soil pre-incorporation of biofumigant biomass.

At the trial site in Lincolnshire (2014-15), the biofumigants were drilled on 31st July 2014. The trial was on a low organic matter, very sandy soil. The level of PCN decline in the fallow plots was 32%. There was no statistically significant effect of any of the treatments on the numbers of viable PCN. However, taking each treatment individually, both *B. juncea* cv. Caliente 99 and *S. alba* cv. IdaGold decreased viable PCN eggs g^{-1} by 40% overall. But the complication of sampling error has meant that following the same calculation, *R. sativus* cv. Bento and *Eruca sativa* cv. Nemat have increased viable PCN eggs g^{-1} by 20%. Supporting the suggested efficacy of the mustards over the *R. sativus* cv. Bento and *Eruca sativa* cv. Nemat , is the ratio of eggs/cyst where the latter showed very little change.

At the Scottish site in Aberdeenshire (2014-15), the biofumigants were drilled on 18th June 2014. There was a general reduction in PCN eggs g^{-1} soil in all treatments. However, the reduction in the plots was statistically significant for *B. juncea* cv. Caliente 99 with a 64% reduction between pre-biofumigant and post-incorporation, compared to a 20% reduction in the untreated fallow plots. When mean number of viable PCN eggs cyst^{-1} was assessed, all treatments (except *B. juncea* cv. Scala) had a significant effect when comparing counts pre-biofumigant and post-incorporation. However, the untreated fallow plots also reduced the number of eggs cyst^{-1}, but only by 17%. *Brassica juncea* cv. Caliente 99 reduced nematode eggs cyst^{-1} by 63%. Whilst the other treatments did not differ significantly from the untreated fallow plots, they did have higher levels of reduction in number of eggs cyst^{-1}: 28% (*B. juncea* cv. Scala); 41% (*S. alba* cv. Ida Gold) and 43% (*R. sativus* cv. Bento).

The positive impact of *B. juncea* cv. Caliente 99 is not specifically on the percentage viability of the eggs, but more on the numbers of viable eggs, which is demonstrated in the significant reductions in nematode eggs cyst^{-1} and subsequent eggs g^{-1} soil.

In 2015-2016 (cultivation experiments), biofumigation was not found to cause a significant reduction in potato cyst nematode population densities in Shropshire, Lincolnshire or Aberdeenshire. The Shropshire site was drilled on 8th September and overwintered due to the delayed drilling in 2015. Previous research has highlighted that biofumigants accumulate a higher concentration of glucosinolates when grown in warmer temperatures and longer day-length and therefore biofumigation performance

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may have been reduced under these conditions. The Lincolnshire and Fife trials were drilled on the 5th August and 10th August, respectively.

In 2016, we assessed the effect of nitrogen and sulphur inputs on biofumigation using *B. juncea* cv Caliente 99. Nitrogen application resulted in significantly greater foliar biomass (fresh and dry weight) whereas sulphur application resulted in a higher biomass of stems and leaves (fresh weight) at the site in Aberdeenshire.

Nitrogen and sulphur applications were found to improve the biofumingant performance of *B. juncea* in suppressing PCN at the site in Shropshire, post incorporation. The trial was drilled on 18th August. The viability of PCN eggs was significantly lower in plots receiving 100-150 kg/ha N and 25-50 kg/ha S than in plots with other treatments. However, there were no statistically significant differences between treatments for viable eggs g^{-1} soil or viable eggs $cyst^{-1}$. Additionally, there were no differences between PCN measurements at the site in Lincolnshire (drilled on 13th August). The site at Aberdeenshire was only used for assessing biomass and glucosinolates.

1.4. Practical recommendations

- The poly-tunnel experiment demonstrated that all the biofumigant species and varieties tested have potential for managing PCN populations. However, further work should be undertaken to generate independent information on the relative efficacy of species/mixtures especially as new mixtures are regularly coming to the market.
- Our field experiments provide further evidence that biofumigation using *Brassica* species can cause a reduction in the viability of encysted PCN eggs. However, results from the field experiments were inconsistent across sites and seasons. Biofumigation is a process affected by many factors including biomass production, glucosinolate accumulation and biomass destruction and incorporation.
- A number of our field experiments were planted later than originally scheduled due to a delay in the harvest of winter wheat. The later sown biofumigant crops (i.e. sowing in late August or September) produced a lower biomass and reductions in PCN viability were not detected. Based on this work, we would encourage growers to sow biofumigants between June and the first week of August.
- Biomass production by *B. juncea* responds to applications of nitrogen. Data from the site at Shropshire would indicate that biofumigation efficiency is improved when *B. juncea* is provided with 100-150 kg/ha N and 25-50 kg/ha S at planting.
- Our experiment investigating maceration and incorporation techniques did not generate any significant results. Biofumigation was generally ineffective during the trial despite the Lincolnshire trial being drilled on the 5th August. Therefore, no recommendations can be made on the choice of implements for biofumigation based on these trials.
- At the time of writing, the analysis of the leaf/stem samples for glucosinolate content has not been completed by the University of York. Once all the results have been received this report and recommendations will be updated as necessary.

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2. INTRODUCTION

Biofumigation in the soil takes place when 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). Glucosinolates (glucose- and sulphur-containing organic anions) and isothiocyanates are the main active compounds involved in biofumigation. Glucosinolates are secondary metabolites produced by certain crops that are hydrolysed by the enzyme myrosinase to form isothiocyanates (Wathelet *et al*., 2004). The interest in biofumigation for the management of soil-borne pests and diseases is due to the toxic effect of isothiocyanates on many soil-borne pathogens (Sarwar *et al.*, 1998).

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

During the past few decades, progress has been made in understanding how to maximize the efficacy of biofumigation by using Brassicaceae crops. This has led to renewed interest in their role in controlling soil-borne nematode pests (Morra and Kirkegaard, 2002). It has been demonstrated by various researchers that biofumigation leads to the significant reduction of various economically important nematode pest populations and the symptoms they cause to crops, with subsequent increases in yield/quality of such crops (see Fourie *et al*., 2016). Although the major body of research done in this regard focused on species of *Globodera*, *Meloidogyne* and *Pratylenchus* (Valdes *et al*., 2012), the review by Fourie *et al*. (2016) also includes literature on other economically important nematode pests that affect potatoes such as *Ditylenchus dipsaci* and *Paratrichodorus minor*.

Potato cyst nematodes (*Globodera pallida* and *G. rostochiensis)* are major, economically important pests for potatoes and occur in potato-cultivated areas worldwide. The cost of managing potato cyst nematode (PCN) in the UK potato industry is estimated to be £26 million per annum and is estimated to double in the absence of the current practice of using granular nematicides (Twining *et al*., 2009). Whilst there are potato cultivars available with resistance to *G. pallida* they are a small proportion of the UK crop. The effective management of PCN, particularly *G. pallida*, is needed to maintain the competitiveness of potato production worldwide.

Current advice offered to growers promotes the integration of two or more management practices, such as the use of partial resistance to *G. pallida*, crop rotation and the use of granular nematicides. However, the management of PCN does require knowledge on the presence of the pest in a field, the species of PCN, and the size of the population, and with issues regarding the limits of detection of PCN being linked to the amount of soil that can be realistically sampled and processed by commercial laboratories, detection of low viable populations of PCN is a problem.

As outlined above, biofumigation is being promoted for the management of soil-borne pests including PCN, and there have been a series of studies investigating the potential use of these crops to manage PCN (Pinto *et al*., 1998; Serra *et al*., 2002; Buskov *et al*., 2002; Lord *et al*., 2001; Aires *et al*., 2009; Ngala *et al*., 2014).

However, many of these reports of beneficial effects of biofumigation against PCN have been conducted *in vitro* or under glasshouse controlled conditions, and whilst there are

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now biofumigant crops being commercially promoted for the management of PCN, there are few reports of experimental data from replicated field trials.

There is a lack of data on the agronomy surrounding the use of biofumigant crops for the management of PCN with contrasting and confusing advice offered on the species and variety of biofumigant crop to be grown, when it should be sown, when it should be incorporated, the best method of maceration and incorporation, what fertilizer inputs are required etc.

For example, Ngala *et al*. (2014) suggested it is preferable to sow biofumigants in the summer months (June-August) following the harvest of crops such as combining peas, oilseed rape, winter barley and winter wheat for a variety of reasons. Biofumigants such as Indian mustard (*B. juncea*) are highly susceptible to sub-zero temperatures associated with ground frosts so late sowing or growing the crop overwinter is not optimal. Also biofumigants produce more biomass when the photoperiod is longer and the temperatures are intermediate as in the summer and early autumn. Brassica species grown in longer days with intermediate temperatures, high light intensity, and dry conditions tend to have the highest total glucosinolate content (Bjorkman *et al*. 2011).

Total glucosinolate concentration increases with increasing sulphur supply as do specific glucosinolates such as sinigrin and glucoraphanin (a major glucosinolate found in oil radish). Most biofumigant seed suppliers recommend sulphur inputs between 25-60 kg/ha. Increasing N inputs can, however, reduce aliphatic and aromatic glucosinolates when low sulphur inputs i.e. 10-20 kg/ha are supplied. Modest inputs of nitrogen (c.100 kg/ha) are likely to have positive effects on biofumigant biomass.

Biomass incorporation is a critical component of biofumigation. There is universal agreement that the above-ground biomass needs thorough pulverisation to enable the greatest release of glucosinolates and myrosinase to produce biocidal concentrations of isothiocyanates. It is recommended that incorporation should take place at mid-flowering as there tends to be higher glucosinolate concentrations in the foliage at this time. The main implement used in biomass maceration is a flail mower. Following maceration, the brassica residues need to be incorporated in quick succession, as the greatest proportion of biocidal volatiles (including isothiocyanates) are released within the first 2 hours after maceration. Incorporation is usually achieved using a rotovator or spader to enable thorough mixing of the soil. Following incorporation, it is advisable to rapidly compress the soil using a heavy roller. This process, should help create a seal and therefore retain volatile isothiocyanates for longer. Incorporation is best undertaken when the soil is moist to improve soil sealing and potentially increase glucosinolate hydrolysis.

This project addressed knowledge gaps in the use of biofumigants used in the UK for the management of PCN, particularly in relation to performance of different biofumigants crops in three areas of the UK (Scotland, Shropshire and Lincolnshire), the method of incorporation and fertilizer requirements, with a view to developing practical guidelines for growers who wish to use biofumigant crops as an option in their PCN management strategy.

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3. Materials and methods

3.1 A poly-tunnel experiment to evaluate biofumigant lines available in Great Britain

Cyst collection

To locate a source of soil, heavily infested with potato cyst nematodes (PCN), 'point' samples were collected from field sites in Shropshire (UK) with a history of PCN. The cysts were extracted from soil samples following standard methods (Shepherd, 1986). For each sample, a sub sample of 25-50 cysts was used to determine the species ratio (Back *et al*., 2006) and egg viability using Meldola's blue stain (Kroese *et al*., 2011). On the basis of this preliminary testing, a large supply of soil (75-100 kg) was collected from a site where (i) PCN eggs appeared to be highly viable (75%+) (ii) a high population density was found (iii) the population contained a high (90%+) proportion of *Globodera pallida*. Cysts were mass extracted using a Fenwick can (Fenwick, 1940) to process 2-3 kg of soil. Species composition and viability was reassessed on three sub samples of the extracted cysts.

Experiment design

The experiment was conducted in a poly-tunnel at Harper Adams University from 17th July- 4th September 2013. The experimental treatments consisted of the 15 biofumigant lines detailed in Table 1 and an untreated control. Each treatment was replicated 5 times and the experimental pots arranged in a randomised block design (see figure 2).

Table 1: *Biofumigant species and cultivars used in a poly-tunnel experiment investigating the effect of incorporated residues from biofumigant species and cultivars on the viability of potato cyst nematodes*

Species	Product name	Supplier
Brassica carinata	Cappuccino	Nickerson Direct (Joordens Zaden)
Brassica juncea	Caliente 99 (ISCI 99)	Plant Solutions Ltd
Brassica juncea	Pacific Gold	AAPS
Brassica juncea	Scala	van Dijke Semo
Brassica juncea	Vitasso	Boston Seeds
Brassica juncea	Keva	Plant Solutions Ltd
Sinapis alba	Ida Gold	AAPS
Sinapis alba	Smash	Nickerson Direct (Joordens Zaden)
Sinapis alba	Loti	Plant Solutions Ltd
Eruca sativa	Trio	Nickerson Direct (Joordens Zaden)
Eruca sativa	Nemat	Plant Solutions Ltd
Raphanus sativus	Bento	Senova (P.H. Petersen)
Raphanus sativus	Doublet	Nickerson Direct (Joordens Zaden)
Raphanus sativus	Anaconda	Joordens Zaden
Raphanus sativus	Terranova	Joordens Zaden

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Experimental procedure

Experimental pots (1.7 litre) were filled with John Innes No.2 sterilised loam, leaving 3 cm free at the top for watering. Small (5 x 4 cm), nylon mesh (250 µm) sachets containing 50 cysts of *G. pallida* were buried to a depth of 15 cm in each pot (Figure 1). In biofumigant treated pots, two seeds were sown at each point of an 'Isosceles triangle' template at a depth of 10 mm (see figure 2). Once the seedlings had emerged they were thinned so that the pot contained 3 seedlings positioned equidistantly from one another. The soil moisture in each experimental pot was kept close to field capacity (35% moisture) throughout the experiment. This was achieved by routine use of a 'Theta' moisture probe (Delta-T Devices, Cambridge, UK) and calculating the quantity of water required per pot. Watering took place 2-3 times a week. Soil temperature was recorded throughout the experiment using 2 x DS1923 hygrochron (iButton®) temperature probes.

Biofumigants were incorporated when the majority of the lines had reached 50% flowering. Initially, plants were removed from their pot and the potting medium was separated into a clear polythene bag. The root ball and underground stem was separated from the above ground foliage. A Viking® GE150 Shredder was used to macerate the above ground foliage and root tissue was roughly cut into c. 3cm length pieces. The chopped material was recombined with soil from the respective experimental pot and thoroughly mixed in a polythene bag for c. 20 seconds. The soil and plant debris was then returned to the pot and the cyst sachet reburied to a depth of 15 cm. The soil was firmed down in each pot and irrigated to pot capacity.

The cyst sachets were collected from the pots and dried at 25°C for 12 hours, eight weeks after incorporation.

Figure 1: *Nylon mesh sachets containing cysts of* Globodera pallida *used in a poly-tunnel experiment investigating the effect of incorporated residues from biofumigant species and cultivars on the viability of potato cyst nematodes*

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Figure 2: *Emergence of brassica seedlings (a) and experimental layout (b) in a poly-tunnel experiment investigating the effect of incorporated residues from biofumigant species and cultivars on the viability of potato cyst nematodes*

Figure 3: *Brassica (biofumigant) foliage, post-maceration in a Viking GE150 Shredder, in a poly-tunnel experiment investigating the effect of incorporated residues from biofumigant species and cultivars on the viability of potato cyst nematodes*

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Assessments

The fresh weight of biofumigants in each pot was measured prior to incorporation. For one replicate of each treatment, whole plants were carefully harvested, preserved following ISO 9167-1:1992 (Determination of glucosinolates content -- Part 1: Method using high-performance liquid chromatography) and sent to the University of York for glucosinolate analysis.

The viability of cysts within the sachets was determined using Meldola's blue stain (Kroese *et al*., 2011).

Analysis of results

Experimental results were analysed using a one way analysis of variance on Genstat 15th Edition (VSN International). Tukey's multiple range test was used to determine if significant differences occured between treatment groups.

3.2. Field experiments to evaluate biofumigant species in contrasting geographical locations in Great Britain (2013-2014)

Background

A total of three replicated field trials were conducted on land known to be infested with PCN in Scotland (**SRUC**), West England (**HAU**) and East England (**Barworth Agriculture Ltd**). Field diaries (planting date, inputs and sample collection) of each experimental site are shown in Tables 2-4.

The experiments were based on an evaluation of four different biofumigant *Brassicas* including *Brassica juncea* (brown mustard), *Sinapis alba* (white mustard), *Eruca sativa* (rocket) and *Raphanus sativus* (oilseed radish) and an untreated control (no biofumigant grown, but rotovated). The four biofumigants were selected as they represented the most promising commercially available biofumigants at the time of the trials (based on the previous experience of the researchers and published studies e.g. Lord *et al.,* 2011). The seed rates and nitrogen and sulphur application rates used were based on those recommended by the suppliers. The incorporation of the biofumigants was undertaken by flailing, followed by rotovator and rolling to seal the soil surface.

Experimental design

Treatments were laid out in a randomised block design. Where possible the blocks of the experiments were laid out to account for local variability in PCN population densities. Each treatment was replicated 5 times and individual plot sizes were 8 drills by 10 metres.

Assessments

Biomass production of each biofumigant was assessed by sampling 2 x 0.5 m^2 per replicate plot prior to maceration/incorporation. The samples were separated into stem, leaf and root biomass and fresh weight and dry weight was recorded.

Potato cyst nematode densities were sampled from the experimental plots at five time points:

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- pre-sowing of biofumigant crops
- pre-incorporation
- post incorporation
- pre-planting of the potato crop (*Pi*) in the following year
- prior to harvesting of the potatoes (*Pf*) – Barworth Agriculture only

Population density was assessed using standard methods, whilst egg/juvenile viability was estimated using Meldola's blue stain (Kroese *et al*., 2011). PCN multiplication (*Pf/Pi*) was calculated by determining the ratio between *Pf* and *Pi*.

Following the potato harvest in 2014, graded yield and tuber quality was assessed.

Soil samples were taken just prior to the planting of the potato crop and analysed for nutrients (N, P and K) and organic matter to establish whether the use of biofumigants leads to additional benefits with regards to nutrient and organic matter status of the soil.

Analysis of results

Experimental results were analysed using a one way analysis of variance on Genstat 15th Edition (VSN International). Tukey's multiple range test was used to determine if significant differences occur between treatment groups.

Table 2: *Inputs made to field experiment 2013-14 (Field Diary) - Staffordshire site (Rugeley), investigating the effect of biofumigant species on potato cyst nematode population densities*

Date	Event
28/08/13	Point samples collected on a 20m grid Soil samples revealed PCN population from 16-67 eggs g^{-1} soil
06/09/13	**Field experiment planted**
04/10/13	Visit to field site indicated poor establishment of *Brassica juncea*
21/11/13	Biofumigant plots flailed and incorporated Soil samples collected for PCN (pre-incorporation) Tissue samples collected for fresh weight & glucosinolate (GSL) analysis
22/01/14	Soil samples for PCN (post incorporation)
04/04/14	Soil samples for PCN (post incorporation) Soil samples for nutrient analysis **Experiment completed**

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Table 3: *Inputs made to field experiment 2013-14 (Field Diary) - Lincolnshire site (Holbeach), investigating the effect of biofumigant species on potato cyst nematode population densities*

Date	Event
26/08/13	**Field experiment planted** Soil samples collected
12/11/13	Soil samples collected for PCN (pre-incorporation) Tissue samples collected for fresh weight & GSL analysis Biofumigant plots flailed and incorporated
26/02/14	Soil samples for PCN (post incorporation)
28/04/14	Soil samples for PCN (*Pi* - pre-planting the potato crop)
23/09/14	Soil samples for PCN (*Pf* – after potatoes) Tuber samples taken for yield/quality estimates **Experiment completed**

Table 4: *Inputs made to field experiment 2013-14 (Field Diary) - Aberdeenshire site (Ethie Barns), investigating the effect of biofumigant species on potato cyst nematode population densities*

Date	Event
06/09/13	Soil samples collected for PCN
09/09/13	**Field experiment planted**
14/11/13	Tissue samples collected for fresh weight & GSL analysis
12/01/14	% ground cover assessed
25/04/14	Soil samples for PCN prior to incorporation Biofumigant plots flailed and incorporated
13/05/14	Soil samples for PCN post incorporation **Experiment completed**

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3.3 Field experiments to evaluate biofumigant species in contrasting geographical locations in Great Britain (2014-2015)

Background

A total of three replicated field trials were conducted on land known to be infested with PCN in Scotland (**SRUC**), West England (**HAU**) and East England (**Barworth Agriculture Ltd**). Field diaries (planting date, inputs and sample collection) of each experimental site are shown in tables 5-7.

The experiments were based on an evaluation of five different biofumigant lines including *Brassica juncea* (brown mustard) cv. Caliente 99, *B. juncea* cv. Scala, *Sinapis alba* (white mustard) cv. Ida Gold, *Eruca sativa* (rocket) cv. Nemat and *Raphanus sativus* (oilseed radish) cv. Bento and an untreated (fallow) control. The five biofumigants were selected on the basis of good performance in previously published research e.g. Lord *et al.,* 2011 and Ngala *et al*., 2014 and performance in unpublished field experiments (Barworth Agriculture). *Eruca sativa* cv. Nemat was excluded from the SRUC site due to a miscommunication. The seed rates and nitrogen and sulphur application rates used were based on those recommended by the suppliers. The incorporation of the biofumigants was undertaken by flailing, followed by rotovator and rolling to seal the soil surface. Fallow (untreated) plots were rotovated at the sites in England and left undisturbed at the site in Scotland. In the following spring, potatoes cv. Desirée were planted over the experimental plots. Each plot was divided (split) with half (two drills) receiving the either Vydate® (a.i. oxamyl) at 55 kg/ha or Nemathorin® (fosthiazate) at 30 kg/ha (Barworth Agriculture Ltd) whilst the other half was left untreated.

Experimental design

Treatments were laid out in a randomised block design. Where possible the blocks of the experiments were positioned to account for local variability in PCN population densities. Each treatment was replicated 5 times and individual plot sizes were 8 drills by 10 metres. At potato planting the experimental plots were modified into split plots with half (two drills) the plot receiving the nematicide Vydate® (a.i. oxamyl) at 55 kg/ha and the other half being left untreated (see Figures 4 and 5). Split plot treatments were randomly allocated.

Assessments

Biomass production of each biofumigant was assessed by sampling 2 x 0.5 m^2 per replicate plot prior to maceration/incorporation. The samples were separated into stem, leaf and root biomass and fresh weight and dry weight was recorded.

Tissue samples (foliage and root) were taken from the biofumigant plots at 6 and 8 weeks after sowing and just prior to biomass incorporation (see Tables 5-7). Four replicates of each biofumigant treatment were sampled. For each plot, 5 plants were carefully collected and separated into leaves, stems and roots. To prevent loss of glucosinolates the tissue samples were either flash frozen in liquid nitrogen or placed

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into a -80°C freezer. Samples were then sent to the University of York for glucosinolate analysis.

Soil samples were taken just prior to the planting of the potato crop and analysed for nutrients (N, P and K) and organic matter to establish whether the use of biofumigants leads to additional benefits with regards to nutrient and organic matter status of the soil. At the HAU site an additional set of samples were taken pre-incorporation of biofumigants.

Potato cyst nematode densities were sampled from the experimental plots at five time points:

- pre-sowing of biofumigant crops
- pre-incorporation
- post incorporation
- pre-planting of the potato crop (*Pi*) in the following year
- prior to harvesting of the potatoes (*Pf*)

Population density was assessed using standard methods, whilst egg/juvenile viability was estimated using Meldola's blue stain (Kroese *et al*., 2011). PCN multiplication (*Pf/Pi*) was calculated by determining the ratio between *Pf* and *Pi*.

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Following the potato harvest in 2015, tuber number, yield of marketable fractions were assessed in each split-plot (1m x 2 drills). A macroscopic assessment of tuber blemishing diseases was undertaken on a sample of 25 tubers from each plot. The severity (disease severity index) of tuber diseases was calculated using the following formula: -

$$DSI = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + (4 \times e) + (5 \times f) + (6 \times g)}{Number\ of\ tubers\ examined} \times \frac{100}{6}$$

Categories: a = 0, b = ≤1%, c = 1-5%, d = 6-10%, e = 11-15%, f = 16-25% g = 25+%

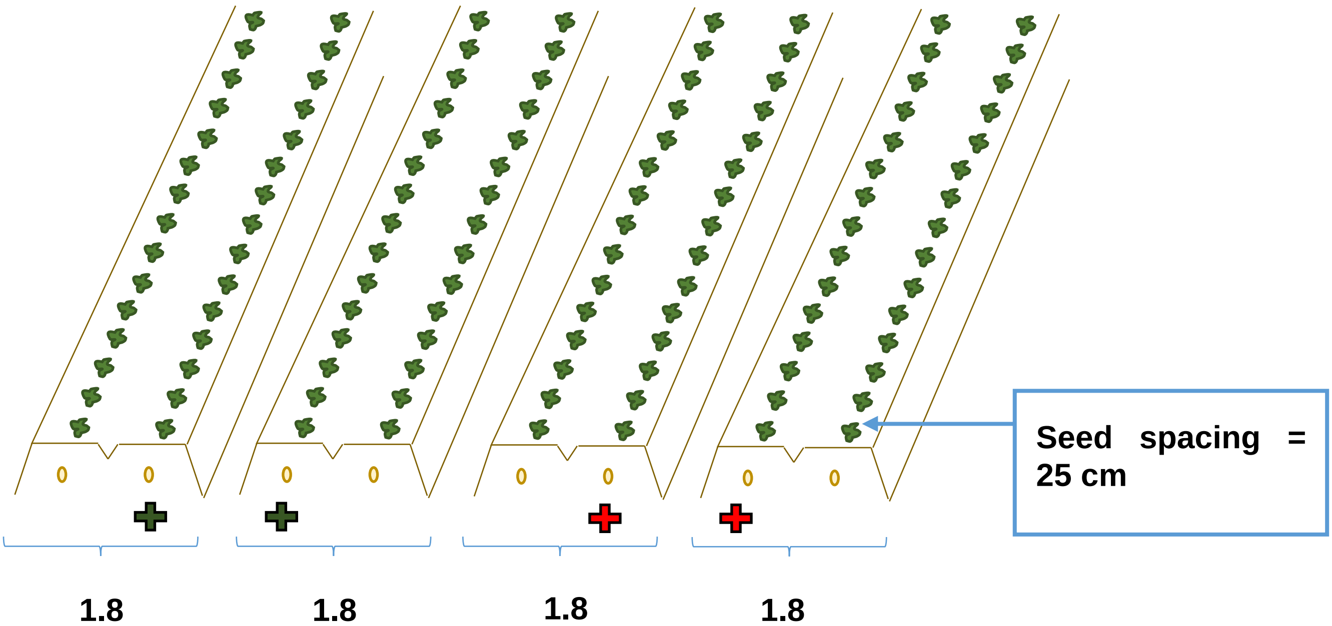

Figure 4: *Schematic overview of an experimental plot (Field Experiments 2014-15) showing bed/ridge layout and the position of treated drills as marked with the + symbol.*

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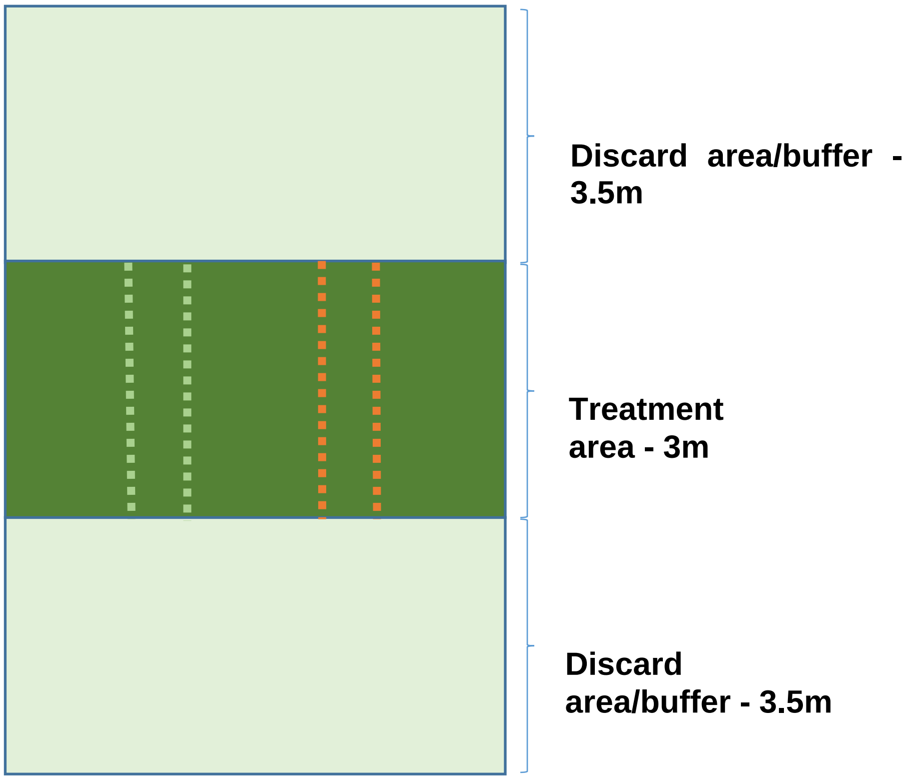

Figure 5: *Schematic overview of an experimental plot (Field Experiments 2014-15). Dashed lines represent the treated drills. Different coloured dashed lines indicate nematicide treated and untreated as determined through the split-plot design*

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Table 5: *Inputs made to field experiment 2014-15 (Field Diary) - Shropshire site (Crudgington), investigating the effect of biofumigant species on potato cyst nematode population densities*

Date	Event
18/07/14	Point samples collected on a 20m grid Soil samples revealed PCN population from 50-148 eggs g^{-1} soil
04/08/14	Second set of soil samples taken for positioning experiment Soil samples revealed PCN population from 52-157 eggs g^{-1} soil
08/08/14	Field plots marked out and recorded with GPS
11/08/14	**Field experiment planted (previous crop = winter barley)** Pre-planting samples collected
03/09/14	Ammonium nitrate (Nitram®) applied at 60 kg/ha
22/09/14	Tissue samples taken for GSL analysis and sent to the University of York
08/10/14	Tissue samples taken for GSL analysis and sent to the University of York
29/10/14	Tissue samples taken for GSL analysis and sent to the University of York
30/10/14	Soil samples taken for PCN analysis (pre-incorporation) and soil nutrients
05/11/14	**Biofumigant incorporation** Tissue samples taken for GSL analysis, plant density count, assessment of fresh and dry weight
14/01/15	Soil samples for PCN (post incorporation)
14/04/15	Soil samples for PCN (pre-planting potatoes) Soil samples for nutrient analysis
20/04/15	**Potatoes planted in field plots** Nematicide (Vydate® (oxamyl) applied at 55 kg/ha to split plots)

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Table 6: *Inputs made to field experiment 2014-15 (Field Diary) - Lincolnshire site (Howell), investigating the effect of biofumigant species on potato cyst nematode population densities*

Date	Event
31.07.14	Pre-planting soil samples collected sent to Harper Adams for assessment of PCN levels
31.07.14	**Field experiment planted**
09.08.14	Fertiliser applied at a rate 70kg/ha N
11.09.14	Tissue samples taken for GSL analysis and stored in -80 freezer for the University of York (stem, root and leaves)
25.09.14	Tissue samples taken for GSL analysis and stored in -80 freezer for the University of York (stem, root and leaves)
14.10.14	Tissue samples taken for GSL analysis and stored in -80 freezer for the University of York (stem, root and leaves)
14.10.14	Soil samples taken for PCN analysis (pre-incorporation) sent to Harper Adams
15.10.14	**Biofumigant incorporation** 2 x 0.5m² biomass samples taken, assessment of fresh and dry weight
09.12.14	PCN soil samples taken (post incorporation) sent to Harper Adams
23.04.15	PCN soil samples taken (Pre-planting potatoes) sent to Harper Adams Soil samples for nutrient analysis sent to SRUC
23.04.15	**Potatoes planted in field plots** Nematicide (Nemathorin applied at 30 kg/ha to split plots)
21.05.15	YARA MILA grower 1000kg/ha applied to field
24.05.15	Full emergence noted.
30.05.15	Calcium Nitrate 600 kg/ha

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Table 7: *Inputs made to field experiment 2014-15 (Field Diary) - Aberdeenshire site (Ethie Barns), investigating the effect of biofumigant species on potato cyst nematode population densities*

Date	Event
23.05.14	Soil samples taken for PCN pre-sowing of biofumigant crops and sent to HAU for assessment – trial plan laid out in field and samples taken from those plots.
18.06.14	**Biofumigant trial planted**
25.09.14	Tissue samples collected for fresh weight and subsequent GSL analysis at York (stored at -80°C)
28.09.14	**Biofumigant trial flailed and incorporated**
25.11.14	Soil samples taken for PCN post-incorporation and sent to HAU for assessment
08.05.15	Pre-planting PCN samples and nutrient soil samples taken
15.05.15	**Potato crop planted and Vydate applied to split plots**
11.09.15	**Potato crop harvested**
18.09.15	Post-harvest PCN samples taken

Analysis of results

The experimental results presented were analysed using general analysis of variance on Genstat 15th Edition (VSN International). Data collected after nematicide application was treated as a split-plot experiment with biofumigant treatments as the main plots and nematicide treatments as sub-plots. Tukey's multiple range test was used to determine whether significant differences occured between treatment groups.

For all data at the Lincolnshire site, the homogeneity of variance was tested by Bartlett's Test. If this test indicated no homogeneity of variance the transformed values were used for analysis of variance. If still no homogeneity of variance was obtained by the transformation, this transformation was cancelled and the statistical analysis should be treated with caution. Assessment data were then analysed using a two-way analysis of variance (ANOVA) on untransformed and transformed data. The probability of no significant differences occurring between treatment means is calculated as the F probability value (p(F)). A mean comparison test was only performed when the treatment probability of F that is calculated during analysis of variance was significant at the observed significance level specified for the mean comparison test. The mean separation letter "a" is assigned to each treatment mean in an assessment data column when a non-significant treatment P(F) is detected. (Student Newman-Keuls' multiple

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comparison test was applied to separate any treatment differences that may be implied by the ANOVA TEST and these are indicated by a letter test; treatment means with no letters in common are significantly different according to the test initiated at the 95% confidence level.)

3.4 Field experiments to compare and evaluate incorporation methods used for biofumigation when targeting potato cyst nematodes (2015-2016)

Background

A total of three replicated field trials were conducted on land known to be infested with PCN in Fife (**SRUC**), Shropshire (**HAU**) and Lincolnshire (**Barworth Agriculture Ltd**). The aim of these experiments was to compare different sequences of biofumigant incorporation (see Table 8) in relation to their suppression of PCN. Based on previously published data on biofumigation and PCN (e.g. Ngala *et al*., 2014) and results from a previous experiment from this project (Scottish site, 2014-15), Indian mustard (*Brassica juncea*) cv. Caliente 99 was selected for the experiment. Experiments were sown between July and September 2015 with the aim of incorporating in November 2015. The seed rates and nitrogen and sulphur application rates used were based on those recommended by the suppliers (Table 8). Due to a later sowing at the site in West England, the experiment was overwintered and incorporated in the following spring. As a precaution the plots were covered with fleece (two layers of 18g fleece, Brinkman (Horticultural Service) UK Ltd) from the 17th December 2015 until the 16th March 2016 (Figure 6). The Indian mustard was incorporated, according to the treatments described in Table 8, at early flowering. In the following spring, potatoes cv. Desirée were planted over the experimental plots. Nemathorin® (fosthiazate) was applied at planting at 30 kg/ha and the plots were maintained following commercial practice.

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Table 8: *Details of field experiments conducted in Fife (**SRUC**), Shropshire (**HAU**) and Lincolnshire (**Barworth Agriculture Ltd**) in 2015-16 to assess the performance of biofumigant incorporation strategies on the suppression of potato cyst nematodes (*Globodera *spp.)*

Treatment number	Cultivation sequence	Seed rate	Fertilizer rates
1	No cover crop - plots rotovated and rolled	-	-
2	Flail A* + Rotovate	8 kg /Ha	100 kg/ Ha N 25 kg/Ha S
3	Flail B* + Rotovate	8 kg /Ha	100 kg/ Ha N 25 kg/Ha S
4	Flail B + Rotovate + Plough	8 kg /Ha	100 kg/ Ha N 25 kg/Ha S
5	Flail B + Spader	8 kg /Ha	100 kg/ Ha N 25 kg/Ha S
6	Flail B + Spader + Plough	8 kg /Ha	100 kg/ Ha N 25 kg/Ha S
7	Flail B + Disc	8 kg /Ha	100 kg/ Ha N 25 kg/Ha S
8	Flail B + Disc + Plough	8 kg /Ha	100 kg/ Ha N 25 kg/Ha S

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Field diaries (planting date, inputs and sample collection) of each experimental site are shown in Tables 9-11.

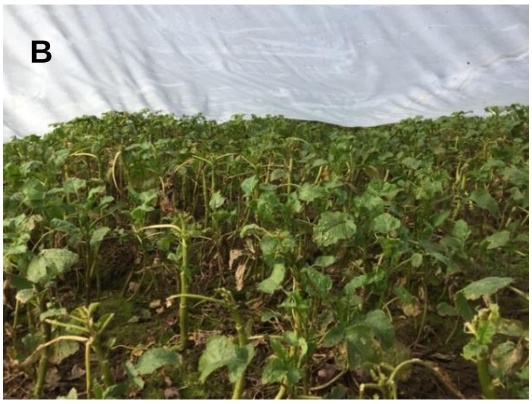

Figure 6: *Fleece used to protect the 2015-16 'cultivations' experiment in Shropshire. Two layers of 18g fleece was used to cover the experimental area on the 17th December 2015 (A). Biofumigants assessed in March 2016 appeared to be free of frost damage (B)*

Experimental design

Treatments were laid out in a randomised block design. Where possible the blocks of the experiments were positioned to account for local variability in PCN population

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densities by using the data on initial PCN populations (*Pi*). Each treatment was replicated 5 times. Plot sizes were 3.6 x 10 m to accommodate 4 potato drills at the later stage of the experiment.

Assessments

The biomass production of each biofumigant was assessed prior to maceration/incorporation by sampling 2 areas of each plot measuring 0.5 m^2. The samples were separated into stem, leaf and root biomass and fresh weight and dry weight was recorded.

Tissue samples (foliage and root) were taken from the biofumigant plots just prior to biomass incorporation. Three replicates of each biofumigant treatment was sampled. For each plot, 5 plants (leaf and stem samples) were carefully collected. To prevent hydrolysis of glucosinolates, the tissue samples were either flash frozen in liquid nitrogen or placed into a -80°C freezer. Samples were then sent to the University of York for analysis.

Soil samples were taken prior to sowing of the biofumigants and before the planting of the potato crop and analysed for nutrients (N, P and K) and organic matter to establish whether the use of biofumigants leads to additional benefits with regards to nutrient and organic matter status of the soil. Soil samples consisted of 4 cores taken with a narrow bladed trowel to a depth of 15 cm following a grid pattern.

Potato cyst nematode densities were sampled from the experimental plots at three time points:

1. pre-sowing of biofumigant crops (*Pi*)
2. pre-incorporation
3. 6 weeks post-incorporation of biofumigants
4. prior to harvesting of the potatoes (*Pf*)

Two sets of pre-incorporation samples were taken at the field site in Shropshire (November 2015 and March 2016) due to the extended growing period of the biofumigant.

Sampling in plots followed a ‘W’ pattern with 40 cores taken to a depth 15-20cm using a cheese corer type auger to provide up to 1kg of soil.

Population density was assessed using standard methods, whilst egg/juvenile viability was estimated using Meldola’s blue stain (Kroese *et al*., 2011).

Following the potato harvest in 2016, tuber number, yield of marketable fractions were assessed in each plot (1m x 2 drills). A macroscopic assessment of tuber blemishing diseases was undertaken on a sample of 25 tubers from each plot. Disease severity was calculated as previously reported.

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Table 9: *Inputs made to field experiment 2015-16 (Field Diary) - Shropshire site (Crudgington), investigating the performance of biofumigant incorporation strategies on the suppression of potato cyst nematodes (*Globodera *spp.)*

Date	Event
20/07/15	Point samples collected on a 20m grid for preliminary PCN analysis
08/09/15	**Field experiment marked out and planted (previous crop = winter barley)** Pre-planting samples (Pi) collected
24/09/15	Fertiliser applied as Single top @120 N/ha (27N, $12SO_3$)
22/09/15	Falcon® (propaquizafop) herbicide applied at 0.5l/ha for barley volunteers
20/11/15	Soil samples taken for PCN analysis (pre-incorporation-1)
26/11/15	Site check
14/12/15	Diquat applied to fallow plots for weeds and volunteers
16/12/15	Slug pellets (ferric phosphate)
17/12/15	Fleece applied to experiment area
6 & 25/01/16	Site check
08/02/16	Site check
16/03/16	Fleece removed
20/04/16	Tissue samples taken for GSL analysis, plant density count, assessment of fresh and dry weight Soil samples for PCN (pre-incorporation-2)
21/04/16	**Biofumigant incorporation**
09/05/16	**Potatoes planted in field plots** Nematicide (Vydate® a.i. oxamyl) applied at 55 kg/ha Soil samples for PCN (pre-planting potatoes/post incorporation)
23/05/16	Pre emergence herbicide – Afalon at 1l/ha, diquat at 2l/ha and Sencorex at 0.5l/ha
09/06/16-01/09/16	Programme for late blight and herbicide management
01/09/16	Canopy desiccation (diquat at 3 l/ha)
07/10/16	**Potatoes harvested**

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Table 10: *Inputs made to field experiment 2015-16 (Field Diary) - Lincolnshire site (Howell fen), investigating the performance of biofumigant incorporation strategies on the suppression of potato cyst nematodes* (Globodera *spp.)*

Date	Event
05/07/15	Prelim PCN point samples taken for siting of trial
03/08/15	Fertilised trial area – Tropicote 15.5N + Calcium YARA MILA 15%N
05/08/15	**Field experiment marked out and planted** Pre-planting PCN samples collected
24/08/15	All plots fully emerged
18/10/15	Pre incorporation PCN samples collected.
18/10/15	Biomass and GLS samples taken
19/10/15	**Biofumigant incorporation**
04/02/16	Soil sampled for PCN (post incorporation).Nutrient samples taken for whole plot and sent to SRUC
13/05/16	**Potatoes planted in field plots** Nematicide (Nemathorin a.i fosthizate) applied at 30 kg/ha Soil samples for PCN (pre-planting potatoes) collected
13/05/16-05/09/16	Programme for late blight and herbicide management
05/09/16	**Potatoes harvested** Soil samples for PCN (post potatoes) (Pf). Split plot A's and B's for storage at Barworth. Whole plots kept at Barworth – for discussion

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Table 11: *Inputs made to field experiment 2015-16 (Field Diary) - Fife site (Culross), investigating the performance of biofumigant incorporation strategies on the suppression of potato cyst nematodes (*Globodera *spp.)*

Date	Event
27/07/15	Point samples collected on a 20m grid for preliminary PCN analysis
10/08/15	**Field experiment marked out and planted (previous crop = winter barley)** Pre-planting PCN samples collected
25/08/15	Fertiliser applied @120 N/ha (27N, $12SO_3$)
22/09/15	Aramo® (tepraloxydim) herbicide applied at 1.0 l/ha for barley volunteers
8/11/15	Tissue samples taken for GSL analysis, plant density count, assessment of fresh and dry weight. Soil samples for PCN (pre-incorporation)
10/11/15	**Biofumigant incorporation**
28/04/16	**Potatoes planted in field plots** Nematicide (Vydate® a.i. oxamyl) applied at 40 kg/ha Soil samples for PCN (pre-planting potatoes/post incorporation)
38/04/16 – 20/10/16	Programme for late blight and herbicide management
20/10/16	**Potatoes harvested**

Analysis of results

The experimental results presented were analysed using a one way analysis of variance on Genstat 15th Edition (VSN International). Tukey's multiple range test was used to determine if significant differences occur between treatment groups.

3.5 Field experiments to evaluate nitrogen and sulphur inputs on Indian mustard (*B. juncea*) cv. Caliente 99 used for suppressing potato cyst nematodes (2016-2017)

Background

A total of three replicated field trials were completed. The sites in Shropshire (**HAU**) and Lincolnshire (**Barworth Agriculture Ltd**) were known to be infested with PCN. The aim of these experiments was to compare the effect of different ratios of nitrogen and sulphur on the glucosinolate accumulation and biomass production of *B. juncea* cv. Caliente 99 and its subsequent effect on potato cyst nematode viability. PCN was not

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present at the site in Fife (**SRUC**) and this experiment was used to assess biomass production/glucosinolate content of the biofumigants.

Experiment design and management

Sites were selected on the basis of having known PCN infestations with the exception of Fife where a PCN infested site was not available. *Brassica juncea* cv. Caliente 99 was sown at a seed rate of 8 kg/ha during August 2016 in experimental plots measuring 3 by 10 metres. Nitrogen (as ammonium nitrate - Nitram) and sulphur (as elemental sulphur) was applied to the plots at the rates shown in Table 12 immediately after sowing. The experiments were set up as a 4 x 3 factorial, randomised block design with 12 treatments and 4 replicates (48 plots).

Table 12: *Details of field experiments conducted in Fife (**SRUC**), Shropshire (**HAU**) and Lincolnshire (**Barworth Agriculture Ltd**) in 2016-17 to assess the effect of nitrogen and sulphur inputs on the biofumigation potential of* Brassica juncea *cv. Caliente 99 and subsequent suppression of potato cyst nematodes (*Globodera *spp.)*

Treatment number	Nitrogen (N) kg/ha	Sulphur (S) kg/ha
1	0	0
2	0	25
3	0	50
4	50	0
5	50	25
6	50	50
7	100	0
8	100	25
9	100	50
10	150	0
11	150	25
12	150	50

Field diaries, showing agronomic inputs for the experimental sites are detailed in Tables 13-15.

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Table 13: *Inputs made to field experiment 2016 (Field Diary) - Shropshire site (Puleston), investigating the effect of nitrogen and sulphur inputs on the biofumigation potential of* Brassica juncea *cv. Caliente 99 and subsequent suppression of potato cyst nematodes (*Globodera *spp.)*

Date	Event
05/08/16	Point samples collected for preliminary PCN analysis
16/08/16	Field experiment marked out
17/08/16	Soil samples collected for PCN and nutrients
18/08/16	**Experiment sprayed (nutrients), cultivated, drilled and rolled**
25/08/16	Site check – even emergence throughout
01+20/09/16	Site checks
06/10/16	Site check – volunteer barley treated with Fusilade Max @ 1l/ha. Spray went on in good conditions
27/10/16	Samples taken – Biomass, GSL analysis and PCN (pre-incorporation)
28/10/16	**Biofumigant incorporation –** flailed, rotovated and rolled
07/12/16	Soil samples taken for PCN (post incorporation)

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Table 14: *Inputs made to field experiment 2016 (Field Diary) - Lincolnshire site investigating the effect of nitrogen and sulphur inputs on the biofumigation potential of* Brassica juncea *cv. Caliente 99 and subsequent suppression of potato cyst nematodes* (Globodera *spp.)*

Date	Event
02/08/16	Point samples collected for preliminary PCN analysis
10/08/16	Cultivated and prepared ground for site
12/08/16	Marked out field experiment. Plots samples for PCN and Nutrient analysis
13/08/16	**Experiment drilled and fertilised**
19/08/16	Site check – full even emergence
+26/09/16 +03/10/16 23/08/17 +10/09/17	Site checks
12/10/16	Samples taken – Biomass, GSL analysis and PCN (pre-incorporation)
13/10/16	**Biofumigant incorporation –** flailed, rotovated and rolled
07/03/17	Soil samples taken for PCN (post incorporation)

Table 15: *Inputs made to field experiment 2016 (Field Diary) - Fife site, investigating the effect of nitrogen and sulphur inputs on the biofumigation potential of* Brassica juncea *cv. Caliente 99.*

Date	Event
19/07/16	Soil samples collected for nutrients
22/07/16	**Experiment sprayed (nutrients), cultivated, drilled and rolled**
28/10/16	Samples taken – Biomass and GSL analysis
31/10/16	**Biofumigant incorporation –** flailed, rotovated and rolled
7/12/16	Soil samples collected for nutrients post-incorporation

Assessments

The biomass production of each biofumigant was assessed prior to maceration/incorporation by sampling 2 areas of each plot measuring 0.5 m^2. The samples were separated into stem, leaf and root biomass and fresh weight and dry weight was recorded.

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Tissue samples (foliage and root) were taken from the biofumigant plots just prior to biomass incorporation. For each plot, 5 plants (leaf and stem samples) were carefully collected. To prevent hydrolysis of glucosinolates, the tissue samples were either flash frozen in liquid nitrogen or placed into a -80°C freezer. Samples were then sent to the University of York for analysis.

Soil samples were taken prior to sowing of the biofumigants and before the planting of the potato crop and analysed for nutrients (N, P and K) and organic matter to establish whether the use of biofumigants leads to additional benefits with regards to nutrient and organic matter status of the soil. Soil samples consisted of 4 cores taken with a narrow bladed trowel to a depth of 15 cm following a grid pattern.

Potato cyst nematode densities were sampled from the experimental plots (not SRUC site) at three time points:

1. pre-sowing of biofumigant crops
2. pre-incorporation
3. 6 weeks post-incorporation of biofumigants

Sampling in plots followed a 'W' pattern with 40 cores taken to a depth 15-20cm using a cheese corer type auger to provide up to 1kg of soil.

Population density was assessed using standard methods, whilst egg/juvenile viability was estimated using Meldola's blue stain (Kroese et al., 2011).

3.6 Estimates for the financial cost of establishing and incorporating a biofumigant crop

To estimate the possible range of costs attributable to a biofumigation crop, two reference publications were used for base lines: The Agricultural Budgeting & Costing Book. No 84 - May 2017; John Nix, Farm Management Pocketbook. 47th edition. By Graham Redman.

The estimated costs for each field task were then validated against figures supplied by four, independent agricultural contractors and grower/contractors, and adjusted accordingly.

3.7 Estimating the carbon footprint associated with biofumigation

This has proved not to be possible with any meaning due to the very nature of 'cover/catch' crops. Estimates can be made for each individual field operation attributed to a specific machine on a specific production unit, applied to a specific field, which would give a very broad range of power usage. In addition, the number of operations to establish a crop can be as few as broadcasting onto a wheat crop two weeks prior to wheat harvest – or as many as a full suite of operations to prepare a fine seed bed.

There is also a considerable discrepancy in the published data for the carbon footprint of the fertilisers that might be used for a biofumigant crop. A further complication is the cost of irrigation.

A more meaningful assessment might be the comparison in costs/savings for a complete rotation that includes biofumigant crops, with one that does not. Potential savings for the complete rotation might include reduced fertiliser use, reduced

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operations due to improved soil structure, reduced pesticides, etc. Of course, this type of assessment would be valid only for an identified production unit.

4. Results

4.1 A poly-tunnel experiment to evaluate biofumigant lines available in Great Britain

Potato cyst nematode viability

Six weeks after the incorporation of biofumigant species a significant reduction ($P<0.001$) in the percentage viability of PCN eggs was recorded. Viability was reduced by 49-65% (based on untreated pots) (Figure 7). However, no significant differences ($P>0.05$) in percentage viability were found between individual species or cultivars of biofumigants. The total number of viable eggs g^{-1} soil was not found to be significantly different between treatments ($P>0.05$).

Fresh weight of biofumigants

Fresh weight of the cultivars differed significantly ($P<0.001$) (Figure 8). *Raphanus sativus* generally had a higher biomass than other species. Vitasso (*Brassica juncea*) produced the highest biomass of all the species tested.

Glucosinolate analysis

Results of glucosinolate analysis are presented in Table 16. Keva (sample 6a) was found to have a profile more closely associated with *S. alba* than *B. juncea*.

Other observations

Powdery mildew (*Erysiphe cruciferarum*) was found abundantly on (*B. juncea*) cultivars, though less frequently on cv. Vitasso. Temperature in the poly-tunnel was high on a number of days (up to 45°C) (Figure 9).

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Table 16: Glucosinolate profile of the biofumigant lines used in the poly-tunnel experiment at Harper Adams University. Data courtesy of Tim Dohney-Adams, University of York

		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

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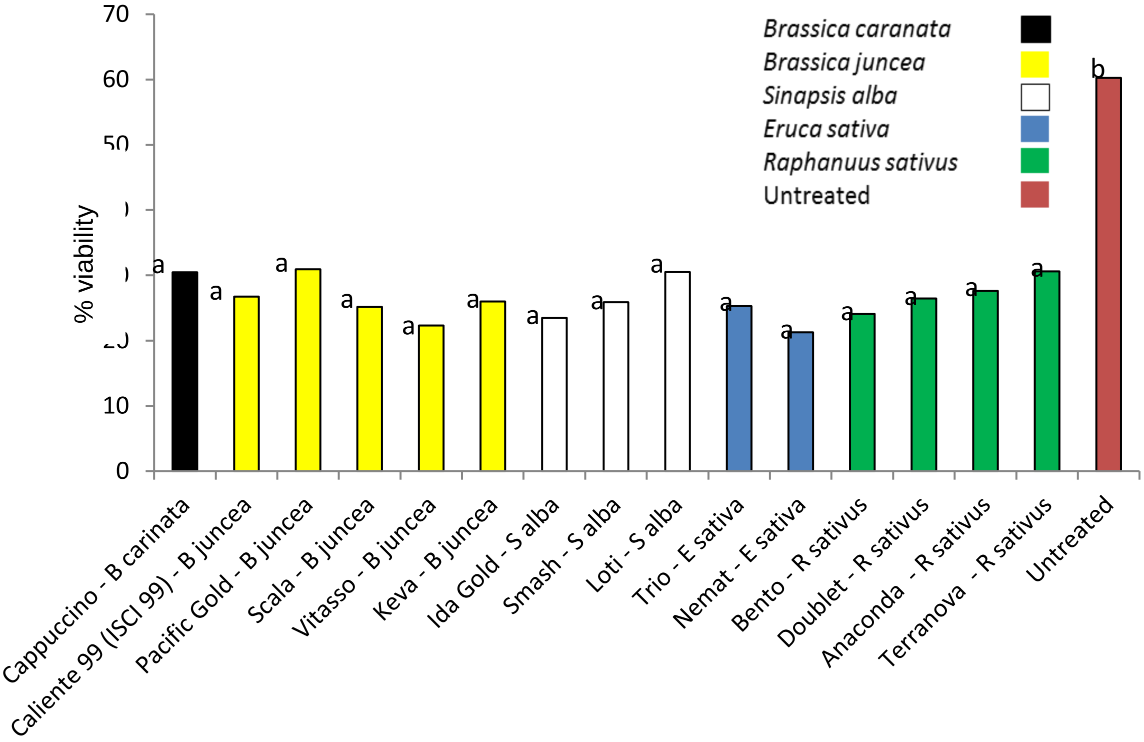

Figure 7: *Percentage viability of potato cyst nematode eggs, six weeks after the incorporation of brassica residues in a poly-tunnel experiment evaluating biofumigant lines available in Great Britain. Letters show significant differences according to Tukey's multiple range test;* P *=0.05. SED = 5.79, %CV = 32 df =79*

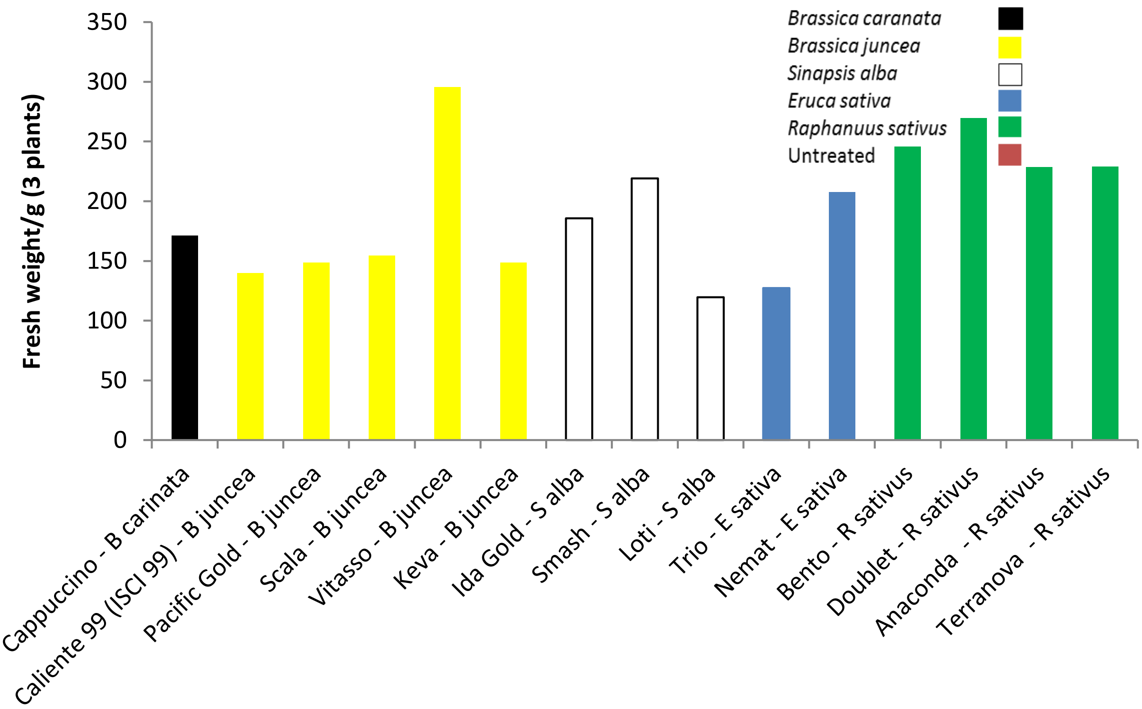

Figure 8: *Fresh weight (g) of biofumigant species grown in a poly-tunnel experiment evaluating biofumigant lines available in Great Britain. SED = 32.19, %CV = 24 df =79*

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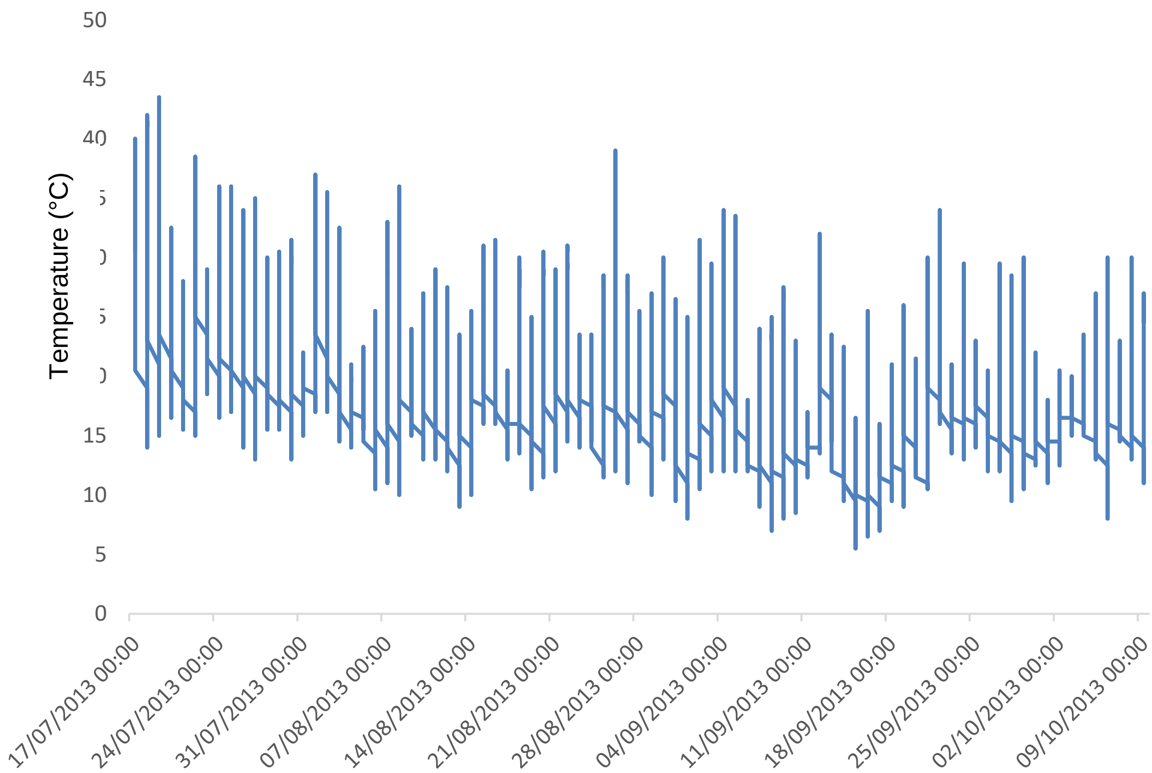

Figure 9: *Soil temperature recorded from in a poly-tunnel experiment evaluating biofumigant lines available in Great Britain*

4.2. Field experiments to evaluate biofumigant species in contrasting geographical locations in Great Britain (2013-2014)

At all sites, brassica biomass was minimal due to the later sowing dates; 26th August - 6th September 2013. The biomass achieved is compared with other data in the discussion. Sowing was delayed at each site due to later harvested winter crops such as oilseed rape. Poor weather in the autumn of 2012 resulted in abnormally late sowing dates and this in turn affected the availability of sites for the experiments.

<u>Shropshire site:</u>

Brassica cover crops (biofumigants) had no significant effect (P>0.05) on the viable potato cyst nematode population densities (viable eggs g^{-1} soil) either pre or post incorporation (Table 17). The same finding was found for % egg viability (P>0.05) (Table 18).

Brassica biomass was generally very low for all of the species assessed (Table 19). No significant differences were found between foliar biomass (fresh and dry weight) (P>0.05). However, significant differences were found between fresh root weights (P=0.004) with *Raphanus sativus* cv. Bento producing the greatest biomass. A similar finding was also observed for root dry weight, with *Raphanus sativus* cv. Bento and *Sinapis alba* cv. Ida-Gold producing the greatest biomass.

Soil was sampled for nutrients, 21 weeks after incorporation of the brassicas.

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Lincolnshire site:

Brassica cover crops (biofumigants) had no significant effect (P>0.05) on potato cyst nematode population densities (eggs g^{-1} soil) either pre or post incorporation (Table 20).

Brassica biomass was generally very low for all of the species assessed (Table 21). Significant differences (P<0.05) were found between foliar and root weights with *Raphanus sativus* cv. Bento producing the greatest biomass.

Total potato yield from field plots which were previously cover cropped with *Brassica juncea* (Caliente 99), *Raphanus sativus* (Bento) *Sinapis alba* (Ida Gold), *Eruca sativa* (Nemat) or left fallow was not significantly different (P>0.05) (Figure 10).

Aberdeenshire site:

Brassica cover crops (biofumigants) had no significant effect (P>0.05) on the viable potato cyst nematode population densities (viable eggs g^{-1} soil) either pre or post incorporation (Table 22).

Brassica biomass was very low for all of the species assessed apart from *Raphanus sativus* cv. Bento radish (Table 23). Significant differences were found between foliar biomass (fresh and dry weight) with the Bento radish and other treatments (P=0.009). Significant differences were found between fresh root weights (P=0.03) with *Raphanus sativus* cv. Bento producing the greatest biomass.

Table 17: *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 2013-14 – Staffordshire site. Samples collected pre-sowing, pre, incorporation and post-incorporation of the brassica cover crops*

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*)	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

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Table 18: *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 2013-14 – Staffordshire site. Samples collected pre-sowing, pre, incorporation and post-incorporation of the brassicas*

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 19: *Fresh and dry weight (g/m^2) of foliar and root biomass from brassica cover crops from field experiment 2013-14 (Staffordshire site) 10 WAP. Letters denote significant differences according to Tukeys multiple range test (P=0.05)*

Treatment	Fresh weight (g/m2) of foliar biomass	Dry weight (g/m2) of foliar biomass	Fresh weight (g/m2) of root biomass	Dry weight (g/m2) of root biomass
Untreated	-	-	-	-
Caliente 99 (*Brassica juncea*)	732	102.74	104a	21.86a
Ida Gold (*Sinapis alba*)	2048	223.04	208ab	46.8b
Bento (*Raphanus sativus*)	1788	188.36	372b	56.68b
Nemat (*Eruca sativa*)	1552	157.6	98a	24.52a
Significance (*P*-value)	NS	NS	0.004	0.019
SED	272.5	22.01	32.9	4.26
%CV	56.3	41.52	53.1	33.97

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Table 20: *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 2013-14 – Lincolnshire site. Samples collected pre-sowing, pre, incorporation and post-incorporation*

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)
Untreated	20.4	5.2	6.2	9.6
Caliente 99 (*Brassica juncea*)	13.8	4.0	7.6	11.2
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

Table 21: *Fresh and dry weight (g/m^2) of foliar and root biomass from brassica cover crops from field experiment 2013-14 (Lincolnshire site) 11 WAP. Letters denote significant differences according to Tukeys multiple range test (P=0.05)*

Treatment	Fresh weight (g/m^2) of foliar biomass	Dry weight (g/m^2) of foliar biomass	Fresh weight (g/m^2) of root biomass	Dry weight (g/m^2) of root biomass
Untreated	-	-	-	-
Caliente 99 (*Brassica juncea*)	794a	106a	115.3b	28.3a
Ida Gold (*Sinapis alba*)	808a	223ab	81.3a	26.5a
Bento (*Raphanus sativus*)	1332b	285b	199.3c	76.6b
Nemat (*Eruca sativa*)	1016ab	115a	92.2ab	18.5a
Significance (*P*-value)	0.012	0.019	<0.001	<0.001
SED	0.15	0.06	10.95	7.37
%CV	23.9	48.1	14.2	31.1

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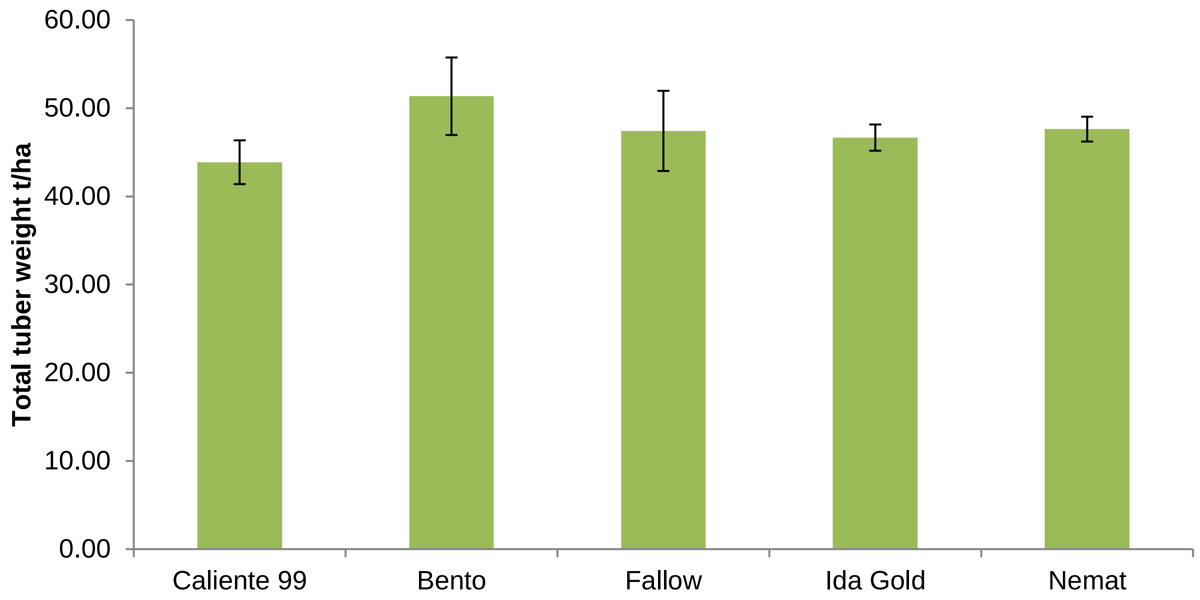

Figure 10: *Potato tuber weight (t/ha) recovered from field plots which were previously cover cropped with* Brassica juncea *(Caliente 99),* Raphanus sativus *(Bento)* Sinapis alba (Ida Gold), Eruca sativa (Nemat) *or left fallow (Field Experiment 2013-14: Lincolnshire site). Error bars show the standard error of the mean (SEM)*

Table 22: *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 2013-14 – Scotland site. Samples collected pre-sowing, pre-incorporation and post-incorporation of the brassica cover crops*

Treatment	Viable eggs g^{-1} soil pre-sowing (September 2013)	Viable eggs g^{-1} soil pre-incorporation (March 2013)	Viable eggs g^{-1} soil post-incorporation (April 2014)
Untreated	14	15	15
Caliente 99 (*Brassica juncea*)	19	17	20
Ida Gold (*Sinapis alba*)	20	20	23
Bento (*Raphanus sativus*)	15	12	9
Nemat (*Eruca sativa*)	33	15	13
Significance (*P*-value)	NS	NS	NS
SED	8.11	8.4	5.79
%CV	49.8	42.2	52.6

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Table 23: *Fresh (g/m^2) of foliar and root biomass from brassica cover crops from field experiment 2013-14 (Scottish site) 10 WAP. Letters denote significant differences according to Tukeys multiple range test (P=0.05)*

Treatment	Fresh weight (g/m^2) of foliar biomass	Fresh weight (g/m^2) of root biomass
Caliente 99 (*Brassica juncea*)	16a	2a
Ida Gold (*Sinapis alba*)	80b	10b
Bento (*Raphanus sativus*)	350c	20b
Nemat (*Eruca sativa*)	24a	2a
Significance (*P*-value)	0.009	0.03
SED	83.5	4.5
%CV	66.7	44.7

4.3 Field experiments to evaluate biofumigant species in contrasting geographical locations in Great Britain (2014-2015)

Shropshire (Crudgington) site:

In general, the 2014 growing season favoured biomass production by biofumigants at the Crudgington site (Shropshire). Biofumigants were sown at an optimal time (11^{th} August) in moist soil conditions. According to the local weather station (Shawbury - meteorological office), there was 89.2mm rainfall and temperatures between 10.2-19.0 °C during August 2014. In contrast, September was much drier with only 17mm of rainfall whilst the temperatures (9.1-19.4°C) were similar to those recorded in August. Biofumigant cover crops were grown for approximately 12 weeks.

Overall, potato cyst nematode population densities appeared to decline over the assessment period (Table 24). No significant differences in viable PCN eggs g^{-1} soil were found between the treatments pre-sowing and post-incorporation of biofumigants or pre-planting of potatoes. However, a significant difference in the viable PCN eggs g^{-1} soil was observed pre-incorporation of the biofumigants ($P<0.05$). According to the least significant difference (LSD = 61.7) all biofumigants except cv. Caliente 99 caused a significant reduction in viable PCN eggs g^{-1} soil when compared to the fallow (untreated) plots, with reductions ranging between 46 - 59%. Two-way analysis of variance (Table 25) based on assessments taken from pre-incorporation to pre-planting of potatoes indicated a significant difference between treatments (P=0.011) and the sampling time (P=0.013). Tukey's post hoc test indicated that cultivation and incorporation of both *R. sativus* cv. Bento and *B juncea* cv. Scala caused significant reductions in viable PCN eggs g^{-1} soil when compared to the fallow treatment.

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Biofumigant treatments appeared to be causing a reduction in the percentage of viable PCN eggs (Table 26). However, one way analysis of variance did not show any significant differences in the percentage of viable PCN eggs between the treatments pre-sowing, pre-incorporation and post-incorporation of biofumigants and pre-planting of potatoes. Two-way analysis of variance (Table 27) based on assessments taken from pre-incorporation to pre-planting of potatoes indicated a significant difference between treatments (P=0.014) and the sampling time (P<0.001). Tukey's post hoc test indicated that cultivation and incorporation of *E. sativa* cv. Nemat caused significant reductions in % of viable PCN eggs and viable PCN eggs g^{-1} soil when compared to the fallow treatment.

One way analysis of variance did not show any significant differences in the number of viable eggs $cyst^{-1}$ between the treatments pre-sowing, pre-incorporation and post-incorporation of biofumigants and pre-planting of potatoes (Table 28). Two-way analysis of variance (Table 29) based on assessments taken from pre-incorporation to pre-planting of potatoes indicated a significant difference between treatments (P=0.033) and the sampling time (P=0.002). Tukey's post hoc test indicated that cultivation and incorporation of *R. sativus* cv. Bento caused significant reductions in viable PCN eggs $cyst^{-1}$ when compared to the fallow treatment.

General analysis of variance (two factors, split plot design) for the number of viable PCN eggs g^{-1} soil and the viable eggs $cyst^{-1}$, sampled post potato production (Pf), revealed no differences between biofumigant or nematicide treatments (Tables 30 and 32). However, the same analysis conducted on the percentage of viable PCN eggs highlighted a significant difference (P = 0.014) between plots treated and untreated with the nematicide Vydate® (Oxamyl) (Table 31). Surprisingly, the viability of eggs appeared to be slightly higher in Vydate® treated plots. The multiplication rate (Pf/Pi), however, was not found to differ significantly between treatments (Table 33).

As might be expected, the fresh weight of biomass produced by the biofumigant lines varied quite considerably, particularly where different tissue types were compared (see Table 34). Significant differences were observed between leaf (P=0.007) and root tissue (P=0.005) but no difference was found between the fresh weight of stems for the treatments studied (P>0.05). *Eruca sativa* cv. Nemat was found to produce notably higher leaf and root biomass than the other biofumigants and this was significant according to Tukey's post hoc test.

The dry weight of biomass produced by the biofumigant lines was less divergent than fresh weights (Table 35). No significant differences were found between the dry weight of stem and leaf tissue (P>0.05) but a significant difference was found between root biomass (P=0.028) for the treatments studied.

Glucosinolate values are presented in Table 36. In general, dominant glucosinolates found in the foliage i.e. sinigrin in *B. juncea*, sinalbin in *S. alba*, glucoraphanin in oilseed radish, had higher concentrations than observed in the polytunnel study.

Blemishing diseases (black scurf, black dot, silver scurf and common scab) found on the progeny tubers (Desirée) were generally unaffected by biofumigant and nematicide treatments, with the exception of black dot disease severity (DSI), which was significantly (P=0.001) higher in Vydate® treated plots (Tables 37-44).

As might be expected, potato tuber yield (total and the 45-65mm fraction) was significantly higher in nematicide treated than untreated plots (P<0.05) (Tables 46 and 48; Figure 11). Biofumigant treatments were not found to significantly affect either tuber

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yield or number (Tables 45-48) whereas the application of nematicide did not affect tuber number (Tables 45 and 47).

Table 24: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Samples collected pre-sowing, pre-incorporation, post-incorporation of the brassica cover crops and pre-planting of potatoes*

Treatment	Viable eggs g^{-1} soil pre-sowing (August 2014)	Viable eggs g^{-1} soil pre-incorporation (October 2014)	Viable eggs g^{-1} soil post-incorporation (January 2015)	Viable eggs g^{-1} soil pre-planting potatoes (April 2015)
Untreated	127	153.2	70.4	108.4
Caliente 99 (*Brassica juncea*)	115	115.8	61.7	89.6
Scala (*Brassica juncea*)	76	82.3	45.5	58.9
Ida Gold (*Sinapis alba*)	125	62.6	80.1	84.4
Bento (*Raphanus sativus*)	99	67.9	52.7	54.3
Nemat (*Eruca sativa*)	116	80.1	47.0	77.4
Significance (*P*-value)	**NS**	**0.05**	**NS**	**NS**
SED	**36.9**	**29.71**	**22.3**	**31.6**
%CV	**53.2**	**50.2**	**59.2**	**63.4**

Table 25: *Two way analysis of variance of viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Based on data collected pre and post-incorporation of the brassica cover crops and pre-planting of potatoes. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Mean viable eggs g^{-1} soil (pre-incorporation to pre-planting potatoes)
Untreated	110.6a
Caliente 99 (*Brassica juncea*)	89.0ab
Scala (*Brassica juncea*)	62.3b
Ida Gold (*Sinapis alba*)	75.7ab
Bento (*Raphanus sativus*)	58.3b
Nemat (*Eruca sativa*)	68.1ab
Significance (*P*-value) *-Treatment*	**0.013**
Significance (*P*-value) *-Sampling time*	**0.011**
Significance (*P*-value) *-Treatment * sampling time*	**0.564**
SED (treatment)	**36.9**
%CV	**53.2**

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Table 26: *The viability of potato cyst nematode eggs (%) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Samples collected pre-sowing, pre-incorporation, post-incorporation of the brassica cover crops and pre-planting of potatoes*

Treatment	% PCN eggs viable pre-sowing (August 2014)	% PCN eggs viable pre-incorporation (October 2014)	% PCN eggs viable post-incorporation (January 2015)	% PCN eggs viable pre-planting potatoes (April 2015)
Untreated	81.7	79.9	63.0	72.8
Caliente 99 (*Brassica juncea*)	82.0	68.8	46.8	59.3
Scala (*Brassica juncea*)	80.0	69.5	57.4	55.8
Ida Gold (*Sinapis alba*)	79.3	65.0	57.5	62.3
Bento (*Raphanus sativus*)	81.6	64.1	50.5	58.5
Nemat (*Eruca sativa*)	84.4	58.0	46.5	55.9
Significance (*P*-value)	**NS**	**NS**	**NS**	**NS**
SED	**4.26**	**7.96**	**6.76**	**10.61**
%CV	**8.3**	**18.6**	**19.9**	**27.6**

Table 27: *Two way analysis of variance of the viability of potato cyst nematode eggs (%) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Based on data collected pre and post-incorporation of the brassica cover crops and pre-planting of potatoes. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	% PCN eggs viable (pre-incorporation to pre-planting potatoes)
Untreated	71.9 a
Caliente 99 (*Brassica juncea*)	58.3 ab
Scala (*Brassica juncea*)	60.9 ab
Ida Gold (*Sinapis alba*)	61.6 ab
Bento (*Raphanus sativus*)	57.6 ab
Nemat (*Eruca sativa*)	53.5 b
Significance (*P*-value) *-Treatment*	**0.014**
Significance (*P*-value) *-Sampling time*	**<0.001**
Significance (*P*-value) *-Treatment * sampling time*	**NS**
SED (treatment)	**5.00**
%CV	**22.6**

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Table 28: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Samples collected pre-sowing, pre-incorporation, post-incorporation of the brassica cover crops and pre-planting of potatoes*

Treatment	Number of viable potato cyst nematode eggs cyst^{-1} pre-sowing (August 2014)	Number of viable potato cyst nematode eggs cyst^{-1} pre-incorporation (October 2014)	Number of viable potato cyst nematode eggs cyst^{-1} post-incorporation (January 2015)	Number of viable potato cyst nematode eggs cyst^{-1} pre-planting potatoes (April 2015)
Untreated	171	200	96.4	131
Caliente 99 (*Brassica juncea*)	159	155	88.8	112
Scala (*Brassica juncea*)	129	125	83.8	84
Ida Gold (*Sinapis alba*)	176	100	107.0	103
Bento (*Raphanus sativus*)	161	106	79.6	77
Nemat (*Eruca sativa*)	164	109	63.2	101
Significance (*P*-value)	**NS**	**NS (0.071)**	**NS**	**NS**
SED	**40.9**	**35.0**	**23.55**	**38.4**
%CV	**40.4**	**41.7**	**43.1**	**60**

Table 29: *Two way analysis of variance of the number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Based on data collected pre and post-incorporation of the brassica cover crops and pre-planting of potatoes. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Number of viable potato cyst nematode eggs cyst^{-1} (pre-incorporation to pre-planting potatoes)
Untreated	142.5 a
Caliente 99 (*Brassica juncea*)	118.6 ab
Scala (*Brassica juncea*)	97.5 ab
Ida Gold (*Sinapis alba*)	103.3 ab
Bento (*Raphanus sativus*)	87.5 b
Nemat (*Eruca sativa*)	91.8 ab
Significance (*P*-value) *-Treatment*	**0.033**
Significance (*P*-value) *-Sampling time*	**0.002**
Significance (*P*-value) *-Treatment * sampling time*	**NS**
SED (treatment)	**18.15**
%CV	**46.6**

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Table 30: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Samples collected post sampling of potatoes (Pf). Potato plots were split and either treated with Vydate (oxamyl) or left untreated*

Biofumigant	No Vydate	Vydate	Mean
Untreated	508	322	415
Caliente 99 (*Brassica juncea*)	444	502	473
Scala (*Brassica juncea*)	583	305	444
Ida Gold (*Sinapis alba*)	455	494	474
Bento (*Raphanus sativus*)	702	578	640
Nemat (*Eruca sativa*)	414	435	425
Mean	518	439	
Grand Mean	479		
Significance (P-value): Biofumigant (B)	0.505	SED: Biofumigant	123.8
Significance (P-value): Nematicide (N)	0.172	SED: Nematicide	55.6
Significance (P-value):B*N	0.419	SED: B*N	156.9
CV%	45		

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Table 31: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Samples collected post sampling of potatoes. Potato plots were split and either treated with Vydate (oxamyl) or left untreated. Values shown were transformed using ArcSin. Values in parentheses are the actual means*

Biofumigant	No Vydate	Vydate	Mean
Untreated	86.07 (98.98)	85.36 (99.14)	85.72 (99.06)
Caliente 99 (*Brassica juncea*)	85.26 (99.09)	88.17 (99.75)	86.72 (99.42)
Scala (*Brassica juncea*)	83.33 (98.22)	90.00 (100)	86.66 (99.11)
Ida Gold (*Sinapis alba*)	86.42 (99.02)	88.89 (99.81)	87.66 (99.42)
Bento (*Raphanus sativus*)	88.02 (99.70)	85.83 (99.05)	86.93 (99.38)
Nemat (*Eruca sativa*)	83.96 (97.89)	88.10 (99.45)	86.03 (98.67)
Mean	85.51 (98.82)	87.73 (99.53)	
Grand Mean	86.62 (99.18)		
Significance (P-value): Biofumigant (B)	0.931	SED: Biofumigant	1.910
Significance (P-value): Nematicide (N)	0.014	SED: Nematicide	0.839
Significance (P-value):B*N	0.061	SED: B*N	2.400
CV%	3.8		

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Table 32: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Samples collected post sampling of potatoes. Potato plots were split and either treated with Vydate (oxamyl) or left untreated*

Biofumigant	No Vydate	Vydate	Mean
Untreated	210.8	161.0	185.9
Caliente 99 (*Brassica juncea*)	193.4	265.4	229.4
Scala (*Brassica juncea*)	235.6	217.6	226.6
Ida Gold (*Sinapis alba*)	216.2	236.8	226.5
Bento (*Raphanus sativus*)	259.6	271.8	265.7
Nemat (*Eruca sativa*)	194.2	252.0	223.1
Mean	218.3	234.1	
Grand Mean	226.2		
Significance (P-value): Biofumigant (B)	0.690	SED: Biofumigant	45.68
Significance (P-value): Nematicide (N)	0.353	SED: Nematicide	16.68
Significance (P-value):B*N	0.319	SED: B*N	54.05
CV%	28.6		

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Table 33: *Multiplication rates of potato cyst nematodes (Pf/Pi) from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Shropshire site. Potato plots were split and either treated with Vydate (oxamyl) or left untreated*

Biofumigant	No Vydate	Vydate	Mean
Untreated	4.9	3.4	4.1
Caliente 99 (*Brassica juncea*)	9.4	10.9	10.2
Scala (*Brassica juncea*)	34.4	17.4	25.9
Ida Gold (*Sinapis alba*)	7.8	12.6	10.2
Bento (*Raphanus sativus*)	32.7	26.6	29.7
Nemat (*Eruca sativa*)	43.6	45.9	44.8
Mean	22.1	19.5	
Grand Mean	20.8		
Significance (P-value): Biofumigant (B)	0.704	SED: Biofumigant	28.18
Significance (P-value): Nematicide (N)	0.376	SED: Nematicide	2.96
Significance (P-value):B*N	0.338	SED: B*N	28.64
CV%	55.0		

Table 34: *Fresh weight (g/m²) of foliar and root biomass from brassica cover crops from field experiment 2014-15 (Shropshire site) 12 WAP. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Fresh weight - leaves (g/m²)	Fresh weight - stems (g/m²)	Fresh weight - roots (g/m²)	Total fresh weight (g/m²)
Untreated	-	-	-	-
Caliente 99 (*Brassica juncea*)	539.5 a	970.3	210.2 b	1720.0
Scala (*Brassica juncea*)	505.7 a	807.8	152.0 ab	1465.6
Ida Gold (*Sinapis alba*)	491.4 a	652.8	83.1 a	1227.2
Bento (*Raphanus sativus*)	1077.4 ab	955.7	220.3 b	2253.4
Nemat (*Eruca sativa*)	1416.76 b	917.2	231.6 b	2565.5
Significance (*P*-value)	**0.007**	**NS**	**0.005**	**0.041**
SED	**259.4**	**171.07**	**37.26**	**437.34**
%CV	**50.9**	**31.4**	**32.8**	**37.5**

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Table 35: *Dry weight (g/m²) of foliar and root biomass from brassica cover crops from field experiment 2014-15 (Shropshire site) 12 WAP. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Dry weight - leaves (g/m²)	Dry weight - stems (g/m²)	Dry weight - roots (g/m²)	Total dry weight (g/m²)
Untreated	-	-	-	-
Caliente 99 (*Brassica juncea*)	68.7	140.7	45.5 b	254.9
Scala (*Brassica juncea*)	77.3	152.9	34.2 ab	264.4
Ida Gold (*Sinapis alba*)	115.2	170.8	23.4 a	309.4
Bento (*Raphanus sativus*)	110.5	101.4	42.6 ab	254.6
Nemat (*Eruca sativa*)	138.4	134.1	45.0 b	317.4
Significance (*P*-value)	**NS**	**NS**	**0.028**	**NS**
SED	**26.2**	**21.88**	**7.01**	**51.66**
%CV	**41.6**	**24.7**	**29.1**	**29.2**

Table 36: *Glucosinolate type and concentration in the leaves, stems and roots of* Brassica juncea *(a),* Sinapsis alba *(b),* Raphanus sativus *(c) and* Eruca sativa *(d) sampled 12 WAP field experiment 2014-15 (Shropshire site). Values are typically mean values of 5 replicates*

(a)

		Glucosinolate content (µmol g^{-1} dry weight)	
Species & cultivar	**Tissue type**	**Sinigrin**	**Gluconasturtiin**
***B. juncea* Caliente 99**	*Leaf*	35.75	0.57
	stem	9.73	0.47
	roots	2.53	4.44
***B. juncea* Scala**	*Leaf*	30.69	0.35
	stem	4.30	0.23
	roots	2.03	3.31

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(b)

Species & cultivar	Tissue type	Glucosinolate content (µmol g^{-1} dry weight) Sinalbin	Glucotropaeolin	Glucobrassicin	Gluconasturtiin
S. alba **Ida Gold**	*Leaf*	42.83	3.68	1.38	0.13
	stem	6.12	0.30	0.17	0.24
	roots	9.93	0.87	0.39	3.84

(c)

Species & cultivar	Tissue type	Glucosinolate content (µmol g^{-1} dry weight) Glucoraphanin	Glucoraphasatin	Glucobrassicin
R. sativus **Bento**	*Leaf*	16.00	1.87	1.98
	stem	3.83	4.65	1.52
	roots	0.99	13.85	1.07

(d)

Species & cultivar	Tissue type	Glucosinolate content (µmol g^{-1} dry weight) Glucoraphanin	Glucoerucin	Mercaptobutyl GSL
E. sativa **Nemat**	*Leaf*	3.34	1.13	0.47
	stem	1.13	1.73	0.12
	roots	0.69	2.56	0

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Table 37: *The incidence of black dot (*Colletotrichum coccodes*) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site). Data transformed using ArcSin – Actual means in parentheses*

Biofumigant	No Vydate	Vydate	Mean
Untreated	22.78 (15.00)	23.03 (15.33)	22.90 (15.17)
Caliente 99 (*Brassica juncea*)	23.30 (14.60)	23.68 (16.13)	23.06 (15.37)
Scala (*Brassica juncea*)	21.79 (14.00)	23.46 (15.87)	22.63 (14.93)
Ida Gold (*Sinapis alba*)	23.19 (15.53)	23.52 (15.93)	23.36 (15.73)
Bento (*Raphanus sativus*)	23.30 (15.67)	23.21 (15.53)	23.26 (15.60)
Nemat (*Eruca sativa*)	22.37 (14.53)	22.60 (14.80)	22.49 (14.67)
Mean	22.65 (14.89)	23.25 (15.60)	
Grand Mean	22.95 (15.24)		
Significance (P-value): Biofumigant (B)	0.818	**SED: Biofumigant**	0.737
Significance (P-value): Nematicide (N)	0.051	**SED: Nematicide**	0.294
Significance (P-value):B*N	0.490	**SED: B*N**	0.896
CV%	5.0		

Table 38: *Black dot (*Colletotricum coccodes*) severity (disease index) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site)*

Biofumigant	No Vydate	Vydate	Mean
Untreated	53.5	66.7	60.1
Caliente 99 (*Brassica juncea*)	64.7	79.1	71.9
Scala (*Brassica juncea*)	67.4	86.9	77.2
Ida Gold (*Sinapis alba*)	73.8	76.5	75.2
Bento (*Raphanus sativus*)	78.9	75.6	77.3
Nemat (*Eruca sativa*)	57.6	70.1	63.8
Mean	66.0	75.8	
Grand Mean	70.9		
Significance (P-value): Biofumigant (B)	0.202	SED: Biofumigant	8.11
Significance (P-value): Nematicide (N)	0.001	SED: Nematicide	2.73
Significance (P-value):B*N	0.199	SED: B*N	9.39
CV%	14.9		

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Table 39: *Black scurf (*Rhizoctonia solani*) incidence on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site). Data transformed using ArcSin – Actual means in parentheses*

Biofumigant	No Vydate	Vydate	Mean
Untreated	9.76 (3.33)	9.49 (3.60)	9.62 (3.47)
Caliente 99 (*Brassica juncea*)	8.55 (3.00)	11.78 (5.07)	10.16 (4.03)
Scala (*Brassica juncea*)	13.31 (5.93)	11.13 (5.13)	12.22 (5.53)
Ida Gold (*Sinapis alba*)	7.07 (2.13)	6.99 (2.07)	7.03 (2.10)
Bento (*Raphanus sativus*)	9.35 (3.00)	9.42 (3.53)	9.38 (3.27)
Nemat (*Eruca sativa*)	11.97 (4.67)	7.13 (1.93)	9.55 (3.30)
Mean	10.00 (3.68)	9.32 (3.56)	
Grand Mean	9.66 (3.62)		
Significance (P-value): Biofumigant (B)	0.309	SED: Biofumigant	2.073
Significance (P-value): Nematicide (N)	0.634	SED: Nematicide	1.407
Significance (P-value):B*N	0.696	SED: B*N	3.199
CV%	56.4		

Table 40: *Black scurf (*Rhizoctonia solani*) severity (disease index) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site)*

Biofumigant	No Vydate	Vydate	Mean
Untreated	5.53	5.33	5.43
Caliente 99 (*Brassica juncea*)	4.00	7.00	5.50
Scala (*Brassica juncea*)	14.20	10.60	12.40
Ida Gold (*Sinapis alba*)	4.13	3.80	3.97
Bento (*Raphanus sativus*)	4.20	7.47	5.83
Nemat (*Eruca sativa*)	8.53	4.07	6.30
Mean	6.77	6.38	
Grand Mean	6.57		
Significance (P-value): Biofumigant (B)	0.080	SED: Biofumigant	2.742
Significance (P-value): Nematicide (N)	0.803	SED: Nematicide	1.543
Significance (P-value):B*N	0.611	SED: B*N	3.829
CV%	90.9		

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Table 41: *Common scab (*Streptomyces *spp.) incidence on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site). Data transformed using ArcSin – Actual means in parentheses*

Biofumigant	No Vydate	Vydate	Mean
Untreated	21.89 (13.93)	22.44 (14.60)	22.16 (14.27)
Caliente 99 (*Brassica juncea*)	23.15 (15.47)	22.47 (14.67)	22.81 (15.07)
Scala (*Brassica juncea*)	20.80 (12.93)	22.69 (14.93)	21.74 (13.93)
Ida Gold (*Sinapis alba*)	22.99 (15.27)	22.22 (14.33)	22.61 (14.80)
Bento (*Raphanus sativus*)	22.01 (14.07)	22.62 (14.80)	22.32 (14.43)
Nemat (*Eruca sativa*)	22.03 (14.13)	23.26 (15.60)	22.64 (14.87)
Mean	22.15 (14.30)	22.62 (14.82)	
Grand Mean	22.38 (14.56)		
Significance (P-value): Biofumigant (B)	0.579	**SED: Biofumigant**	0.627
Significance (P-value): Nematicide (N)	0.367	**SED: Nematicide**	0.503
Significance (P-value):B*N	0.610	**SED: B*N**	1.073
CV%	8.7		

Table 42: *Common scab (*Streptomyces *spp.) severity (disease index) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site)*

Biofumigant	No Vydate	Vydate	Mean
Untreated	34.5	37.6	36.0
Caliente 99 (*Brassica juncea*)	35.7	33.0	34.3
Scala (*Brassica juncea*)	36.7	41.8	39.3
Ida Gold (*Sinapis alba*)	45.0	40.9	43.0
Bento (*Raphanus sativus*)	32.7	44.8	38.7
Nemat (*Eruca sativa*)	40.6	49.8	45.2
Mean	37.5	41.3	
Grand Mean	39.4		
Significance (P-value): Biofumigant (B)	0.077	SED: Biofumigant	3.76
Significance (P-value): Nematicide (N)	0.308	SED: Nematicide	3.65
Significance (P-value):B*N	0.764	SED: B*N	7.35
CV%	35.8		

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Table 43: *Silver scurf (*Helminthosporium solani*) incidence on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site). Data transformed using ArcSin – Actual means in parentheses*

Biofumigant	No Vydate	Vydate	Mean
Untreated	5.73 (1.13)	1.32 (0.13)	3.53 (0.63)
Caliente 99 (*Brassica juncea*)	3.47 (0.53)	5.28 (0.93)	4.38 (0.73)
Scala (*Brassica juncea*)	2.92 (0.47)	2.75 (0.40)	2.84 (0.43)
Ida Gold (*Sinapis alba*)	6.36 (1.80)	4.05 (0.87)	5.20 (1.33)
Bento (*Raphanus sativus*)	0.94 (0.13)	3.36 (0.67)	2.15 (0.40)
Nemat (*Eruca sativa*)	4.63 (1.27)	5.19 (1.20)	4.91 (1.23)
Mean	4.01 (0.89)	3.66 (0.70)	
Grand Mean	3.83 (0.79)		
Significance (P-value): Biofumigant (B)	0.307	SED: Biofumigant	1.500
Significance (P-value): Nematicide (N)	0.649	SED: Nematicide	0.759
Significance (P-value):B*N	0.126	SED: B*N	1.995
CV%	76.7		

Table 44: *Silver scurf (*Helminthosporium solani*) severity (disease index) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site)*

Biofumigant	No Vydate	Vydate	Mean
Untreated	2.13	0.33	1.23
Caliente 99 (*Brassica juncea*)	0.67	1.33	1.00
Scala (*Brassica juncea*)	0.67	0.67	0.67
Ida Gold (*Sinapis alba*)	4.27	1.33	2.80
Bento (*Raphanus sativus*)	0.27	2.20	1.23
Nemat (*Eruca sativa*)	2.13	2.07	2.10
Mean	1.69	1.32	
Grand Mean	1.51		
Significance (P-value): Biofumigant (B)	0.240	SED: Biofumigant	0.920
Significance (P-value): Nematicide (N)	0.528	SED: Nematicide	0.572
Significance (P-value):B*N	0.212	SED: B*N	1.352
CV%	147.2		

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Table 45: *Total tubers/m² produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site).*

Biofumigant	No Vydate	Vydate	Mean
Untreated	28.48	27.27	27.87
Caliente 99 (*Brassica juncea*)	29.00	28.37	28.69
Scala (*Brassica juncea*)	27.85	29.22	28.54
Ida Gold (*Sinapis alba*)	28.74	28.85	28.80
Bento (*Raphanus sativus*)	25.59	27.15	26.37
Nemat (*Eruca sativa*)	26.89	29.63	28.26
Mean	27.76	28.41	
Grand Mean	28.09		
Significance (P-value): Biofumigant (B)	0.943	SED: Biofumigant	2.641
Significance (P-value): Nematicide (N)	0.485	SED: Nematicide	0.924
Significance (P-value):B*N	0.820	SED: B*N	3.088
CV%	12.7		

Table 46: *Total yield t/ha produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site)*

Biofumigant	No Vydate	Vydate	Mean
Untreated	26.10	31.57	28.83
Caliente 99 (*Brassica juncea*)	32.90	40.88	36.89
Scala (*Brassica juncea*)	31.33	38.56	34.95
Ida Gold (*Sinapis alba*)	29.53	43.33	36.43
Bento (*Raphanus sativus*)	31.10	33.86	32.48
Nemat (*Eruca sativa*)	29.91	41.70	35.81
Mean	30.14	38.32	
Grand Mean	34.23		
Significance (P-value): Biofumigant (B)	0.607	SED: Biofumigant	5.068
Significance (P-value): Nematicide (N)	<0.001	SED: Nematicide	1.665
Significance (P-value):B*N	0.445	SED: B*N	5.831
CV%	18.8		

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Table 47: *Total tubers/m² at 45-65mm produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site)*

Biofumigant	No Vydate	Vydate	Mean
Untreated	16.93	15.16	16.04
Caliente 99 (*Brassica juncea*)	16.78	16.52	16.65
Scala (*Brassica juncea*)	17.11	17.63	17.37
Ida Gold (*Sinapis alba*)	16.52	18.89	17.70
Bento (*Raphanus sativus*)	15.15	16.37	15.76
Nemat (*Eruca sativa*)	18.85	18.19	18.52
Mean	16.89	17.12	
Grand Mean	17.01		
Significance (P-value): Biofumigant (B)	1.999	SED: Biofumigant	0.734
Significance (P-value): Nematicide (N)	0.759	SED: Nematicide	0.759
Significance (P-value):B*N	2.392	SED: B*N	0.687
CV%	17.3		

Table 48: *Total weight (t/ha) of potatoes cv. Desirée at 45-65mm grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site)*

Biofumigant	No Vydate	Vydate	Mean
Untreated	18.37	21.26	19.81
Caliente 99 (*Brassica juncea*)	21.59	24.75	23.17
Scala (*Brassica juncea*)	21.04	23.87	22.45
Ida Gold (*Sinapis alba*)	21.21	26.47	23.84
Bento (*Raphanus sativus*)	22.68	22.67	22.67
Nemat (*Eruca sativa*)	22.70	26.81	24.76
Mean	21.27	24.30	
Grand Mean	22.78		
Significance (P-value): Biofumigant (B)	0.766	SED: Biofumigant	3.329
Significance (P-value): Nematicide (N)	0.002	SED: Nematicide	0.859
Significance (P-value):B*N	0.629	SED: B*N	3.646
CV%	14.6		

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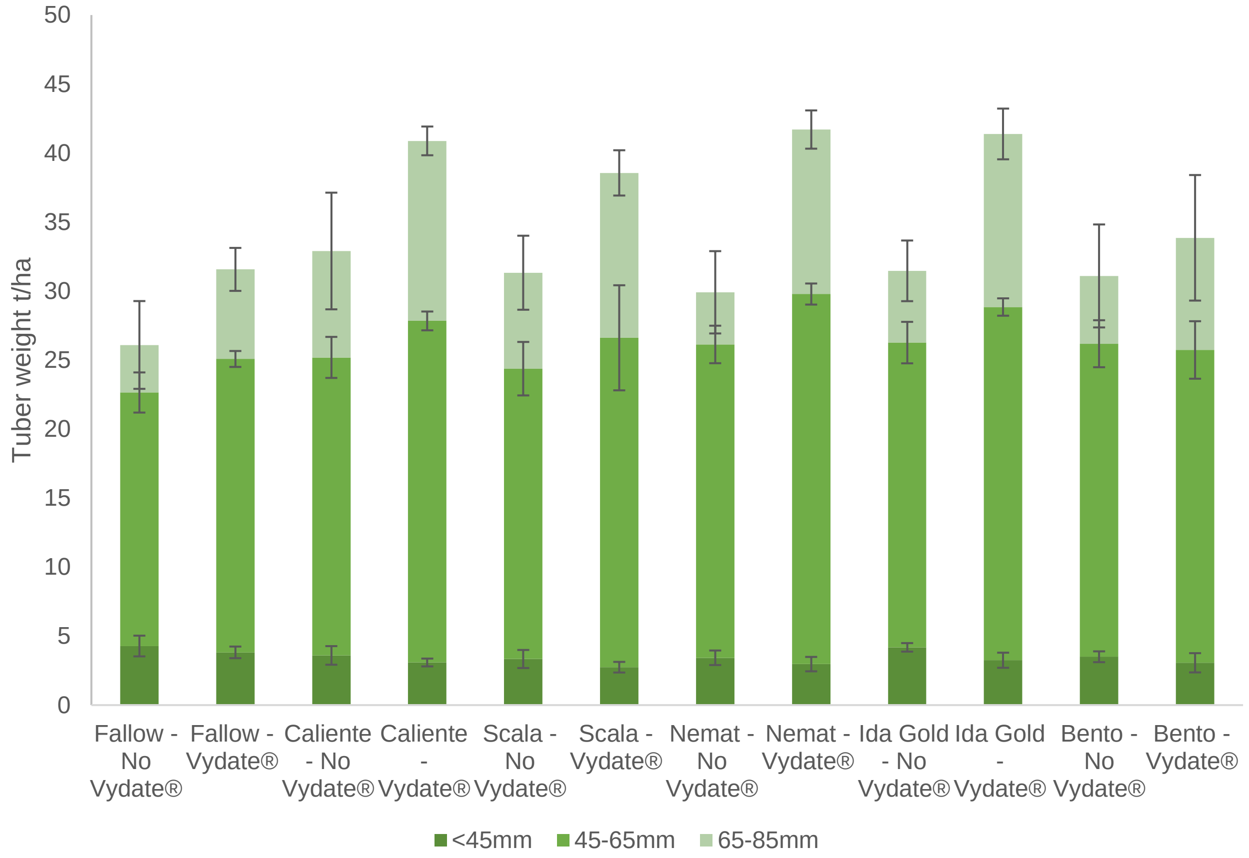

Figure 11: *Yield fractions (t/ha) of potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Shropshire site). Error bars show the standard error of the mean*

Lincolnshire site:

In August, the local weather station recorded 61.6mm rainfall and temperatures between 11.5-20.0°C. In contrast, September was much drier with only 9.6mm of rainfall whilst the temperatures remained at 11-19.2°C. The tinytag datalogger at the field site recorded daytime soil temperatures on the day of planting at 27°C; the night time soil temperatures dropping to 17°C. Throughout August, daytime soil temperatures were between 18-26°C and night time soil temperatures between 8-14°C. Biofumigant cover crops were grown for approximately 10 weeks.

There was a moderately consistent decrease in percentage viability of eggs (Table 50) over the growing period of the biofumigant crop. From a statistical analysis stand-point, there was no effect on the number of viable PCN eggs g^{-1} soil or eggs $cyst^{-1}$ by any of the treatments (Tables 49/51 and 52 respectively). The use of the nematicide Nemathorin (fosthiazate) significantly reduced the final population (*Pf*) of PCN post potatoes (*P*<0.001) (Table 55).

Of particular relevance to this site is the soil type. Much of our previous work has been undertaken on loams, predominantly silty loam and some on high organic soils. This site is very much a sand and although the crops were successful and above ground

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biomass was high, much of the highly volatile ITCs may have been lost to the atmosphere at maceration/incorporation.

This has previously been an issue with chemical fumigants such as Telone (1,3 dichloropropene). In addition, the maceration was particularly effective with the plant material being reduced to a more amorphous state before incorporation with the intention of maximising the effect of the flail operation. Forward speed of the equipment was slow and the hammers were nearly new. Although the accumulated biomass for each of the plant treatments reflected well grown commercial crops (*R. sativus* cv. Bento yielded greater than 80t ha-1), the benefits may have been lost in the maceration process even though incorporation was as immediate as practically possible. This degree of maceration of above ground material is not likely to be a situation experienced in the commercial sector but of course, this will depend on available equipment.

Also for consideration at this site, is the relatively high soil temperatures found down to a depth of 18 cm during the summer which may well have had an impact on the PCN cysts found nearer the soil surface, particularly those plots where there was less or no ground cover.

The biomass of the biofumigants at this trial was high, Bento yielding over 80t/ha in total fresh weight, it was significantly greater than all other varieties. All other varieties yielded between 50-52t/ha in total fresh weight. There was no significant difference between these varieties. The greatest fresh weight of root biomass was produced by Bento and Caliente 99 (Table 53).

The Pf viable eggs g^{-1} soil, eggs $cyst^{-1}$ and % viable eggs (Tables 55-57) values show there wasn't a significant difference in PCN populations post a potato crop between the biofumigant treatments. The use of a nematicide did significantly reduce *Pf* viable eggs g^{-1} soil, eggs $cyst^{-1}$ and % viable eggs (Tables 55-57).

Multiplication rate (Pf/Pi) of PCN was unaffected by biofumigant treatments (Table 58). However, Nemathorin (fosthiazate) application resulted in a significant reduction (P= 0.01) of the Pf/Pi.

Biofumigation did not result in a significant reduction in the blemishing diseases black dot, common scab or silver scurf (Tables 59-61). However, there was a significant reduction in the disease severity (DSI) for black dot in plots where Nemathorin was applied (P =0.013) (Table 59).

Potato tuber yield (total t/ha) was significantly higher in nematicide-treated than untreated plots (P<0.001) but no significant difference was seen in the 45-65mm fraction. Biofumigant treatments were not found to significantly affect either tuber yield or number (Tables 62-65).

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Table 49: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Lincolnshire site. Samples collected pre-sowing, pre-incorporation and post incorporation of the brassica cover crops. Treatments with the same letters are not significantly different according to Student-Newman-Keuls (P=0.05)*

Treatment	Viable eggs g $^{-1}$ soil pre-sowing (July 2014)	Viable eggs g $^{-1}$ soil pre-incorporation (October 2014)	Viable eggs g $^{-1}$ soil post-incorporation (December 2014)
Untreated	44.64 a	49.40 a	30.34 a
Caliente 99 (*Brassica juncea*)	44.26 a	30.24 a	25.40 a
Scala (*Brassica juncea*)	49.16 a	33.58 a	38.24 a
Ida Gold (*Sinapis alba*)	56.10 a	31.74 a	33.90 a
Bento (*Raphanus sativus*)	43.84 a	42.76 a	49.92 a
Nemat (*Eruca sativa*)	38.04 a	49.42 a	49.18 a
Significance (*P*-value)	NS	NS	NS
SD	14.11	22.00	15.35
CV%	30.67	55.68	40.59

Table 50: *The viability of potato cyst nematode eggs (%) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Lincolnshire site. Samples collected pre-sowing, pre-incorporation and post incorporation of the brassica cover crops. Treatments with the same letters are not significantly different according to Student-Newman-Keuls (P=0.05)*

Treatment	% PCN eggs viable pre-sowing (July 2014)	% PCN eggs viable pre-incorporation (October 2014)	% PCN eggs viable post-incorporation (December 2014)
Untreated	65.34 a	49.40 a	30.34 a
Caliente 99 (*Brassica juncea*)	68.90 a	30.24 a	25.40 a
Scala (*Brassica juncea*)	72.30 a	33.58 a	38.24 a
Ida Gold (*Sinapis alba*)	77.38 a	31.74 a	33.90 a
Bento (*Raphanus sativus*)	72.86 a	42.76 a	49.92 a
Nemat (*Eruca sativa*)	66.08 a	49.42 a	49.18 a
Significance (*P*-value)	NS	NS	NS
SD	8.67	9.21	11.85
CV%	12.31	15.26	20.94

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Table 51: *Transformed data of column 2 (Viable eggs g^{-1}soil pre-incorporation) from Table 13 using Log (TL), square root (TS) and Arscine square root percent (TA). The data still failed the Bartletts test of homogeneity so should be treated with caution*

Treatment	Viable eggs g $^{-1}$ soil pre-incorporation	Viable eggs g $^{-1}$ soil pre-incorporation	Viable eggs g $^{-1}$ soil pre-incorporation
ARM action code	TS[3]	TL[3]	TA[3]
Untreated	47.29 a	45.34 a	49.77 a
Caliente 99 (*Brassica juncea*)	28.49 a	27.03 a	29.40 a
Scala (*Brassica juncea*)	31.33 a	29.29 a	32.51 a
Ida Gold (*Sinapis alba*)	31.71 a	31.69 a	31.72 a
Bento (*Raphanus sativus*)	42.55 a	42.35 a	42.70 a
Nemat (*Eruca sativa*)	43.27 a	37.28 a	51.19 a
Significance (*P*-value)	NS	NS	NS
SD	1.65	0.22	14.06
CV%	27.03	14.68	36.17

Table 52: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Lincolnshire site. Samples collected pre-sowing, pre-incorporation and post incorporation of the brassica cover crops. Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)*

Treatment	Number of viable potato cyst nematode eggs cyst^{-1} pre-sowing (July 2014)	Number of viable potato cyst nematode eggs cyst^{-1} pre-incorporation (October 2014)	Number of viable potato cyst nematode eggs cyst^{-1} post-incorporation (December 2014)
Untreated	111.60 a	91.80 a	66.20 a
Caliente 99 (*Brassica juncea*)	106.58 a	82.78 a	73.32 a
Scala (*Brassica juncea*)	130.73 a	84.40 a	102.95 a
Ida Gold (*Sinapis alba*)	144.65 a	85.80 a	96.99 a
Bento (*Raphanus sativus*)	105.20 a	94.80 a	102.40 a
Nemat (*Eruca sativa*)	108.80 a	106.14 a	107.80 a
Significance (*P*-value)	NS	NS	NS
SD	27.10	29.64	25.71
CV%	22.98	32.6	28.07

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Table 53: *Fresh weight (g/m²) of foliar and root biomass plus stem number per m² from brassica cover crops from field experiment 2014-15 (Lincolnshire site). Means followed by same letter do not significantly differ (P=0.05, Student-Newman-Keuls)*

Treatment	Fresh weight - stems (g/m²)	Fresh weight - leaves (g/m²)	Fresh weight - roots (g/m²)	Total fresh weight (g/m²)	Stem number/m²
Untreated	0 c	0 c	0 c	0 c	0 d
Caliente 99 (*Brassica juncea*)	3162 a	1936 b	421 a	5099 b	51.2 b
Scala (*Brassica juncea*)	3058 a	1968 b	120 c	5026 b	47.2 b
Ida Gold (*Sinapis alba*)	3049 a	2006 b	290 b	5050 b	37.6 b
Bento (*Raphanus sativus*)	3746 a	4425 a	430 a	8171 a	48.8 b
Nemat (*Eruca sativa*)	1522 b	3672 ab	163 c	5195 b	69.2 a
Significance (*P*-value)	0.001	0.001	0.001	0.001	0.001
SD	5.50	10.47	0.55	14.94	9.23
CV	22.73	44.85	23.44	31.41	21.8

Table 54: *Dry weight (g/m²) of foliar and root biomass from brassica cover crops from field experiment 2014 (Lincolnshire site). Means followed by same letter do not significantly differ (P=0.05, Student-Newman-Keuls)*

Treatment	Dry weight -stems (g/m²)	Dry weight - leaves (g/m²)	Dry weight - roots (g/m²)	Total dry weight (g/m²)
Untreated	0.0 c	0.0 c	0.0 d	0.0 c
Caliente 99 (*Brassica juncea*)	422 a	232 b	82 a	737 b
Scala (*Brassica juncea*)	415 a	236 b	23 c	675 b
Ida Gold (*Sinapis alba*)	404 a	234 b	57 b	696 b
Bento (*Raphanus sativus*)	499 a	535 a	84 a	1118 a
Nemat (*Eruca sativa*)	204 b	441 ab	32 c	678 b
Significance (*P*-value)	0.001	0.001	0.001	0.001
SD	0.71	1.24	0.11	1.90
CV	22.1	44.57	23.68	29.28

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Table 55: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Lincolnshire site. Samples collected post sampling of potatoes (Pf). Potato plots were split and either treated with Nemathorin* 10G *(Fosthiazate) or left untreated*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	80.2	29.7	55.0
Caliente 99 (*Brassica juncea*)	104.6	41.6	73.1
Scala (*Brassica juncea*)	119.7	39.5	79.6
Ida Gold (*Sinapis alba*)	82.9	29.6	56.2
Bento (*Raphanus sativus*)	78.9	73.2	76.1
Nemat (*Eruca sativa*)	120.4	30.8	75.6
Mean	97.8	40.7	
Grand Mean	69.3		
Significance (P-value): Biofumigant (B)	0.738	SED: Biofumigant	20.63
Significance (P-value): Nematicide (N)	<0.001	SED: Nematicide	10.75
Significance (P-value):B*N	0.319	SED: B*N	27.79
CV%	60.1		

Table 56: *Viable population densities of potato cyst nematodes (viable eggs per cyst) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Lincolnshire site. Samples collected post sampling of potatoes (Pf). Potato plots were split and either treated with Nemathorin* 10G *(Fosthiazate) or left untreated*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	96.4	59.2	77.8
Caliente 99 (*Brassica juncea*)	111.2	88.0	99.6
Scala (*Brassica juncea*)	117.2	81.4	99.3
Ida Gold (*Sinapis alba*)	99.0	55.2	77.1
Bento (*Raphanus sativus*)	86.6	80.6	83.6
Nemat (*Eruca sativa*)	129.2	75.0	102.1
Mean	106.6	73.2	
Grand Mean	89.9		
Significance (P-value): Biofumigant (B)	0.647	SED: Biofumigant	20.08
Significance (P-value): Nematicide (N)	0.002	SED: Nematicide	9.60
Significance(P-value):B*N	0.764	SED: B*N	26.07
CV%	41.3		

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Table 57: *Viable population densities of potato cyst nematodes (% viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or left fallow in field experiment 2014-15 – Lincolnshire site. Samples collected post sampling of potatoes (Pf). Potato plots were split and either treated with Nemathorin* 10G *(Fosthiazate) or left untreated*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	65.18	54.34	59.76
Caliente 99 (*Brassica juncea*)	72.52	62.65	67.59
Scala (*Brassica juncea*)	66.60	63.05	64.82
Ida Gold (*Sinapis alba*)	64.14	57.84	60.99
Bento (*Raphanus sativus*)	67.64	55.67	61.65
Nemat (*Eruca sativa*)	72.22	58.95	65.58
Mean	68.05	58.75	
Grand Mean	63.40		
Significance (P-value): Biofumigant (B)	0.395	SED: Biofumigant	4.123
Significance (P-value): Nematicide (N)	<0.001	SED: Nematicide	1.770
Significance (P-value):B*N	0.615	SED: B*N	5.138
CV%	10.8		

Table 58: *Multiplication rates of potato cyst nematodes (Pf/Pi) from field plots with Brassica juncea cv. Caliente 99, Brassica juncea cv. Scala, Sinapis alba cv. Ida Gold, Raphanus sativus cv. Bento, Eruca sativa cv. Nemat or left fallow in field experiment 2014-15 – Lincolnshire site. Potato plots were split and either treated with Nemathorin (fosthiazate) or left untreated. Data transformed using square root - backtransformed means in parentheses*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	1.804 (3.82)	1.157 (1.58)	1.481 (2.70)
Caliente 99 (*Brassica juncea*)	1.828 (3.45)	1.140 (1.46)	1.484 (2.46)
Scala (*Brassica juncea*)	2.051 (4.41)	1.476 (2.32)	1.764 (3.36)
Ida Gold (*Sinapis alba*)	1.603 (2.94)	1.815 (3.80)	1.709 (3.38)
Bento (*Raphanus sativus*)	1.973 (4.33)	1.696 (3.90	1.835 (4.10)
Nemat (*Eruca sativa*)	2.112 (5.61)	1.074 (1.16)	1.593 (3.38)
Mean	1.895 (4.10)	1.393 (2.37)	
Grand Mean	1.644 (3.23)		
Significance (P-value): Biofumigant (B)	0.828	SED: Biofumigant	0.3210
Significance (P-value): Nematicide (N)	0.010	SED: Nematicide	0.1853
Significance (P-value):B*N	0.502	SED: B*N	0.4539
CV%	43.7		

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Table 59: *Black dot (*Colletotricum coccodes*) severity (disease index) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (fosthiazate), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Lincolnshire site)*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	8.98	7.46	8.22
Caliente 99 (*Brassica juncea*)	8.28	7.60	7.94
Scala (*Brassica juncea*)	7.12	7.28	7.20
Ida Gold (*Sinapis alba*)	13.62	5.86	9.74
Bento (*Raphanus sativus*)	10.84	3.28	7.06
Nemat (*Eruca sativa*)	12.72	5.20	9.22
Mean	10.26	6.20	
Grand Mean	8.23		
Significance (P-value): Biofumigant (B)	0.901	SED: Biofumigant	2.708
Significance (P-value): Nematicide (N)	0.013	SED: Nematicide	1.564
Significance (P-value):B*N	0.454	SED: B*N	3.830
CV%	73.6		

Table 60: *Common scab (*Streptomyces spp.*) severity (disease index) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (fosthiazate), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Lincolnshire site)*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	15.10	13.58	14.34
Caliente 99 (*Brassica juncea*)	8.12	5.58	6.85
Scala (*Brassica juncea*)	13.86	11.06	12.46
Ida Gold (*Sinapis alba*)	13.98	10.92	12.45
Bento (*Raphanus sativus*)	10.38	9.50	9.94
Nemat (*Eruca sativa*)	10.94	10.90	10.92
Mean	12.06	10.26	
Grand Mean	11.16		
Significance (P-value): Biofumigant (B)	0.122	SED: Biofumigant	2.693
Significance (P-value): Nematicide (N)	0.252	SED: Nematicide	1.555
Significance (P-value):B*N	0.992	SED: B*N	3.809
CV%	54.0		

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Table 61: *Silver scurf (*Helminthosporium solani*) severity (disease index) on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Lincolnshire site)*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	11.92	17.86	14.89
Caliente 99 (*Brassica juncea*)	15.86	9.72	12.79
Scala (*Brassica juncea*)	11.30	13.90	11.30
Ida Gold (*Sinapis alba*)	8.64	13.06	10.85
Bento (*Raphanus sativus*)	11.32	7.12	9.22
Nemat (*Eruca sativa*)	12.82	12.96	12.89
Mean	11.88	12.44	
Grand Mean	12.21		
Significance (P-value): Biofumigant (B)	0.441	SED: Biofumigant	2.780
Significance (P-value): Nematicide (N)	0.776	SED: Nematicide	3.235
Significance (P-value):B*N	0.210	SED: B*N	7.923
CV%	50.9		

Table 62: *Total tubers th/ha produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (Fosthiazate), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Lincolnshire site).*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	248.4	222.5	235.5
Caliente 99 (*Brassica juncea*)	254.7	238.4	224.4
Scala (*Brassica juncea*)	248.7	313.1	274.1
Ida Gold (*Sinapis alba*)	211.2	237.5	280.9
Bento (*Raphanus sativus*)	232.2	218.4	225.3
Nemat (*Eruca sativa*)	280.9	267.2	246.6
Mean	246.0	249.5	
Grand Mean	247.8		
Significance (P-value): Biofumigant (B)	0.292	SED: Biofumigant	30.01
Significance (P-value): Nematicide (N)	0.834	SED: Nematicide	16.52
Significance (P-value):B*N	0.599	SED: B*N	41.46
CV%	25.8		

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Table 63: *Total yield t/ha produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (Fosthiazate), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Lincolnshire site).*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	25.0	34.7	29.9
Caliente 99 (*Brassica juncea*)	23.6	37.2	30.4
Scala (*Brassica juncea*)	23.6	46.3	34.9
Ida Gold (*Sinapis alba*)	17.9	38.4	28.1
Bento (*Raphanus sativus*)	20.8	35.6	28.2
Nemat (*Eruca sativa*)	27.1	43.7	35.4
Mean	23.0	39.3	
Grand Mean	31.1		
Significance (P-value): Biofumigant (B)	0.519	SED: Biofumigant	4.91
Significance (P-value): Nematicide (N)	<0.001	SED: Nematicide	2.43
Significance (P-value):B*N	0.685	SED: B*N	6.46
CV%	30.2		

Table 64: *45-65mm yield t/ha produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (Fosthiazate), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Lincolnshire site).*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	13.59	13.95	13.77
Caliente 99 (*Brassica juncea*)	13.32	13.64	13.48
Scala (*Brassica juncea*)	12.75	17.45	15.10
Ida Gold (*Sinapis alba*)	11.16	13.17	12.17
Bento (*Raphanus sativus*)	13.90	13.76	13.83
Nemat (*Eruca sativa*)	14.95	15.95	15.45
Mean	13.28	14.65	
Grand Mean	13.97		
Significance (P-value): Biofumigant (B)	0.652	SED: Biofumigant	2.050
Significance (P-value): Nematicide (N)	0.282	SED: Nematicide	1.247
Significance (P-value):B*N	0.882	SED: B*N	2.978
CV%	34.6		

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Table 65: *45-65mm tuber number th/ha produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (Fosthiazate), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Lincolnshire site).*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	159.4	133.0	146.2
Caliente 99 (*Brassica juncea*)	165.2	133.7	149.4
Scala (*Brassica juncea*)	156.8	152.5	154.7
Ida Gold (*Sinapis alba*)	139.6	128.6	134.1
Bento (*Raphanus sativus*)	159.4	125.8	142.6
Nemat (*Eruca sativa*)	178.9	149.8	164.4
Mean	159.9	137.2	
Grand Mean	148.6		
Significance (P-value): Biofumigant (B)	0.677	SED: Biofumigant	18.43
Significance (P-value): Nematicide (N)	0.056	SED: Nematicide	11.26
Significance (P-value):B*N	0.963	SED: B*N	26.83
CV%	29.3		

<u>Aberdeenshire (Ethie Barns) site:</u>

Overall, potato cyst nematode population densities declined in all treatments over the assessment period (Table 66). No significant differences in mean viable PCN eggs g^{-1} soil were found between the treatments pre-sowing and post-incorporation of biofumigants. However, a significant difference in the viable PCN eggs g^{-1} soil was observed within the Caliente 99 treatment (P=0.009) pre-sowing and post-incorporation: means of 125.76 (SED ± 47.27) and 43.17 (SED ± 33.71) respectively. This was the only treatment to demonstrate a significant reduction in viable PCN eggs g^{-1} soil.

The Caliente 99 treatment also had a significantly higher % reduction in mean viable PCN eggs g^{-1} soil pre-sowing and post-incorporation of biofumigants, when compared to the untreated (Table 66, P=0.033, ANOVA on ARCSIN transformed data). None of the other treatments had a significant difference in % reduction in mean viable PCN eggs g^{-1} soil pre-sowing and post-incorporation of biofumigants, when compared to the Untreated.

Similarly there was a general decline in all treatments in the mean number of viable potato cyst nematode eggs $cyst^{-1}$ (Table 67). No significant differences in mean number of viable potato cyst nematode eggs $cyst^{-1}$ were found between the treatments pre-sowing and post-incorporation of biofumigants. However, there were significant differences observed in the number of viable potato cyst nematode eggs $cyst^{-1}$ within the same treatments pre-sowing and post-incorporation (except for Scala, P=0.079).

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The percentage of viable PCN eggs pre-sowing and post-incorporation of biofumigants, were similar across all treatments (Table 68). The percentage of viable eggs dropped from ~ 80%-90% to ~45%-52% after incorporation of the treatments. This also included the fallow plots (which were ploughed in the same way as the treated plots), where there was a similar drop in percentage of viable eggs – 91% to 51%. There were no significant differences in percentage of viable PCN eggs found between the treatments pre-sowing and post-incorporation of biofumigants (Table 68).

Two-way analysis of variance (Tables 69, 70 and 71) based on assessments taken from pre-incorporation to pre-planting of potatoes did not demonstrate any significant differences between treatments and the sampling time in terms of mean viable eggs^{-1}, mean % of viable eggs and mean No. of eggs cyst^{-1} respectively.

The fresh and dry weight of biomass produced by the biofumigant lines varied quite considerably, particularly where different tissue types were compared (see Tables 72 and 73). *R. sativus* cv. Bento produced the greatest fresh and dry weight in terms of leaves, stems and roots out of all the treatments (Tables 72 and 73), with *B. juncea* cv. Caliente 99 the least. Significant differences (P<0.001) were observed between leaf, root and stem tissue for the treatments studied.

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Table 66: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Samples collected pre-sowing and post-incorporation of the brassica cover crops*

Treatment	Mean viable eggs g^{-1} soil pre-sowing (May 2014)	Mean viable eggs g^{-1} soil post-incorporation (November 2014)	Mean viable eggs g^{-1} soil pre-planting potatoes (May 2015)
Untreated	80.29	58.95	76.4
Caliente 99 (*Brassica juncea*)	125.76	43.17	74.2
Scala (*Brassica juncea*)	97.93	76.41	57.0
Ida Gold (*Sinapis alba*)	80.55	43.21	53.4
Bento (*Raphanus sativus*)	100.23	56.25	67.2
Significance (*P*-value)	NS	NS	NS
SED	47.27	33.71	45.98
%CV	19.21	24.67	55.67

Table 67: *The number of viable potato cyst nematode eggs cyst $^{-1}$ sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Samples collected pre-sowing, and post-incorporation of the brassica cover crops*

Treatment	Number of viable potato cyst nematode eggs $cyst^{-1}$ pre-sowing (May 2014)	Number of viable potato cyst nematode eggs $cyst^{-1}$ post-incorporation (November 2014)	Mean % reduction in viable potato cyst nematode eggs $cyst^{-1}$
Untreated	100.4	67	17.44
Caliente 99 (*Brassica juncea*)	118.4	43.8	63.59*
Scala (*Brassica juncea*)	91.8	58	28.18
Ida Gold (*Sinapis alba*)	93	49.4	41.08
Bento (*Raphanus sativus*)	73.4	38.4	43.16
Significance (*P*-value)	NS	NS	NS
SED	39.99	27.0	42.74
%CV	17.03	22.17	44.92

* Indicates significant difference in % reduction compared to the Untreated (ANOVA using ARCSIN transfomed % data)

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Table 68: *The viability of potato cyst nematode eggs (%) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Samples collected pre-sowing, post-incorporation of the brassica cover crops and pre-planting of potatoes*

Treatment	% PCN eggs viable pre-sowing (May 2014)	% PCN eggs viable post-incorporation (November 2014)	% PCN eggs viable pre-planting potatoes (May 2015)
Untreated	91.46	51.42	93.81
Caliente 99 (*Brassica juncea*)	87.27	51.88	96.28
Scala (*Brassica juncea*)	84.90	51.98	95.56
Ida Gold (*Sinapis alba*)	83.91	47.18	95.16
Bento (*Raphanus sativus*)	85.34	45.35	93.45
Significance (*P*-value)	NS	NS	NS
SED	7.37	7.59	4.0865
%CV	3.45	6.23	3.92

Table 69: *Two way analysis of variance of viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento or left fallow in field experiment 2014-15 – Aberdeenshire site. Based on data collected post-incorporation of the brassica cover crops and pre-planting of potatoes. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Mean viable eggs g^{-1} soil (post-incorporation to pre-planting potatoes)
Untreated	67.67a
Caliente 99 (*Brassica juncea*)	58.69a
Scala (*Brassica juncea*)	66.71a
Ida Gold (*Sinapis alba*)	48.31a
Bento (*Raphanus sativus*)	61.73a
Significance (*P*-value) *-Treatment*	0.473
Significance (*P*-value) *-Sampling time*	0.180
Significance (*P*-value) *-Treatment * sampling time*	0.301
SED (treatment)	20.515
%CV	43.53

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Table 70: *Two way analysis of variance of the viability of potato cyst nematode eggs (%) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Based on data collected post-incorporation of the brassica cover crops and pre-planting of potatoes. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	% PCN eggs viable (pre-incorporation to pre-planting potatoes)
Untreated	71.62 a
Caliente 99 (*Brassica juncea*)	74.08 a
Scala (*Brassica juncea*)	73.77 a
Ida Gold (*Sinapis alba*)	71.17 a
Bento (*Raphanus sativus*)	69.40 a
Significance (*P*-value) -*Treatment*	0.636
Significance (*P*-value) -*Sampling time*	<0.001
Significance (*P*-value) -*Treatment * sampling time*	0.833
SED (treatment)	18.65
%CV	33.22

Table 71: *Two way analysis of variance of the number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Based on data collected post-incorporation of the brassica cover crops and pre-planting of potatoes. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Number of viable potato cyst nematode eggs cyst^{-1} (post-incorporation to pre-planting potatoes)
Untreated	43.53a
Caliente 99 (*Brassica juncea*)	34.04a
Scala (*Brassica juncea*)	39.30a
Ida Gold (*Sinapis alba*)	36.68a
Bento (*Raphanus sativus*)	31.55a
Significance (*P*-value) -*Treatment*	0.323
Significance (*P*-value) -*Sampling time*	0.001
Significance (*P*-value) -*Treatment * sampling time*	0.055
SED (treatment)	15.73
%CV	54.67

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Table 72: *Fresh weight (g/m²) of foliar and root biomass from brassica cover crops from field experiment 2014-15 (Aberdeenshire site) 11 WAP. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Fresh weight - leaves (g/m^2)	Fresh weight - stems (g/m^2)	Fresh weight - roots (g/m^2)	Total fresh weight (g/m^2)
Untreated	-	-	-	-
Caliente 99 (*Brassica juncea*)	194.34a	375.86a	86.00a	656.2a
Scala (*Brassica juncea*)	285.91a	366.7a	50.00b	702.6a
Ida Gold (*Sinapis alba*)	250.9a	416.68ab	44.61b	712.2a
Bento (*Raphanus sativus*)	687.86b	489.51b	100.86a	1278.2b
Significance (*P*-value)	0.001	0.001	0.001	0.001
SED %CV	72.74 63.38	41.70 13.56	13.27 38.92	121.9 35.24

Table 73: *Dry weight (g/m²) of foliar and root biomass from brassica cover crops from field experiment 2014-15 (Aberdeenshire site) 11 WAP. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

Treatment	Dry weight - leaves (g/m^2)	Dry weight - stems (g/m^2)	Dry weight - roots (g/m^2)	Total dry weight (g/m^2)
Untreated	-	-	-	-
Caliente 99 (*Brassica juncea*)	24.099a	54.682a	18.70a	97.48a
Scala (*Brassica juncea*)	42.989b	69.758b	11.204b	123.95ab
Ida Gold (*Sinapis alba*)	59.418c	111.678c	12.455b	183.55c
Bento (*Raphanus sativus*)	71.992c	51.193a	20.359a	143.54b
Significance (*P*-value)	0.001	0.001	0.001	0.001
SED %CV	8.314 41.94	6.74 38.72	3.122 28.89	16.92 26.4

After potatoes were planted following the biofumigant crops, there were no significant effects due to the biofumigant crop noted in terms of the subsequent mean No. of viable eggs g^{-1} soil, % viability of eggs and eggs cyst-^{1} post-potato harvest (Tables 74, 75 and 76). There was a significant effect of the use of nematicide (Vydate) on reducing the number of viable eggs g^{-1} (*P*=0.017) post-potato harvest (Table 74).

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There were no significant effects due to the biofumigant crop noted in the multiplication rates of potato cyst nematodes (*Pf/Pi*) post-potato harvest (Table 77). There was a significant effect of the use of nematicide (Vydate) on reducing the multiplication rates of potato cyst nematodes (*Pf*/*Pi*) post-potato harvest (*P*=0.045, Table 77).

After potatoes were planted following the biofumigant crops, there were no significant effects due to the biofumigant or use of nematicide (Vydate) noted in terms of disease incidence (common scab and black scurf) recorded on the harvested potato crop (Tables 78 and 79).

After potatoes were planted following the biofumigant crops, there were no significant effects due to the biofumigant noted in terms of total tubers/m^2 or total yield/ha on the harvested potato crop (Tables 80 and 81). There was a significant impact of the use of a nematicide (Vydate) on total tubers/m^2 and total yield/ha (*P*=0.0001 and 0.001 respectively, Tables 80 and 81).

Table 74: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Samples collected post sampling of potatoes (Pf). Potato plots were split and either treated with Vydate (oxamyl) or left untreated*

Biofumigant	No Vydate	Vydate	Mean
Untreated	281.5	208.5	245.0
Caliente 99 (*Brassica juncea*)	254.0	205.5	229.8
Scala (*Brassica juncea*)	223.0	165.0	194.0
Ida Gold (*Sinapis alba*)	83.6	68.6	190.3
Bento (*Raphanus sativus*)	209.0	171.5	205.8
Mean	238.3	187.5	
Grand Mean	212.9		
Significance (P-value): Biofumigant (B)	0.378		
Significance (P-value): Nematicide (N)	0.017		
Significance (P-value):B*N	0.977		
CV%	34.4		

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Table 75: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Samples collected post sampling of potatoes. Potato plots were split and either treated with Vydate (oxamyl) or left untreated. Values shown were transformed using ArcSin. Values in parentheses are the actual values.*

Biofumigant	No Vydate	Vydate	Mean
Untreated	82.81 (92.63)	85.24 (95.95)	84.04 (94.29)
Caliente 99 (*Brassica juncea*)	81.84 (91.36)	82.54 (92.38)	82.27 (91.87)
Scala (*Brassica juncea*)	81.46 (90.83)	83.46 (93.55)	82.51 (92.19)
Ida Gold (*Sinapis alba*)	85.76 (96.67)	80.87 (90.26)	84.17 (93.47)
Bento (*Raphanus sativus*)	78.44 (86.89)	75.74 (83.80)	77.39 (85.35)
Mean	82.11 (91.68)	81.74 (91.19)	
Grand Mean	81.92 (91.40)		
Significance (P-value): Biofumigant (B)	0.161		
Significance (P-value): Nematicide (N)	0.869		
Significance (P-value):B*N	0.667		
CV%	9.32		

Table 76: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Samples collected post sampling of potatoes. Potato plots were split and either treated with Vydate (oxamyl) or left untreated*

Biofumigant	No Vydate	Vydate	Mean
Untreated	200.4	187.6	194.0
Caliente 99 (*Brassica juncea*)	219.4	180.8	200.1
Scala (*Brassica juncea*)	168.2	193.2	180.7
Ida Gold (*Sinapis alba*)	228.0	146.0	187.0
Bento (*Raphanus sativus*)	112.4	149.4	130.9
Mean	185.7	171.4	
Grand Mean	178.5		
Significance (P-value): Biofumigant (B)	0.188		
Significance (P-value): Nematicide (N)	0.466		
Significance (P-value):B*N	0.309		
CV%	39.6		

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Table 77: *Multiplication rates of potato cyst nematodes (Pf/Pi) from field plots with* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento, or left fallow in field experiment 2014-15 – Aberdeenshire site. Potato plots were split and either treated with Vydate (oxamyl) or left untreated*

Biofumigant	No Vydate	Vydate	Mean
Untreated	3.8	2.8	3.3
Caliente 99 (*Brassica juncea*)	3.4	2.8	3.1
Scala (*Brassica juncea*)	4.1	3.0	3.5
Ida Gold (*Sinapis alba*)	5.7	4.1	4.9
Bento (*Raphanus sativus*)	3.7	2.9	3.3
Mean	4.3	3.0	
Grand Mean	3.6		
Significance (P-value): Biofumigant (B)	0.172		
Significance (P-value): Nematicide (N)	0.045		
Significance (P-value):B*N	0.984		
CV%	50.93		

Table 78: *Common scab (*Streptomyces spp.*) incidence on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Aberdeenshire site). Data transformed using ArcSin – Actual means in parentheses*

Biofumigant	No Vydate	Vydate	Mean
Untreated	20.9 (20.67)	22.3 (22.0)	21.6 (21.3)
Caliente 99 (*Brassica juncea*)	18.8 (18.6)	29.6 (28.9)	24.2 (23.8)
Scala (*Brassica juncea*)	27.1 (26.5)	39.1 (38.1)	33.1 (32.3)
Ida Gold (*Sinapis alba*)	20.5 (20.3)	23.0 (22.8)	21.8 (21.5)
Bento (*Raphanus sativus*)	26.3 (25.8)	24.9 (24.4)	25.6 (25.1)
Mean	22.2 (22.4)	26.9 (27.2)	
Grand Mean	25.2 (24.8)		
Significance (P-value): Biofumigant (B)	0.136		
Significance (P-value): Nematicide (N)	0.107		
Significance (P-value):B*N	0.556		
CV%	45.1		

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Table 79: *Black scurf (*Rhizoctonia solani*) incidence on potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Aberdeenshire site). Data transformed using ArcSin – Actual means in parentheses*

Biofumigant	No Vydate	Vydate	Mean
Untreated	9.29 (9.08)	8.87 (8.80)	9.08 (8.94)
Caliente 99 (*Brassica juncea*)	6.37 (6.30)	9.73 (9.68)	8.05 (7.99)
Scala (*Brassica juncea*)	5.26 (5.24)	17.29 (17.12)	11.27 (11.18)
Ida Gold (*Sinapis alba*)	5.10 (5.00)	4.82 (4.80)	4.92 (4.90)
Bento (*Raphanus sativus*)	8.54 (7.34)	4.26 (8.48)	7.96 (7.91)
Mean	10.00 (3.68)	9.32 (3.56)	
Grand Mean	8.26 (8.18)		
Significance (P-value): Biofumigant (B)	0.787		
Significance (P-value): Nematicide (N)	0.315		
Significance (P-value):B*N	0.705		
CV%	2.7		

Table 80: *Total tubers/m² produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Aberdeenshire site).*

Biofumigant	No Vydate	Vydate	Mean
Untreated	27.2	34.4	30.8
Caliente 99 (*Brassica juncea*)	24.0	31.4	27.7
Scala (*Brassica juncea*)	25.2	38.4	31.8
Ida Gold (*Sinapis alba*)	23.8	34.4	29.1
Bento (*Raphanus sativus*)	18.0	32.0	25.0
Mean	23.6	34.1	
Grand Mean	28.9		
Significance (P-value): Biofumigant (B)	0.504		
Significance (P-value): Nematicide (N)	0.0001		
Significance (P-value):B*N	0.879		
CV%	35.5		

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Table 81: *Total yield t/ha produced by potatoes cv. Desirée grown in field plots with or without nematicide treatment (oxamyl), previously cropped with brassica species or left fallow (untreated). Field experiment 2014-15 (Aberdeenshire site).*

Biofumigant	No Vydate	Vydate	Mean
Untreated	3.76	9.59	6.68
Caliente 99 (*Brassica juncea*)	1.66	7.38	4.52
Scala (*Brassica juncea*)	4.04	12.57	8.30
Ida Gold (*Sinapis alba*)	3.56	10.87	7.21
Bento (*Raphanus sativus*)	3.17	6.59	4.88
Mean	3.24	9.40	
Grand Mean	6.32		
Significance (P-value): Biofumigant (B)	0.150		
Significance (P-value): Nematicide (N)	0.001		
Significance (P-value):B*N	0.632		
CV%	77.7		

4.4 Field experiments to compare and evaluate incorporation methods used for biofumigation when targeting potato cyst nematodes

<u>Shropshire (Crudgington) site:</u>

The field site at Crudgington, Shropshire was planted much later than planned due to wet weather occurring in July and August 2015 which caused a delay in the harvest of Winter Barley. As a result, the experiment was overwintered and incorporated on the 21st April 2016. Figure 12 displays meteorological data from the local weather station in Shawbury. In general the season was mild with very few frosts. November (2015), December (2015) and January (2016) each received over 80mm of rainfall. Plots with *B. juncea* cv. Caliente 99 were grown for a period of 35 weeks and were protected by fleece from 17/12/2015 until the 16/03/2016 (approximately 13 weeks).

Potato cyst nematode viable eggs g^{-1} soil (Table 82), viable eggs per cyst (Table 83) and % egg viability (Table 84) were not found to differ significantly between the treatments at any of the assessment timings (pre-sowing, pre-incorporation (1 and 2) and post incorporation of *B. juncea* cv. Caliente 99). Mean viable eggs g^{-1} soil appeared to decline in most treatments with progressive sampling dates.

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Foliar biomass of *B. juncea*, pre-incorporation (35 WAP), (Table 85) was not found to differ significantly between the treatment plots and the mean for the experimental area was 19.36 t/ha ±4.42 (SD). Analysis of the fresh and dry weight of *B. juncea* stems, leaves and roots (Tables 85 and 86) did not reveal any significant differences between treatment plots, except for the dry weight of leaves, where a significant difference was found between treatment plots (P = 0.018).

Blemising diseases, tuber number and tuber yield were not found to differ significantly between treatments (P > 0.05) (Tables 87-89 and Figure 13) . Whilst not significantly different, it appeared that potato tuber yield was higher and blemishing diseases were lower (black scurf, silver scurf and common scab) in field plots where *B. juncea* had been macerated with a flail at a faster forward speed (6 – 9 kph) and incorporated using a rotovator.

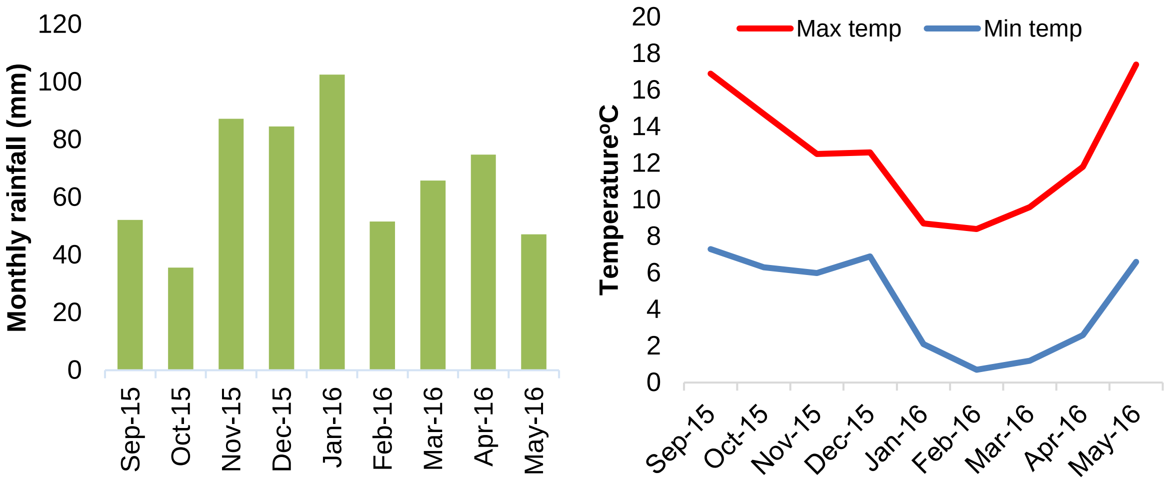

Figure 12: *Meterological data for the Shropshire site based on Met Office historical weather station data (Shawbury)*

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Table 82: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots (Shropshire site) with and without* Brassica juncea *cv. Caliente 99*. *Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Soil samples for PCN collected pre-sowing and pre and post-incorporation of Caliente 99. Values have been transformed using square root (back transformed mean values in parentheses)*

Treatment	Viable eggs g^{-1} soil pre-sowing (September 2015)	Viable eggs g^{-1} soil pre-incorporation (November 2015)	Viable eggs g^{-1} soil pre-incorporation (April 2016)	Viable eggs g^{-1} soil post-incorporation (May 2016)
No cover crop - plots rotovated and rolled	7.22 (69.4)	5.95 (44.9)	5.63 (51.7)	6.37 (57.2)
Flail A* + Rotovate	7.35 (70.4)	5.94 (45.0)	6.45 (48.6)	5.87 (47.0)
Flail B* + Rotovate	6.24 (58.3)	5.73 (48.7)	5.25 (39.5)	4.42 (37.6)
Flail B + Rotovate + Plough	6.51 (58.6)	6.86 (65.4)	6.71 (68.8)	6.27 (50.7)
Flail B + Spader	7.62 (79.5)	7.02 (61.2)	5.98 (47.1)	6.63 (56.4)
Flail B + Spader + Plough	6.39 (61.6)	6.50 (60.2)	5.78 (53.3)	5.67 (43.0)
Flail B + Disc	6.55 (55.3)	5.94 (40.7)	5.94 (44.2)	5.21 (32.5)
Flail B + Disc + Plough	6.86 (67.0)	6.46 (63.0)	6.72 (65.3)	7.75 (90.6)
Significance (*P*-value)	0.221	0.848	0.889	0.119
SED	0.587	0.992	1.171	1.037
%CV	13.6	24.9	30.6	27.2

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 84: *The viability of potato cyst nematode eggs (%) sampled from field plots (Shropshire site) with and without* Brassica juncea *cv. Caliente 99. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Soil samples for PCN collected pre-sowing and pre and post-incorporation of Caliente 99. Values are arcsine transformed (back transformed mean values in parentheses)*

Treatment	% PCN eggs viable pre-sowing (September 2015)	% PCN eggs viable pre-incorporation (November 2015)	% PCN eggs viable pre-incorporation (April 2016)	% PCN eggs viable post-incorporation (May 2016)
No cover crop - plots rotovated and rolled	85.3 (99.1)	72.6 (89.1)	66.4 (81.5)	69.7 (85.6)
Flail A* + Rotovate	75.8 (90.5)	67.8 (84.9)	76.4 (94.1)	62.1 (77.1)
Flail B* + Rotovate	78.6 (95.4)	72.9 (90.7)	72.7 (89.2)	58.8 (71.1)
Flail B + Rotovate + Plough	83.7 (98.3)	71.4 (89.2)	67.3 (83.7)	66.5 (83.3)
Flail B + Spader	80.8 (96.7)	74.5 (92.5)	69.6 (87.0)	66.7 (84.0)
Flail B + Spader + Plough	77.0 (93.1)	68.5 (84.1)	58.6 (73.1)	63.1 (78.8)
Flail B + Disc	83.8 (98.2)	66.1 (83.1)	68.6 (85.7)	65.7 (81.8)
Flail B + Disc + Plough	82.9 (97.7)	77.6 (93.6)	71.1 (88.7)	68.7 (86.7)
Significance (*P*-value)	**0.349**	**0.187**	**0.450**	**0.370**
SED	**4.54**	**4.30**	**7.38**	**4.83**
%CV	**8.9**	**9.5**	**17.0**	**11.7**

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 83: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Shropshire site) with and without* Brassica juncea *cv. Caliente 99. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Soil samples for PCN collected pre-sowing and pre and post-incorporation of Caliente 99*

Treatment	Number of viable potato cyst nematode eggs cyst^{-} pre-sowing (September 2015)	Number of viable potato cyst nematode eggs cyst^{-} pre-incorporation (November 2015)	Number of viable potato cyst nematode eggs cyst^{-} pre-incorporation (April 2016)	Number of viable potato cyst nematode eggs cyst^{-} post-incorporation (May 2016)
No cover crop - plots rotovated and rolled	78.0	80.8	81.4	87.7
Flail A* + Rotovate	97.5	78.6	90.4	70.4
Flail B* + Rotovate	140.8	96.5	90.4	67.6
Flail B + Rotovate + Plough	70.3	118.1	101.6	83.3
Flail B + Spader	97.8	101.1	89.9	86.6
Flail B + Spader + Plough	58.2	98.4	87.4	74.9
Flail B + Disc	63.8	66.0	72.8	49.0
Flail B + Disc + Plough	75.0	85.7	107.1	109.2
Significance (*P*-value)	0.131	0.714	0.960	0.425
SED	28.23	28.42	29.02	24.45
%CV	52.4	49.6	50.9	49.2

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 85: *Fresh weight of foliar and root biomass from brassica cover crops from field experiment 2015-16 (Shropshire site) 35 WAP*

Treatment	Fresh weight of leaves/g (10 plants)	Fresh weight of stems/g (10 plants)	Fresh weight of roots/g (10 plants)	Total fresh weight (t/ha)
No cover crop - plots rotovated and rolled	-	-	-	-
Flail A* + Rotovate	65.3	135.5	21.37	19.60
Flail B* + Rotovate	43.3	109.1	16.73	17.76
Flail B + Rotovate + Plough	48.9	123.1	18.68	20.12
Flail B + Spader	52.7	126.6	18.96	20.32
Flail B + Spader + Plough	41.6	89.7	13.98	18.96
Flail B + Disc	43.1	95.9	16.29	18.32
Flail B + Disc + Plough	55.9	127.9	20.88	20.56
Significance (*P*-value)	0.053	0.179	0.183	0.954
SED	7.71	19.25	2.919	2.997
%CV	24.3	26.4	25.5	24.5

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Table 86: *Dry weight of foliar and root biomass from brassica cover crops from field experiment 2015-16 (Shropshire site) 35 WAP*

Treatment	Dry weight of leaves/g (10 plants)	Dry weight of stems/g (10 plants)	Dry weight of roots/g (10 plants)
No cover crop - plots rotovated and rolled	-	-	-
Flail A* + Rotovate	11.02	22.20	3.98
Flail B* + Rotovate	7.06	19.20	3.30
Flail B + Rotovate + Plough	7.98	21.08	3.56
Flail B + Spader	9.02	21.74	3.52
Flail B + Spader + Plough	6.80	15.30	2.68
Flail B + Disc	6.74	16.20	2.94
Flail B + Disc + Plough	8.56	20.84	3.70
Significance (*P*-value)	0.018	0.229	0.405
SED	1.207	3.199	0.611
%CV	23.4	25.9	28.6

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 87: *The incidence of blemishing diseases on potatoes cv. Desirée grown in field plots (Shropshire site) previously cropped with Brassica juncea cv. Caliente 99 or left fallow. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc.. Data transformed using ArcSin – Actual means in parentheses*

Treatment	Black scurf (*Rhizoctonia solani*)	Silver scurf (*Helminthosporium solani*)	Black dot (*Colletotricum coccodes*)	Common scab (*Streptomyces scabei*)	Netted scab (*Rhizoctonia solani*)
No cover crop - plots rotovated and rolled	55.0 (65.6)	22.6 (17.2)	29.1 (25.1)	42.8 (46.4)	26.1 (19.8)
Flail A* + Rotovate	54.0 (59.3)	23.6 (18.7)	31.5 (28.7)	45.0 (50.0)	24.0 (17.2)
Flail B* + Rotovate	43.7 (48.2)	11.4 (6.8)	32.5 (30.6)	35.3 (38.8)	19.4 (15.2)
Flail B + Rotovate + Plough	46.3 (50.8)	27.7 (26.1)	30.4 (30.9)	39.5 (40.6)	22.9 (19.2)
Flail B + Spader	62.3 (72.8)	10.0 (4.1)	33.9 (32.4)	36.7 (37.6)	13.4 (8.8)
Flail B + Spader + Plough	50.4 (59.0)	21.3 (15.3)	22.3 (17.6)	42.5 (46.4)	26.8 (21.0)
Flail B + Disc	52.8 (62.5)	17.1 (10.7)	18.9 (11.2)	53.5 (63.7)	30.8 (27.5)
Flail B + Disc + Plough	57.8 (65.9)	18.4 (12.3)	34.2 (32.2)	40.8 (42.8)	21.8 (16.7)
Significance (*P*-value)	0.513	0.305	0.279	0.354	0.252
SED	8.87	7.65	6.88	7.40	6.30
%CV	26.6	63.6	37.4	27.8	43.0

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 88: *The severity of blemishing diseases (DSI) on potatoes cv. Desirée grown in field plots (Shropshire site) previously cropped with Brassica juncea cv. Caliente 99 or left fallow. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc.. Data transformed using ArcSin – Actual means in parentheses*

Treatment	Black scurf (*Rhizoctonia solani*)	Silver scurf (*Helminthosporium solani*)	Black dot (*Colletotricum coccodes*)	Common scab (*Streptomyces scabei)*
No cover crop - plots rotovated and rolled	25.8	6.44	9.3	14.20
Flail A* + Rotovate	20.5	8.22	12.2	13.06
Flail B* + Rotovate	16.6	3.10	10.6	9.20
Flail B + Rotovate + Plough	16.7	9.15	13.6	10.99
Flail B + Spader	24.1	1.23	11.8	8.61
Flail B + Spader + Plough	20.0	6.07	5.6	12.11
Flail B + Disc	23.6	5.04	4.3	21.40
Flail B + Disc + Plough	28.2	5.00	12.7	11.96
Significance (*P*-value)	0.523	0.524	0.278	0.078
SED	6.28	3.849	4.20	3.894
%CV	45.3	110.0	66.3	48.5

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Table 89: *The number and yield of potatoes cv. Desirée grown in field plots (Shropshire site) previously cropped with Brassica juncea cv. Caliente 99 or left fallow. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc.*

Treatment	Total tubers per plot	Yield t/ha
No cover crop - plots rotovated and rolled	53.8	7.41
Flail A* + Rotovate	53.2	7.64
Flail B* + Rotovate	74.6	11.20
Flail B + Rotovate + Plough	57.6	8.20
Flail B + Spader	63.4	8.82
Flail B + Spader + Plough	50.4	6.20
Flail B + Disc	56.4	8.70
Flail B + Disc + Plough	58.6	7.71
Significance (*P*-value)	0.556	0.479
SED	11.65	2.100
%CV	31.5	40.3

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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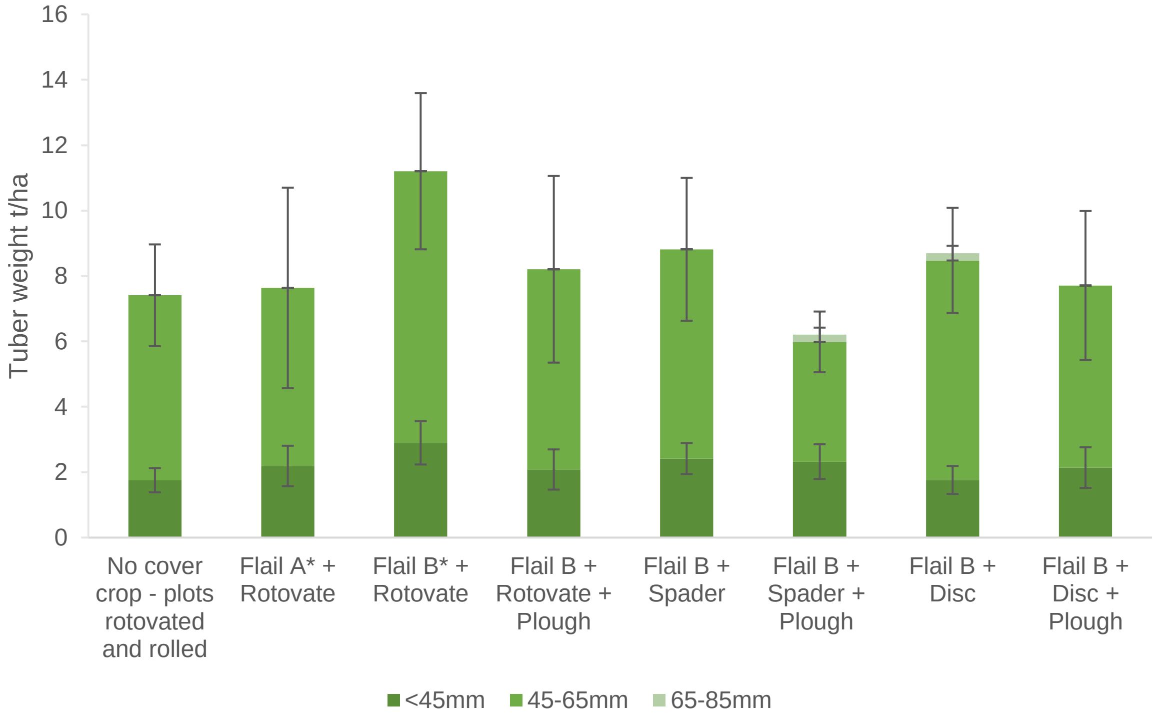

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Figure 13: *Yield fractions (t/ha) of potatoes cv. Desirée grown in field plots (Shropshire site) previously cropped with Brassica juncea cv. Caliente 99 or left fallow. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Error bars show the standard error of the mean*

Table 90: *Influence of incorporation method on the mean Extractable Nitrate N (mg/kg DM) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Shropshire site).*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	28.6ab	23.7a
Flail A + Rotovate	28.2ab	23.4a
Flail B + Rotovate	21.8ab	23.5a
Flail A + Rotovate + Plough	21.0b	30.0a
Flail A + Spader	16.1b	27.4a
Flail A + Spader + Plough	25.3ab	25.0a
Flail A + Disc	18.3b	29.8a
Flail A + Disc + Plough	36.1a	37a
Significance (*P*-value)	0.003	NS
SED	7.8	7.1
%CV	36.51	29.58

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Table 91: *Influence of incorporation method on the mean Extractable Phosphorus (mg/l) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Shropshire site).*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	15.4d	17.6b
Flail A + Rotovate	15.9cd	19.2ab
Flail B + Rotovate	15.6cd	19.5ab
Flail A + Rotovate + Plough	16.5cd	20.1ab
Flail A + Spader	17.3bcd	20.3ab
Flail A + Spader + Plough	18.0bc	21.8a
Flail A + Disc	19.3b	19.5ab
Flail A + Disc + Plough	22.0a	18.6ab
Significance (*P*-value)	0.001	0.014
SED	2.09	1.62
%CV	13.73	9.50

Table 92: *Influence of incorporation method on the mean Extractable Potassium (mg/l) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Shropshire site).*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	174.2a	182.0a
Flail A + Rotovate	157.2a	186.6a
Flail B + Rotovate	168.2a	173.4a
Flail A + Rotovate + Plough	151.4a	181.6a
Flail A + Spader	157.0a	198.6a
Flail A + Spader + Plough	162.0a	212.6a
Flail A + Disc	150.4a	183.6
Flail A + Disc + Plough	158.6a	197.4a
Significance (*P*-value)	NS	NS
SED	16.8	28.99
%CV	12.07	17.59

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Table 92: *Influence of incorporation method on the mean organic matter (mg/kg) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Shropshire site)*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	837.7a	815.7a
Flail A + Rotovate	844.8a	822.5a
Flail B + Rotovate	840.4a	818.3a
Flail A + Rotovate + Plough	835.6a	813.7a
Flail A + Spader	842.2a	820.1a
Flail A + Spader + Plough	840.0a	817.9a
Flail A + Disc	840.9a	818.8a
Flail A + Disc + Plough	840.5a	818.4a
Significance (*P*-value)	NS	NS
SED	5.36	5.67
%CV	0.33	0.79

Fife (Culross) site:

The field site was following an early-harvested winter barley site, where the grower was wanting to put in a mustard crop to tackle areas of poor yield seen over several years in a range of crops that was considered to be due to free-living nematode damage. Due to the very short notice given by the grower regarding the incorporation of his whole field of mustard, it was not possible to get a spader (only one available in Scotland, and based in north east Scotland) in time before the grower wanted to incorporate. Consequently, only flail and rotavate was possible at this site.

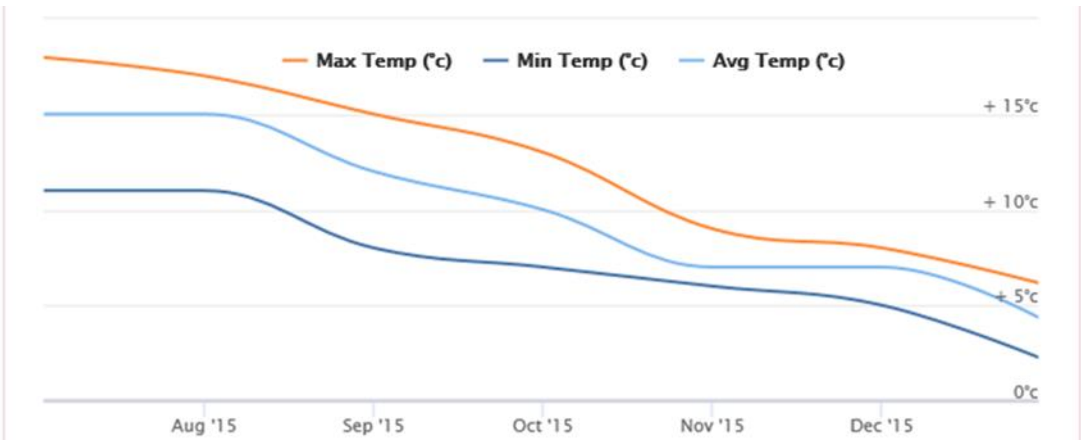

Figure 14: *Maximum, minimum and average air temperatures at Fife trial site in 2015*

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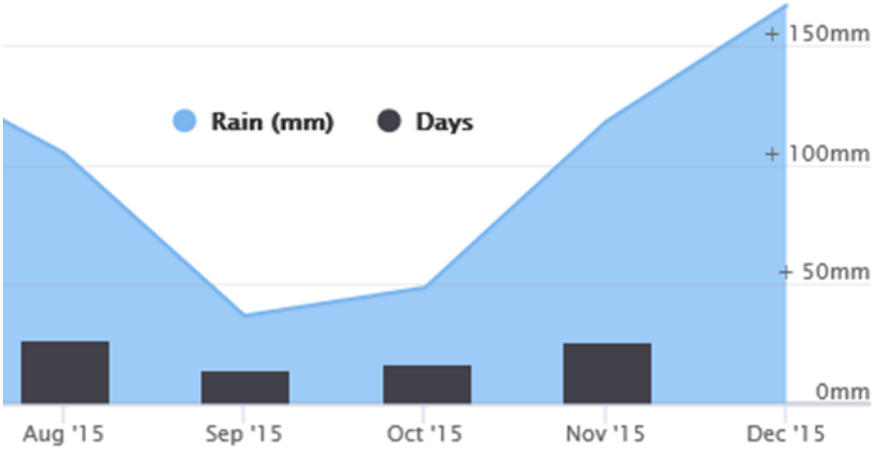

Figure 15: *Rainfall at Fife trial site in 2015*

The *B. juncea* biofumigant crop was in the ground for 14 weeks from sowing (10th August) to incorporation (10th November). Temperature and rainfall during this period are presented in Figures 14 and 15 respectively.

The soil nutrient levels taken pre-incorporation of the biofumigant and pre-planting of the potatoes are summarised in Tables 94 to 97 below. There were no significant effects noted from the incorporation method used, and in most cases the levels of dry matter and nutrients in the soil declined from pre-incorporation of the biofumigant to the time of potato planting.

Table 94: *Influence of incorporation method on the mean Extractable Nitrate N (mg/kg DM) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Fife site).*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	19.3a	10.6a
Flail A* + Rotovate	19.5a	9.7a
Flail B* + Rotovate	21.7a	9.4a
Flail B* + Rotovate + Plough	23.1a	9.5a
Significance (*P*-value)	NS	NS
SED	10.4	2.1
%CV	40.5	17.6

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Table 95: *Influence of incorporation method on the mean Extractable Phosphorus (mg/l) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Fife site).*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	24.9a	21.8a
Flail A* + Rotovate	25.8a	24.6a
Flail B* + Rotovate	26.2a	28.8a
Flail B* + Rotovate + Plough	27.6a	23.9a
Significance (*P*-value)	NS	NS
SED	14.0	14.0
%CV	43.8	46.0

Table 96: *Influence of incorporation method on the mean Extractable Potassium (mg/l) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Fife site).*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	260.4a	231.1a
Flail A* + Rotovate	245.2a	283.1a
Flail B* + Rotovate	184.2a	227.1a
Flail B* + Rotovate + Plough	244.6a	242.9a
Significance (*P*-value)	NS	NS
SED	104.5	84.7
%CV	36.4	28.0

Table 97: *Influence of incorporation method on the mean organic matter (mg/kg) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Fife site).*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	868.6a	811.0a
Flail A* + Rotovate	867.4a	817.8a
Flail B* + Rotovate	869.0a	813.6a
Flail B* + Rotovate + Plough	869.3a	809.0a
Significance (*P*-value)	NS	NS
SED	2.1	9.3
%CV	0.2	0.9

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Whilst potato cyst nematode viable eggs g^{-1} soil (Table 98) and viable eggs per cyst (Table 99) appeared to decline in all treatments with progressive sampling dates, they were not found to differ significantly between the pre-sowing and post incorporation of *B. juncea* cv. Caliente 99 whichever incorporation method was used.

Foliar, stem and root biomass of *B. juncea*, pre-incorporation (Tables 100 and 101) was not found to differ significantly between the treatment plots.

Table 98: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots (Fife site) with and without* Brassica juncea *cv. Caliente 99. Biofumigant plants macerated using two flail speeds and incorporated with a rotovator, and/or plough disc. Soil samples for PCN collected pre-sowing and post-incorporation of Caliente 99.*

Treatment	Viable eggs g^{-1} soil pre-sowing (August 2015)	Viable eggs g^{-1} soil post-incorporation (November 2015)
No cover crop - plots rotovated and rolled	58.75	49.50
Flail A* + Rotovate	75.25	60.50
Flail B* + Rotovate	83.75	66.25
Flail B + Rotovate + Plough	75.00	65.75
Significance (*P*-value)	NS	NS
SED	32.23	29.87
%CV	32.08	35.91

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Table 99: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Fife site) with and without* Brassica juncea *cv. Caliente 99. Biofumigant plants macerated using two flail speeds and incorporated with a rotovator, and/or plough disc. Soil samples for PCN collected pre-sowing and post-incorporation of Caliente 99.*

Treatment	Number of viable eggs cyst^{-1} pre-sowing (August 2015)	Number of viable eggs cyst^{-1} post-incorporation (November 2015)
No cover crop - plots rotovated and rolled	318.0	248.8
Flail A* + Rotovate	362.0	290.5
Flail B* + Rotovate	366.3	322.0
Flail B + Rotovate + Plough	309.3	313.0
Significance (*P*-value)	NS	NS
SED	79.8	113.9
%CV	17.1	28.2

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 100: *Fresh weight of foliar and root biomass from brassica cover crops from field experiment 2015-16 (Fife site) 14 WAP*

Treatment	Fresh weight of leaves/g (10 plants)	Fresh weight of stems/g (10 plants)	Fresh weight of roots/g (10 plants)	Total fresh weight (t/ha)
No cover crop - plots rotovated and rolled	-	-	-	-
Flail A* + Rotovate	84.9	189.7	23.5	26.3
Flail B* + Rotovate	73.6	181.8	21.8	25.0
Flail B + Rotovate + Plough	78.2	184.7	22.3	25.2
Significance (*P*-value)	NS	NS	NS	NS
SED	14.7	23.3	4.4	4.5
%CV	11.7	7.9	12.3	11.2

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Table 101: *Dry weight of foliar and root biomass from brassica cover crops from field experiment 2015-16 (Fife site) 14 WAP*

Treatment	Dry weight of leaves/g (10 plants)	Dry weight of stems/g (10 plants)	Dry weight of roots/g (10 plants)
No cover crop - plots rotovated and rolled	-	-	-
Flail A* + Rotovate	13.69	32.20	4.52
Flail B* + Rotovate	12.43	29.80	4.31
Flail B + Rotovate + Plough	12.83	30.23	4.38
Significance (*P*-value)	NS	NS	NS
SED	2.5	6.7	0.8
%CV	12.3	13.6	1.2

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 102: *The number and yield of potatoes cv. Desirée grown in field plots (Fife site) previously cropped with Brassica juncea cv. Caliente 99 or left fallow. Biofumigant plants macerated using two flail speeds and incorporated with a rotovator, and/or plough*

Treatment	Total tubers th/ha	Yield t/ha
No cover crop - plots rotovated and rolled	645	30.9
Flail A* + Rotovate	780	40.4
Flail B* + Rotovate	705	34.4
Flail B + Rotovate + Plough	745	40.4
Significance (*P*-value)	0.521	0.219
SED	159.5	12.9
%CV	20.4	16.8

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

<u>Lincolnshire (Howell Fen) site:</u>

The Howell Fen site was fully irrigated with unlimited access to a reservoir and situated in a free draining, sandy loam soil. The crop was grown from August 2015 until incorporation in October 2015 and was irrigated as required with a target of 15 ml per week. Fertiliser was applied pre-drilling and incorporated. Background data on temperature, rainfall and soil characteristics is displayed in Table 103 and Figures 16-19.

The assessments of potato cyst nematode in soil samples taken from the plots gave no significant differences between treatments at any of the timings, (pre-sowing, pre-incorporation (Tables 108-110) and post incorporation of *B. juncea* cv. Caliente 99). The assessments included viable eggs g^{-1} soil, viable eggs per cyst and % egg viability. There was considerable variation in the PCN results and even after transforming the data, there were no clear indications of differences between the treatments, even though the crop biomass was moderately successful across all the plots with a mean of 56.61t/ha (SD 16.36). All the PCN data required transformation (Log X+1 or square root X +0.5).

There were no agronomic problems with the crop, such as ingress of weeds, pests or disease. Analysis of the fresh and dry weight of the leaves and roots (Tables 111 and 112) did not reveal any significant differences between treatment plots.

Unfortunately, spaders are not commonly used in the East Midlands and a used one was sourced specifically for the trial. The design was archaic but functioned moderately well.

A Desirée potato crop was planted in May 2016, post the biofumigant crop. The site was fully irrigated with unlimited access to a reservoir and situated in a free draining, sandy loam soil. The assessments of potato cyst nematode in soil samples were taken from the plots pre and post the potato crop. The crop was maintained throughout the season and a full blight programme was applied when necessary. The *Pf* assessment showed lower eggs g^{-1} soil values in treatments with Flail B/Disc/Roll and Flail B/Spader/Roll (Table 113). The crop produced a greater yield in the cultivation methods of the Flail B/Rotovate/Roll. The lowest yield was produced in the Fallow plots.

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Blemising diseases, were not found to differ significantly between treatments ($P > 0.05$) (Table 115). Whilst not significantly different, it appeared that black scurf incidences were higher in field plots where *B. juncea* had not been grown.

Table 103: *Soil analysis pre-drilling of the 2015 experiment at Howell Fen*

pH	P	P	K	K	Mg	Mg
	Mg/ml	Index	Mg/ml	Index	Mg/ml	Index
7.1	28.2	3	190	2+	66	2

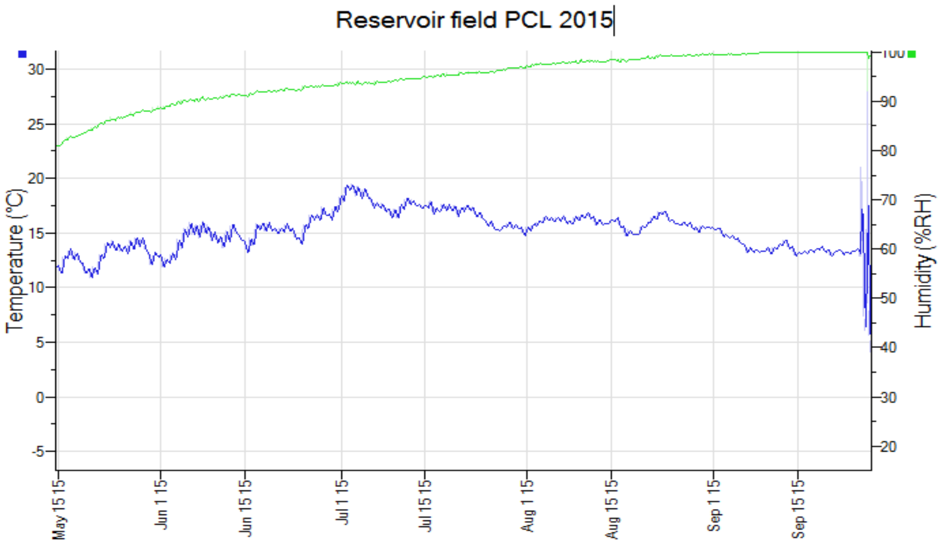

Figure 16: *Data logger soil temperature at the Howell Fen site in 2015*

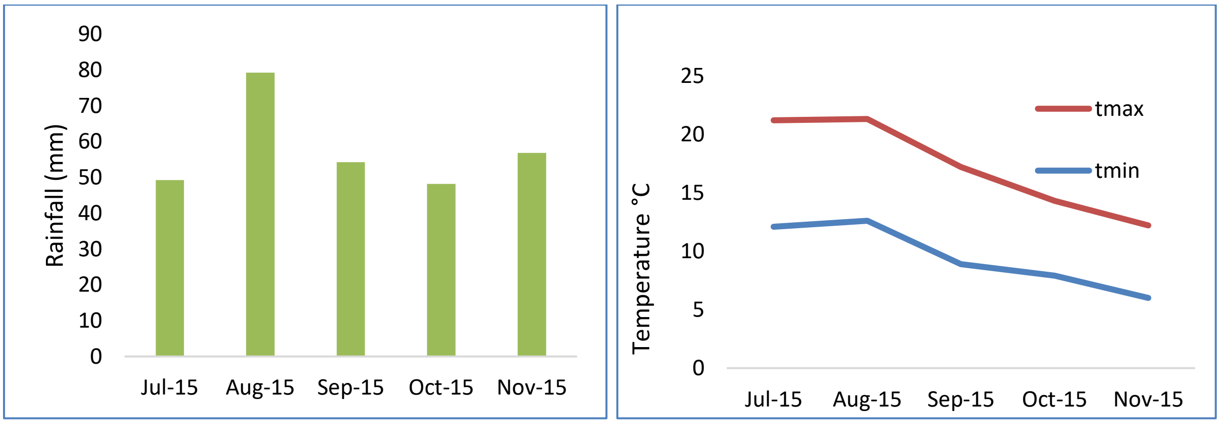

Figure 17: *Meterological data for the Lincolnshire site based on Met Office historical weather station data (Waddington)*

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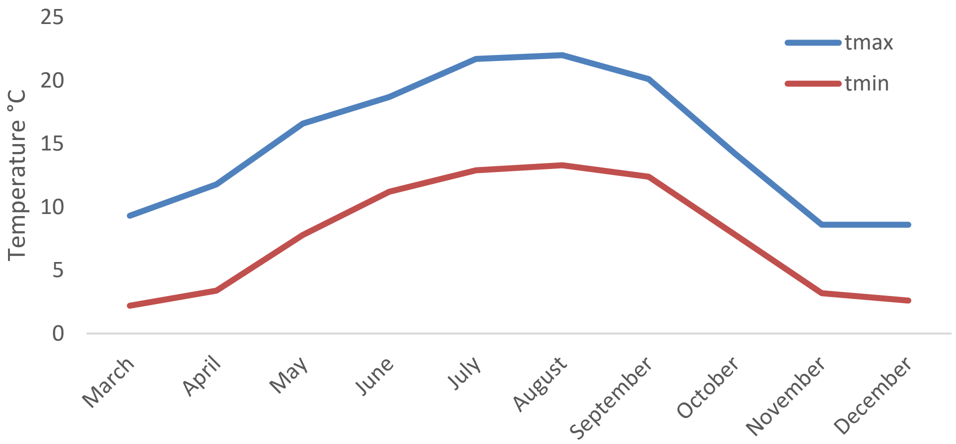

Figure 18: *Meterological data for the Lincolnshire site based on Met Office historical weather station data (Waddington) 2016*

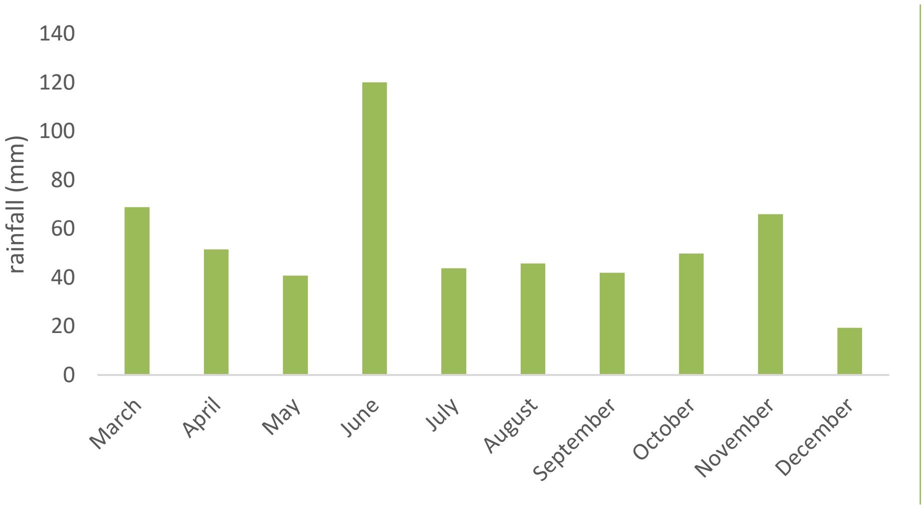

Figure 19: *Meterological data for the Lincolnshire site based on Met Office historical weather station data (Waddington) 2016*

Table 104: *Influence of incorporation method on the mean Extractable Nitrate N (mg/kg DM) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Lincolnshire (Howell Fen) site.*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	59.86a	8.54a
Flail A + Rotovate	18.24b	9.98a
Flail B + Rotovate	18.21b	9.11a
Flail A + Rotovate + Plough	19.75b	8.83a
Flail A + Spader	27.00b	8.87a
Flail A + Spader + Plough	12.10b	9.04a
Flail A + Disc	19.37b	7.83a
Flail A + Disc + Plough	8.36b	8.31a
Significance (*P*-value)	0.001	NS
SED	17.55	1.28
%CV	88.16	16.70

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Table 105: *Influence of incorporation method on the mean Extractable Phosphorus (mg/l) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Lincolnshire (Howell Fen) site.*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	29.20a	28.04a
Flail A + Rotovate	27.34a	23.76a
Flail B + Rotovate	28.36a	26.34a
Flail A + Rotovate + Plough	28.10a	28.78a
Flail A + Spader	44.30a	41.42a
Flail A + Spader + Plough	34.92a	35.78a
Flail A + Disc	40.12a	38.42a
Flail A + Disc + Plough	21.12a	21.31a
Significance (*P*-value)	NS	NS
SED	16.34	15.84
%CV	59.22	59.74

Table 106: *Influence of incorporation method on the mean Extractable Potassium (mg/l) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Lincolnshire (Howell Fen) site.*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	264.6a	185.8a
Flail A + Rotovate	294.6a	218.6a
Flail B + Rotovate	277.4a	267.8a
Flail A + Rotovate + Plough	208.4a	214.8a
Flail A + Spader	254.3a	229.8a
Flail A + Spader + Plough	234.8a	242.8a
Flail A + Disc	216.8a	193.8a
Flail A + Disc + Plough	181.4a	192.6a
Significance (*P*-value)	NS	NS
SED	74.8	51.8
%CV	35.65	27.27

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Table 107: *Influence of incorporation method on the mean organic matter (mg/kg) pre-incorporation of the biofumigant in 2015 and pre-planting of potatoes in 2016 (Lincolnshire (Howell Fen) site.*

Incorporation method	Pre-incorporation	Pre-potatoes
No cover crop - plots rotovated and rolled	987.3a	894.2a
Flail A + Rotovate	987.1a	891.2a
Flail B + Rotovate	985.7a	898.7a
Flail A + Rotovate + Plough	987.5a	894.0a
Flail A + Spader	987.8a	889.0a
Flail A + Spader + Plough	986.4a	896.0a
Flail A + Disc	988.6a	893.6a
Flail A + Disc + Plough	987.8a	894.6a
Significance (*P*-value)	NS	NS
SED	1.73	6.22
%CV	0.20	0.80

Table 108: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots (Lincolnshire site) with and without* Brassica juncea *cv. Caliente 99. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Soil samples for PCN collected pre-sowing and pre and post-incorporation of Caliente 99 and pre planting of a Desiree potato crop.*

Treatment	Viable eggs g^{-1} soil pre-sowing (August 2015)	Viable eggs g^{-1} soil pre-incorporation (October 2015)	Viable eggs g^{-1} soil post-incorporation (February 2016)	Viable eggs g^{-1} soil pre-planting (May 2016)
Flail A + Rotovate	15.64	26.60	22.85	25.06
Flail B + Rotovate	9.966	14.86	12.26	15.88
Flail B + Rotovate + Plough	20.83	37.58	22.98	17.46
Flail B + Spader	14.54	14.70	15.41	11.34
Flail B + Spader + Plough	14.36	18.61	14.11	18.61
Flail B + Disc	25.69	24.05	17.91	23.07
Flail B + Disc + Plough	25.09	25.45	27.95	20.74
Fallow	13.66	36.20	24.07	19.68
Significance (P-value)	0.872	0.336	0.422	**0.876**
SED	0.225	0.885	0.68	**0.17**
%CV	36.49	35.84	30.7	**26.67**

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 109: *The viability of potato cyst nematode eggs (%) sampled from field plots (Lincolnshire site) with and without* Brassica juncea *cv. Caliente 99. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Soil samples for PCN collected pre-sowing and pre and post-incorporation of Caliente 99 and pre planting of a Desiree potato crop*

Treatment	% PCN eggs viable pre-sowing (August 2015)	% PCN eggs viable pre-incorporation (October 2015)	% PCN eggs viable post-incorporation (February 2016)	% PCN eggs viable pre-planting (May 2016)
Flail A + Rotovate	94.57	85.99	83.00	79.32
Flail B + Rotovate	88.73	75.12	73.13	80.17
Flail B + Rotovate + Plough	83.74	70.55	80.93	83.88
Flail B + Spader	94.91	73.02	81.57	63.51
Flail B + Spader + Plough	94.73	82.46	84.52	78.9
Flail B + Disc	94.77	87.92	83.78	78.72
Flail B + Disc + Plough	94.92	81.58	77.44	78.37
Fallow	93.66	90.06	86.22	77.37
Significance (P-value)	0.5987	0.5204	0.5717	0.8761
SED	0.0247	0.4749	0.29	0.344
%CV	2.51	10.53	6.42	26.67

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Table 110: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Lincolnshire site) with and without* Brassica juncea *cv. Caliente 99. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Soil samples for PCN collected pre-sowing and pre and post-incorporation of Caliente 99 and pre planting of a Desiree potato crop.*

Treatment	Number of viable potato cyst nematode eggs cyst^{-} pre-sowing (August 2015)	Number of viable potato cyst nematode eggs cyst^{-} pre-incorporation (October 2015)	Number of viable potato cyst nematode eggs cyst^{-} pre-incorporation (April 2016)	Number of viable potato cyst nematode eggs cyst^{-} post-incorporation (May 2016)
Flail A + Rotovate	49.91	57.51	60.91	56.06
Flail B + Rotovate	36.71	43.88	45.18	52.73
Flail B + Rotovate + Plough	43.64	59.69	61.54	54.15
Flail B + Spader	41.45	47.82	44.65	28.96
Flail B + Spader + Plough	49.56	48.15	37.67	41.81
Flail B + Disc	60.48	69.68	47.35	51.02
Flail B + Disc + Plough	54.54	46.13	64.75	47.00
Fallow	38.04	75.92	57.14	44.28
Significance (P-value)	0.9313	0.7295	0.5964	0.8809
SED	0.1445	1.0835	0.863	0.1561
%CV	17.27	28.93	23.82	18.65

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 111: *Fresh weight of foliar and root biomass from brassica cover crops from field experiment 2015-16 (Lincolnshire site)*

Treatment	Fresh weight of stem/g (10 plants)	Fresh weight of leaves/g (10 plants)	Fresh weight of roots/g (10 plants)	Total fresh weight t/ha
Flail A + Rotovate	270.58	277.70	32.05	55.45
Flail B + Rotovate	254.34	256.44	28.6	55.58
Flail B + Rotovate + Plough	275.64	282.04	32.25	56.19
Flail B + Spader	292.36	291.82	32.06	49.99
Flail B + Spader + Plough	268.98	254.26	32.44	56.29
Flail B + Disc	276.94	286.04	35.83	64.84
Flail B + Disc + Plough	279.24	272.74	31.06	57.97
Fallow	0.00	0.00	0.00	0.00
Significance (P-value)	0.1734	0.2097	0.2641	0.8208
SED	10.006	12.97	2.033	8.1908
%CV	7.3	9.45	12.69	28.9

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Table 112: *Dry weight of foliar and root biomass from brassica cover crops from field experiment 2015-16 (Lincolnshire site)*

Treatment	Dry weight of stem/g (10 plants)	Dry weight of leaves/g (10 plants)	Dry weight of roots/g (10 plants)
Flail A + Rotovate	40.700	36.95	5.724
Flail B + Rotovate	39.940	34.58	5.294
Flail B + Rotovate + Plough	44.020	38.18	5.992
Flail B + Spader	46.760	40.26	6.228
Flail B + Spader + Plough	40.960	38.30	6.230
Flail B + Disc	42.270	37.50	6.416
Flail B + Disc + Plough	42.460	40.48	5.894
Fallow	0.00	0.00	0.000
Significance (P-value)	0.0705	0.0529	0.3865
SED	1.73	1.4375	0.4013
%CV	8.15	7.56	13.45

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 113: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from field plots (Lincolnshire site) with and without* Brassica juncea *cv. Caliente 99*. *Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Soil samples for PCN collected post planting of a Desiree potato crop (Pf).*

Treatment	Viable eggs g^{-1} soil Pf (Oct 2016)
Flail A + Rotovate	**56.6**
Flail B + Rotovate	**54.8**
Flail B + Rotovate + Plough	**44.4**
Flail B + Spader	**38.0**
Flail B + Spader + Plough	**65.4**
Flail B + Disc	**59.2**
Flail B + Disc + Plough	**38.6**
Fallow	**62.6**
Significance (P-value)	**0.633**
SED	**17.51**
%CV	**52.8**

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

Table 114: *The number and yield of potatoes cv. Desirée grown in field plots (Lincolnshire site) previously cropped with Brassica juncea cv. Caliente 99 or left fallow. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc.*

Treatment	Total tubers th/ha	Yield t/ha
No cover crop - plots rotovated and rolled	763	38.6
Flail A* + Rotovate	726	41.7
Flail B* + Rotovate	878	53.0
Flail B + Rotovate + Plough	837	42.3
Flail B + Spader	767	42.6
Flail B + Spader + Plough	867	46.0
Flail B + Disc	783	40.2
Flail B + Disc + Plough	862	45.8
Significance (*P*-value)	0.582	0.284
SED	89.9	5.57
%CV	17.5	20.1

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

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Table 115: *The incidence of blemishing diseases on potatoes cv. Desirée grown in field plots (Lincolnshire site) previously cropped with Brassica juncea cv. Caliente 99 or left fallow. Biofumigant plants macerated using two flail speeds and incorporated with various implement selections including a rotovator, spader, plough and disc. Black scurf data transformed using SQRT – Actual means in parentheses*

Treatment	Black scurf (*Rhizoctonia solani*)	Silver scurf (*Helminthosporium solani*) Mean prediction figures	Black dot (*Colletotricum coccodes*) Mean prediction figures	Common scab (*Streptomyces scabei)* Mean prediction figures
No cover crop - plots rotovated and rolled	3.99 (20.5)	15.96	9.459	4.001
Flail A* + Rotovate	0.91 (2.5)	17.36	13.799	0.881
Flail B* + Rotovate	1.46 (5.3)	16.56	10.139	4.441
Flail B + Rotovate + Plough	1.79 (5.6)	9.90	8.079	4.441
Flail B + Spader	0.90 (2.9)	14.84	8.339	6.041
Flail B + Spader + Plough	2.14 (7.0)	14.48	11.459	4.855
Flail B + Disc	2.29 (8.9)	15.33	7.000	8.825
Flail B + Disc + Plough	1.84 (8.5)	6.82	5.479	2.441
Significance (*P*-value)	0.293 (0.086)	0.279	0.434	0.477
SED	1.226 (5.65)	4.544	3.611	3.302
%CV	101.1 (116.7)	50.96	60.39	116.98

* Flail A. forward speed = 2.5 – 3.5 kph, Flail B. forward speed = 6 – 9 kph

1.5 Field experiments to evaluate nitrogen and sulphur inputs on Indian mustard (*B. juncea*) cv. Caliente 99 used for suppressing potato cyst nematodes (2016-2017)

Shropshire site:

The field experiment was planted on the 18th August 2016 and macerated/incorporated on the 28th October 2016. Based on meteorological data from the local weather station (Shawbury), mean monthly rainfall was lower than average during August-October (Figure 20); 80-100mm would typically be expected. Temperatures were mild during August and September (11.9-21.4 °C) and became cooler in October (6.5-14 °C) (Figure 20).

Population densities of PCN (viable eggs g^{-1} soil, % egg viability and viable eggs $cyst^{-1}$) were not found to differ significantly between treatments when sampled pre-sowing (Pi) and pre-incorporation of *B. juncea* cv. Caliente 99 (Tables 116 to 121). However, the

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viability of PCN eggs (% viability) was significantly affected by both sulphur and nitrogen when PCN were sampled 6 weeks after the maceration and incorporation of *B. juncea* (Table 123).

In addition, a significant interaction was seen between nitrogen and sulphur on egg viability. Increasing sulphur inputs resulted in reduced PCN egg viability when nitrogen inputs were not included. In general, an input of 25 kg/ha of sulphur in the presence of nitrogen (50-150 kg/ha) resulted in a reduction PCN egg viability. Increasing the sulphur input to 50kg/ha in conjunction with nitrogen had little additional effect on PCN egg viability. Measurements of viable eggs g^{-1} soil and viable eggs $cyst^{-1}$ were not found to significantly differ between treatments when PCN were sampled 6 weeks after the maceration and incorporation of *B. juncea* (Tables 122 and 124).

Fresh and dry weight of *B. juncea* leaves and stems were significantly increased by nitrogen but were unaffected by sulphur (Tables 125-128; Figure 21**).** Fresh and dry weight of *B. juncea* roots were unaffected by the treatments (Tables 129 and 130. Foliage of *B. juncea* (Tables 131 and 132) was approximately 3.4 fold higher in plots receiving 150 kg/ha N and 0 S (30.54 t FW ha) than in plots receiving 0 N or S (9.07 t FW ha).

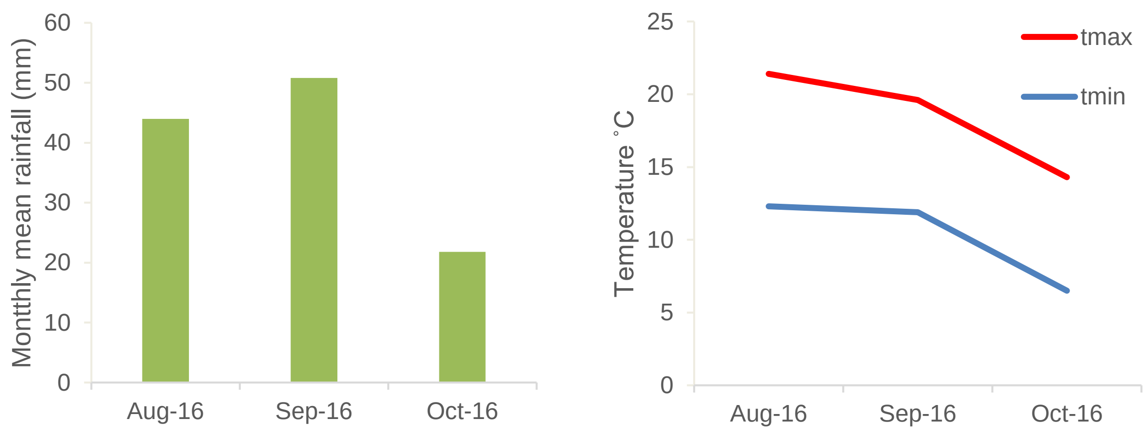

Figure 20: *Meterological data (rainfall and temperature) for the Shropshire site (2016) based on Met Office historical weather station data (Shawbury)*

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Table 116: *Viable population densities of potato cyst nematodes (viable eggs g^{-1} soil) sampled from field plots (Shropshire site), pre-planting of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Values have been transformed using square root – actual values in parentheses*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	3.84 (15)	4.07 (17)	3.16 (11)	3.69 (14)
50	3.04 (10)	3.44 (12)	3.23 (11)	3.24 (11)
100	2.75 (8)	2.94 (10)	3.95 (16)	3.21 (11)
150	3.70 (15)	3.53 (14)	3.14 (10)	3.46 (13)
Mean	3.33 (12)	3.50 (13)	3.37 (12)	
Grand Mean	3.40 (12)			
Significance (P-value): N	0.514		SED: N	0.358
Significance (P-value): S	0.861		SED: S	0.310
Significance (P-value): N*S	0.301		SED: N*S	0.620
CV%	25.8			

Table 117: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots (Shropshire site), pre-planting of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Values have been transformed using ArcSin – actual values in parentheses*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	79.2 (96)	78.8 (96)	81.4 (97)	79.8
50	76.9 (93)	82.2 (98)	79.6 (95)	79.6
100	82.1 (98)	86.0 (98)	82.2 (98)	83.4
150	78.8 (94)	77.5 (95)	83.3 (97)	79.9
Mean	79.3	81.1	81.6	
Grand Mean	80.7			
Significance (P-value): N	0.503		SED: N	2.94
Significance (P-value): S	0.620		SED: S	2.54
Significance (P-value): N*S	0.843		SED: N*S	5.09
CV%	8.9			

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Table 118: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Shropshire site), pre-planting of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*.

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	50.8	56.5	40.7	49.4
50	36.5	52.1	43.6	44.1
100	30.2	40.8	57.1	42.7
150	65.1	45.2	35.2	48.5
Mean	45.6	48.6	44.2	
Grand Mean	46.1			
Significance (P-value): N	0.842		SED: N	8.83
Significance (P-value): S	0.838		SED: S	7.65
Significance (P-value): N*S	0.218		SED: N*S	15.3
CV%	46.9			

Table 119: *Viable population densities of potato cyst nematodes (viable eggs g^{-1} soil) sampled from field plots (Shropshire site), pre-incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Values have been transformed using ArcSin – actual values in parentheses*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	4.18 (18)	3.64 (14)	3.28 (12)	3.70 (15)
50	2.83 (8)	3.59 (13)	3.79 (15)	3.40 (12)
100	2.85 (8)	3.76 (16)	3.97 (17)	3.53 (13)
150	2.68 (7)	2.98 (10)	4.43 (21)	3.37 (14)
Mean	3.13 (10)	3.49 (13)	3.87 (16)	
Grand Mean	3.50 (13)			
Significance (P-value): N	0.865		SED: N	0.432
Significance (P-value): S	0.159		SED: S	0.274
Significance (P-value): N*S	0.254		SED: N*S	0.747
CV%	30.2			

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Table 120: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots (Shropshire site), pre-incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Values have been transformed using square root – actual values in parentheses*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	80.01 (96)	76.02 (94)	79.43 (96)	78.48
50	71.84 (90)	76.70 (94)	78.08 (95)	75.54
100	81.14 (97)	81.37 (97)	78.17 (96)	80.23
150	72.08 (90)	76.53 (93)	79.81 (97)	76.14
Mean	76.27	77.65	78.87	
Grand Mean	77.60			
Significance (P-value): N	0.114		SED: N	2.092
Significance (P-value): S	0.367		SED: S	1.812
Significance (P-value): N*S	0.259		SED: N*S	3.624
CV%	6.6			

Table 121: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Shropshire site), pre-incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	54.8	49.2	43.3	49.1
50	40.2	50.9	52.8	48.0
100	36.1	54.3	62.4	50.9
150	28.8	40.8	74.1	47.9
Mean	40.0	48.8	58.2	
Grand Mean	49.0			
Significance (P-value): N	0.987		SED: N	9.42
Significance (P-value): S	0.099		SED: S	8.16
Significance (P-value): N*S	0.326		SED: N*S	16.31
CV%	47.1			

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Table 122: *Viable population densities of potato cyst nematodes (viable eggs g*$^{-1}$ *soil) sampled from field plots (Shropshire site), 6 weeks post incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Values have been transformed using square root – actual values in parentheses*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	3.71 (13.76)	3.67 (13.47)	3.26 (10.63)	3.55 (12.60)
50	3.11 (9.67)	3.68 (13.54)	3.57 (12.75)	3.45 (11.90)
100	3.19 (10.18)	3.91 (15.29)	4.16 (17.31)	3.75 (14.06)
150	3.78 (14.29)	3.51 (15.29)	3.46 (11.97)	3.58 (12.82)
Mean	3.45 (11.90)	3.69 (13.62)	3.61 (13.03)	
Grand Mean	3.58 (12.82)			
Significance (P-value): N	0.788		SED: N	0.298
Significance (P-value): S	0.638		SED: S	0.258
Significance (P-value): N*S	0.484		SED: N*S	0.517
CV%	20.4			

Table 123: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots (Shropshire site), 6 weeks post incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting. Values have been transformed using ArcSin – actual values in parentheses. Means with same letter are not significantly different according to Tukeys multiple range test (*P = 0.05*)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	75.05 (93.0)bc	69.29 (87.1)abc	64.93 (81.8)ab	69.76a
50	66.75 (84.4)ab	67.77 (85.4)abc	69.81 (86.8)abc	68.11ab
100	81.47 (97.0)c	58.60 (72.7)a	63.57 (80.0)ab	67.88ab
150	69.70 (86.9)abc	59.26 (73.7)a	56.75 (69.5)a	61.91b
Mean	73.24b	63.73a	63.76a	
Grand Mean	66.91			
Significance (P-value): N	0.011		SED: N	2.333
Significance (P-value): S	<0.001		SED: S	2.020
Significance (P-value): N*S	0.004		SED: N*S	4.041
CV%	8.5			

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Table 124: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Shropshire site), 6 weeks post incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	62.7	63.7	56.8	61.1
50	44.8	50.8	57.6	51.1
100	49.7	56.4	54.0	53.4
150	56.8	53.7	51.2	53.9
Mean	53.5	56.2	54.9	
Grand Mean	54.9			
Significance (P-value): N	0.587		SED: N	7.58
Significance (P-value): S	0.920		SED: S	6.57
Significance (P-value): N*S	0.953		SED: N*S	13.13
CV%	33.8			

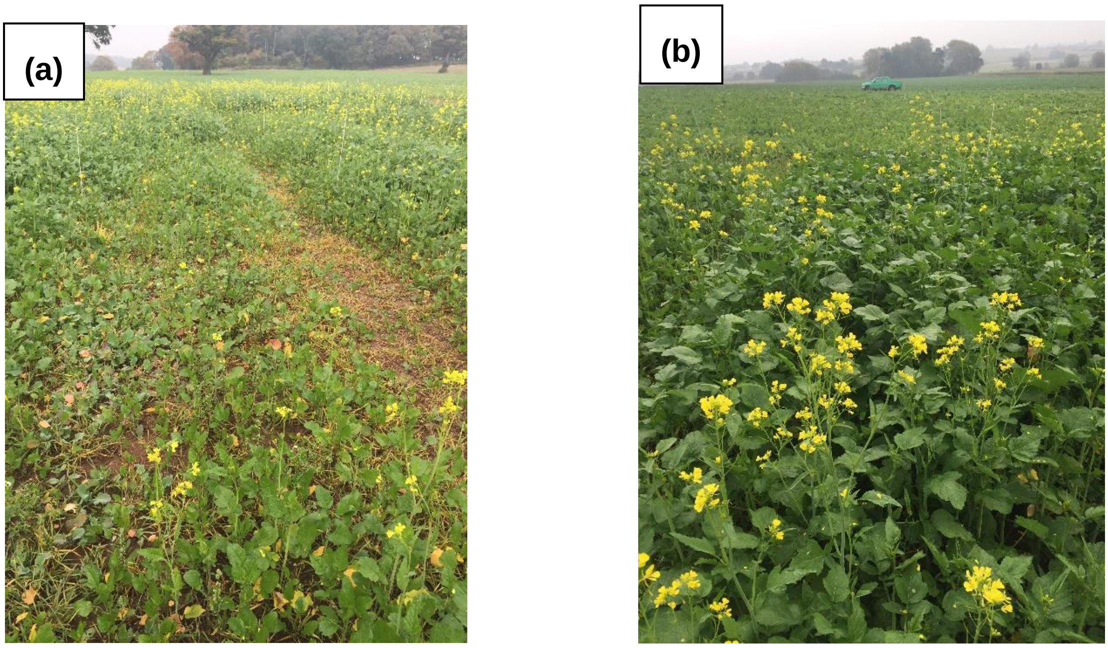

Figure 21: *Growth of* Brassica juncea *cv. Caliente 99 in field plots treated with (a) no nitrogen or sulphur or (b) 150 kg/ha N and 25 kg/ha S. Field experiment 2016 (Shropshire site) at c. 10 WAP*

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Table 125: *Fresh weight of leaves (g m^2) sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	509	603	623	578
50	680	604	833	706
100	906	927	931	921
150	1125	719	906	917
Mean	805	713	823	
Grand Mean	780			
Significance (P-value): N	0.009		SED: N	111.1
Significance (P-value): S	0.482		SED: S	96.2
Significance (P-value): N*S	0.570		SED: N*S	192.4
CV%	34.9			

Table 126: *Dry weight of leaves (g m^2) sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	84.5	95.4	97.1	92.3a
50	104.8	95.9	129.3	110.0ab
100	136.2	147.2	137.0	140.1b
150	159.0	109.0	139.3	135.8b
Mean	121.1	111.9	125.7	
Grand Mean	119.6			
Significance (P-value): N	0.008		SED: N	14.80
Significance (P-value): S	0.553		SED: S	12.82
Significance (P-value): N*S	0.553		SED: N*S	25.63
CV%	30.3			

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Table 127: *Fresh weight of stems (g m^2) sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05).*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	398	818	878	698a
50	1126	940	1436	1167b
100	1446	1684	1507	1546b
150	1929	1254	1656	1613b
Mean	1225	1174	1369	
Grand Mean	1256			
Significance (P-value): N	<0.001		SED: N	191.7
Significance (P-value): S	0.484		SED: S	166.0
Significance (P-value): N*S	0.289		SED: N*S	332.1
CV%	37.4			

Table 128: *Dry weight of stems (g m^2) sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	66.4	118.8	127.0	104.1a
50	146.1	134.5	197.3	159.3b
100	186.6	206.0	175.1	189.2b
150	205.3	151.3	196.5	184.4b
Mean	151.1	152.6	174.0	
Grand Mean	159.2			
Significance (P-value): N	<0.001		SED: N	18.75
Significance (P-value): S	0.302		SED: S	16.24
Significance (P-value): N*S	0.162		SED: N*S	32.48
CV%	28.8			

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Table 129: *Fresh weight of roots (g m^2) sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	191	279	225	232
50	244	203	263	237
100	260	275	251	262
150	284	218	231	244
Mean	245	244	243	
Grand Mean	244			
Significance (P-value): N	0.781		SED: N	31.4
Significance (P-value): S	0.998		SED: S	27.2
Significance (P-value): N*S	0.464		SED: N*S	54.4
CV%	31.6			

Table 130: *Dry weight of roots (g m^2) sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	58.1	68.3	67.9	64.8
50	70.6	63.7	79.4	71.2
100	79.4	82.8	74.6	78.9
150	78.9	61.6	71.3	70.6
Mean	71.8	69.1	73.3	
Grand Mean	71.4			
Significance (P-value): N	0.213		SED: N	6.52
Significance (P-value): S	0.756		SED: S	5.65
Significance (P-value): N*S	0.513		SED: N*S	11.30
CV%	22.4			

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Table 131: *Fresh weight of foliage (g m²) sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP. Values in parentheses are the same means expressed as FW t/ha. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	907 (9.07)	1422 (14.22)	1501 (15.01)	1276a
50	1805 (18.05)	1545 (15.45)	2269 (22.69)	1873ab
100	2352 (23.52)	2611 (26.11)	2438 (24.38)	2467b
150	3054 (30.54)	1973 (19.73)	2561 (25.61)	2530b
Mean	2030	1888	2192	
Grand Mean	2037			
Significance (P-value): N	<0.001		SED: N	299.2
Significance (P-value): S	0.507		SED: S	259.1
Significance (P-value): N*S	0.366		SED: N*S	518.3
CV%	36			

Table 132: *Dry weight (g m²) of foliage sampled from brassica cover crops from field experiment 2016 (Shropshire site) 11 WAP. Values in parentheses are the same means expressed as DW t/ha. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	151 (1.51)	214 (2.14)	224 (2.24)	196a
50	251 (2.51)	230 (2.30)	327 (3.27)	269ab
100	323 (3.23)	353 (3.53)	312 (3.12)	329b
150	364 (3.64)	260 (2.60)	336 (3.36)	320b
Mean	272	265	300	
Grand Mean	279			
Significance (P-value): N	<0.001		SED: N	32.7
Significance (P-value): S	0.437		SED: S	28.3
Significance (P-value): N*S	0.297		SED: N*S	56.7
CV%	28.7			

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Fife site:

The field experiment was planted on the 22nd July 2016 and macerated/incorporated on the 31st October 2016. The growing conditions for the Caliente 99 mustard allowed rapid establishment of the crop, with ample rainfall throughout the summer months (Figures 22 and 23). Temperatures cooled in September and this did have an impact on the growth of the mustard, and this is reflected in the biomass of the plant when it reached early flowering.

There were significant effects through the use of differing rates and combinations of Nitrogen and Sulphur on the fresh weights of leaves, stems and roots at the Fife trial site (Tables 133, 134, 135 and 136).

The impact of nitrogen and sulphur rate was significant on the fresh weight of the leaves (P = 0.001, P = 0.001 respectively, Table 133) and stems (P = 0.001, P = 0.015, respectively, Table 134), with increasing rates of nitrogen and/or sulphur tending to increase the fresh weights.

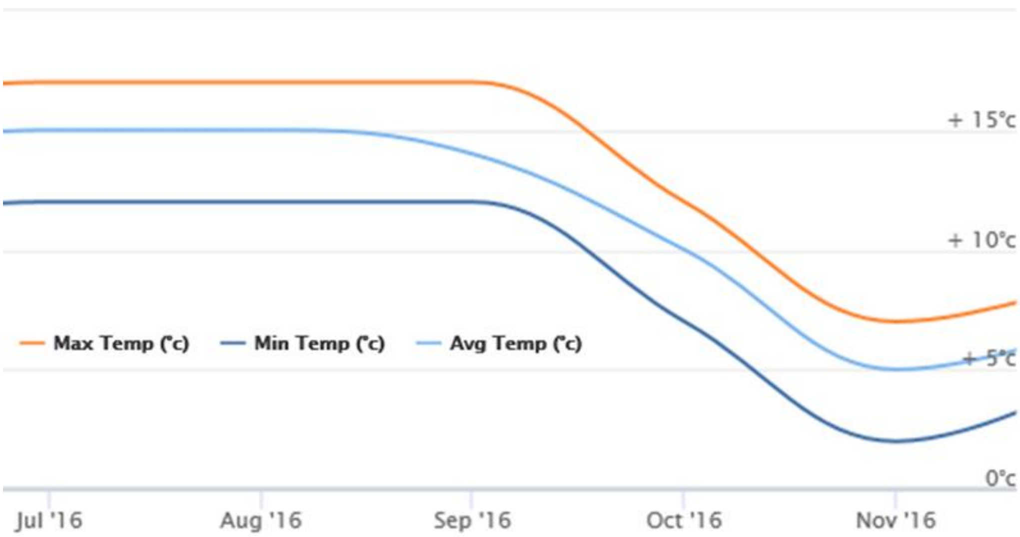

Figure 22: *Maximum, minimum and average air temperatures at Fife trial site in 2016*

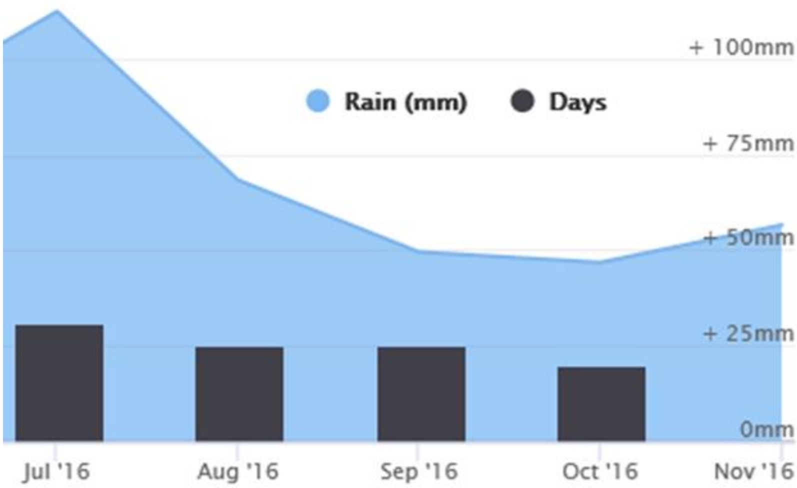

Figure 23: *Ranfall data for Fife trial site in 2016*

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Table 133: *Fresh weight of leaves (g m^2) sampled from brassica cover crops from field experiment 2016 (Fife site) 14 WAP*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	58	60	79	66
50	126	172	168	156
100	263	289	334	295
150	487	555	586	542
Mean	234	269	292	
Grand Mean	265			
Significance (P-value): N	0.001			
Significance (P-value): S	0.001			
Significance (P-value): N*S	0.494			
CV%	71			

Table 134: *Fresh weight of stems (g m^2) sampled from brassica cover crops from field experiment 2016 (Fife site) 14 WAP*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	115	82	121	106
50	208	280	264	251
100	411	473	565	483
150	778	837	794	803
Mean	378	418	436	
Grand Mean	411			
Significance (P-value): N	0.001			
Significance (P-value): S	0.015			
Significance (P-value): N*S	0.053			
CV%	66.6			

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Table 135: *Fresh weight of roots (g m^2) sampled from brassica cover crops from field experiment 2016 (Fife site) 14 WAP*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	19	21	22	20
50	29	26	41	32
100	68	95	122	95
150	161	162	142	155
Mean	69	76	82	
Grand Mean	75			
Significance (P-value): N	0.001			
Significance (P-value): S	0.219			
Significance (P-value): N*S	0.034			
CV%	78			

Table 136: *Fresh weight of foliage (g m^2) sampled from brassica cover crops from field experiment 2016 (Fife site) 14 WAP. Values in paraentheses are the same means as expressed as FW t/h. Treatments with the same letters are not significantly different according to Tukeys multiple range test*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	173 (1.73)	143 (1.43)	200 (2.00)	172a
50	334 (3.34)	452 (4.52)	433 (4.33)	406b
100	674 (6.74)	762 (7.62)	899 (8.99)	778c
150	1333 (13.33)	1391 (13.91)	1380 (13.80)	1345d
Mean	629 (6.29)	687 (6.87)	728 (7.28)	
Grand Mean	675			
Significance (P-value): N	0.001			
Significance (P-value): S	0.004			
Significance (P-value): N*S	0.263			
CV%	68			

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Lincolnshire site:

The field experiment was planted on the 13th August 2016 and macerated/incorporated on the 13th October 2016. Based on meteorological data from the local weather station (Waddington), mean monthly rainfall was 42-49ml/month during August-October (Figure 24). Temperatures were a maximum of 22˚C during August dropping to a maximum of 14.2 ˚C in October 2016 (Figure 25). The site was fully irrigated with unlimited access to a reservoir and situated in a free draining, sandy loam soil.

Population densities of PCN (viable eggs g^{-1} soil, % egg viability and viable eggs $cyst^{-1}$) were not found to differ significantly between treatments when sampled pre-sowing (*Pi*), pre-incorporation and post incorporation of *B. juncea* cv. Caliente 99 (Tables 137 to 145).

Fresh and dry weight of *B. juncea* leaves, stems and roots were significantly increased by nitrogen (*P* <0.001) but were unaffected by sulphur (Tables 146-151). The total foliage of *B. juncea* were significantly increased by nitrogen (*P*<0.001 but were unaffected by sulphur (Tables 152 and 153).

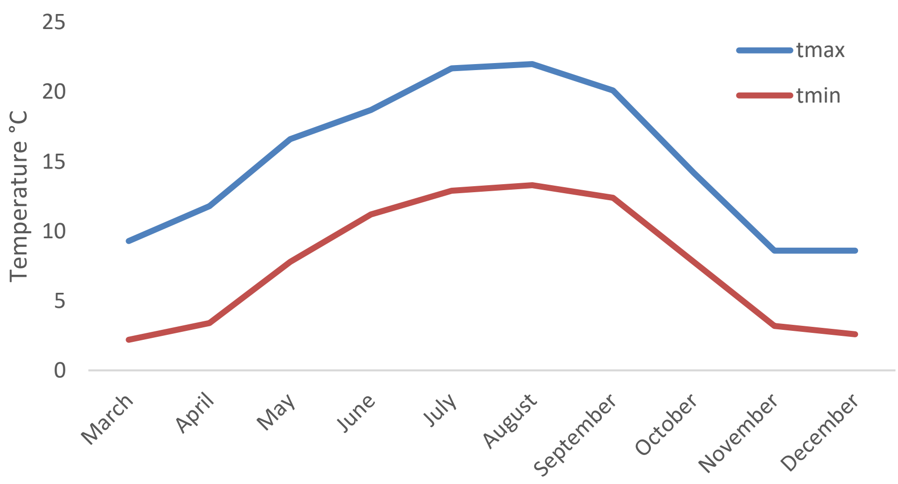

Figure 24: *Meterological data (temperature) for the Lincolnshire site (2016) based on Met Office historical weather station data (Waddington)*

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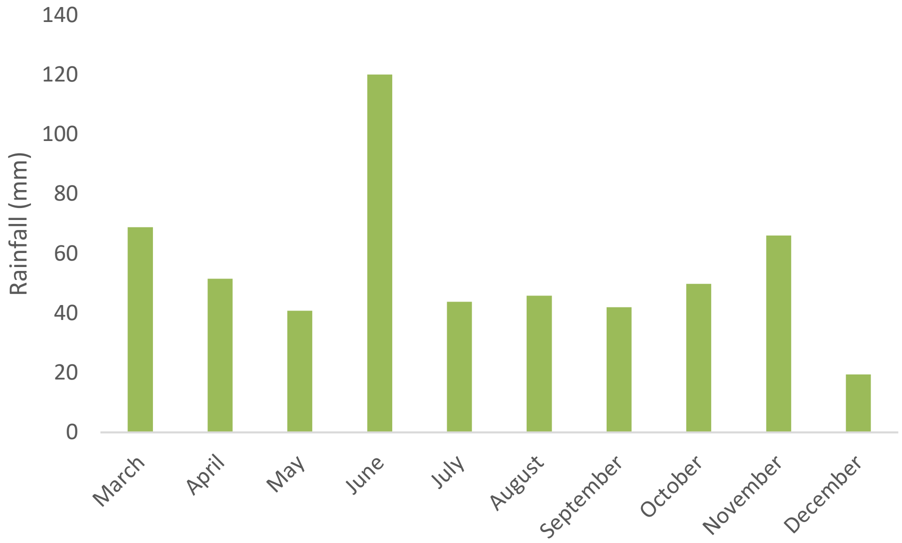

Figure 25: *Meterological data (rainfall) for the Lincolnshire site (2016) based on Met Office historical weather station data (Waddington)*

Table 137: *Viable population densities of potato cyst nematodes (viable eggs g^{-1} soil) sampled from field plots (Lincolnshire site), pre-planting of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	25.4	22.8	21.1	23.1
50	23.4	24.0	13.8	20.4
100	25.1	27.5	24.8	25.8
150	25.0	24.2	18.4	22.5
Mean	24.7	24.6	19.5	
Grand Mean	23.0			
Significance (P-value): N	0.725		SED: N	4.75
Significance (P-value): S	0.359		SED: S	4.11
Significance (P-value): N*S	0.983		SED: N*S	8.22
CV%	50.6			

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Table 138: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots (Lincolnshire site), pre-planting of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	59.3	62.0	60.6	60.6
50	64.2	68.9	61.4	64.8
100	75.2	70.1	67.2	70.9
150	71.6	66.7	64.3	67.5
Mean	67.6	66.9	63.4	
Grand Mean	66.0			
Significance (P-value): N	0.127		SED: N	4.29
Significance (P-value): S	0.486		SED: S	3.71
Significance (P-value): N*S	0.926		SED: N*S	7.43
CV%	15.9			

Table 139: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Lincolnshire site), pre-planting of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	100.7	85.6	57.9	81.4
50	85.0	73.5	49.6	69.4
100	101.3	79.2	66.1	82.2
150	81.6	84.3	72.8	79.6
Mean	92.2	80.6	61.6	
Grand Mean	78.1			
Significance (P-value): N	0.799		SED: N	14.46
Significance (P-value): S	0.062		SED: S	12.52
Significance (P-value): N*S	0.971		SED: N*S	25.04
CV%	45.3			

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Table 140: *Viable population densities of potato cyst nematodes (viable eggs g^{-1} soil) sampled from field plots (Lincolnshire site), pre-incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	22.6	19.4	19.1	20.4
50	17.4	22.6	16.9	19.0
100	17.6	12.9	25.2	18.6
150	23.8	23.5	22.8	23.3
Mean	20.4	19.6	21.0	
Grand Mean	20.3			
Significance (P-value): N	0.604		SED: N	3.86
Significance (P-value): S	0.915		SED: S	3.34
Significance (P-value): N*S	0.603		SED: N*S	6.68
CV%	46.5			

Table 141: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots (Lincolnshire site), pre-incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	64.0	68.4	68.8	67.1
50	51.7	56.5	63.3	57.2
100	61.0	55.9	61.9	59.6
150	59.3	61.0	62.5	60.9
Mean	59.0	60.5	64.1	
Grand Mean	61.2			
Significance (P-value): N	0.066		SED: N	3.67
Significance (P-value): S	0.263		SED: S	3.18
Significance (P-value): N*S	0.849		SED: N*S	6.36
CV%	14.7			

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Table 142: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Lincolnshire site), pre-incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	96.2	69.2	63.1	76.2
50	39.4	75.7	86.0	67.1
100	74.2	43.6	69.1	62.3
150	78.0	68.1	64.9	70.3
Mean	72.0	64.1	70.8	
Grand Mean	69.0			
Significance (P-value): N	0.666		SED: N	11.35
Significance (P-value): S	0.696		SED: S	9.83
Significance (P-value): N*S	0.096		SED: N*S	19.67
CV%	40.3			

Table 143: *Viable population densities of potato cyst nematodes (viable eggs g^{-1} soil) sampled from field plots (Lincolnshire site), 6 weeks post incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	11.0	22.9	23.2	19.0
50	23.5	24.6	10.4	19.5
100	17.4	22.5	20.9	20.3
150	26.8	22.2	25.3	24.8
Mean	19.7	23.1	20.0	
Grand Mean	20.9			
Significance (P-value): N	0.651		SED: N	5.02
Significance (P-value): S	0.690		SED: S	4.35
Significance (P-value): N*S	0.466		SED: N*S	8.70
CV%	58.9			

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Table 144: *The viability (%) of encysted potato cyst nematode eggs sampled from field plots (Lincolnshire site), 6 weeks post incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	59.2	59.9	55.9	58.3
50	65.6	70.0	54.3	63.3
100	59.3	64.3	63.9	62.5
150	69.9	66.2	66.4	67.5
Mean	63.5	65.1	60.1	
Grand Mean	62.9			
Significance (P-value): N	0.114		SED: N	3.64
Significance (P-value): S	0.292		SED: S	3.15
Significance (P-value): N*S	0.475		SED: N*S	6.30
CV%	14.2			

Table 145: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from field plots (Lincolnshire site), 6 weeks post incorporation of* Brassica juncea *cv. Caliente 99. Field plots treated with contrasting proportions of nitrogen and sulphur at planting*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	41.6	65.5	69.5	58.9
50	60.4	77.5	36.0	58.0
100	72.0	52.9	64.5	63.2
150	83.8	69.8	65.7	73.1
Mean	64.5	66.5	58.9	
Grand Mean	63.3			
Significance (P-value): N	0.552		SED: N	9.42
Significance (P-value): S	0.743		SED: S	8.16
Significance (P-value): N*S	0.289		SED: N*S	16.31
CV%	45			

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Table 146: *Fresh weight of leaves (t ha^{-1}) sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05).*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	13.09	18.62	13.46	15.06a
50	20.78	18.41	18.84	19.35b
100	20.91	21.48	21.11	21.17b
150	19.93	22.76	23.00	21.89b
Mean	18.68	20.32	19.10	
Grand Mean	19.37			
Significance (P-value): N	<0.001		SED: N	1.445
Significance (P-value): S	0.406		SED: S	1.252
Significance (P-value): N*S	0.333		SED: N*S	2.504
CV%	18.3			

Table 147: *Dry weight of leaves (t ha^{-1}) sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	1.073	1.055	1.052	1.060a
50	2.207	1.752	1.892	1.951b
100	2.058	2.178	2.058	2.098b
150	1.887	2.290	2.227	2.135b
Mean	1.806	1.819	1.807	
Grand Mean	1.811			
Significance (P-value): N	<0.001		SED: N	0.1547
Significance (P-value): S	0.995		SED: S	0.1340
Significance (P-value): N*S	0.452		SED: N*S	0.2680
CV%	20.9			

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Table 148: *Fresh weight of stems (t ha^{-1}) sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	11.66	13.03	11.00	11.90a
50	16.49	16.10	15.83	16.14b
100	17.64	16.94	18.59	17.73b
150	17.35	19.79	19.65	18.93b
Mean	15.79	16.46	16.27	
Grand Mean	16.17			
Significance (P-value): N	<0.001		SED: N	2.963
Significance (P-value): S	0.859		SED: S	2.566
Significance (P-value): N*S	0.910		SED: N*S	5.132
CV%	22.1			

Table 149: *Dry weight of stems (t ha^{-1}) sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	1.061	1.080	1.340	1.160a
50	1.705	1.683	1.678	1.688b
100	1.863	1.705	1.998	1.855b
150	1.855	2.127	2.055	2.013b
Mean	1.621	1.649	1.768	
Grand Mean	1.679			
Significance (P-value): N	<0.001		SED: N	0.1579
Significance (P-value): S	0.530		SED: S	0.1368
Significance (P-value): N*S	0.892		SED: N*S	0.2735
CV%	23			

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Table 150: *Fresh weight of roots (t ha^{-1}) sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	1.049	0.898	0.752	0.900a
50	0.990	1.170	1.147	1.102a
100	1.471	2.129	1.492	1.697b
150	1.495	2.191	1.525	1.737b
Mean	1.251	1.597	1.229	
Grand Mean	1.359			
Significance (P-value): N	<0.001		SED: N	0.1980
Significance (P-value): S	0.069		SED: S	0.1714
Significance (P-value): N*S	0.517		SED: N*S	0.3429
CV%	35.7			

Table 151: *Dry weight of roots (t ha^{-1}) sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	0.0970	0.1173	0.1100	0.0864
50	0.0970	0.1173	0.1100	0.1081
100	0.1480	0.2087	0.1712	0.1760
150	0.2305	0.2191	0.1525	0.2007
Mean	0.1410	0.1550	0.1325	
Grand Mean	0.1428			
Significance (P-value): N	<0.001		SED: N	0.0223
Significance (P-value): S	0.509		SED: S	0.0193
Significance (P-value): N*S	0.393		SED: N*S	0.0386
CV%	38.3			

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Table 152: *Fresh weight of foliage (t ha^{-1}) sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP. Means with same letter are not significantly different according to Tukeys multiple range test (P = 0.05)*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	24.75	31.64	24.46	26.95a
50	37.27	34.51	34.68	35.49b
100	38.55	38.42	39.70	38.89b
150	37.27	42.55	42.65	40.83b
Mean	34.46	36.78	35.37	
Grand Mean	35.54			
Significance (P-value): N	<0.001		SED: N	2.701
Significance (P-value): S	0.612		SED: S	2.339
Significance (P-value): N*S	0.642		SED: N*S	4.679
CV%	18.6			

Table 153: *Dry weight (t ha^{-1}) of foliage sampled from brassica cover crops from field experiment 2016 (Lincolnshire site) 10 WAP.*

	Sulphur kg/ha			
Nitrogen kg/ha	0	25	50	Mean
0	2.13	2.14	2.39	2.22
50	3.91	3.44	3.57	3.64
100	3.92	3.88	4.06	3.95
150	3.74	4.42	4.28	4.15
Mean	3.43	3.47	3.58	
Grand Mean	3.49			
Significance (P-value): N	<0.001		SED: N	0.293
Significance (P-value): S	0.835		SED: S	0.254
Significance (P-value): N*S	0.800		SED: N*S	0.508
CV%	20.6			

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1.6 Rates of decline

Shropshire site:

The number of viable eggs g^{-1} soil and viable eggs per cyst was found to be significantly lower in treated nematicide plots (P<0.05) (Tables 154 and 156 respectively), however no significant difference between biofumigation treatments was seen. There was no difference in % egg viability between the treatments (Table 155).

Table 154: *Viable population densities of potato cyst nematodes (viable eggs g $^{-1}$ soil) sampled from previous field plots (field experiment 2014-15) of* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or fallow, treated or untreated with the nematicide Vydate (oxamyl)– Shropshire site. Samples collected in October 2016. Values shown were transformed using square root. Values in parentheses are the actual values.*

Biofumigant	No Vydate	Vydate	Mean
Untreated	9.88 (102)	7.80 (62)	8.84 (82)
Caliente 99 (*Brassica juncea*)	16.15 (356)	10.38 (113)	13.26 (235)
Scala (*Brassica juncea*)	9.94 (106)	12.60 (190)	11.27 (148)
Ida Gold (*Sinapis alba*)	12.65 (167)	9.53 (109)	11.09 (138)
Bento (*Raphanus sativus*)	13.30 (197)	10.28 (110)	11.79 (153)
Nemat (*Eruca sativa*)	18.90 (376)	12.80 (203)	15.85 (290)
Mean	13.47 (217)	10.56 (131)	
Grand Mean	12.02 (174)		
Significance (P-value): Biofumigant (B)	0.091	**SED: Biofumigant**	2.333
Significance (P-value): Nematicide (N)	0.036	**SED: Nematicide**	1.347
Significance (P-value):B*N	0.477	**SED: B*N**	3.299
CV%	43.4		

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Table 155: *The viability (%) of encysted potato cyst nematode eggs sampled from previous field plots (field experiment 2014-15) of* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or fallow, treated or untreated with the nematicide Vydate (oxamyl)– Shropshire site. Samples collected in October 2016. Values shown were transformed using ArcSin. Values in parentheses are the actual values.*

Biofumigant	No Vydate	Vydate	Mean
Untreated	86.79 (99)	86.73 (99)	86.76 (99)
Caliente 99 (*Brassica juncea*)	85.86 (99)	86.27 (99)	86.06 (99)
Scala (*Brassica juncea*)	83.09 (98)	86.07 (99)	84.58 (99)
Ida Gold (*Sinapis alba*)	85.16 (99)	82.08 (97)	83.62 (98)
Bento (*Raphanus sativus*)	84.52 (98)	83.54 (98)	84.03 (98)
Nemat (*Eruca sativa*)	87.80 (100)	82.85 (98)	85.33 (99)
Mean	85.54 (99)	84.59 (98)	
Grand Mean	85.06 (99)		
Significance (P-value): Biofumigant (B)	0.680	**SED: Biofumigant**	10
Significance (P-value): Nematicide (N)	0.453	**SED: Nematicide**	30
Significance (P-value):B*N	0.537	**SED: B*N**	5
CV%	5.7		

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Table 156: *The number of viable potato cyst nematode eggs cyst^{-1} sampled from previous field plots (field experiment 2014-15) of* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or fallow, treated or untreated with the nematicide Vydate (oxamyl)– Shropshire site. Samples collected in October 2016..*

Biofumigant	No Vydate	Vydate	Mean
Untreated	82.0	74.0	78.0
Caliente 99 (*Brassica juncea*)	156.8	84.4	120.6
Scala (*Brassica juncea*)	98.0	122.6	110.3
Ida Gold (*Sinapis alba*)	120.8	83.4	102.1
Bento (*Raphanus sativus*)	107.8	81.6	94.7
Nemat (*Eruca sativa*)	179.2	113.8	146.5
Mean	124.1	93.3	
Grand Mean	108.7		
Significance (P-value): Biofumigant (B)	0.130	**SED: Biofumigant**	24.66
Significance (P-value): Nematicide (N)	0.036	**SED: Nematicide**	14.24
Significance (P-value):B*N	0.383	**SED: B*N**	34.87
CV%	50.7		

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Lincolnshire site:

A decline in viable eggs g^{-1} soil was seen in the previously treated nematicide plots (P<0.05) (Table 157), however no significant difference between biofumigation treatments was seen.

Table 157: *Viable population densities of potato cyst nematodes (viable eggs g^{-1} soil) sampled from previous field plots (field experiment 2014-15) of* Brassica juncea *cv. Caliente 99,* Brassica juncea *cv. Scala*, Sinapis alba *cv. Ida Gold,* Raphanus sativus *cv. Bento,* Eruca sativa *cv. Nemat or fallow, treated or untreated with the nematicide Nemathorin (fosthiazate) – Lincolnshire site. Samples collected in August 2016.*

Biofumigant	No Nemathorin	Nemathorin	Mean
Untreated	31.4	8.6	20.0
Caliente 99 (*Brassica juncea*)	19.2	13.4	16.3
Scala (*Brassica juncea*)	17.6	10.0	13.8
Ida Gold (*Sinapis alba*)	23.8	16.8	20.3
Bento (*Raphanus sativus*)	41.6	9.6	25.6
Nemat (*Eruca sativa*)	24.0	14.2	19.1
Mean	26.3	12.1	
Grand Mean	19.2		
Significance (P-value): Biofumigant (B)	0.935	**SED: Biofumigant**	11.33
Significance (P-value): Nematicide (N)	0.029	**SED: Nematicide**	6.11
Significance (P-value):B*N	0.762	**SED: B*N**	15.50
CV%	123.3		

1.7 Estimates for the financial cost of establishing and incorporating a biofumigant crop

The cost of the different operations required to grow and incorporate a biofumigant crop can vary considerably between units. The costs shown are based on contractor charges, which tend to be less variable and might apply to companies that have not factored in the commitment to a biofumigant crop and would be reliant on contractors to fill in the gaps. Therefore, growers and farmer-contractors may cover overheads and

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machinery within the farming business, resulting in potentially lower costs than contractor charges.

Seed

Seed cost per hectare will vary not just with variety and rate, but with the different species; radish having a higher rate (kg/ha) than mustards. (The costs given are for true biofumigant crops, not cover crops).

Insecticides

Observations over nine years have shown that a good biofumigant crop is unlikely to require an insecticide. Turnip sawfly has been the only intermittent problem and a 'hot' mustard is only ever a temporary host.

Variable Inputs

		Low £/ha	Average £/ha	High £/ha		Minimal	Highest (includes radish)
Seed	8.5 – 10kg/ha	65	75	82		55	135
P K	0	0	0	0		-	
Insecticides		0	0	7.5		-	
Nitrogen	100kg	66	66	66		66	66
(Sulphur)	(20kg)	--	(20)	(20)		--	(20)
Cultivations							
	Flat lift	30	36	40		-	40
	Drilling (direct)	36	40	52		36	52
	Flailing	72	80	85		72	85
	Rotovating	80	85	88		80	88
	Rolling	17	20	22		-	22
Applications	Sprays	0	0	12		-	12
	Fertiliser	9	12	15		9	12
		£409	£448	£503.5		£322	£546
+ Sulphur			£468	£523.5			£566

The 'Minimal' cost includes reduced nitrogen, allowing for a perceived 30kg residual from a previous crop. Where additional sulphur is required, Sulphate of Ammonia might be used in conjunction with Ammonium Nitrate.

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1.8 Estimating the carbon footprint associated with biofumigation

a. Potentially, the crop can be grown for as short a period as eight weeks. This could probably keep fertiliser induced field emissions to less than 500 $kgCO_2$ e/ha
b. Other factors such as nitrogen are highly variable depending on source. For example, that produced using energy from hydroelectric compared to Russian gas.

   At the lowest, t CO_2-eqv per t N = 3.3. Therefore, 100kgN results in $kgCO_2$ e/ha = 330

c. Estimated fuel use/ha = 55 litres. This is obviously dependent on HP of the tractors used but assumes a modern 200HP.
d. Importantly, there is considerable evidence that the nitrogen used is captured and held by the fast growing mustards making it available for following crops. This, presumably, negates the nitrogen-manufacture carbon cost directly associated with the biofumigant.

5. DISCUSSION

The GB potato industry regard potato cyst nematodes (PCN) (*Globodera pallida* and *G. rostochiensis*) as intractable pests of high economic importance. Industry attention is fully justified on account of the uncertainty around nematicides and the lack of resistance available in widely grown cultivars. For instance, only two of the top 10 most planted varieties (AHDB, 2016) have partial resistance (3) to *G. pallida.* This species was found in 95% of sites that tested positive for PCN in a survey of England & Wales 2014-2017 (Dybal *et al*., unpublished). Management of PCN relies on using multiple crop protection strategies including, hygiene, legislation, variety choice, chemical control (nematicides) and rotation. Control needs to be both short term (protection of yield) and long term (preventing the elevation of population densities in soil), and applied throughout the rotation.

The use of biofumigation for the management of soil-borne pests and pathogens is of interest worldwide as evidenced by the '6th International Symposium of Biofumigation' held in Stellenbosch, South Africa on the 24-27th July 2016. At this meeting, a common theme was the need to understand the benefits of biofumigation from a holistic farming systems perspective (Kirkegaard, 2016). In the UK, there is a growing shift towards growing cover crops, including biofumigants, under the Ecological Focus Area associated with the Basic Payment Scheme. Biofumigation has previously been shown to be effective at reducing potato cyst nematodes under controlled conditions (Buskov *et al*., 2002; Lord *et al*., 2011; Ngala *et al*., 2015; Wood *et al*., 2017) and in the field (Ngala *et al*., 2014; Watts *et al*., 2015). The aim of this project was to assess the use of biofumigation for the management of potato cyst nematodes in GB potatoes and develop recommendations for its optimal use. Specific areas of investigation have included species/cultivar choice, method of maceration & incorporation and nutrient inputs (nitrogen and sulphur). The results of our experiments have shown that biofumigation can reduce the viability of PCN eggs in agreement with the work conducted by others (E.g. Lord *et al*., 2011; Ngala *et al*., 2014; Watts *et al*., 2015; Wood *et al*., 2017). However, PCN suppression was not consistent between experimental sites and different seasons.

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5.1 Polytunnel experiment conducted at Harper Adams University in 2013

In the poly-tunnel experiment, biofumigants had a similar suppressive effect towards PCN but there were no differences between the species and cultivars tested. This is surprising given the contrasting glucosinolate profiles associated with these species (Rosa *et al*., 1997) (Table 16). Lord *et al*. (2011) showed that *B. juncea* lines containing high concentrations of 2-propenyl GSL were the most effective against PCN, causing 95% mortality of the encysted eggs of *G. pallida*. Further repetition of the study is required to support the results observed.

5.2 Field experiments to compare the biofumigant potential of Brassica species - 2013-14

The results of the field experiments conducted in 2013-14 were inconclusive due to the later than expected planting dates. Due to shorter day length and lower temperature, the biomass recorded in the experiments is generally much lower than reported elsewhere. For instance, *B. juncea* (Caliente 99) was found to produce 1.03 and 1.06 t/ha dry matter in field experiments conducted in Staffordshire and Lincolnshire respectively. However, in three years of field experiments conducted in France, *B. juncea* dry matter was approximately 2.5 -4 t/ha (Motisi *et al*., (2009).

5.3 Field experiments to compare the biofumigant potential of Brassica species - 2014-15

In 2014-15, a series of field experiments were conducted in separate locations (Lincolnshire, Aberdeenshire and Shropshire) to assess the performance of biofumigant species. At the site in Crudgington (Shropshire), a reduction in viable PCN eggs g^{-1} soil, eggs $cyst^{-1}$ and percentage egg viability were recorded at the site in Crudgington, Shropshire when assessments made pre-incorporation onwards were considered together, although there were different outcomes depending on the method of assessment used. A significant reduction in viable PCN eggs g^{-1} soil was caused by *B. juncea* cv. Scala and *R. sativus* cv. Bento whereas *E. sativa* cv. Nemat caused a reduction in percentage egg viability. Each method of assessment has its advantages and disadvantages. For instance the number of viable eggs g^{-1} soil takes into account any eggs that may have decomposed but is subject to considerable variation due to inconsistencies that occur at each point of sampling. Devine *et al*. (1999) recommends expressing results on the basis of eggs per cyst to overcome this issue, particularly when monitoring PCN decline. In our study, *R. sativus* cv. Bento was found to cause a significant reduction in the viable number of eggs $cyst^{-1}$ when the assessments made pre-incorporation onwards were considered in the same analysis.

At Crudgington, differences were observed between the fresh weights of brassica biomass assessed 12 WAP. *Eruca sativa* cv. Nemat and *R. sativus* cv. Bento produced the highest leaf and root biomass (fresh weight). Whilst biomass is not the only factor affecting biofumigation success (Kirkegaard & Sawar, 1998), it may have contributed to the reduction in PCN observed for these lines at this site. During this reporting period, we did not receive results on glucosinolate analysis from the University of York. Since glucosinolates are an integral component of the biofumigation process, such data may facilitate further interpretation of the present results.

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At the Scottish site in Aberdeenshire (2014-15), there was a general reduction in all treatments in PCN eggs g^{-1} soil. However, the reduction in the *B. juncea* cv. Caliente 99 plots was statistically significant at a 64% reduction between pre-biofumigant and post-incorporation, compared to a 20% reduction in the untreated fallow plots. The other *B. juncea* cultivar in the trial - cv. Scala – performed no better than the untreated fallow plots in terms of reducing PCN eggs g^{-1} soil. When mean number of viable potato cyst nematode eggs $cyst^{-1}$ was assessed, as suggested by Devine *et al* (1999), all treatments (except *B. juncea* cv. Scala) had a significant effect when comparing counts pre-biofumigant and post-incorporation. However, the Untreated fallow plots also reduced the number of eggs $cyst^{-1}$, but only by 17%. *B. juncea* cv. Caliente 99 reduced nematode eggs $cyst^{-1}$ by 63%. Whilst the other treatments did not statistically differ from the untreated fallow plots, they did have higher levels of reduction in number of eggs $cyst^{-1}$: 28% (*B. juncea* cv. Scala); 41% (*S. alba* cv. Ida Gold) and 43% (*R. sativus* cv. Bento). It should be noted that there were similar reductions in percentage of viable eggs across all treatments including the untreated fallow plots. Consequently the positive impact of *B. juncea* cv. Caliente 99 is not specifically on the percentage viability of the eggs, but more on the numbers of viable eggs, which is demonstrated in the significant reductions in nematode eggs $cyst^{-1}$ and subsequent eggs g^{-1} soil.

At the Scottish site, the most effective brassica crop in terms of reducing PCN was *B. juncea* cv. Caliente 99 despite having the lowest fresh and dry weight biomass out of all the treatments. Unfortunately the samples for glucosinolate analysis were held up in transit to York and subsequently were unusable, so we are unable to determine whether the *B. juncea* cv. Caliente 99 in Scotland had relatively high glucosinolate levels, despite having relatively low dry weight biomass - < 1 t ha^{-1}.

5.4 Field experiments investigating crop maceration and incorporation methods - 2014-15

In 2015, biofumigation with *B. juncea* cv Caliente 99 did not cause a significant reduction in potato cyst nematodes (viable eggs g^{-1} soil, percentage viability or viable eggs $cyst^{-1}$) at the site in Shropshire. The cultivation of *B. juncea* during the winter months, with lower UV light, solar radiation and temperature, is likely to have resulted in a lower accumulation of glucosinolates (GSLs). Although this argument needs to be qualified with GSL analysis of preserved tissue samples, there is evidence from the literature to support this hypothesis. For example, Ngala *et al*. (2014) also found that PCN populations were unaffected when biofumigant plants were grown from September until April. Furthermore, glucosinolate concentration was much lower when *B. juncea* was cultivated from September to April as opposed to July to November. Björkman *et al.* (2011) describes how light (intensity, duration and quality) and temperature can affect glucosinolate concentration. Biofumigants grown during autumn and winter are typically exposed to lower temperatures, shorter day length, lower light intensity and greater availability of soil moisture, which reduces total glucosinolate concentration (Björkman *et al*., 2011). Whilst conditions at the Shropshire site were generally mild, the majority of these factors have to be taken into account.

Although the experiment undertaken in Shropshire did not show any effects of biofumigation using different incorporation procedures, other work conducted at Harper Adams University has provided useful information on the maceration and incorporation of biofumigants in relation to PCN. Watts *et al*. (2015) evaluated maceration using a V-

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tine flail topper or roll conditioner in combination with an array of cultivation strategies (spading, rotovating and ploughing), and found that incorporation implement did not affect the performance of biofumigation in the suppression of PCN. However, using the V-tine flail topper, rather than a roll conditioner, resulted in a significantly higher reduction of potato cyst nematode viability.

The trial undertaken at Fife was unable to compare the full range of different incorporation strategies due to the unavailability of a Spader. However, there was no difference in viable eggs g^{-1} soil, percentage viability or viable eggs $cyst^{-1}$ between the experimental treatments at any of the sampling dates.

The results from the Lincolnshire trial site were disappointing, particularly as the crop biomass was representative of what can be achieved in this area when irrigation is possible. There may be three contributing factors; rapid crop growth and development, no pest or disease pressure, a poor sealing soil type. A rapidly growing crop may not accumulate sufficient glucosinolates for the purpose of biofumigation and without disease or pest pressure, the levels will not be stimulated. Lastly, and perhaps most importantly, the sandy soil did not seal well and the smell of the isothiocyanates was very noticeable after incorporation. Although the soil was moist at the time of incorporation, it may be that irrigating post incorporation is a consideration for these soil types.

5.5 Field experiments investigating nitrogen and sulphur inputs on biofumigation efficacy of Indian mustard (*Brassica juncea*) cv. Caliente 99 - 2016

The field experiments conducted in 2016 evaluated contrasting nitrogen (N) and sulphur (S) on *B. juncea* biomass, glucosinolate accumulation, and most importantly, the suppression of PCN. All three experiments demonstrated the response of *B. juncea* to nitrogen inputs - typically 100-150 kg/ha produced the greatest biomass. This is similar to the recommendation given by Tozers Seeds Ltd, the UK distributor of Caliente 99/199. Tozers Seeds (Tozers Seeds, 2017) recommend applying N at a rate of 120-140 kg/ha, depending on soil type and previous cropping. Clearly it is important to consider the soil nitrogen supply (soil mineral N) when making decisions on N application. Increasing doses of S significantly increased the fresh weight of leaves, with and without N inputs, at the site in Aberdeenshire. However, *B. juncea* biomass was generally unaffected by S inputs at the sites in Lincolnshire or Shropshire.

Whilst biomass is important, it is not the only factor that affects biofumigation success. Glucosinolate accumulation (data not available at present) has an important bearing on the products of hydrolysis, particularly ITC production. Schonhof *et al*. (2007) examined N and S inputs in broccoli (*Brassica oleracea* var. *italica*) and found that glucosinolate concentration was higher with insufficient N or lower with insufficient S. More specifically, methionine derived glucosinolates (mainly glucoraphanin and glucobrassicin) were found to decrease with increasing N when S was applied at 10-20 kg/ha.

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Suppression of PCN in relation to the nutrient treatments applied to *B. juncea* was observed at the site in Shropshire with the assessment of % viable eggs at 6 weeks after biofumigant incorporation. Here N, S and the interaction between N and S had a significant effect on the viability of PCN. The results indicate that an N input of 100-150 kg/ha and an S input of 25 kg/ha provided the best control. In Lincolnshire, N and S inputs applied to *B. juncea* did not cause a reduction in PCN viability while the site in Aberdeenshire was not PCN infested.

5.6 Blemishing diseases, potato yield and PCN decline

Blemishing diseases, potato yield and PCN decline were unaffected by biofumigant treatments in this project. However, there were some indications that biofumigation may be suppressive against blemishing diseases (black scurf, silver scurf and black dot), particularly in the 2015-2016 experiments on maceration and incorporation (Lincolnshire and Shropshire). Previous work has demonstrated beneficial effects of biofumigation and isothiocynates on blemishing diseases/pathogens in the field/glasshouse (Larkin and Griffin, 2007) and under *in-vitro* conditions (Taylor et al., 2014) respectively.

6. Conclusions

- **Sowing date:** The date of sowing biofumigants has a large influence over their efficacy due to biomass production, glucosinolate accumulation and in some cases the soil temperature at the time of incorporation; lower soil temperatures may influence the volatility of isothiocynates and other hydrolysis products. In our field experiments Indian mustard (*B. juncea*) cv. Caliente 99 produced lower foliar biomass (E.g. 0.18 t FW ha at the site in Aberdeenshire in 2013) when sown later (end of August or early September). This was particularly evident in 2013-14 (Shropshire, Aberdeenshire and Lincolnshire) and in 2015-16 (Shropshire – overwintered) when the experiments followed crops such as winter wheat. Interestingly, PCN suppression did not occur in these experiments. On this basis, biofumigants, such as *B. juncea*, need to be sown in early summer i.e. June or July. In this respect, biofumigation is more suited to growers who produce winter barley, combining peas or oilseed rape.

- **Cultivar choice:** In the polytunnel experiment conducted at HAU, all of the biofumigant species and cultivars caused a reduction in the viability of PCN eggs. In this experiment, the biofumigants were incorporated at the same time, when the majority had reached 50% flowering. Future studies of this nature should consider macerating and incorporating each species/cultivar when they have reached early flowering (25-50% flowering). This may provide a fairer comparison as each species has reached a similar growth stage. A separate BBSRC HAPI project (BB/K020706/1 - Establishing biofumigation as a sustainable pesticide replacement for control of soil-borne pests and pathogens in potato and horticultural crops) has investigated glucosinolate accumulation in biofumigant species at different growth stages. Initial data confirmed peak GSL accumulation at the onset of flowering in species such as *B. juncea*.

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The field experiment conducted in Shropshire in 2014-15 provided some further evidence that Indian mustard (*B. juncea*) cv. Scala, oilseed radish (*Raphanus sativus*) cv. Bento and *Eruca sativa* cv. Nemat could reduce PCN viable eggs g^{-1} soil, viable eggs per cyst or % egg viability. However, at the site in Aberdeenshire, *B. juncea* cv. Caliente 99 plots caused a 64% reduction in PCN eggs g^{-1} soil between pre-biofumigant and post-incorporation whereas the cultivar Scala had no effect. It is difficult to compare glucosinolate profiles between species as the respective ITCs can vary in their toxicity. However, *in-vitro* studies conducted by Wood *et al*. (2017) have demonstrated the toxicity of allyl isothiocynate (2-propenyl ITC) on PCN under *in-vitro* conditions. Concentrations of 25-50 ppm caused 100% mortality of *G. pallida* juveniles while concentrations above 50 ppm caused a reduction in both hatch and egg viability.

- **Maceration and incorporation:** Unfortunately, it is not possible to make any recommendations on maceration/incorporation technique as PCN suppression was not observed in our study. However, studies conducted by Watts *et al*. (2015) indicate that machinery used for maceration is more important than the choice of incorporation method.

- **Nitrogen and sulphur inputs:** Data from the site in Shropshire indicates that S needs to be provided with N inputs to get the best suppression of PCN. This data indicated that biofumigation efficiency (reduction of PCN egg viability) is improved when *B. juncea* cv. Caliente 99 is provided with 100-150 kg/ha N and 25-50 kg/ha S at planting. However, this is a limited data set and further work is required to confirm the results. Additionally, supporting data on glucosinolate concentration would help support the finding.

- **PCN assessments:** Due to the patchy distribution of potato cyst nematodes, field experiments of this nature can be challenging even when experiments are blocked according to soil densities. Viable eggs g^{-1} soil is perhaps the most variable measurement. Eggs per cyst gives an indication of egg loss, while % viability provides a fairly comparative assessment of egg viability. For further experiments on biofumigation and PCN, it would be advisable to use buried 'cyst sachets' to reduce the inherent variation associated with soil sampling while providing a better estimate of eggs per cyst and % egg viability.

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